

# **Evaluation of Preservation Methods for Nutrient Species Collected by Automatic Samplers**

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## **ABSTRACT**

Automatic samplers are a common method of data collection for numerous monitoring projects in the South Florida region and elsewhere. Although total phosphorus (TP) is the primary parameter of interest within this region, nitrogen species such as ammonia nitrogen ( $\text{NH}_4\text{-N}$ ), nitrate+nitrite nitrogen ( $\text{NO}_x\text{-N}$ ), and total kjeldahl nitrogen (TKN) are also collected and analyzed. Federal and state quality assurance guidelines require nutrient samples to be preserved by acidification with  $\text{H}_2\text{SO}_4$  to a  $\text{pH}<2$  and stored immediately at  $4^\circ\text{C}$ . However, the remoteness of many sampling locations in South Florida makes it difficult to supply electricity for the refrigeration of samples collected by autosamplers. In addition, the use of propane-powered refrigerated autosamplers is a costly and ineffective solution in the South Florida climate. Consequently, while samples collected at these remote locations are routinely pre-preserved with acid, they are not cooled to  $4^\circ\text{C}$  for a period from one to seven days. This study evaluated if a statistically significant difference ( $\alpha=.05$ ) existed between concentrations of nitrogen species from a common source sample that was either: processed immediately; refrigerated to  $4^\circ\text{C}$  for seven days; or not refrigerated for seven days. In all cases, the collected sample was pre-preserved by adding 1ml of 50%  $\text{H}_2\text{SO}_4$  to each 1-liter discrete sample container before each 7 day testing period. Differences in concentrations of the calculated parameter total nitrogen (TN) were also investigated.

Analyses using the Wilcoxon Signed-Rank Test showed no significant differences among the three treatment groups for  $\text{NO}_x\text{-N}$ , TKN, TN and TP. Significant differences were observed when the  $\text{NH}_4\text{-N}$  samples that were processed immediately were paired with  $\text{NH}_4\text{-N}$  samples that were left unrefrigerated or refrigerated for seven days. Information from this study can be used by researchers and managers in evaluating the usefulness of nutrient water quality data that is collected when sample refrigeration is not available.

## INTRODUCTION

Standardized procedures for the preservation of nutrient water quality samples have been adopted by numerous environmental regulatory agencies. These procedures have undergone changes over the years and methods that were once thought imperative to preservation have since been revealed to provide no added benefit to sample integrity. Techniques intended to preserve water quality samples are not absolute and are limited in their ability to delay and control the biological and chemical changes that begin immediately after sample collection. However, methodologies used for the collection of water quality samples should ensure that no significant changes in sample composition occur prior to analysis (American Public Health Association, 1992). Several recommended techniques for the preservation of nutrient water quality samples are routine to monitoring programs, but these requirements can be difficult to implement under natural conditions and may restrict collection efforts in certain remote locations. The South Florida Water Management District (SFWMD) is continuously challenged with striking a balance between fulfilling these preservation requirements and complying with provisions of legally mandated sampling or research based objectives. With over 1350 active monitoring sites, the SFWMD has an extensive surface water quality monitoring network that spans a wide variety of ecosystems and field conditions. Factors such as site location, AC power availability, and budgetary considerations for employee time and instrumentation costs have prompted the SFWMD to undertake various special studies to determine if alternatives to sample processing and preservation requirements are possible. Viable solutions to accommodating substantial and diverse data needs, while still maintaining the highest standard of data quality, is the ultimate goal of these types of investigations.

The U.S. Environmental Protection Agency (EPA) and the U.S. Geological Survey (USGS) prescribe similar standard methods for the preservation of surface water samples. These preservation methods are based on the constituents to be analyzed and may include combinations of the following techniques: filtering of samples; the addition of biocides (e.g.  $\text{H}_2\text{SO}_4$ ); and the chilling of samples to  $4^\circ\text{C}$  (U.S. EPA, 1993; and USGS, 1999). The SFWMD incorporates these standards into a Comprehensive Quality Assurance Plan (CQAP) that is approved annually by the Florida Department of Health. The currently approved technique for the

preservation of samples to be analyzed for ammonia nitrogen ( $\text{NH}_4\text{-N}$ ), nitrate+nitrite nitrogen ( $\text{NO}_x\text{-N}$ ), total kjeldahl nitrogen (TKN) and total phosphorus (TP) requires acidification with  $\text{H}_2\text{SO}_4$  to a  $\text{pH} < 2$  and the immediate cooling of samples to at least  $4^\circ\text{C}$  (Florida Department of Environmental Protection, 1992; SFWMD, 1999). Surface water grab samples are preserved by these techniques unequivocally, but the cooling of discrete samples collected by automatic sampler can be delayed from 1 to 7 days. Samples collected by automatic sampler are pre-acidified with 1ml of 50%  $\text{H}_2\text{SO}_4$  (to every 1-liter discrete bottle), but the cooling of discrete samples does not take place until the sample bottles are collected out of the units and processed by a field technician. This method of pre-acidification without immediate refrigeration is acceptable to the Florida Department of Environmental Protection (FDEP) when TP is the only parameter to be analyzed and only if the samples are collected by an automatic sampler. Historically, samples collected by autosamplers with no available on-site refrigeration have also been analyzed for  $\text{NH}_4\text{-N}$ ,  $\text{NO}_x\text{-N}$  and TKN. This creates a discrepancy between the required protocol and the SFWMD's current collection method.

To address this issue, the SFWMD developed a validation study to assess the effects of non-refrigeration on samples analyzed for  $\text{NH}_4\text{-N}$ ,  $\text{NO}_x\text{-N}$ , and TKN. Total nitrogen (TN) concentrations, which are calculated from these parameters, were also investigated. Concentrations for TN were calculated by adding the corresponding mean values of TKN and  $\text{NO}_x\text{-N}$  for each test event at each study site. Total phosphorus concentrations also were analyzed to ensure that the approved methodology of pre-acidification without refrigeration was, in fact, achieving the quality standards desired. This study was designed with the support and cooperation of the FDEP.

### **Objectives**

To assess the validity of data from acidified, non-refrigerated samples, the following objectives were established:

- 1) To determine if concentrations of  $\text{NH}_4\text{-N}$ , TKN,  $\text{NO}_x\text{-N}$ , TN and TP differ significantly between samples cooled immediately to  $4^\circ\text{C}$  and samples left at ambient temperatures for seven days.

- 2) To statistically quantify the effectiveness of the current method of pre-acidification without on-site refrigeration in maintaining the integrity of these sample components;
- 3) To utilize the results of this study to update future monitoring protocols and preservation techniques so that they assure compliance with state and federal requirements (e.g. eliminate the collection of parameters that can not be collected without significant degradation; provide an alternative means of preservation; or move to amend the parameter lists for samples collected under legal mandate); and
- 4) Increase the validity of the SFWMD water quality database by reflecting the results of the study to either include or flag the period of record nitrogen data collected by a non-refrigerated methodology (flagged data is marked in the database with a caveat that it has not passed certain quality assurance criteria).

### **Preservation Method Studies**

Previous investigations into preservation methods for water quality nutrient collections have been diverse in their goals and methodologies. Extensive information regarding the suitability and effectiveness of acid preservation, sample holding times and freezing versus chilling of nutrient samples has been documented. The work of Patton and Truitt (1995) was responsible for changing the long-standing method of adding mercury (II) chloride to samples collected for nutrient analysis, to the now widely accepted use of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). The findings of Patton and Gilroy (1998) were critical in assessing holding time constraints on samples for nutrient determinations, and also validated concerns over the acidification of samples to be analyzed for nitrite. Several studies have addressed the need of refrigeration versus freezing as a method of preservation (Philbert, 1973; Thayer, 1970), but very limited information is available on studies dealing with no refrigeration as an option in method comparisons.

The reliance on chilling or freezing of all types of water quality samples has been an intrinsic component to preservation methodology and the option of not immediately chilling samples analyzed for nutrient components has been tested only on a limited basis. Fishman et al. (1986) evaluated preservation methods on nutrient samples and concluded that samples left at ambient temperatures for 16 days did not maintain stability with respect to nitrogen and phosphorus species. These samples were not preserved with any acid

treatments. A similar study was developed specifically for nutrient samples collected by automated samplers. Kotlash and Chessman (1998) found that variability among treatments was dependent on the initial concentration of the nutrient sampled, as well as the method of preservation. The non-refrigerated samples were analyzed after six days and were not preserved by acidification. The comparative studies reviewed differed from the conditions under which the SFWMD collects pre-acidified, unrefrigerated automatic samples that are analyzed for nutrients. Field holding times, the presence and/or type of acidification, and in some cases the sample characteristics, were not comparable to those present in our study.

A large amount of literature also is devoted to holding times as a factor in sample preservation. These studies specifically looked at preservation methods in relation to the period of time between sample collection and sample analysis (Harmonised Monitoring, 1984; Salley et al., 1986; Turtola, 1989). It should be understood that our study was not intended to evaluate recovery rates between sample retrieval and laboratory analysis. It is our assertion that once the samples are collected from the sampling units, the preservation techniques employed maintain a sufficient stability of the analytes until analyses are performed by the laboratory. The preservation techniques always include the addition of  $H_2SO_4$  to maintain a  $pH < 2$  and refrigeration to  $4^\circ C$ . The current maximum allowable laboratory holding time for all the parameters investigated is 28 days after the sample is field processed and placed at  $4^\circ C$ . The time between submittal to the lab and actual laboratory analysis was 3 to 5 days for all samples used in this study.

## **History**

The SFWMD has utilized automatic samplers to collect nutrient parameters since 1978 and this mode of sample collection is incorporated in the design of numerous monitoring projects. There are currently 60 automatic sampling units in operation within SFWMD boundaries and 48 of these units collect samples that are analyzed for at least one of the nitrogen species investigated in this study. These devices have proven to be a cost-effective way of obtaining representative water quality data over a set period of time. Autosampler use is also advantageous because the units supply a means of monitoring water quality on a nearly continual basis without employee presence and they can be programmed for specific monitoring needs such as flow

proportionality. Despite these advantages, autosamplers are expensive to purchase, can require high maintenance, and are limited by a site's logistical capacity to accommodate a sampling unit. Many SFWMD monitoring sites are in remote locations with no source of commercial power. Using rechargeable batteries and/or solar panels that are able to supply just enough energy to keep the sampling units powered has generally solved this obstacle. However, providing an on-site mechanism for the cooling of samples collected by autosampler has only been achieved on a limited basis. Attempts to instrument several remotely located autosamplers with propane powered refrigerators was abandoned after repeated maintenance and reliability problems. The success of any refrigerator, either electrical or propane, to maintain temperatures of 4°C when exposed to the extreme climate of South Florida, has also been sporadic. Due to these constraints, it has not been a feasible option to supply refrigeration to all of the sites that utilize autosamplers. Of the 60 automatic sampling units currently deployed, only 8 are equipped with refrigeration. All of these units are located at water control structures where appropriate shelter and power are available.

### **Current Autosampler Protocols**

To reap the potential employee-hour-reduction benefits of autosampler use, sample collection from all SFWMD autosampler units is performed on a weekly basis (i.e. every seven days) except for non-routine collections (e.g. mandated special event sampling at certain water control structures). The SFWMD uses automatic samplers to collect two primary types of samples: composite and discrete. Composite samples represent collections of aliquots into a 11 or 19 liter (3 or 5 gallon) jug that is housed in an on-site refrigerated unit. The autosamplers currently configured to collect composite samples are all connected to the operating pumps of certain structures. Composite samples are collected only at sites where power and storage are available for the refrigeration unit. Discrete samples are typically collected at sites where commercial power is not available or where daily concentrations are desired. Autosamplers deployed for discrete sampling contain up to 24, 1-liter, discrete sampling bottles. Each discrete bottle is pre-acidified with 1ml of 50% H<sub>2</sub>SO<sub>4</sub>. Units collecting discrete samples are programmed to collect samples on a fixed time interval or configured with a flow proportional trigger. The 1-liter discrete bottles containing samples are then composited into a single sample for analysis. All analyses are conducted by the SFWMD Water Chemistry

Laboratory in accordance with the analytical methods detailed in the CQAP (SFWMD, 1999). Discrete samples are also processed according to the CQAP, in all aspects except one: the samples prepared for analysis of NO<sub>x</sub>-N and NH<sub>4</sub>-N are not filtered because these samples have been pre-acidified and there are concerns about the safety of filtering samples containing acid. Theoretically, because NO<sub>x</sub>-N is an anion and due to the low acidity in the sample container, it should not be sorbed with any particulates. Also, samples historically collected within the South Florida system do not generally contain a significant amount of particulates so sorption problems with NH<sub>4</sub>-N would not be considered significant. The SFWMD addressed the filtration issue in a test project that showed no significant difference ( $\alpha = .05$ ) between concentrations of NO<sub>x</sub>-N and NH<sub>4</sub>-N in filtered versus un-filtered samples that had been pre-acidified to a pH<2 (Grosser, 1997).

## **METHODOLOGY**

### **Site Logistics**

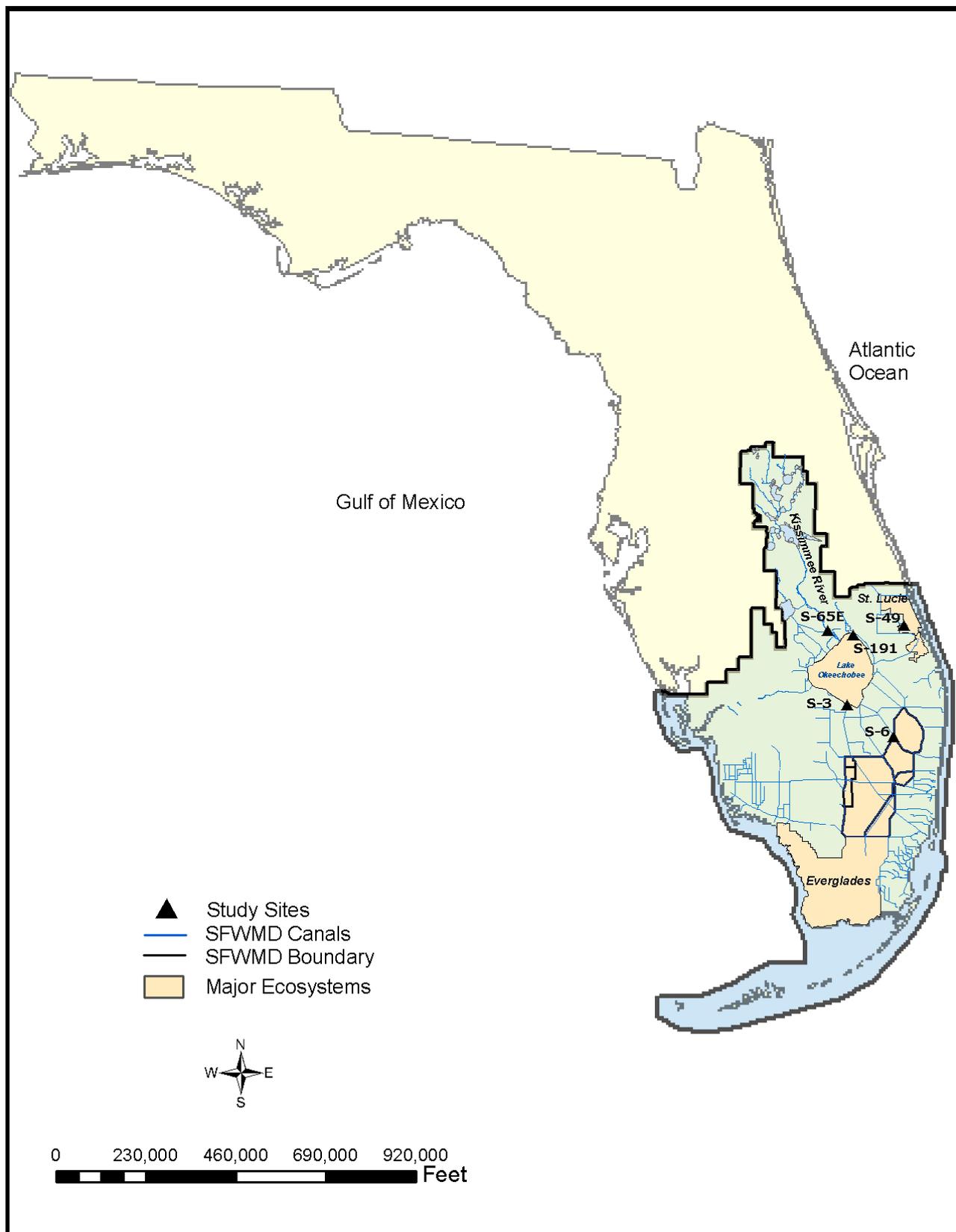
The FDEP and SFWMD agreed that a five-site sample design would be adequate to meet the statistical requirements of this study (Figure 1).

Selection Criteria: The following criteria were developed to select potential study sites:

- 1) Selected sites were established autosampler locations that are currently part of the monitoring network.  
This allowed for period of record data review and provided on-site existing equipment;
- 2) Sites were representative of the potential range of nutrient concentrations found historically within SFWMD boundaries; and
- 3) Potential sites had the space to accommodate a refrigerator and an additional autosampler housing compartment in a secure location that provided protection from vandalism and extreme weather such as lightning and high winds. An AC power source was also required to power the refrigerators.

### **Instrumentation**

Each site was equipped with an autosampler housing compartment (base) and a reliable refrigeration unit, as well as the established on-site functional autosampler unit. The functional autosamplers continued to be used



**Figure 1.** Study site locations within the South Florida Water Management District (SFWMD) area.

for their current monitoring projects and were taken off line only for the amount of time it took for the study samples to be collected. A maximum/minimum digital thermometer was placed in each refrigerator and in each autosampler-housing compartment to record the range of air temperatures experienced by both sets of samples over each seven-day exposure period. The thermometers were equipped with an outdoor sensor that was strung into the refrigerators, allowing for inside and outside (ambient) temperature recordings. Two microprocessor-based dataloggers that tracked air temperature over time were also used. The Thermasense™ units allowed air temperatures to be tracked at five minute intervals for the seven day period. These units were placed only inside the refrigerators and their use was rotated among the sites.

### **Sample Generation and Processing**

Whenever possible, one test event per month was performed at each site. Each test event lasted for seven days. Test events consisted of a sample generation (day one) and sample collection (day seven) component. During the sample generation, seven liters of water were collected from the established on-site autosampler. This volume of water was collected in a continuous manner and represented a single source sample. Six pre-acidified (1 ml of 50% H<sub>2</sub>SO<sub>4</sub>), one-liter discrete bottles were then filled with 800 milliliter portions of the main source sample. This was done immediately after the main sample was collected in order to prevent any settling of suspended solids. Two of the bottles were placed in the refrigerator units and cooled to 4°C and two bottles were placed in the non-functioning autosampler base compartment and left at ambient temperature. Both sets of bottles were left uncapped. The two remaining bottles were then processed, placed on ice and taken to the lab for analysis. The samples processed immediately represent a best case scenario for obtaining the most representative nutrient concentration values at the moment of sampling and were coded as “grab simulation” data. The refrigerated and non-refrigerated samples were left at the field sites for a period of seven days. This interval represents the current maximum time that discrete automatic samples would normally be left in the field without refrigeration. The sample collection component for each test event was performed seven days later. The non-refrigerated samples were retrieved from the autosampler housing compartments and processed first. The refrigerated samples were then taken from the refrigerator and processed. Sample processing for all three treatment groups was consistent with the current SFWMD

methods for discrete automatic sample collections (SFWMD, 1999). Air temperature readings were also recorded and the Thermasense™ dataloggers were brought back to the office and downloaded.

### **Quality Assurance / Quality Control**

Quality control (QC) samples were generated for each test event in accordance with the CQAP (SFWMD, 1999). The following QC samples were collected:

- a) 3 replicate regular test samples for each parameter/per test condition/per site
- b) 1 equipment blank for each parameter taken at the beginning of a day when sample generation occurred
- c) 1 spiked sample of known concentration for each parameter/per test condition
- d) 2 field blanks for each parameter / per refrigerated and non-refrigerated test condition

Quality assurance protocols dealing with data review were followed in accordance with the methods outlined in the CQAP (SFWMD, 1999). A Relative Percent Difference (RPD) formula was used to calculate the precision of the three replicate samples analyzed for each treatment group, during each test event:

$$\text{RSD} = \frac{\text{abs} ([A]-[B])}{[A]+[B]} \times 200 \quad \text{where A and B are the analytical concentrations for two of the replicates being compared}$$

RPD values were calculated among all three replicates to determine any outliers. Any RPD >40% resulted in the outlying value being excluded from the data set. Concentration data points reported at the minimum detection limit for that given parameter were also excluded from the data set.

The guideline of acceptability for the equipment and field blank samples was set at <2 times the minimum detection limit (MDL) for each nitrogen component. Any equipment blank >2 times the MDL, for any parameter, would result in the flagging of all samples for that event and the scheduling of a replacement test event at that site. Samples associated with field blanks from the same treatment group reading >2 times the MDL were also excluded from the data set. The target limit for accuracy of the “control” spiked samples was 90-110% of the known true value. Spiked samples falling outside of the 90-110% recovery range were not

flagged from the data set, but information relating to lab accuracy, analytical bias and treatment condition effects on known concentration values were derived from % Recovery statistics.

## **Data Analysis**

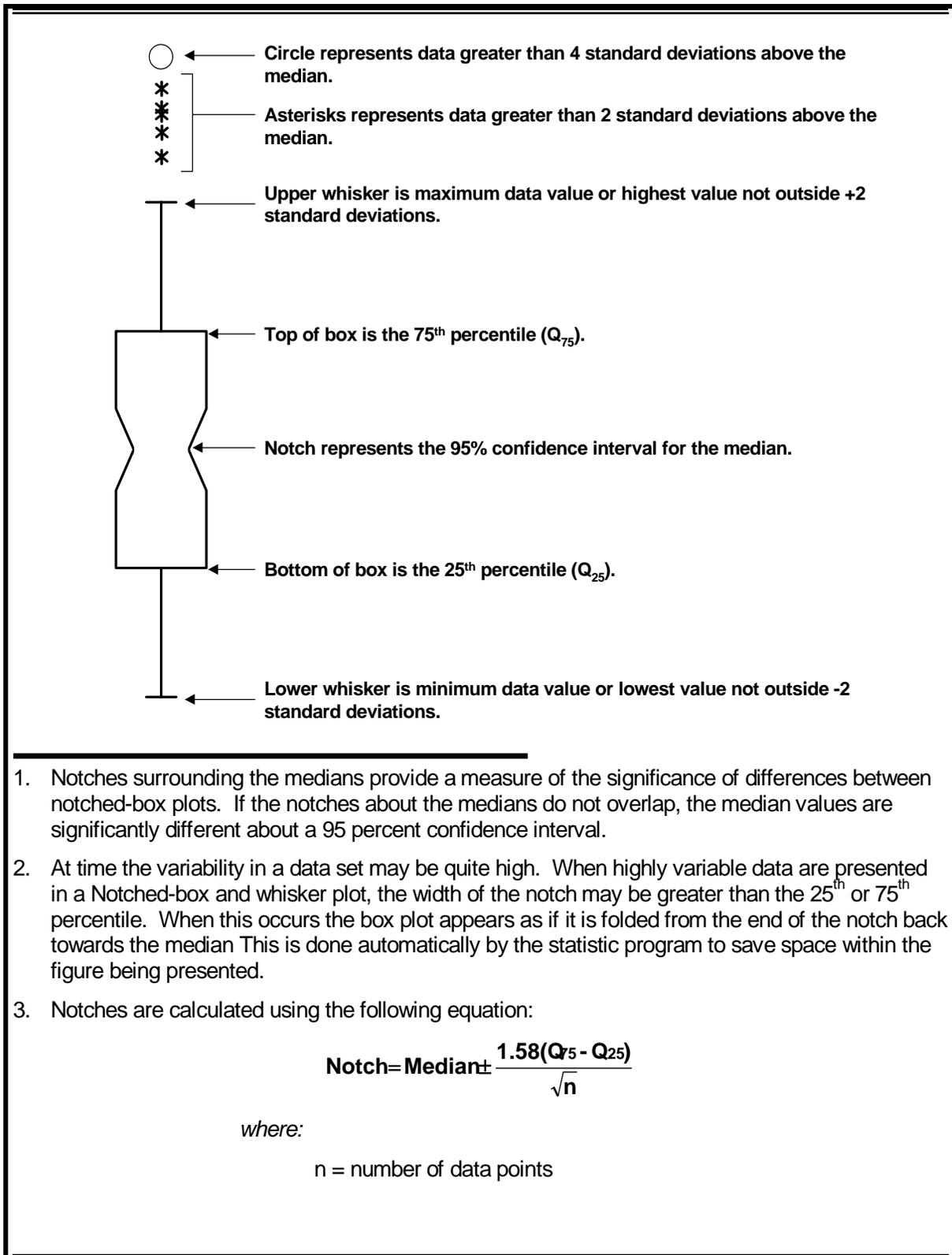
Test sample nutrient concentration data that met the QA/QC requirements of this study were tested for normality within each treatment condition. The Shapiro-Wilk's W-test, the Lillifor's test and the Kolmogorov-Smirnov tests for normal distribution were applied to the data set. The results indicated that the data were not normally distributed for any of the parameters ( $p < 0.0001$ ). Parameter specific notched-box and whisker plots were generated to show the general distribution of the entire data set between treatment groups (Figure 2). The replicate test samples for each specific test event, at each specific study site, were then averaged among their treatment groups and used for further data comparisons. This was done to eliminate the bias of pairing singular concentration values whose apparent specific relationship is really only a factor of how the data were reported and not how the samples were processed. The Wilcoxon Signed-Rank test was determined to be the most appropriate way to analyze the site specific pairings of the mean concentration values of each treatment group, for each test event. The Wilcoxon Signed-Rank test uses the sign and the magnitude of the rank of differences between specific pairs of measurements and was used to determine if the differences found between the mean values of each treatment group pairing were statistically significant from zero (0) (Ott, 1984). Statistical evaluations were performed using SAS and corroborated using Systat. All statistical analyses were evaluated at a 95% Confidence Level (C.L.). The following hypotheses were tested:

**H<sub>0</sub>** = The population distribution of differences between grab simulation, non-refrigerated, and refrigerated (to 4°C) test condition concentration values is symmetrical about zero (0) for all parameters (NH<sub>4</sub>-N, NO<sub>x</sub>, TKN, TN and TP).

**H<sub>A</sub>** = The population distribution of differences between grab simulation, non-refrigerated, and refrigerated (to 4°C) test condition concentration values is not symmetrical about zero (0).

**Level of Significance:**  $\alpha = 0.05$

The means of the test concentration data for each of the treatments were also plotted by linear regression analysis. The intercept and r values for relationships between treatments groups were calculated for each nutrient component.



**Figure 2.** Description of notched-box and whisker plot used for this study.

## RESULTS

Problems with power supply, scheduling and a malfunctioning security system resulted in missed test events at two of the sites. In an effort to ensure that the findings of this study could be applied to other SFWMD monitoring projects, and in order to represent the range of seasonal and environmental conditions that are experienced in this region, the study was continued for a longer period than originally planned. The study lasted for 14 months from October 1998 through November 1999, with a total of 65 test events being conducted among the five sites. This resulted in the determination of 2,196 environmental sample concentration ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_x$ , TKN and TP) data points among all three of the test treatments. A total of 71 data values (3.2%) were flagged after failing the quality assurance evaluations detailed previously. The breakdown of applied data points accumulated for each nutrient species is as follows:  $\text{NH}_4\text{-N}$  = 511,  $\text{NO}_x$  = 541, TKN = 547 and TP = 526. All data are presented in milligrams per litre (mg/l) for the specified compound form. Wide ranges of concentration levels for all nutrients were realized among the five study sites. The grab simulated concentrations of  $\text{NH}_4\text{-N}$  ranged from 0.009 mg/l to 0.791 mg/l (Table I) and TKN values had the largest range between the grab simulated and unrefrigerated groups (0.8 mg/l to 5.2 mg/l). The mean value for all  $\text{NH}_4\text{-N}$  concentrations generated from non-refrigerated samples was 12.5% greater than the average of grab simulated  $\text{NH}_4\text{-N}$  concentrations. All other parameters showed general statistical agreement between each of the three treatment groups.

Although the scope of this study did not include the collection of additional parameters at the time of sample collection, ancillary data including pH, dissolved oxygen, alkalinity and specific conductance were available for four of the five study sites (Table II). These data were collected during established routine monitoring visits to the sites. Since the data were not taken in conjunction with this study, they do not reflect the exact water quality conditions at the time samples for this study were collected. The data can however be used to characterize the general conditions present at these four study sites. The values coincide with the collection of data over the eleven month period during which this study was conducted (October 1998 – November 1999). Conductivity and alkalinity showed the greatest variation between the four study sites. Mean water quality values for dissolved oxygen, temperature and pH were similar at the four sites during the study period.

The overall distribution of the 2,125 concentrations points were compiled into notched-box and whisker plots for each parameter and compared against each treatment group (Figures 3 and 4). The distribution of ammonia samples exhibited the only visible differences among treatment groups. Individual concentrations of  $\text{NH}_4\text{-N}$  and TP had the greatest number of data points outside the range of 4 standard deviations above the median. The minimum concentration values for all four parameters (among all treatment groups) were within negative (-) 2 standard deviations below the median. The notched medians for TP and  $\text{NO}_x\text{-N}$  were positioned in almost identical locations on the plots for all three treatment groups.

Matched pairs of  $\text{NH}_4\text{-N}$  mean concentration values between grab simulated and unrefrigerated samples were significantly different (Wilcoxon signed-rank,  $p < 0.001$ ) for data collected at all five study sites (Table III). The presence of refrigeration for seven days was not an effective means of delaying changes to  $\text{NH}_4\text{-N}$  concentrations when compared to samples processed immediately ( $p < 0.001$ ). Comparisons of  $\text{NH}_4\text{-N}$  concentrations in samples kept under refrigerated conditions also did not compare well with samples left unrefrigerated for seven days ( $p < 0.001$ ). The pairing of  $\text{NO}_x$  concentrations from samples left unrefrigerated with those processed immediately resulted in no significant differences (SAS:  $p = 0.803$ ). There was a mere 1% chance that differences observed for  $\text{NO}_x$  concentrations in samples processed immediately with those refrigerated for seven days were real and not a coincidence of random sampling (SAS:  $p = 0.993$ ). Calculated TKN values resulted in acceptable agreement between unrefrigerated samples and grab simulation samples (SAS:  $p = 0.341$ ). TKN concentrations were most correlated for tests between refrigerated samples and samples processed immediately (SAS:  $p = 0.805$ ). The null hypothesis could not be rejected when concentrations of TN were compared between any of the three treatment groups. The exposure of samples to any of the three treatment conditions had no real effect on the matched pairs of TP concentrations. There was a 93% chance that the differences observed between TP concentrations analyzed from samples that had been left unrefrigerated with samples that had been processed immediately were real (SAS:  $p = 0.066$ ).

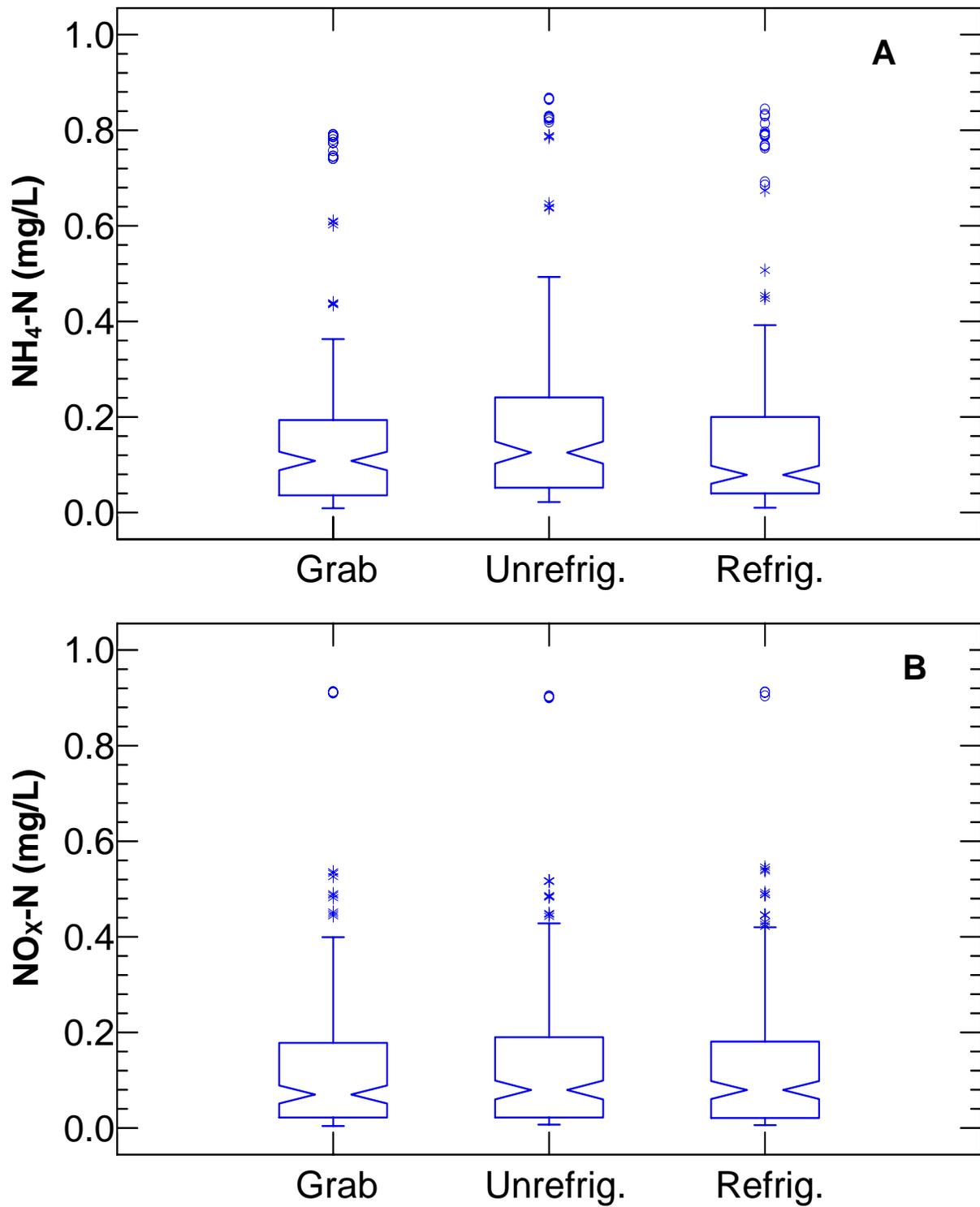
Linear regression analyses between each treatment group also were plotted from mean test event concentrations. Although significant differences were found in the Wilcoxon Signed-Rank analysis

Table I. General statistical characteristics of all nutrient concentrations (mg/l) collected among the three treatment groups.

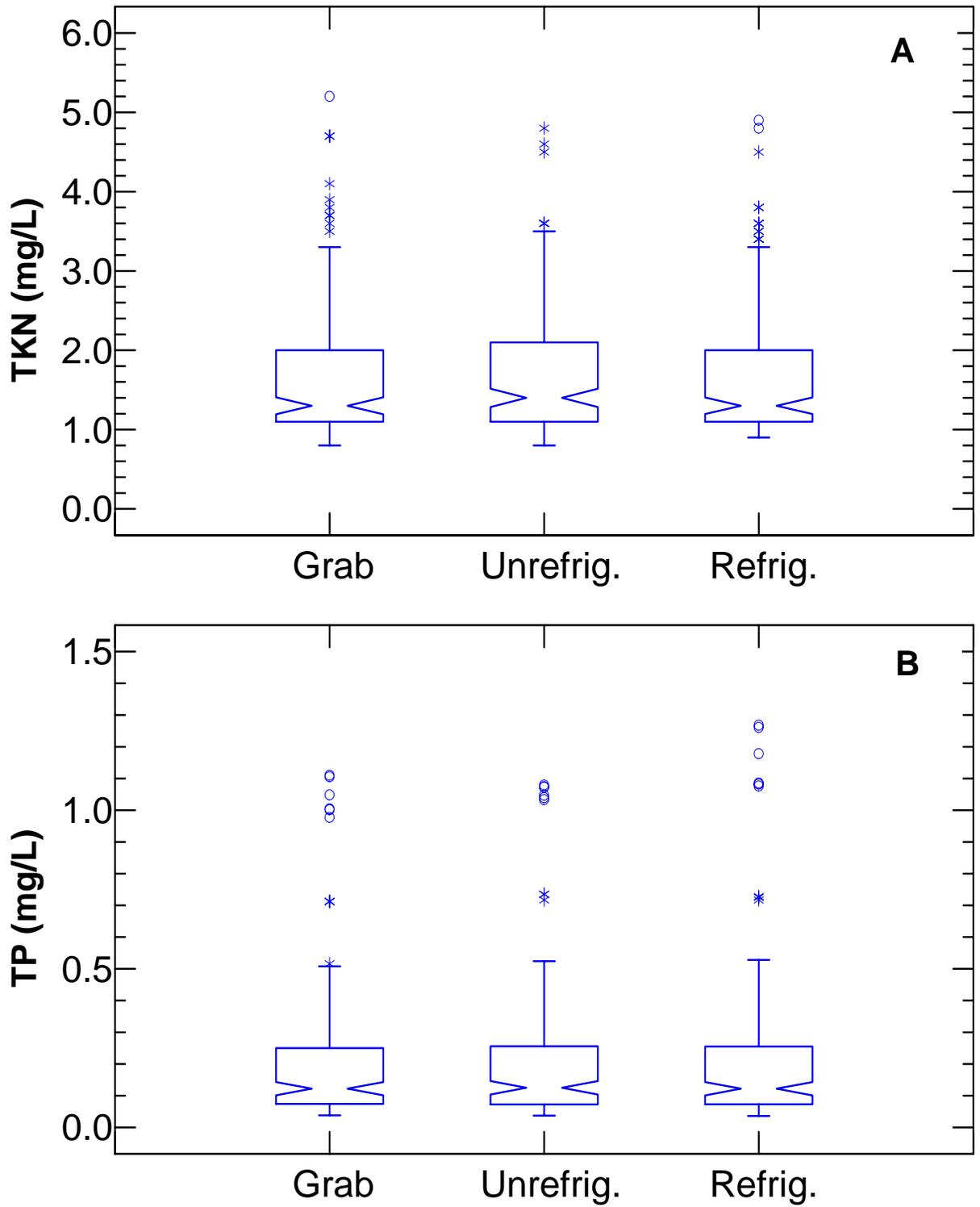
	n	Min.	Max.	Std. Dev.	Variance	Mean	Median	Skewness	MDL
<b>NH<sub>4</sub>-N</b>									<b>0.009 mg/l</b>
Grab Simulation	<b>164</b>	<b>0.009</b>	<b>0.791</b>	<b>0.203</b>	<b>0.042</b>	<b>0.169</b>	<b>0.108</b>	<b>1.979</b>	
Non-Refrigerated	<b>166</b>	<b>0.022</b>	<b>0.867</b>	<b>0.216</b>	<b>0.047</b>	<b>0.193</b>	<b>0.125</b>	<b>1.979</b>	
Refrigerated	<b>181</b>	<b>0.010</b>	<b>0.844</b>	<b>0.208</b>	<b>0.043</b>	<b>0.167</b>	<b>0.079</b>	<b>2.103</b>	
<b>NO<sub>x</sub></b>									<b>0.004 mg/l</b>
Grab Simulation	<b>174</b>	<b>0.004</b>	<b>1.063</b>	<b>0.205</b>	<b>0.042</b>	<b>0.153</b>	<b>0.072</b>	<b>2.598</b>	
Non-Refrigerated	<b>183</b>	<b>0.007</b>	<b>1.082</b>	<b>0.203</b>	<b>0.042</b>	<b>0.156</b>	<b>0.087</b>	<b>2.570</b>	
Refrigerated	<b>184</b>	<b>0.006</b>	<b>1.088</b>	<b>0.205</b>	<b>0.042</b>	<b>0.155</b>	<b>0.092</b>	<b>2.594</b>	
<b>TKN</b>									<b>0.5 mg/l</b>
Grab Simulation	<b>176</b>	<b>0.8</b>	<b>5.2</b>	<b>0.875</b>	<b>0.765</b>	<b>1.7</b>	<b>1.3</b>	<b>1.54</b>	
Non-Refrigerated	<b>186</b>	<b>0.8</b>	<b>4.8</b>	<b>0.837</b>	<b>0.701</b>	<b>1.7</b>	<b>1.4</b>	<b>1.44</b>	
Refrigerated	<b>185</b>	<b>0.9</b>	<b>4.9</b>	<b>0.838</b>	<b>0.702</b>	<b>1.7</b>	<b>1.3</b>	<b>1.58</b>	
<b>TP</b>									<b>0.004 mg/l</b>
Grab Simulation	<b>167</b>	<b>0.038</b>	<b>1.110</b>	<b>0.186</b>	<b>0.034</b>	<b>0.190</b>	<b>0.117</b>	<b>2.656</b>	
Non-Refrigerated	<b>179</b>	<b>0.037</b>	<b>1.079</b>	<b>0.184</b>	<b>0.034</b>	<b>0.195</b>	<b>0.121</b>	<b>2.483</b>	
Refrigerated	<b>180</b>	<b>0.036</b>	<b>1.085</b>	<b>0.184</b>	<b>0.034</b>	<b>0.194</b>	<b>0.122</b>	<b>2.514</b>	
<b>TN</b>									<b>0.5 mg/l</b>
Grab Simulation	<b>59</b>	<b>0.9</b>	<b>5.0</b>	<b>1.008</b>	<b>1.016</b>	<b>1.9</b>	<b>1.5</b>	<b>1.46</b>	
Non-Refrigerated	<b>62</b>	<b>1.0</b>	<b>4.8</b>	<b>0.968</b>	<b>0.937</b>	<b>1.9</b>	<b>1.5</b>	<b>1.47</b>	
Refrigerated	<b>62</b>	<b>1.0</b>	<b>4.8</b>	<b>0.965</b>	<b>0.932</b>	<b>1.9</b>	<b>1.5</b>	<b>1.53</b>	

Table II. Ancillary water quality parameters collected at S191, S3, S6 and S65E during routine monitoring conducted during the same time period as the study (October 1998 – November 1999).

	Conductivity (umhos/cm)	Temperature (°C )	Dissolved Oxygen (mg/l)	Alkalinity (mg/l)	pH (units)
<b>S191</b>					
n	<b>27</b>	<b>27</b>	<b>27</b>	<b>27</b>	<b>25</b>
Minimum Value	<b>227</b>	<b>17.23</b>	<b>1.72</b>	<b>29.23</b>	<b>5.31</b>
Maximum Value	<b>1436</b>	<b>29.75</b>	<b>8.87</b>	<b>106.80</b>	<b>7.90</b>
Mean	<b>676</b>	<b>24.98</b>	<b>5.15</b>	<b>71.27</b>	<b>6.97</b>
<b>S3</b>					
n	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>13</b>
Minimum Value	<b>416</b>	<b>21.68</b>	<b>2.42</b>	<b>102</b>	<b>6.12</b>
Maximum Value	<b>1390</b>	<b>32.01</b>	<b>7.54</b>	<b>320.4</b>	<b>8.00</b>
Mean	<b>854</b>	<b>25.46</b>	<b>4.39</b>	<b>201.54</b>	<b>7.37</b>
<b>S6</b>					
n	<b>65</b>	<b>65</b>	<b>62</b>	<b>20</b>	<b>63</b>
Minimum Value	<b>449</b>	<b>18.22</b>	<b>1.50</b>	<b>130.80</b>	<b>6.59</b>
Maximum Value	<b>1412</b>	<b>31.67</b>	<b>13.10</b>	<b>424.40</b>	<b>8.26</b>
Mean	<b>1069</b>	<b>25.01</b>	<b>4.36</b>	<b>324.53</b>	<b>7.45</b>
<b>S65E</b>					
n	<b>31</b>	<b>31</b>	<b>28</b>	<b>34</b>	<b>31</b>
Minimum Value	<b>114</b>	<b>19.29</b>	<b>0.24</b>	<b>17.50</b>	<b>5.34</b>
Maximum Value	<b>237</b>	<b>32.11</b>	<b>10.00</b>	<b>50.78</b>	<b>8.70</b>
Mean	<b>163</b>	<b>26.05</b>	<b>4.82</b>	<b>32.38</b>	<b>7.08</b>



**Figure 3.** Notched-box and whisker plots of all concentration values for Grab Simulated, Unrefrigerated and Refrigerated treated samples analyzed for (A) NH<sub>4</sub>-N and (B) NO<sub>x</sub>-N.



**Figure 4.** Notched-box and whisker plots of all concentration values for Grab Simulated, Unrefrigerated and Refrigerated treated samples analyzed for (A) TKN and (B) TP.

Table III. Statistical evaluation of the significance of paired-differences for the five parameters of interest using the Wilcoxon Signed-Rank test. The evaluation was performed using SAS and Systat with a Confidence Level of 95%. The test statistic for each program, as well as p-values are reported below.

Parameter	Comparison	No. of Samples Compared	Mean of Differences	SAS Analysis		Systat Analysis		Significant Difference (?)
				Sign Rank Stat	P-Value	Z-Stat	P-Value	
NH <sub>4</sub> -N	UnRefrg vs. Grab	52	0.028	679.5	<0.001	6.184	<0.001	Yes
	UnRefrg vs. Refrg	57	-0.016	-711.5	<0.001	-5.784	<0.001	Yes
	Grab vs. Refrg	57	0.012	604.5	<0.001	5.248	<0.001	Yes
NO <sub>x</sub> -N	UnRefrg vs. Grab	59	0.001	32.5	0.803	0.225	0.822	No
	UnRefrg vs. Refrg	62	<0.001	-55.5	0.596	-0.517	0.605	No
	Grab vs. Refrg	59	<0.001	-1.0	0.993	-0.034	0.973	No
TKN	UnRefrg vs. Grab	59	0.014	80.0	0.341	1.346	0.178	No
	UnRefrg vs. Refrg	62	-0.018	-64.0	0.245	-1.525	0.127	No
	Grab vs. Refrg	59	-0.007	-18.0	0.805	-0.183	0.855	No
TN	UnRefrg vs. Grab	59	0.012	61.0	0.453	0.787	0.431	No
	UnRefrg vs. Refrg	62	-0.023	-74.0	0.141	-1.335	0.182	No
	Grab vs. Refrg	59	-0.014	-42.0	0.563	-0.46	0.646	No
TP	UnRefrg vs. Grab	54	0.001	190.0	0.066	1.878	0.06	No
	UnRefrg vs. Refrg	57	-0.001	-125.5	0.172	-1.072	0.284	No
	Grab vs. Refrg	54	<0.001	131.0	0.222	1.149	0.251	No

Grab: Grab Simulation Sample  
 Refrg: Refrigerated Sample  
 UnRefrg: Unrefrigerated Sample

of NH<sub>4</sub>-N data concentrations in each of the treatment groups, the linear relationship between these groups is well-fitted, even for the grab simulated versus unrefrigerated data ( $r = 0.996$ ) (Figure 5). Robust linear agreement was found for comparisons between all treatment groups for NO<sub>x</sub>, TKN, TN and TP (Figure 6-9). Regression relationships between non-refrigerated and refrigerated samples were not only the most correlated for TP ( $r = 0.999$ ) and NO<sub>x</sub>-N ( $r = 0.999$ ), but the intercept of these regressions were essentially equal to zero. The pairing of refrigerated and grab simulated concentrations for all five parameters generally produced the best fit.

Quality control samples had good precision throughout the study period. No equipment blanks had analyte contamination >2 times the MDL. There were four non-refrigerated and three refrigerated field blanks that exceeded the QA requirement for NH<sub>4</sub>-N detection. One non-refrigerated field blank for TP had an unacceptable value. The analysis of the control “spike” samples resulted in a 100% acceptable recovery rate (90-110%) for all treatment groups of NO<sub>x</sub>-N samples. There were a total of three non-refrigerated and two refrigerated spikes for NH<sub>4</sub>-N that did not meet the recovery criteria. TKN had the poorest spike recovery performance, with six non-refrigerated and eight refrigerated samples falling outside the recovery range.

## DISCUSSION

The results of this study reveal that representative concentration data for NO<sub>x</sub>-N, TKN, TN and TP can be obtained from automatic sampling units when no refrigeration is available over a seven day period, bearing in mind that the samples were always pre-acidified to a pH < 2 throughout that seven day period. The data for NH<sub>4</sub>-N obtained in this study rejected the null hypothesis and resulted in significant differences between comparisons of all three treatment groups. Representative data for NH<sub>4</sub>-N could not be obtained when samples were left in the field for seven days, regardless of the presence of refrigeration. This reflects the notion that ammonia concentrations are more unstable than other nutrient parameters, due to the rapid progression of the nitrogen cycle under natural conditions. There may however be a shorter time period that ammonia concentrations in samples left in the field with or without refrigeration could remain significantly unchanged from samples processed immediately after collection. The differences of means calculated

### Mean NH<sub>4</sub>-N

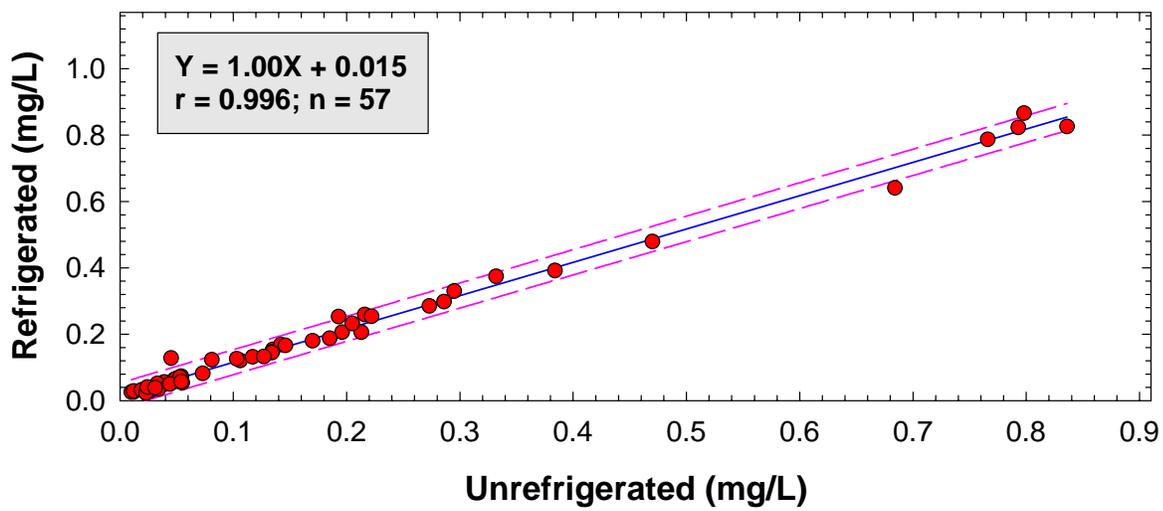
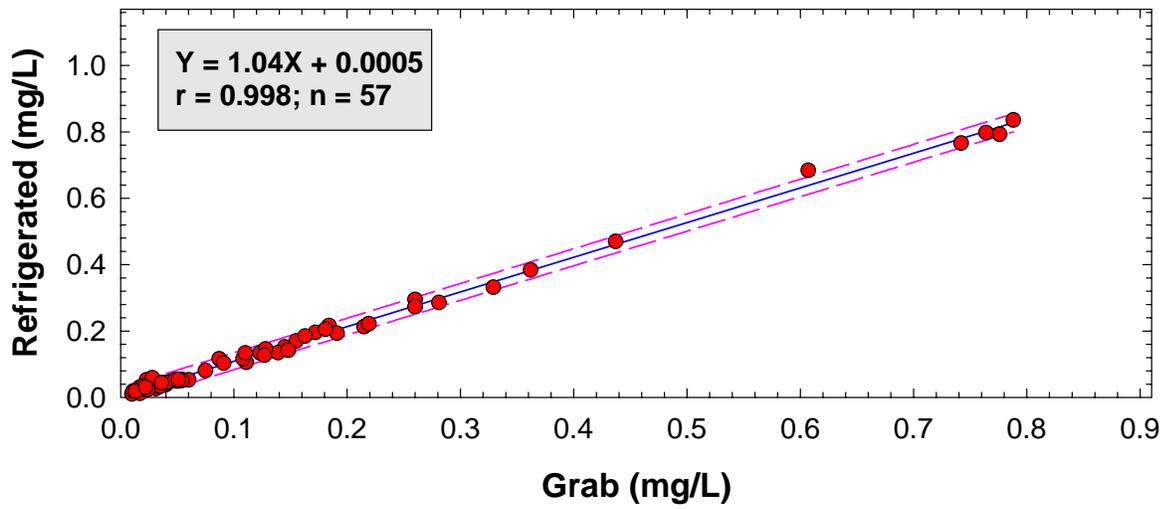
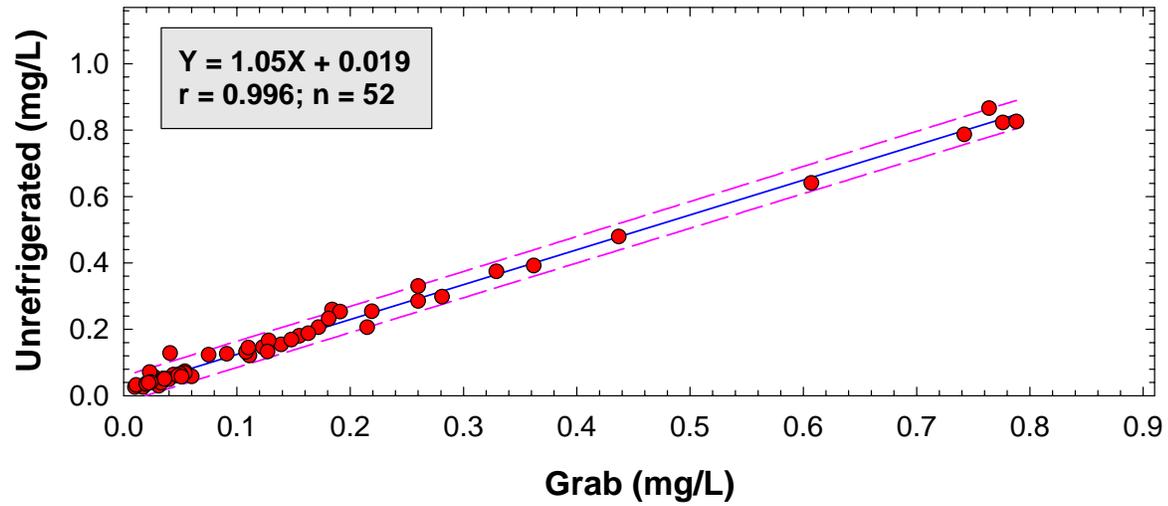
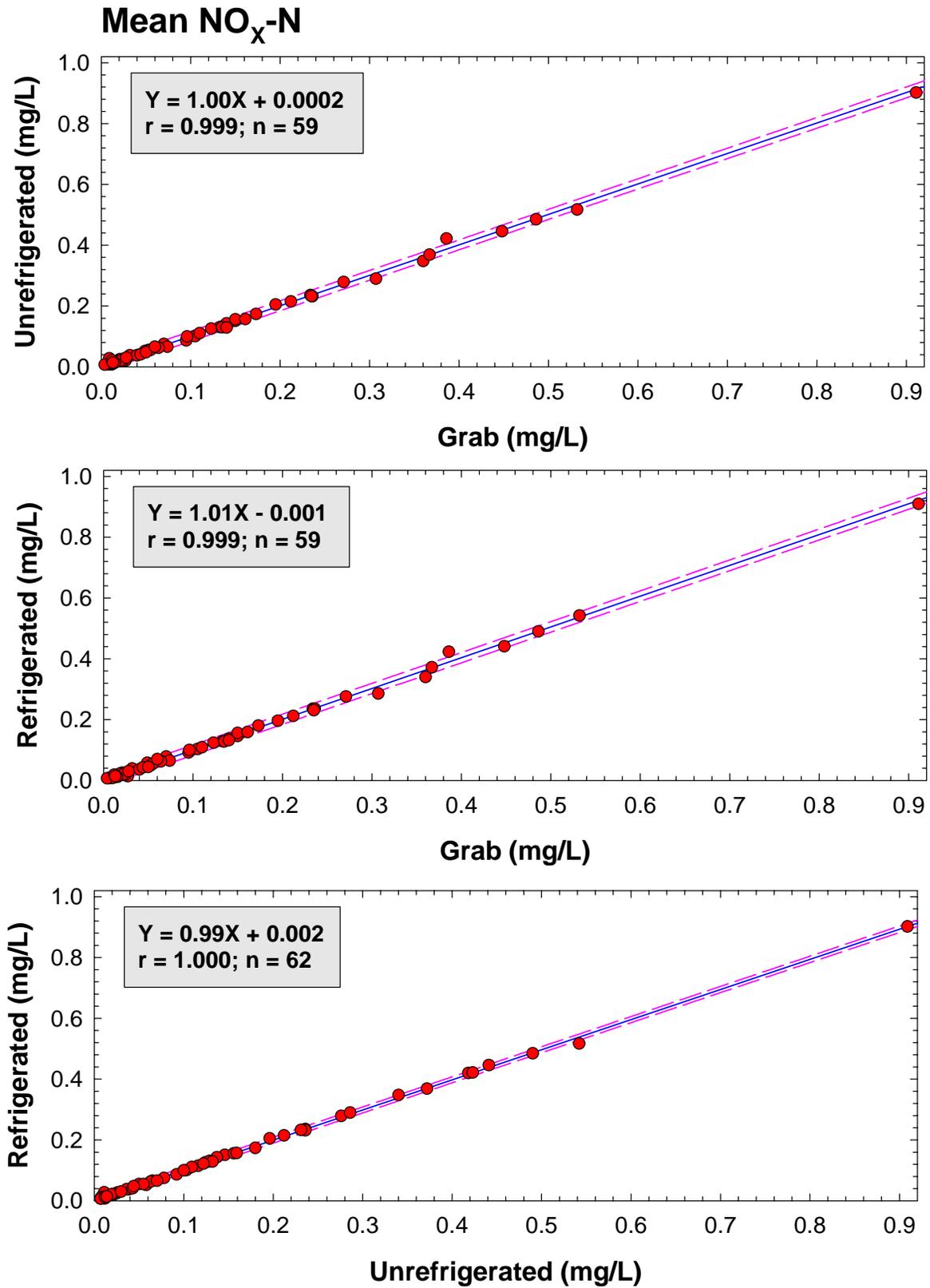


Figure 5. Linear regression of Grab, Unrefrigerated and Refrigerated samples for NH<sub>4</sub>-N.



**Figure 6.** Linear regression of Grab, Unrefrigerated and Refrigerated samples for NO<sub>x</sub>-N.

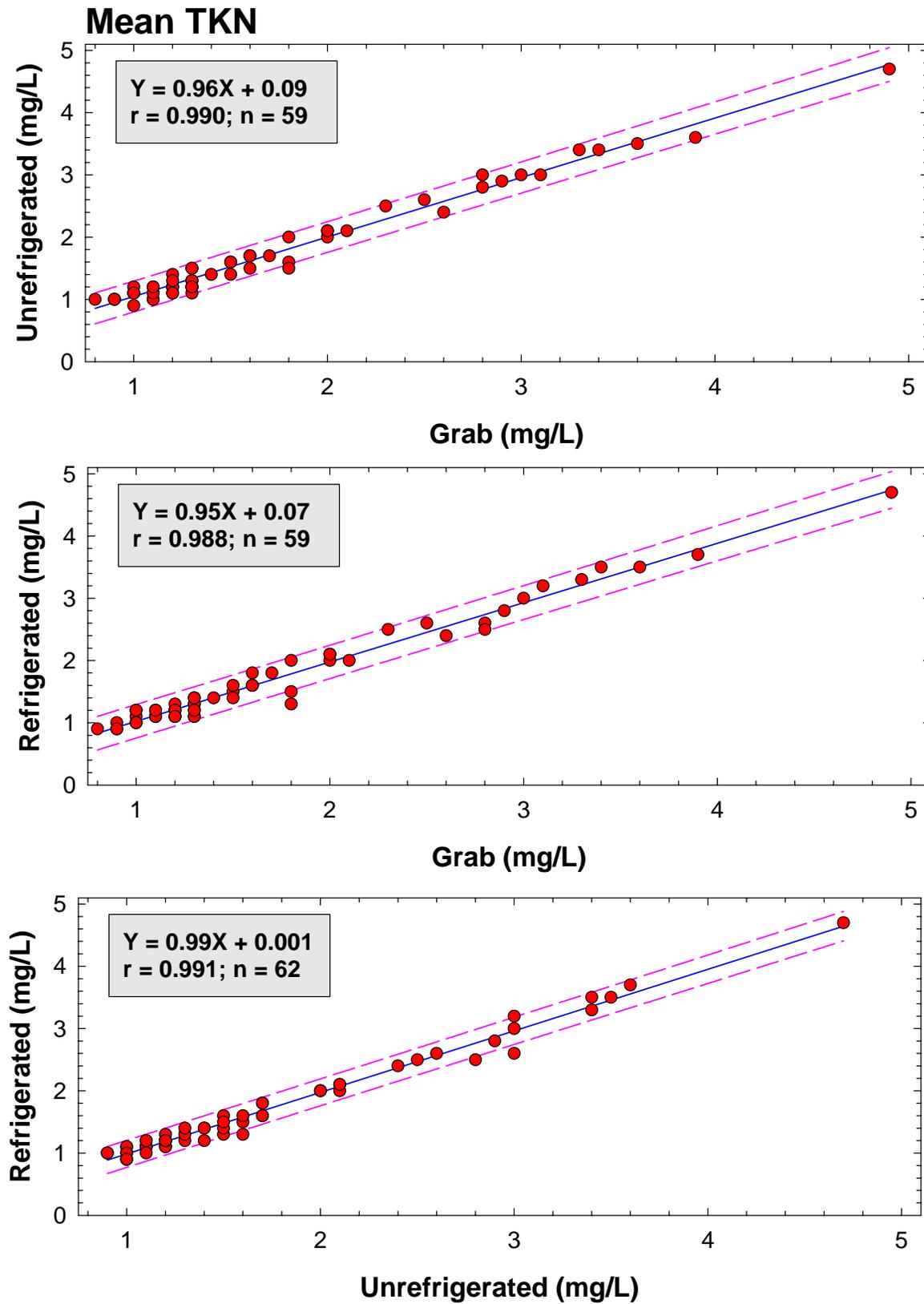


Figure 7. Linear regression of Grab, Unrefrigerated and Refrigerated samples for TKN.

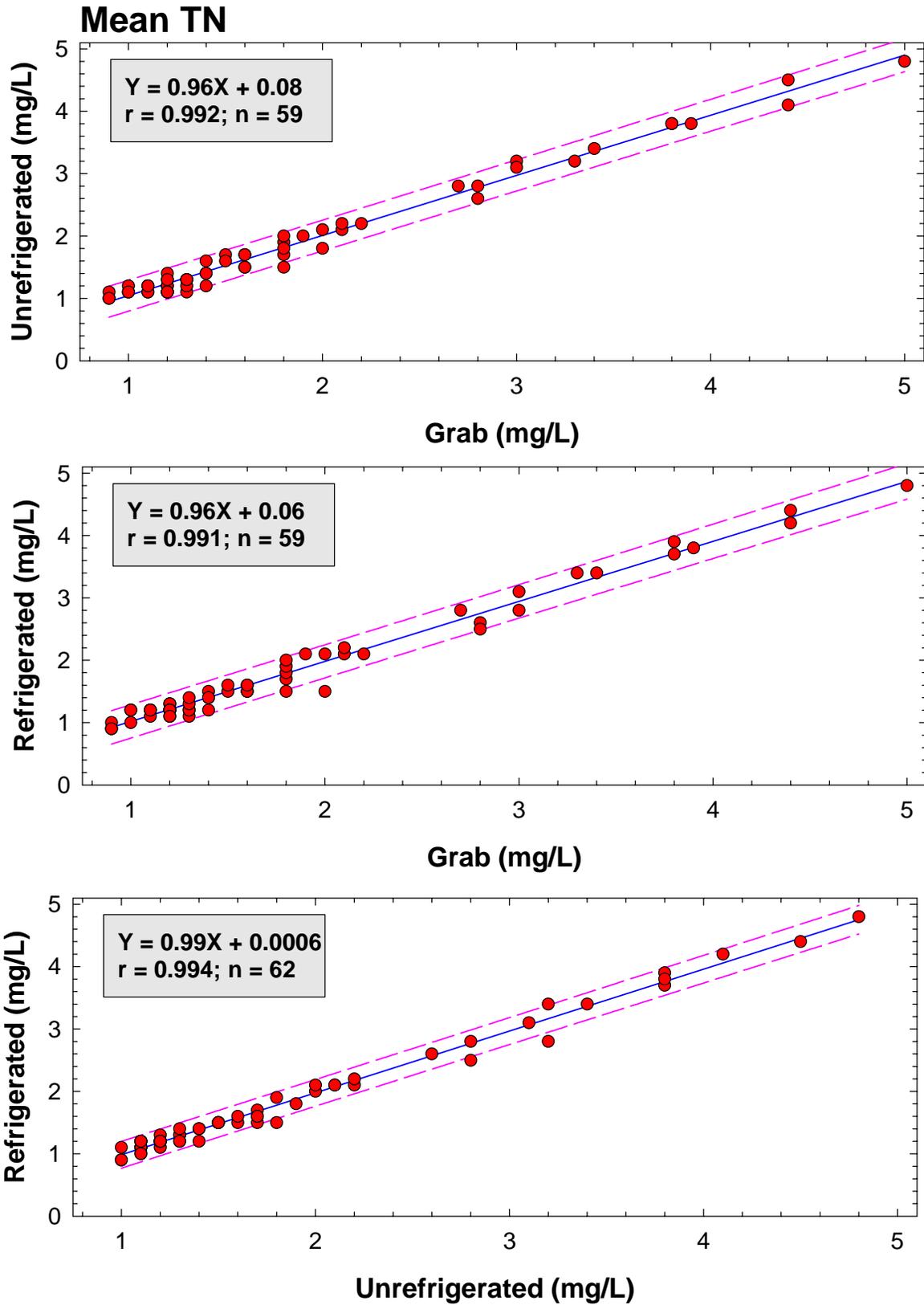
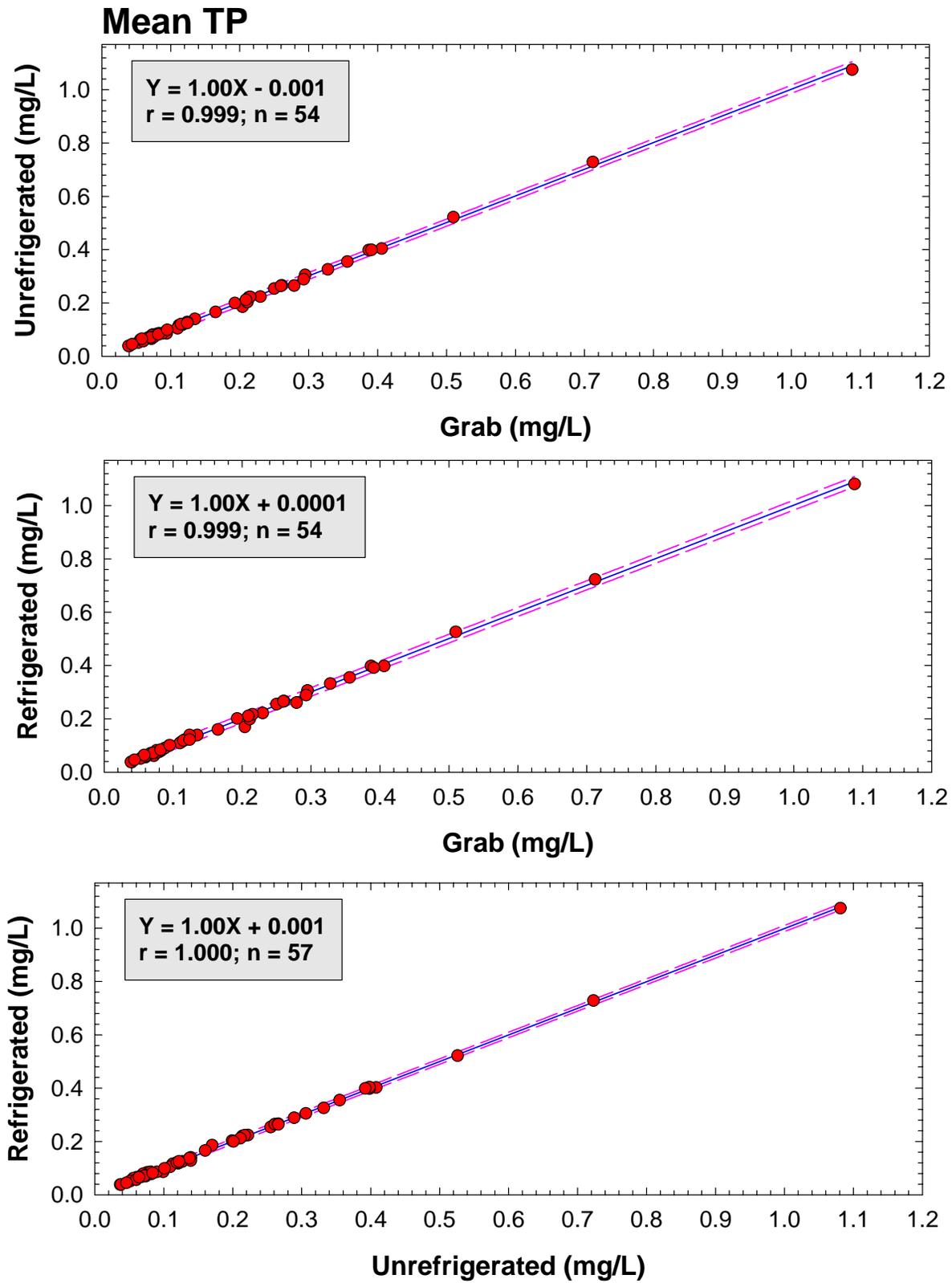


Figure 8. Linear regression of Grab, Unrefrigerated and Refrigerated samples for TN.



**Figure 9.** Linear regression of Grab, Unrefrigerated and Refrigerated samples for TP.

for all three  $\text{NH}_4\text{-N}$  data groups were well above the MDL for this parameter, indicating a relatively large difference between each of the specific paired concentrations for each of the treatment groups. This was reflected in the extremely low p values obtained in the Wilcoxon signed-rank test for ammonia. All the other nutrients analyzed had mean differences less than their respective MDL.

Although the main objective of this study was to determine if there were significant differences between non-refrigerated and refrigerated samples, the added comparison to a base line, grab simulation sample strengthens these findings. In fact, p values generated from the Wilcoxon test revealed that concentrations for parameters such as  $\text{NO}_x\text{-N}$ , TKN and TN had stronger correlation between the non-refrigerated and grab simulation samples, than they did when concentrations from non-refrigerated samples were compared to samples that were refrigerated. The concentration data for these parameters did not show any general trends of increased or decreased concentrations after a lack of refrigeration for seven days. The concentration values for the non-refrigerated and refrigerated data were equally distributed about the concentrations obtained from the grab simulation samples and had a favorable measure of variation, as indicated in the extremely high p values for these parameters. In contrast, concentrations of  $\text{NH}_4\text{-N}$  increased over the seven day period and the non-refrigerated samples consistently had higher values than the grab simulation and refrigerated samples in the same test event period (non-refrigerated > refrigerated > grab simulated). The p values obtained when comparing the TP concentration data between the three treatment groups give the impression that large differences existed between the means of these groups. However, the mean TP data were extremely uniform across the three treatment groups and the lack of variation forced the Wilcoxon test into attributing more significance to the few instances when even moderate variation between treatment groups did occur.

Strong linear relationships also existed between all nitrogen species and total phosphorus concentrations in samples collected under grab simulated conditions and samples left un-refrigerated for seven days in an enclosed autosampler base compartment. Concentrations of  $\text{NH}_4\text{-N}$ , and TKN showed the highest variability between the three treatment groups, but were still closely correlated. Comparisons between treatment groups, for concentrations of  $\text{NO}_x\text{-N}$  and TP, exhibited the most solid relationships on linear regression plots. Linear

regression plots between individual treatment groups showed no visible trend for stronger correlation at either high or low concentration limits for any of the parameters. This indicates that representative data for nutrient parameters can be obtained by this applied method (no refrigeration and pH<2 for seven days) throughout a wide range of ambient nutrient conditions and therefore over a potentially broad class of water body ecosystems. The use of nutrient data collected by autosamplers without the presence of refrigeration could be considered for areas that have similar water characteristics as those analyzed from the South Florida region. Microbial changes to nitrogen components were sufficiently suppressed and significant nitrogen cycling was delayed over the seven-day exposure period. Although it has been found that acidification alone can be suitable for the maintenance of nitrogen forms (Kotlash and Chessman, 1998), our study did not include samples without H<sub>2</sub>SO<sub>4</sub> additions, so it is not possible to determine if the biocide was the main reason for this stabilization. Water quality traits such as organic levels, pH and nutrient concentration loading may also play a role in the maintenance of samples without refrigeration.

Total nitrogen values showed good general agreement among all treatment groups and TN recovery for the non-refrigerated samples did not appear to be affected by the inconsequential loss and gains of other nitrogen components occurring in the autosampler bases. This could prove to be a potential benefit if water quality monitoring objectives move toward information goals that are driven by Total Maximum Daily Load (TMDL) mandates. Understanding the sources of impacts to the biological state of fresh and coastal water bodies that have been subjected to accelerated eutrophication has been an important focus of the national TMDL regulatory program (U.S. EPA, 1999). Total phosphorus and total nitrogen have been targeted as two of the primary nutrients of concern for assessing eutrophication issues. The ability to collect flow proportional samples for these parameters may be largely driven by budgetary constraints. The option of collecting scientifically valid TP and TN data, without the added cost of refrigeration would be a helpful step in achieving these monitoring program objectives.

This study also emphasized the need to use environmental samples when testing water quality preservation methods. The data collected from the “spiked” control samples were originally to be included in the same

statistical analyses as the environmental samples. However, early data analysis revealed that the samples prepared with de-ionized water were not comparable with environmental samples. Comparisons between treatment groups for control sample concentrations did not exhibit any of the relationships similar to those found for the environmental sample data. This is most likely caused by the absence of natural buffering influences that can not be expressed in samples prepared with de-ionized water. It is recommended that any future studies of field preservation methods be conducted using real time environmental samples only.

## CONCLUSIONS

This study indicates that the SFWMD methods for the collection of nutrient data by automatic samplers are sufficiently maintaining the integrity of the  $\text{NO}_x\text{-N}$  and TKN nitrogen species being sampled, and the current approved method for collections of TP by automatic sampler are also meeting data quality expectations. Sampling methods for the collection of  $\text{NH}_4\text{-N}$  should incorporate the shortest possible time frame from collection to processing. Samples exposed for seven days were not able to maintain concentrations of  $\text{NH}_4\text{-N}$  significantly unchanged from samples collected on day one. The use of  $\text{H}_2\text{SO}_4$  to maintain a pH level  $< 2$  was not sufficient to buffer these samples from significant changes, even when refrigeration was used. Changes to sampling protocols and flagging of  $\text{NH}_4\text{-N}$  data collected under these conditions should be initiated by the SFWMD. A future study to address the exact time period that samples to be analyzed for ammonia can remain in the field (with or without refrigeration) could be useful if mandates for  $\text{NH}_4\text{-N}$  collections can not be changed to reflect these method requirements.

Additional tests for alternative sampling methods should continue to be pursued by organizations with the resources and need to conduct such studies. Support of alternative sampling method studies can be fostered through scientific review by oversight agencies and by method change approvals if the findings are accepted. Water quality managers should investigate the cost savings and information opportunities that can be gained through remote sampling when the added burden of refrigeration is eliminated.

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