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# Evaluation of storage strategies and techniques for the hydrochemical analysis of nutrients in Atlantic Ocean coastal water samples

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**HRDAMY001**

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# Abstract

Filtered and unfiltered samples of coastal seawater from the Atlantic Ocean (Muizenberg and Llandudno beaches, Cape Town) representing a non upwelling region and an upwelling region respectively were stored at -20$^0$C. Deviations in the concentrations of silicate, inorganic phosphate, nitrite, nitrate and urea from the initial concentrations determined on collection of the seawater samples were monitored at intervals over a twelve week storage period.

1. Chemical analysis of filtered and non-filtered water samples on the day of collection demonstrated that filtration affected the concentration of nutrients in the samples. In three of the eight filtered seawater samples the filtrate contained a lower concentration of nutrients than the corresponding non-filtered seawater samples. Sample t-tests indicated that these differences were not significant. The study shows that no single storage strategy for the determination of all nutrients in seawater can be recommended and that the filtering/storage strategy used should be adapted depending on the nature of the nutrient to be analysed.

The mean inorganic phosphate concentrations present on sampling at Llandudno and Muizenberg beach ranged from approximately 0.4 µmol to 0.7 µmol $L^{-1}$. Filtered frozen samples for inorganic phosphate determinations stored well for twelve weeks with minor deviations in concentration from the initial concentration obtained at week 0 being recorded. This strategy showed less variability over the twelve week trial than shown with

non-filtered samples and is the recommended strategy for the preservation of inorganic phosphate. Ideally samples should be analysed within the first 2 weeks of storage.

The mean dissolved silicate concentrations present on sampling at Llandudno and Muizenberg beach ranged from approximately 9 µmol to 14 µmol $L^{-1}$. Neither of the two storage strategies explored during this study proved ideal as fluctuations in the level of dissolved silicate were noted using both storage regimes. Where immediate analysis is not possible the strategy which provided the least variability in this study i.e. storage of non-filtered samples at -20°C is recommended.

The mean nitrite concentrations present on sampling at Llandudno and Muizenberg beach ranged from approximately 0.4 µmol to 0.7 µmol $L^{-1}$. Ideally samples (filtered and non-filtered) should be analysed within the first 2 weeks of storage as further delays in analysis resulted in fluctuations in the nitrite concentration.

The mean dissolved urea concentrations present in non-filtered water on sampling at Llandudno was 0.2 µmol $L^{-1}$ with 1.5 µmol $L^{-1}$ present in the sample taken at Muizenberg beach. The major feature of the preservation study on variations in the concentration of urea in stored water over time is the relative stability of samples stored frozen for periods for periods of up to 8 weeks.

A second study on ten Atlantic Ocean coastal water samples collected at sites on the Cape Peninsula compared analytical techniques used to determine silicate and dissolved nitrate concentrations in seawater. For inorganic silicate analysis a modification of the manual method of Grasshoff (1976) and an automated flow injection analysis (FIA) method were used. The FIA method yielded concentrations that were on average two times higher in

seven out of the ten analyses. For the determination of dissolved nitrate concentrations two manual methods (differing in the method of reduction of nitrate to nitrite) were used: shaking the sample with cadmium pellets and passing the sample through a cadmium column. In both cases the effect of using Tris and ammonium chloride ($NH_4Cl$) buffers was determined. In addition nitrate concentrations were determined using FIA. Dissolved nitrate concentrations determined by the FIA and the cadmium column method (using both Tris and $NH_4Cl$ buffers) differed significantly whereas those obtained using the shaking method were frequently low or negative. The shaking method used to determine nitrate concentrations is not recommended.

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| | |
|---|---|
| FIA | Flow injection analysis |
| g | gram |
| $H_3PO_4$ | ortho-phosphoric acid |
| $HgCl_2$ | mercuric chloride |
| L | Liter |
| mg | milligram |
| mol | molar |
| n | nano |
| $NH_4Cl$ | ammonium chloride |
| Tris | tris(hydroxymethyl)aminomethane |

# Chapter 1: Literature review

## 1.1 Introduction

The ocean is the largest water body on Earth with 71% of the surface of the earth being covered by the oceans (Millero, 2006). The ocean interacts with the atmosphere and land masses through complex cycles of biogeochemical and hydrological processes. Understanding the chemical composition and processes of the ocean is necessary because of the role played by the ocean in regulating changes in the global environment. Such understanding is vital as we now live in a period of rapid global change. A wide range of measurements is required to understand oceanic processes. Chemical oceanographers have investigated the distribution of biogeochemically active elements, metals and organic pollutants and have developed sophisticated analytical techniques.

Seawater, a slightly alkaline (pH 7.5 – 8.4) aqueous solution, is a complex mixture of inorganic and organic compounds (Millero and Sohn, 1992). Most of the elements in the periodic table of elements are found in seawater (Millero, 2006). Sodium (55% w/v), chloride (31%), sulfate (8%), magnesium (4%), calcium (1%) and potassium (1%) are the major ionic components of seawater (Munn, 2011). Fourteen elements (O, H, Cl, Na, Mg, S, Ca, K, Br, C, Sr, B, Si and F) are found in concentrations greater than 1 ppm. Bruland (1983) classified the elements in seawater into three classes, based on concentration:

Major elements: 0.05 to 750 mmol $L^{-1}$

Minor elements: 0.05 to 50 µmol $L^{-1}$

Trace elements: 0.05 to 50 µmol $L^{-1}$

Many of the minor and trace elements are involved in inorganic and biological reactions in seawater (Bruland, 1983).

Traditionally in chemical oceanography the term “nutrient element” was applied to the biolimited elements phosphorus, inorganic nitrogen compounds and silicon (Spencer, 1975). As nutrient elements in the ocean are elements which are involved in a number of biochemical reactions in the upper ocean (such as the production of organic matter by

photosynthesis) a number of other constituents of seawater are also nutrient elements. For example, in certain areas of the ocean, iron plays a role in controlling the growth of marine phototrophs (Martin *et al*., 1994). Millero (2006) assessed the availability of and need by phytoplankton for various elements by analysing the chemical composition of phytoplankton and comparing the results to the concentration of the elements in average seawater (Table 1.1). From this crude assessment it is evident that phosphorus, inorganic nitrogen compounds, iron and silicon are elements that limit productivity in the oceanic environments.

Table 1.1 Distribution of elements in phytoplankton (N) and seawater (A): a measure of availability to need (A/N) (after Millero, 2006)

| **Element** | **N(g/100g)** | **A(g/m$^3$)** | **A/N** |
|---|---|---|---|
| **Na** | 3 | 10750 | 3600 |
| **K** | 1 | 390 | 390 |
| **Mg** | 0.4 | 1300 | 300 |
| **Ca** | 0.5 | 416 | 830 |
| **P** | 0.6 | 0.030 | 0.05 |
| **N** | 5 | 0.3 | 0.06 |
| **Si**[a] | 10 | 0.5 | 0.05 |
| **Si**[b] | 0.5 | 0.5 | 1 |
| **Fe**[a] | 0 | 0.05 | 1.3 |
| **Fe**[b] | 0.001 | 0.00005 | 0.05 |

[a] Diatoms

[b] Phytoplankton

## 1.2 The analysis of constituents in seawater

Critical steps in the analysis of seawater are the sampling strategy, sample treatment and storage. Marine analytical chemists must ensure that samples collected for a study represent the properties of the study area (i.e. that two samples collected from the same water mass are not discriminable from each other) and that the sample is stable (i.e. retains the properties of interest from the point of collection to the final analytical measurement). Communities of microorganisms (including phytoplankton, bacteria, protists, and zooplankton) are present in samples of seawater (Munn, 2011). In untreated water samples these communities remain biochemically active and influence the distribution of organic matter, nutrients and trace metals.

## 1.3 Filtration of seawater

Seawater is composed of numerous components which are found in a number of phases (Millero, 2006). In the study of ocean processes oceanographers differentiate between dissolved matter and particulate matter. Measurements of the concentrations and fluxes of the dissolved matter in seawater are among the most important factors in the study of ocean processes (Millero, 2006).

Although the boundary between dissolved and particulate fractions is a continuum, the fraction of seawater that passes through a filter with a pore size of 0.45 µm is commonly known as the dissolved phase. The difference between "dissolved" and "particulate" is an empirical distinction and reflects the size of the filter used in the sample preparation. Many bacteria and viruses pass through filters with a pore size of 0.45 µm (Munn, 2011). The dissolved phase may also contain colloidal material with a size range of 1 to 200 nm (Koike *et al.*, 1990; Wells and Goldberg, 1993). Colloids are those materials that pass through a 0.45 µm filter but are not dissolved. Organic colloids include the complex sugars while inorganic colloids include iron hydroxides. Because it is technically challenging to separate the colloidal particles, bacteria and viruses (Millero, 2006; Munn, 2011) the separation of

dissolved and particulate fractions through filters with defined pore sizes is still the most commonly used technique in oceanography. Dissolved solutes pass through a 0.45 µm filter and may be inorganic and organic in nature. The nutrient elements are members of the inorganic dissolved solutes (Grasshoff, 1976).

In order to differentiate between constituents in the dissolved and particulate phases in seawater a variety of filtration techniques is employed. Filtration also serves to remove large particles that may interfere with the detectors used in analytical systems from the solution and prevents the leaching of analytes from particles into the solution during sample preservation and storage (Kremling and Brügmann, 1999). Filtration of oceanic waters is generally unnecessary as the concentration of suspended particulate material is low (Millero, 2006). Coastal and estuarine waters and oceanic surface waters where high production is experienced (e.g. during phytoplankton blooms) are subjected to filtration processes.

Seawater samples are filtered as soon after collection as possible. A variety of filters and filtration techniques are available. The choice of filter and filtration technique is determined by the requirements of the analysis.

### 1.3.1 Depth and sieve filters

Filters are grouped into two main types: depth and sieve filters (Grasshoff, 1976; Higson, 2003; Karl and Proctor 2007). Depth filters are manufactured from cellulose, glass fibres or metal oxides and the pore sizes are not well defined. Depth filtration depends on the physical entrapment of particulate matter. Sieve filters have well defined pore sizes and are manufactured from plastic films (cellulose acetate, cellulose nitrate, polycarbonate *etc.*). The volume of sample that can be filtered on sieve filters is normally less than that which can be filtered on depth filters (Karl and Proctor 2007). For the determination of nutrients in seawater a common choice is the glass fibre filter disk with a nominal pore size of ~ 1µm (Kirkwood, 1996).

## 1.3.2 Filtration techniques

Common filtration techniques employed by chemical oceanographers include vacuum filtration, pressure filtration and tangential flow filtration (Kirkwood, 1996).

### 1.3.2.1 Vacuum filtration

In vacuum filtration, filtration is facilitated by suction with a vacuum pump (Kirkwood, 1996, Munn 2011). A vacuum filtration unit has a filter support made of sintered glass or ceramic material. A low vacuum pressure is used to avoid the rupture of cells and the consequent leaching of dissolved material from within the cells onto the filter. The number of transfer steps in the procedure, during which the sample is exposed to the ambient air and to a number of different materials, poses a contamination risk.

### 1.3.2.2 Pressure filtration

In pressure filtration the water sample is pressurized and forced through an in-line filter (Kirkwood, 1996; Munn, 2011). Pure air or, if the redox state of the water must be preserved to prevent precipitation of components, an inert gas is used to create the pressure. Filter holders manufactured from various plastic materials (Teflon®, polypropylene, polycarbonate) are used for pressure filtration. In some systems the water sampler can be pressurized and the filter holder and receiving bottle directly connected. This system requires fewer transfer steps and minimises the potential contamination risks associated with vacuum filtration procedures.

### 1.3.2.3 Tangential flow filtration

Tangential flow filtration (TFF) is a sophisticated filtration technique that allows the separation of colloidal particles from the dissolved phase by filtration of 10–100 L of seawater (Munn, 2011). In pressure and vacuum filtration the sample flows perpendicular

to the filter surface and the filter rapidly becomes clogged with particles. In TFF the sample flows parallel to the filter surface and the colloidal particles become more concentrated as the filtrate passes through the pores of the filter. This system is ideal for use when an accurate and precise size separation in a much diluted sample is required. Laboratory-scale systems are available (e.g. the Millipore PELLICON system).

## 1.4 Storage and preservation of samples

Nutrients in sea water samples need to be analysed or stabilised as soon after collection as possible (Grasshoff, 1976). The ideal method for nutrient analysis is an *in situ* analysis (Kremling and Brügmann, 1999). Following filtration of sea water samples bacteria and micro- and nano plankton still present in the samples will continue to metabolize bioreactive elements and organic matter. The concentrations of nutrients in samples that have not been preserved alter rapidly due to biological activity (Munn, 2011). In addition, the material of the storage vessels may interact with and/or absorb some constituents of the sample. Sample composition may also be altered by photolytic breakdown of the organic compounds in the sample and by pH induced changes in the speciation of inorganic constituents (Millero, 2006). When samples that have not been preserved are eventually analysed the results may not represent the situation in the body of water being studied.

Immediate *in situ* analysis of nutrients in seawater is generally not possible as instrumentation used for analyses may not be present at the points of sample collection on research vessels or on the shoreline. For this reason researchers have developed methods for the storage and preservation of water samples. Various methods of preservation are used to preserve the integrity of the sample. These include the use of refrigeration, freezing, quick freezing in liquid nitrogen, mercuric chloride poisoning and pasteurization (Kremling *et al*., 2007). However because each analyte has its own reaction chemistry (Kirkwood, 1996; Higson, 2003) no single preservation strategy will be suitable for the preservation of all constituents of seawater.

### 1.4.1 Non-frozen refrigeration

Refrigeration (<8°C) of coastal sea waters is possible if the analysis of the nutrients in the samples occurs within two hours of sampling (Kirkwood, 1996). Samples are refrigerated in the dark in order to minimise biological activity. For open water or deep oceanic samples where the biological activity is lower samples may be refrigerated for a maximum of 10 hours (Kirkwood, 1996).

Subsamples for specific analyses are required (Kremling *et al*., 2007). For nitrite analysis, seawater samples with ammonia levels of <1 µmol $L^{-1}$ can be refrigerated for up to 3 hours with no changes in the nitrite concentrations occurring. For silicate analysis the subsample is acidified with sulphuric acid to pH 2.5 before refrigeration to prevent polymerisation. Ammonium chloride buffer (0.2M) is added to the subsample for nitrate analysis to prevent biological activity from occurring during refrigeration. Samples for dissolved phosphate analysis are not well preserved at $4^0$C (Kremling *et al*., 2007).

### 1.4.2 Freezing

Freezing of nutrient samples to -$20^0$C is a common method of storing and preserving water samples (Thayer, 1970; DeGobbis, 1973; Venrick and Hayward, 1985; Chapman and Mostert, 1990; Avanzino and Kennedy, 1993; Aminot and Kirkwood, 1995; Dore *et al*., 1996; Fellman *et al*., 2008; Segura-Noguera *et al*., 2011). The method prevents biological activity and concomitant changes in the concentrations of nutrients in the samples before analyses of the samples can be performed. Despite the many studies conducted to gauge the effect of freezing on nutrient samples no unequivocal conclusion has been drawn. Contradictory results are evident for the effects of freezing of seawater samples for phosphate and ammonium concentration determinations. No statistical differences in the nitrate and silicate concentrations in fresh and frozen seawater samples were found, but only if the concentration of the latter was < 60 µmol $L^{-1}$.

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### 1.4.3 Quick freezing in liquid nitrogen

Quick freezing in liquid nitrogen prior to storage at -20°C is the method of choice for many researchers when nutrient samples must be stored for long periods (MacDonald and McLaughlin, 1982; Venrick and Hayward, 1985; Gardolinski *et al*., 2001). Freezing of nutrient samples must occur soon after filtration to prevent residual biological transformations. This is particularly important in the case of ammonia and nitrite analyses since these compounds undergo rapid biotransformations.

Polymerization of dissolved inorganic silicate in samples of low salinity or high silicon concentrations (>120 µmol $L^{-1}$ of Si) during freezing has been noted (Venrick and Hayward, 1985). To overcome this sufficient time (>24h) for depolymerisation to occur must be allowed in the thawing process. Alternatively a separate aliquot for silicate analysis may be stored under acidic conditions (pH=2.5) (Venrick and Hayward, 1985).

### 1.4.4 Mercuric chloride poisoning

Poisoning chemicals, including sulphuric acid, chloroform and mercuric chloride ($HgCl_2$), have been widely used to poison the organisms responsible for metabolizing the bioreactive elements and organic matter present in filtered seawater samples. $HgCl_2$, which has been used for decades to preserve nutrients, acts by binding to the sulphydryl groups on proteins thereby inhibiting enzyme activity (Kirkwood, 1992).

The recommended dosage for $HgCl_2$ is dependent on the concentration of organic matter and the microbial load in the sample. Kattner (1999) was able to preserve inorganic nutrients such as nitrate, silicate and phosphate for up to 2 years with 105 mg $L^{-1}$ of $HgCl_2$ but reported that ammonia analyses of the stored samples were unreliable. High $HgCl_2$ concentrations interfere when nitrate analyses are conducted using copperized cadmium reductors but reports on the critical level vary. Kirkwood (1992) reported that levels of >59 mg $L^{-1}$ were detrimental whereas Kremling and Wenck (1986) found levels of >10 mg $L^{-1}$ to interfere. Concentrations of < 10 mg $L^{-1}$ were insufficient to preserve the integrity of unfiltered Atlantic Ocean water samples at $4^0$C for longer than 2 weeks. Kirkwood (1992)

was able to preserve the nitrate in filtered North Sea samples at room temperature for 2 weeks using 20 mg $L^{-1}$ $HgCl_2$.

The use of $HgCl_2$ for the preservation of nutrient samples has become less popular due to the need to concurrently measure the mercury concentrations in seawater at sub-nanogram levels per litre. These measurements are not possible when any form of mercury is used on research vessels. In addition the environmental impacts of mercury are contributing to the decline in its status as a standard preservation technique (Kattner, 1999).

### 1.4.5 Pasteurization

Pasteurization is a method which uses heat to preserve sea water samples to be used for nutrient analysis. Pasteurization has two major advantages: no chemical additions (preservatives) are required and no specialized equipment is necessary for the preservation of samples after pasteurization. Samples can be stored at room temperature for many months prior to analysis (Aminot and Kerouel, 1997; Aminot and Kerouel, 1998). Pasteurization requires incubators/ovens which are present in most laboratories and allows direct treatment of samples in plastic bottles (Aminot and Kerouel, 1997; Aminot and Kerouel, 1998; Daniel *et al.*, 2012).

Pasteurization at 80 ± 3°C for 2 hours followed by storage of the water samples at ambient temperature successfully preserved the integrity of the nutrients in seawater for several years (Aminot and Kerouel, 1998). Nitrate, nitrite, phosphate and silicate levels in seawater samples were unaltered during storage. After pasteurization at 65°C and preservation for 1 year nitrate and nitrite concentrations showed insignificant changes, but under the same conditions slight changes in ammonia concentrations were reported. Ammonia levels in pasteurized water were more consistent when pasteurisation was performed at temperatures between 80-85°C. Phosphate concentrations varied when heat was applied to the water samples but these changes can be limited by reducing the pH to 7 before pasteurisation at temperatures of between 80-85°C (Aminot and Kerouel, 1998; Kremling and Brügmann, 1999; Daniel *et al*., 2012).

## 1.5 Analytical methods to determine nutrients in seawater

Three major categories of analytical methods for the determination of nutrients in seawater exist (Kremling *et al*., 2007). Manual methods are those where each sample is analysed individually for each variable. Automated methods have been developed where automated versions of the manual methods are used and allow for the simultaneous analysis of the required variables. On contact with seawater sensors provide a visible signal which represents the concentration of the analyte. Sensors are potentially ideal nutrient analysers as they provide *in situ* readings without the use of chemical treatments. Currently the sensors available for marine research are not considered accurate enough for the direct detection of nutrients in seawater (Hansen and Koroleff, 1999; Kremling *et al*., 2007).

### 1.5.1 Determination of inorganic phosphate

In seawater phosphorus exists as ions of ortho-phosphoric acid, $H_3P0_4$**.** Approximately 10% of the inorganic phosphate in seawater is present as $PO_4^{3-}$ while the remainder exists as $HPO_4^{2-}$ (Strickland and Parsons, 1968; Stumm and Morgan, 1981).

Procedures for the determination of the inorganic phosphate concentrations in seawater are based on the reaction of the phosphate ions with molybdate in acidic medium producing a yellow molybdophosphate (Grasshoff, 1976). If low concentrations of phosphate are present a further reduction of Mo(VI) to Mo(V) is performed producing intensely colored molybdenum blue compounds which improve the sensitivity of the analysis. Ascorbic acid and antimony ions are commonly employed as reductants in a two-step reaction (Murphy and Riley, 1962). In the first step inorganic phosphate ions react with ammonium molybdate and antimony while in the second step ascorbic acid is used as the reductant. The blue phosphomolybdic complex is stable for hours and salinity does not affect the depth of the colour. The minimum concentration of phosphorous that is detectable at 880 nm is 0.01 µmol $L^{-1}$ and absorbances are a linear function of the concentration up to 28 µmol $L^{-1}$. Higher phosphate concentrations can be measured using wavelengths of 600-770 nm but a 30% loss in sensitivity is experienced.

A high sensitivity method which is able to detect up to 0.0004 µmol $L^{-1}$ dissolved inorganic phosphate concentrations in seawater uses an extraction procedure (Strickland and Parsons, 1968). The phosphomolybic acid complex is extracted in a solvent (isobutanol or *n*-hexane) before reduction in the organic phase.

### 1.5.2 Determination of dissolved inorganic silicate

Marine phytoplankton communities include a large proportion of diatoms. Silicon is a major constituent of these diatoms and is also found in some marine algae, fungi and sponges (Munn, 2011). The concentration of suspended silicone in sea water varies from trace amounts to several mg $L^{-1}$. Careful sampling and preservation of seawater used for silicate analysis is required. Contact with glassware and glass fibre filters results in contamination of the seawater samples by silicate originating in these items. If samples are frozen, the silicate in low-salinity samples may polymerise. Generally storage of separate sub-samples for silicate analysis under acidic conditions (pH=2.5) is required (Venrick and Hayward, 1985).

To determine the concentration of dissolved silicon compounds in seawater ammonium molybdate is added to an acid sample. The silicate ions react with the molybdate and form a yellow silicomolybdate complex (Grasshoff, 1976). This complex exists in two isomeric forms. At low pH's (0.8 – 2.5) the unstable β- silicomolybdate forms. At pH 3.5 – 4.5 a more stable α- isomer forms. To overcome the problems associated with the low intensity yellow colour formed by both silicomolybdate isomers methods involving further reduction of the isomers to dark blue complexes have been developed. Sulphite (Strickland and Parsons, 1968) and ascorbic acid (Koroleff, 1971) are commonly used reducing agents. Oxalic acid may be added to eliminate phosphate interference. (Koroleff, 1976; Truesdale and Smith, 1975; Truesdale and Smith, 1976).

The detection limit of reactions in which further reduction of the silicomolybdate isomers occurs is ~0.03 µmol $L^{-1}$. The method has been adapted for analysis using an autoanalyzer.

## 1.5.3 Determination of dissolved nitrite and nitrate

### 1.5.3.1 Determination of nitrite

When the microbial process of denitrification occurs in seawater at low oxygen levels nitrite is produced. Nitrite is also excreted by phytoplankton when an excess of nitrate and phosphate stimulates a heavy bloom of plankton (Martin, 1968). The concentration of nitrite in seawater is generally low (<O.l pmol $L^{-1}$).

The standard spectrophotometric method for the determination of nitrite in seawater determines nitrite by the reaction of nitrite under acidic conditions with an aromatic amine (sulphanilamide hydrochloride) which leads to the formation of a diazonium compound which is coupled with a second aromatic amine (N-(1-napthyl)-ethylene-diamine dihydrochloride) (Bendschneider and Robinson, 1952). The resultant azo dye is quantified by spectrophotometry. The amount of azo dye formed is proportional to the initial nitrite concentration over a wide range of concentrations (0-10 µmol $L^{-1}$). Flow-analysis versions of the method have been tested which have been able to analyse spiked natural seawater samples from 0 – 100 nmol $L^{-1}$ with a resolution of 1 nmol $L^{-1}$ (Hansen and Koroleff, 1999).

Anion exchange chromatography offers a method for analysing nitrates present in extremely low concentrations (nanomole levels) in seawater (Wada and Hattori, 1971). In this method the azo dye formed from nitrite and N-(1-napthyl)-ethylene-diamine dihydrochloride is adsorbed in an anion exchange resin. The dye is subsequently eluted from the column with 60% acetic acid and measured spectophotometrically at 550 nm.

#### 1.5.3.2 Determination of nitrate

In the ocean dissolved elemental nitrogen is the most abundant form of nitrogen followed by nitrate, ammonia, nitrite and dinitrogen oxide (Stumm and Morgan, 1981). The final oxidation product of nitrogen compounds in seawater is nitrate.

Five methods for the determination of nitrate in seawater were reviewed by Spencer and Brewer (1970). Many are not sensitive enough to determine the low concentrations present in surface waters or are subject to interference by other constituents in the seawater. A method involving the nitration of an organic compound (2,6-xylenol) is insensitive and is subject to interference from the chlorides present in seawater (Hartley and Asai, 1963). A polarographic method in which nitrate is catalytically reduced in the presence of uranyl ions is difficult and is not suitable for shipboard analysis (Grasshoff, 1976). A spectrophotometric method in which the nitrates in seawater containing 50% concentrated sulphuric acid form nitrosyl chloride is hazardous and insensitive (Armstrong, 1963). Ion electrodes have been used successfully to determine the nitrate concentrations in fresh water (Whitfield and Jagner, 1981) but interference by chloride present in seawater makes this method unsuitable for use in marine environments.

The most commonly used and most sensitive assays for the determination of nitrate in seawater use cadmium/copper or zinc powders to reduce nitrate to nitrite (Grasshoff, 1976). The nitrite is then detected as an azo dye as previously described for the determination of nitrite in seawater. The nitrite originally present in the seawater must be determined and subtracted from the total amount of nitrite detected (Hansen and Koroleff, 1999). Flow injection analysis (FIA) has been used to automate the determination of nitrate (Johnson and Petty, 1982). Using copperised cadmium to reduce nitrate to nitrite and determining the concentration of the azo dye formed, more than 75 analyses per hour were achieved. The detection limit of this procedure was 0.1 µmol $L^{-1}$. Precision of greater than 1% was noted at concentrations of nitrate exceeding 10 µmol $L^{-1}$.

Ultraviolet spectrometry has been used to determine high levels of nitrates (0.5 – 1 mmol $L^{-1}$) (Mertens and Massart, 1971). An acidified sample was filtered through a 0.5 µm filter and the extinction was measured against a blank at 210 nm. Nitrate in the blank was reduced to ammonia by boiling samples with Raney nickel for 30 minutes and stirring for 90 minutes at $90^0$C. The concentration of nitrate was obtained from a calibration curve.

A chemiluminescent method was able to detect nanomolar quantities of nitrate (and nitrite) in seawater with a precision of ±2 nmol $L^{-1}$ (Walters *et al*., 1987). The nitrate was reduced to nitric oxide. The gaseous nitric oxide was carried in a helium carrier gas flow into a nitrogen oxide analyzer where it reacted with ozone to form nitrogen dioxide. The photon released by nitrogen dioxide on return to its ground state was detected by a photomultiplier. The integrated output from the photomultiplier over the time taken by the nitric oxide to be purged from the sample is proportional to the amount of nitrate in the sample. The equipment is compact and sturdy but the procedure is slow, averaging 10-12 samples per hour.

### 1.5.4 Determination of dissolved urea

Urea is found in significant concentrations in the near-shore and offshore surface waters. Quantification of both dissolved inorganic and organic nitrogen fluxes is necessary to interpret the complex nitrogen cycle in these waters. Because urea plays a role as an excretion product of protein metabolism, functions in osmoregulation and is a nitrogen source for phytoplankton growth it cannot be ignored by biological oceanographers (Goeyens *et al*., 1998). McCarthy (1970) and Kaufman *et al*. (1983) have shown that even when dissolved inorganic species such as nitrate and ammonium are present in excess, urea is preferentially taken up.

Urea concentrations are determined using either indirect or direct methods. The indirect urease method is based on the hydrolysis of urea by urease. The end product of the reaction, ammonium, is assayed using standard procedures (McCarthy, 1970). Price and

Harrison (1987) reported that due to urease inhibition this indirect method underestimated urea concentrations.

A colorimetric method based on the reaction between urea and diacetylymonoxime is currently the method of choice. The absorbance of the resultant imidazolene which forms a red complex with thiosemicarbazide is determined at 520 nm. This method has a detection limit of 0.10 µmol $L^{-1}$ (Goeyens *et al.*, 1998).

## 1.6 Aims and objectives

Because nutrients and other bioactive compounds in seawater samples may be rapidly metabolised by phytoplanton and may undergo other physical and chemical changes it is preferable to analyse the samples for nutrient compounds within a few hours after the sample has been collected. In many instances this is not a practical option and water samples need to be stored under appropriate storage conditions with the aim of minimising alterations in the amount of nutrient to be measured.

The objectives of this study are to:

- Determine the effects of different storage procedures on coastal seawater samples with respect to accuracy (compared to immediate analysis) and precision.

- Compare the manual methods of Grasshoff (1976) and automated flow injection analysis (FIA) methods to determine silicate and dissolved nitrate concentrations in seawater.

# Chapter 2: Methods

## 2.1 Sample sites

Bulk Atlantic Ocean surface water samples (2000 ml) were collected in plastic containers from two sites: Surfers Corner in Muizenberg, False Bay and Llandudno beach (Figure 2.1). Both sites are located in Cape Town, South Africa and represent a non upwelling region and an upwelling region respectively. Samples for the determination of dissolved inorganic phosphates were collected on 09 July 2012, for dissolved nitrites and nitrates on 12 July 2012, for dissolved inorganic silicates on 13 July 2012 and for dissolved urea on 24 July 2012.


Figure 2.1. A map of South Africa displaying the two Atlantic Ocean sample sites: Surfers Corner in Muizenberg, False Bay and Llandudno beach, Cape Town (www.google.com).

## 2.2 Subsampling programme

Bulk water samples were immediately transported to the Chemistry Laboratory, Department of Oceanography, University of Cape Town where subsamples were prepared. Fifty per cent (1000 ml) of each sample was filtered while the remainder of the bulk sample (1000 ml) remained unfiltered. The filtered and unfiltered subsamples were stored at -20$^0$C.

## 2.3 Filtration procedures

Filtration was carried out using a minimum number of procedures to avoid the risk of contamination associated with multiple procedures and transfers. The internationally accepted delineation between dissolved and particulate matter in seawater is achieved by filtration through a 0.45 μm filter (Riley, 1975). Filters of the correct pore size were unavailable. Samples for phosphate, urea, nitrite and nitrate analyses were filtered using 47mm micro-glass fibre filter papers with a pore size of 0.7 μm (Lasec, South Africa). The samples for silicate analyses were filtered using a Stericup™ vacuum driven disposable polystyrene filtration system which contains a polyether sulfone membrane of pore size 0.22μm. All filters were prewashed with deionised water and then with the sample prior to use.

## 2.4 Storage strategies for the analysis of nutrients

The filtered and non-filtered subsamples were stored in 50 ml aliquots in Nalgene polypropylene tubes at -20$^0$C. Chemical analysis of the seawater for the nutrients silicate, inorganic phosphate, nitrite, nitrate and urea was performed immediately after collection and after 1 week, 2 weeks, 3 weeks, 4 weeks, 8 weeks and 12 weeks of storage. Frozen samples were thawed prior to use. As silicon tends to polymerise when frozen (Venrick and Hayward, 1985) aliquots for silicate analysis were allowed to stand for 3 hours after thawing prior to analysis.

## 2.5 Determination of nutrients

The methods used follow the procedures in the Honours/ Taught Masters Practical Course and Laboratory Analyses Manual provided by the Department of Oceanography, University of Cape Town. These are a modification of the procedures published by Grasshoff (1976).

### 2.5.1 Determination of dissolved inorganic phosphorus

**Reagents**

**Ammonium molybdate solution.** Ammonium paramolybdate ($(NH_4)_6Mo_7O_{24}.4H_20$) (7.5g) was dissolved in distilled water (250 ml). The solution was stored in a glass bottle.

**Sulphuric acid solution.** Concentrated sulphuric acid (70 ml) was dissolved in deionised water (450 ml) and stored in an amber glass bottle.

**Ascorbic acid solution.** Ascorbic acid ($C_6H_8O_6$) (13.5 g) was dissolved in deionised water (250 ml) and stored in a dark glass bottle at $4^0$C.

**Potassium antimonyl-tartrate solution.** Potassium antimonyl-tartrate ($K(SbO)C_6H_4O_6.4H_2O$) (0.34 g) was dissolved in deionised water (250 ml) and stored in a glass bottle.

**Mixed reagent.** The mixed reagent was made from the above stock solutions. Whilst mixing molybdate solution (10 ml) was added to the sulphuric acid solution (25 ml). Thereafter ascorbic acid solution (10 ml) and potassium antimonyl-tartrate solution (5 ml) were added and the reagent was stored in a dark glass bottle. The reagent is stable for several months.

**Phosphate standard stock solution.** Anhydrous potassium dihydrogen phosphate ($K_2HPO_4$) (0.816 g) was dissolved in deionised water (1000 ml). The solution was stored in a dark bottle over chloroform (1 ml).

**6 µmol $L^{-1}$ phosphate standard working solution.** The phosphate standard stock solution (0.2 ml) was dissolved in deionised water (200 ml) to make a 6 µmol $L^{-1}$ working standard. This solution was made on the day of the experiment.

**Method**

A range of standards (0-5.4 µmol $L^{-1}$) was made by diluting the 6 µmol $L^{-1}$ working standard with deionised water. Samples were analysed in triplicate. Mixed reagent (0.5 ml) was added to the standards (5 ml), samples (5 ml) and a reagent blank (5 ml deionised water). The solutions were vortexed and allowed to react for 1.5 hours. The absorbances at 880 nm were determined using a Spectronic™ Helios™ Epsilon™ spectrophotometer.

A standard curve for inorganic phosphate (absorbance at 880 nm versus concentration) was plotted and used to determine the concentration of inorganic phosphate in the samples (Appendix 4).

**Statistical analysis**

Results were statistically analysed using a One Way Repeated Measures ANOVA test followed by a Holm-Sidak multiple comparisons test ($\alpha = 0.05$) (section 2.7).

## 2.5.2 Determination of dissolved inorganic silicate

**Reagents**

All reagents were made and stored in polyethylene bottles.

**Acid molybdate solution.** Ammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) (38 g) was dissolved in deionised water (300 ml). The solution was stored in a polyethylene bottle.

**4.5 mol $L^{-1}$ sulphuric acid solution.** Sulphuric acid (density 1.84 $kg.L^{-1}$) (250 ml) was added to deionised water (750 ml). The solution was allowed to cool and made up to 1 L with deionised water. The solution was stored in a polyethylene bottle.

**Mixed reagent.** The mixed reagent was made from the above stock solutions. Acid molybate solution (300 ml) was added to the 4.5 mol $L^{-1}$ sulphuric acid solution (300 ml). The solution was stored in a polyethylene bottle.

**Oxalic acid solution.** Oxalic acid dehydrate ($(COOH)_2.2H_2O$) (10 g) was dissolved in deionised water (100 ml). The saturated solution was stored in a plastic bottle.

**Ascorbic acid solution.** Ascorbic acid ($C_6H_8O_6$) (1.4 g) was dissolved in deionised water (100 ml) and stored in a dark glass bottle at $4^0$C.

**Silicate standard stock solution (5 mmol $L^{-1}$).** Disodium hexaflurosilicate ($Na_2SiF_6$) (0.96 g) was dissolved in deionised water in a plastic 1000 ml volumetric flask and diluted to volume. The solution was stored in a polyethylene bottle.

**Silicate standard working solution (100 µmol $L^{-1}$).** The silicate standard stock solution (20 ml) was diluted in deionised water (1000 ml) to make a 100 µmol $L^{-1}$ working standard.

**Method**

The analyses were performed in plastic test tubes. Samples were analysed in triplicate. A range of standards (0-60 µmol $L^{-1}$ ) was made by diluting the 100 µmol $L^{-1}$ working standard with deionised water. Mixed reagent (0.2 ml) was added to the standards (5 ml), samples (5 ml) and a reagent blank (5 ml deionised water). The solutions were vortexed. After 10 minutes oxalic acid solution (0.2 ml) was added followed immediately by ascorbic acid solution (0.2 ml). The tubes were vortexed. The absorbance was determined after 60 minutes at 810 nm using a Spectronic™ Helios™ Epsilon™ spectrophotometer. A standard curve for silicate (absorbance at 810 nm versus concentration) was plotted and used to determine the concentration of silicate in the samples (Appendix 4). The concentrations of the silicate samples were multiplied by 1.15 to account for the salt error factor.

**Statistical analysis**

Results were statistically analysed using a One Way Repeated Measures ANOVA test followed by a Holm-Sidak multiple comparisons test ($\alpha = 0.05$) (section 2.7).

## 2.5.3 Determination of dissolved nitrite and nitrate

**Reagents**

**Sulfanilimide solution.** Sulfanilamide (1 g) was dissolved in concentrated hydrochloric acid (10 ml) and deionised water (60 ml). This solution was diluted to 100 ml with deionised water. The solution was stored in a glass bottle.

**n-(1-naphthyl)-ethylenediamine dihydrochloride (NEDI) solution.** NEDI (0.1 g) was dissolved in deionised water (100 ml) of deionised water. The solution was stored in a dark glass bottle at $4^0$C.

**4.7 mol** $L^{-1}$ **Ammonium chloride buffer.** Ammonium chloride (25 g) was dissolved in deionised water (100 ml). The pH of the solution was adjusted to 8.5 with 1 N sodium hydroxide.

**Nitrite standard stock solution (5 mmol** $L^{-1}$**).** Anhydrous analytical grade sodium nitrite was dried at 110°C for 1 hour. Sodium nitrite (0.345 g) was dissolved in deionised water (1000 ml). The solution was stored over chloroform (1 ml) in a dark glass bottle.

**Nitrite working standard solution (5 µmol** $L^{-1}$**).** Nitrite standard stock solution (0.2 ml) was dissolved in deionised water (200 ml). This solution was diluted to make a range of standards.

**Nitrate standard stock solution (5 µmol** $ml^{-1}$**).** Anhydrous analytical grade sodium nitrate was dried at 110°C for an hour. Sodium nitrate (0.425 g) was dissolved in deionised water (1000 ml). The solution was stored over chloroform (1 ml) in a dark glass bottle.

**Cadmium granules.**

**For preparation of the cadmium reductor.** Acetone, 10% hydrochloric acid, copper sulphate solution.

**Cadmium column activator**. Potassium nitrate ($KNO_3$) (0.5055 g) was dissolved in deionised water (500 ml) to make a 10 mmol $L^{-1}$ solution. 1 ml of this solution was diluted with deionised water (99 ml) to make a 100 µmol $L^{-1}$ solution.

**Method**

**Nitrite concentration determination**

Samples were analysed in triplicate. A range of standards (0-1 µmol $L^{-1}$) was made by diluting the 5 µmol $L^{-1}$ nitrite working standard solution with deionised water.

Samples and standards (5 ml) were analysed in glass test tubes. An azo-dye was allowed to develop in the test tubes after the addition of sulfanilimide stock solution (0.1 ml) to all tubes. The samples were mixed and left to react for 2-8 minutes. NEDI stock solution (0.1 ml) was added to the test tubes. The solutions were vortexed. The absorbance was determined after 1.5 hours at 540 nm using a Spectronic™ Helios™ Epsilon™ spectrophotometer. A standard curve for nitrite (absorbance at 540 nm versus concentration) was plotted and used to determine the concentration of nitrite in the samples (Appendix 4).

**Nitrate concentration determination**

As no copperised cadmium columns were initially available, nitrate in the seawater samples was reduced using the "shake method". The cadmium reductor was prepared as follows: cadmium granules (2 teaspoons) were placed in a glass beaker and washed in acetone before rinsing in deionised water. The cadmium granules were freed from oxides by rinsing in a dilute hydrochloric acid solution followed by deionised water. The granules were vigorously shaken in a copper sulphate solution and prior to rinsing with deionised water. The reductor was activated by washing with the cadmium column activator, a potassium nitrate (100 µmol $L^{-1}$) solution, before a final rinse with deionised water.

Samples were analysed in triplicate. A range of standards (0-30 µmol $L^{-1}$) was made by diluting the 5 mmol $L^{-1}$ nitrate working standard solution with deionised water. 50 ml of each concentration was produced. Aliquots (25 ml) of sample and standard were placed in

glass beakers and ammonium chloride buffer (0.5 ml) was added. Samples and standards were individually added to the beaker containing the activated cadmium granules. The contents of the beaker were shaken for 1 minute before 5 ml of the reduced test solution was dispensed into glass test tubes for further analysis. The cadmium pellets were thoroughly washed with deionised water in between samples.

An azo-dye was allowed to develop in the test tubes after the addition of sulfanilimide stock solution (0.1 ml) to all tubes. The samples were mixed and left to react for 2-8 minutes. NEDI stock solution (0.1 ml) was added to the test tubes. The solutions were vortexed. The absorbance was determined after 1.5 hours at 540 nm using a Spectronic™ Helios™ Epsilon™ spectrophotometer. A standard curve for nitrite (absorbance at 540 nm versus concentration) was plotted and used to determine the concentration of nitrite in the samples (Appendix 4). These readings represent the reduced nitrate plus the ambient nitrite in the seawater sample. The concentration of nitrite from the unreduced samples (i.e. the ambient nitrite, see nitrite concentration determination above) was deducted from these values.

**Statistical analysis**

Results were statistically analysed using a One Way Repeated Measures ANOVA test followed by a Holm-Sidak multiple comparisons test ($\alpha = 0.05$) (Section 2.7).

## 2.5.4 Determination of dissolved urea

**Reagents**

**Urea standard stock solution (10**µmol $L^{-1}$ **urea N $ml^{-1}$).** Urea crystals ($CO(NH_2)_2$) (0.1503 g) were dissolved in distilled water (500 ml).

**Sodium chloride solution.** Sodium chloride (300 g) was dissolved in distilled water (1000 ml).

**Reagent A.** Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) (70 g) was added to distilled water (60 ml) and dissolved as far as possible. Concentrated sulphuric acid (1000 ml) was slowly added.

**Reagent B.** Manganese II chloride tetrahydrate ($MnCl_2.4H_20$) (31.1 g) and potassium nitrate ($KNO_3$) (0.62 g) were dissolved in distilled water (100 ml).

**Reagent C** Diacetyl monoxime ($CH_3COC(NOH)CH_3$) (1.165 g) and semi carbazide (0.0136 g) were added to 50% ethanol (25 ml) and Reagent B (25 ml).

**Method**

Samples were analysed in triplicate. A range of standards (10 µmol $L^{-1}$ urea N $ml^{-1}$) was made by diluting the urea standard stock solution with deionised water.

Samples and standards (5 ml) were pipetted into glass test tubes. Sodium chloride solution (1 ml) was added to each test tube, followed by Reagent A (0.71 ml). The contents were vortexed. Thereafter Reagent C (0.2 ml) was added to each test tube. The tubes were covered in foil and placed in an oven at 75°C for 3-3.5 hours. The test tubes were cooled under tap water to ambient room temperature and were left to equilibrate for 30 minutes. Absorbances were determined at 520 nm using a Spectronic™ Helios™ Epsilon™ spectrophotometer. A standard curve for urea N (absorbance at 520 nm versus concentration) was plotted and used to determine the concentration of urea N in the samples (Appendix 4).

**Statistical analysis**

Results from Llandudno were statistically analysed using a One Way Repeated Measures ANOVA test followed by a Holm-Sidak multiple comparisons test ($\alpha = 0.05$). Results from Muizenberg were analysed using a Friedman Repeated Measures ANOVA test followed by a Holm-Sidak multiple comparisons test ($\alpha = 0.05$) (section 2.7).

## 2.6 Comparison of methodologies for the analysis of nutrients in seawater

### 2.6.1 Sample sites

Atlantic Ocean surface water samples (1000 ml) were collected in plastic containers from ten sites: Surfers Corner in Muizenberg (2), Llandudno beach (2), Kalk Bay (1), Hout Bay beach (2), Glen Beach (1) and Sea Point (2) (Figure 2.2). All sites are located in Cape Town, South Africa.


Figure 2.2. A map of the South Africa displaying the Atlantic Ocean sample sites: Surfers Corner in Muizenberg (2), Llandudno beach (2), Kalk Bay (1), Hout Bay beach (2), Glen Beach (1) and Sea Point (2) (www.google.com).

## 2.6.2 Determination of dissolved inorganic silicate

Methods employed for the determination of silicate concentrations in sea water were compared using the seawater samples collected in section 2.6.1. Immediately after collection the water samples were filtered using a Stericup vacuum driven disposable plastic filtration system with a pore size of 0.22 μm. The samples were stored on ice during transport to the laboratory. Two analytical methods were compared: the first method was the manual method described in section 2.5.2. The second automated method determined the silicate in the samples by FIA. The analyses were performed in plastic test tubes. Samples were analysed in triplicate.

### 2.6.2.1 Determination of silicate using the manual method

The manual method described in section 2.5.2 was used to determine the concentration of silicate in the ten sea water samples.

### 2.6.2.2 Determination of silicate by flow injection analysis

Samples were analysed using a QuikChem® 8500 Automated Ion Analyzer (Hach Company, U.S.A.). QuickChem® method 31-1114-27-1-D for the determination of silicate in seawater was followed to determine the silicate concentrations in the ten seawater samples. Reagents and standards were prepared according to the manual. This method differs from the manual method (Koroleff, 1971) in that stannous chloride instead of ascorbic acid is used to reduce the yellow silicomolybdate complex.

**Statistical analysis**

Results were statistically analysed using a One Way ANOVA test followed by a Holm-Sidak multiple comparisons test ($\alpha = 0.05$) (section 2.7).

## 2.6.3 Determination of dissolved nitrite and nitrate

Two manual and one automated method for the determination of dissolved nitrate and nitrite concentrations in sea water were compared using the ten seawater samples collected in section 2.6.1. Immediately after collection the water samples were filtered using a

Stericup vacuum driven disposable plastic filtration system with a pore size of 0.22 μm. The samples were stored on ice during transport to the laboratory. Three analytical methods were performed to determine the dissolved nitrate concentrations in the samples: the first method was the manual method (the "shaking" method) described in section 2.5.3. The second manual method used a column to contain the cadmium granules (the "cadmium column method") thereby controlling the conversion of nitrate to nitrite. The third automated method determined the dissolved nitrate and nitrite in the samples by FIA. Samples were analysed in triplicate.

#### 2.6.3.1 Determination of dissolved nitrite and nitrate using the shaking method

The manual method described in section 2.5.3 was used to determine the concentration of dissolved nitrate and nitrite in the ten sea water samples.

#### 2.6.3.2 Determination of dissolved nitrite and nitrate using the cadmium column

**Reagents**

With the exception of the cadmium reductor, reagents were prepared as described for the shaking method in section 2.5.3.

**Cadmium column kit**

This comprised of

- a column of copperized cadmium
- acetone and a dilute hydrochloric acid (HCl) solution (dilute concentrated HCl 1:5 in deionised water)
- a peristaltic pump and tubing

**Preparation of the cadmium column**

The cadmium reductor column was prepared as follows: cadmium granules (2 teaspoons) were placed in a glass beaker and washed in acetone before rinsing in deionised water. The cadmium granules were freed from oxides by rinsing in a dilute hydrochloric acid solution followed by deionised water. The granules were vigorously shaken in a copper sulphate

solution. The reductor column was filled with deionised water prior to loading with the cadmium granules. The reductor was activated by washing with the cadmium column activator, a potassium nitrate (100 µmol $L^{-1}$) solution, before a final rinse with deionised water.

**Method**

**Nitrite concentration determination**

Nitrite concentrations were determined as described for the shaking method in section 2.5.3.

**Nitrate concentration determination**

Samples were analysed in triplicate. A range of nitrate standards was prepared as described for the shaking method in section 2.5.3.

Aliquots (50 ml) of sample and standard were placed in glass beakers and ammonium chloride buffer (1.0 ml) was added. Reduction of the nitrates in each sample was achieved by passing the sample through the cadmium reductor column. A fraction (25 ml) of each sample was used for rinsing the column prior to the collection of aliquots (5 ml) for analysis. The cadmium pellets in the column were thoroughly washed with deionised water in between samples.

The reduced samples were further analysed in glass test tubes. An azo-dye was allowed to develop in the test tubes after the addition of sulfanilimide stock solution (0.1 ml) to all tubes. The samples were mixed and left to react for 2-8 minutes. NEDI stock solution (0.1 ml) was added to the test tubes. The solutions were vortexed. The absorbance was determined after 1.5 hours at 540 nm using a Spectronic™ Helios™ Epsilon™ spectrophotometer. A standard curve for nitrite (absorbance at 540 nm versus concentration) was plotted and used to determine the concentration of nitrite in the samples (Appendix 4). These readings represent the reduced nitrate plus the ambient nitrite in the seawater sample. The concentration of nitrite from the unreduced samples (i.e. the

ambient nitrite, see nitrite concentration determination above) was deducted from these values.

#### 2.6.3.3 Determination of dissolved nitrate and nitrite by flow injection analysis

Samples were analysed using a QuikChem® 8500 Automated Ion Analyzer (Hach Company, U.S.A.). QuickChem® method 31-107-04-1-E for the determination of nitrate and nitrite in seawater was followed to determine the nitrate and nitrite concentrations in the ten seawater samples. Reagents and standards were prepared according to the manual. This method is an automated variant of the manual method of Koroleff (1971).

### 2.6.4 Comparison of two buffers used for the determination of dissolved nitrate and nitrite in seawater

Grasshoff (1976) prescribes the use of ammonium chloride ($NH_4Cl$) as both a buffer and a complexant during the analysis of nitrate in seawater. In this study the effect of substituting Tris(hydroxymethyl)aminomethane (Tris) buffer for $NH_4Cl$ buffer was investigated. The ten filtered seawater samples collected in section 2.6.1 were used for the comparison. Two analytical methods were performed to determine the dissolved nitrate concentrations in the samples: the first method was the "shaking" method described in section 2.5.3. The second method was the "cadmium column method" described in section 2.6.3.2. Samples were analysed in triplicate. Using each of these methods the effect of using $NH_4Cl$ and Tris as a buffer was investigated.

**Reagents**

**$NH_4Cl$ buffer:** prepared as described in section 2.5.3.

**Tris(hydroxymethyl)aminomethane (Tris) buffer.** 1 mol $L^{-1}$ TRIS buffer (30 ml) was added to deionised water (70 ml) to make a 0.3 mol $L^{-1}$ solution. The pH of the solution was adjusted to 8.5 with 1 N sodium hydroxide.

**Method**

The shaking method described in section 2.5.3 was used to determine the concentration of dissolved nitrate and nitrite in the ten sea water samples in the presence of $NH_4Cl$ or Tris buffers. The buffers (0.5 ml) were added to samples and standards (25 ml) prior to exposure to the cadmium granules.

An alternative method, the "cadmium column method" described in section 2.6.3.2 was also used to determine the concentration of dissolved nitrate and nitrite in the ten sea water samples in the presence of ammonium chloride or Tris buffers. The buffers (1.0 ml) were added to samples and standards (50 ml) prior to passage through the cadmium column.

## 2.7 Statistical analysis

The results in this study were statistically analysed using SigmaPlot version 12.3 (www.sigmaplot.com/). The results were graphically represented using Matlab version 7.10.0.499 (R2010a) (www.matlab.com/).

### 2.7.1 Normality test

Normally distributed populations are required for all parametric tests. The Shapiro-Wilk normality test (Townend, 2012) was used to determine whether the data being analysed followed a normal distribution. The null hypothesis ($H_0$) for the normality test was that the data was normally distributed. The alternate hypothesis ($H_1$) was that the data did not follow a normal distribution. The null hypothesis was rejected (i.e. the alternate hypothesis was accepted) when the P value obtained was less than equal to the value of alpha ($\alpha$ = 0.05). If the outcome was 'pass', the data is distributed following a normal distribution. If

the outcome was 'fail', the data does not follow the normal distribution. Normally distributed populations are required for all parametric tests.

### 2.7.2 Levene's equal variance test

This test determined whether variances of different samples were equal or unequal. The null hypotheis ($H_0$) for this test was that the variances of the samples were homoscedastic (equal). If the P-value determined from this test was less than a critical value (α=0.05), the differences in sample variances were unlikely to have occured based on random sampling from a population with equal variances. The null hypothesis of equal variances would be rejected and the alternate hypothesis ($H_1$) that there were differences between the variances in a population would be accepted. If Levene's test is positive ($P<0.05$), the variances in the different groups were equal and a parametric test assuming homoscedasticity is required. In this study a One Way Repeated Measures ANOVA was used.

### 2.7.3 One Way Repeated Measures ANOVA test

A One Way Repeated Measures ANOVA test was used to test the effect of storage conditions on the nutrient concentrations in a seawater sample over time. The test analyses the changes in each water sample over the time series as opposed to analysing only the initial (t=0) and final (t=12 weeks) values. By focussing on the changes differences that may occur due to individual responses are disregarded (Townend, 2012). The null hypothesis is that there are no differences amongst treatments. The test is a parametric test that assumes that the treatment effects are normally distributed. Because the One Way Repeated Measures ANOVA tested the hypothesis of no difference between several treatment groups and did not determine which groups were different, a multiple comparison test (the Holm-Sidak method) was used to isolate these differences (section 2.7.5). Where populations were found to be not normally distributed a non parametric test (the Friedman Repeated Measures ANOVA on Ranks Test) was used (section 2.7.4).

### 2.7.4 Holm-Sidak multiple comparisons test

A multiple comparisons test was used following a one-way ANOVA. Many t-tests were performed simultaneously and the results indicated which comparisons are statistically significant. P-values for each comparison were calculated in the same manor as a Fisher's LSD test computes p- values (Townend, 2012). A value for the significance level of alpha ($\alpha = 0.05$) was set. K was equal to the number of comparisons performed, (i=K). The null hypothesis for this test was that there was no difference between the methods used. If the P-value for a particular comparison was more than alpha/i the Holm-Sidak test concluded that none of the comparisons were statistically different (No). If the p-value for a particular comparison was less than alpha/i the Holm-Sidak test concluded that the comparison was statistically different (Yes).

### 2.7.5 Friedman Repeated Measures ANOVA on Ranks

A Friedman Repeated Measures ANOVA on Ranks test was used to statistically analyse data that did not follow a normal distribution pattern. The test compared the effects of different treatments on a group and the treatments are compares. The test is a non parametric test that does not require equal variance.

### 2.7.6 Paired t-test

A paired t-test was used to test the differences between two groups. The data must be normally distributed and the test can only test the difference between two groups. The null hypothesis of the test was that there is no difference between groups. The null hypothesis was rejected if the p-value obtained was less than 0.05 and it was concluded that a statistically significant difference exists between the two groups.

### 2.7.7 One Way ANOVA

A One Way ANOVA test was used to test the differences between the means of several subgroups of a variable thereby producing a p-value for the comparison. If the p-value was less than 0.001, the means of at least two or more of the subgroups were statistically significant i.e. the differences in the mean values between the treatment groups were

greater than would be expected by chance. A multiple comparisons test was necessary to determine which comparisons were statistically significant.

### 2.7.8 Kruskal-Wallis One Way ANOVA on Ranks

The Kruskal-Wallis One Way Analysis of Variance on Ranks was used as a non-parametric alternative to the one-way ANOVA. It performs the same analysis as the One Way ANOVA except that it is based on ranks rather than means.

# Chapter 3: Results

## 3.1 Determination of nutrients

Bulk Atlantic Ocean coastal water samples collected from Surfers Corner, Muizenberg and Llandudno Beach were examined for the effects of storage on the following dissolved inorganic nutrients: phosphate, silicate, nitrite, nitrate and urea. The seawater samples were subjected to two different storage procedures, namely freezing at -20$^0$C (frozen) with and without filtration through a 0.7 µm filter for phosphate, urea, nitrite and nitrate analysis and through a filter with a pore size of 0.22 µm for silicate analysis. Initial concentrations of samples were determined within 3 hours of collection and after six time intervals with a maximum storage time of 12 weeks. At each time interval, subsamples were analysed in triplicate and the mean concentration of the nutrient and the standard error at each point were plotted against time. The results are summarised in Figures 3.1 to 3.8. Results of statistical analyses are provided in Appendices 1-3.

### 3.1.1 Inorganic phosphate

The mean inorganic phosphate concentrations in stored samples of seawater collected at Llandudno Beach as a function of time are presented in Figure 3.1. The standard error within the triplicate samples for each storage strategy is indicated. Results for the sample collected at Muizenberg Beach are shown in Figure 3.2.

#### 3.1.1.1 Llandudno beach

Fluctuations in the phosphate concentrations in stored samples were noted with the overall tendency being an increase in the mean inorganic phosphate concentration over time (Figure 3.1). At the time of sampling (week 0 in all figures) the inorganic phosphate concentration in filtered samples was higher (µ=0.598 µmol $L^{-1}$) than in non-filtered samples (µ=0.389 µmol $L^{-1}$). Between weeks 0 and 2 the phosphate concentration in stored samples remained relatively constant. Between week 4 and 12 the trend of a gradual increase in the concentration of inorganic phosphate became apparent. The concentration obtained for the non-filtered samples at week 3 may be an outlier. The inorganic phosphate concentration in

samples that had been filtered and frozen were least affected over the twelve week trial with no significant variations of the mean concentrations at week 0 and week 12 being evident. Over the twelve week trial the inorganic phosphate concentrations in the non-filtered frozen sample did reveal a significant difference between the samples analysed at week 0 and those analysed at week 12 with values of $p<0.001$ being recorded.

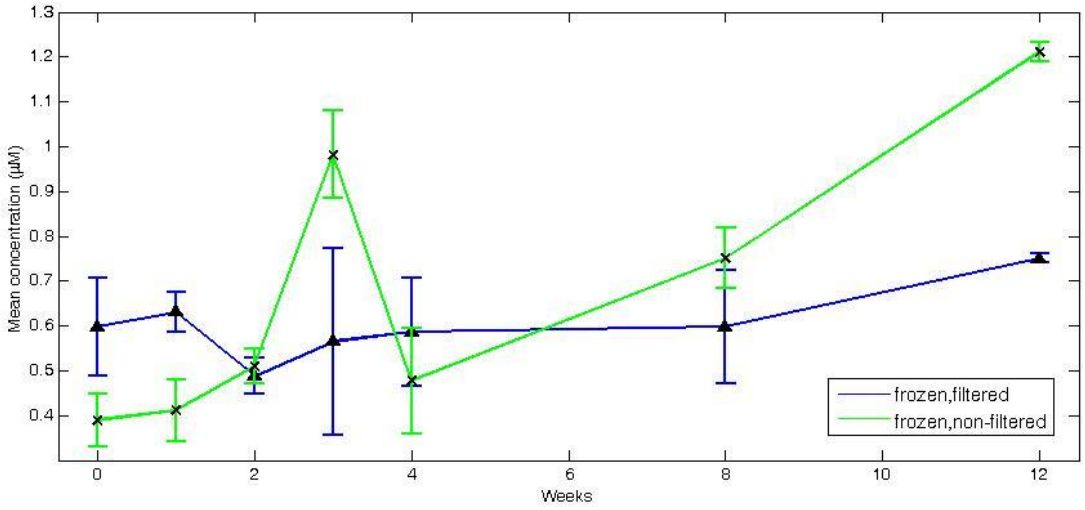


Figure 3.1 Mean inorganic phosphate concentrations in stored samples of coastal water collected at Llandudno beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

When comparing the treatments, there was no statistically significant difference in the week 0 mean inorganic phosphate concentrations of filtered and non-filtered samples despite the higher mean concentration of filtered samples. At week 12, no statistically significant differences in the mean in phosphate concentrations were found between filtered frozen samples and non-filtered frozen samples ($\mu_{ff}=\mu_{fnf}$).

### 3.1.1.2 Muizenberg beach

Fluctuations in the phosphate concentrations in stored samples were again noted with both showing an increase in the mean inorganic phosphate concentration over time (Figure 3.2). At the time of sampling the inorganic phosphate concentration in the non-filtered sample was higher ($\mu$= 0.674 µmol $L^{-1}$) than in the filtered sample ($\mu$= 0.4 µmol $L^{-1}$). As with the water samples taken at Llandudno, fluctuations were noted in the first weeks of the storage period after which an upward trend was noted. Over the twelve week trial the inorganic phosphate concentration in samples that had been filtered and frozen showed no statistically significant difference ($\mu_0=\mu_{12}$ p=0.494). Inorganic phosphate concentrations in the non-filtered frozen samples analysed at week 0 and week 12 were significantly different ($\mu_0\neq\mu_{12}$ p<0.001).

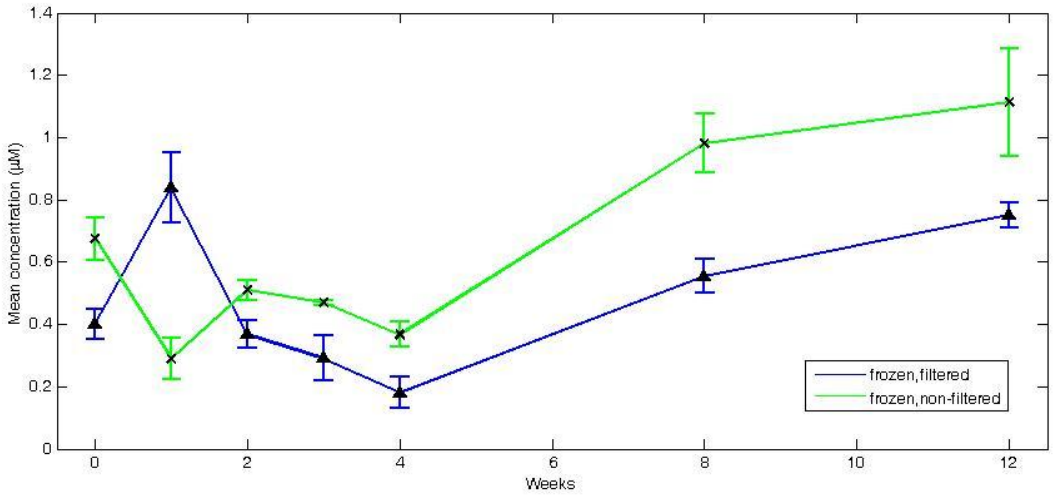


Figure 3.2 Mean inorganic phosphate concentrations in stored samples of coastal water collected at Muizenberg beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

When comparing the treatments, there were no statistically significant differences in the week 0 mean inorganic phosphate concentrations of filtered and non-filtered samples ($\mu_f=\mu_{nf}$) despite the higher mean concentration of non-filtered samples. No significant

difference in mean inorganic phosphate concentrations was observed between the frozen non-filtered and frozen filtered water samples analysed at week 12 (µff=µfnf).

## 3.1.2 Dissolved inorganic silicate

The mean inorganic silicate concentrations in stored samples of seawater collected at Llandudno Beach as a function of time are presented in Figure 3.3. The standard error within the triplicate samples for each storage strategy is indicated. Results for the sample collected at Muizenberg Beach are shown in Figure 3.4.

### 3.1.2.1 Llandudno beach

Fluctuations in the dissolved inorganic silicate concentrations in stored samples were noted with the overall tendency being a decrease in the mean inorganic silicate concentration over the first three weeks followed by an increase in the concentrations (Figure 3.3).

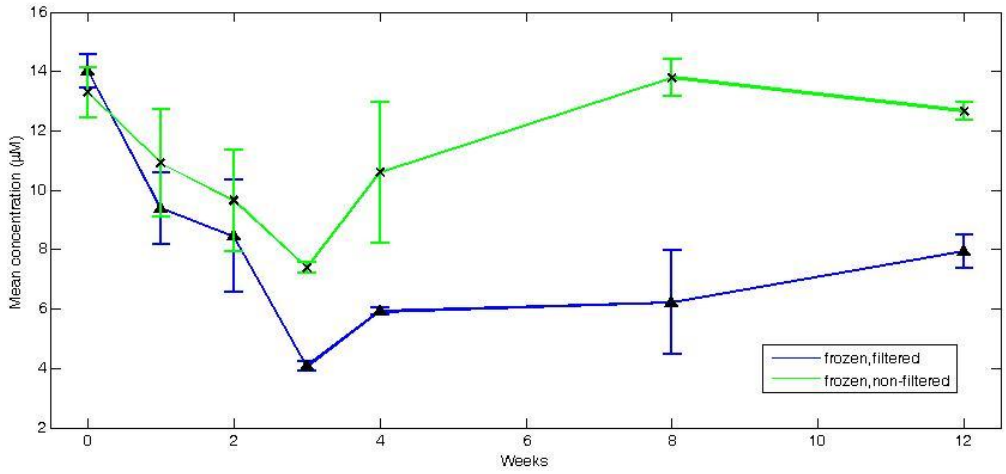


Figure 3.3 Mean dissolved inorganic silicate concentration in stored samples of coastal water collected at Llandudno beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen, non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

At the time of sampling (week 0) the inorganic silicate concentration in filtered samples was higher (µ=14.019 µmol $L^{-1}$) than in non-filtered samples (µ=13.296 µmol $L^{-1}$) (Figure 3.3). These differences were not statistically significant. Between week 0 and week 3, the mean inorganic silicate concentrations in filtered frozen samples decreased significantly from

μ=14.019 μmol $L^{-1}$ to μ=4.062 μmol $L^{-1}$ ($p<0.001$) and in non-filtered frozen samples decreased significantly from μ=13.296 μmol $L^{-1}$ to μ=7.389 μmol $L^{-1}$ ($p<0.001$). No significant difference in the dissolved inorganic silicate concentration was noted between week 0 and week 12 analyses of both the filtered and non-filtered frozen samples.

### 3.1.2.2 Muizenberg beach

In the water sampled at Muizenberg beach no clear trend in the dissolved inorganic silicate concentrations over time was noted (Figure 3.4). Values for both filtered and non-filtered frozen samples fluctuated over the 12 week storage period. Smaller fluctuations in the concentration of silicate in the non-filtered samples were recorded. The standard errors within the triplicate samples at each point were in many cases large.

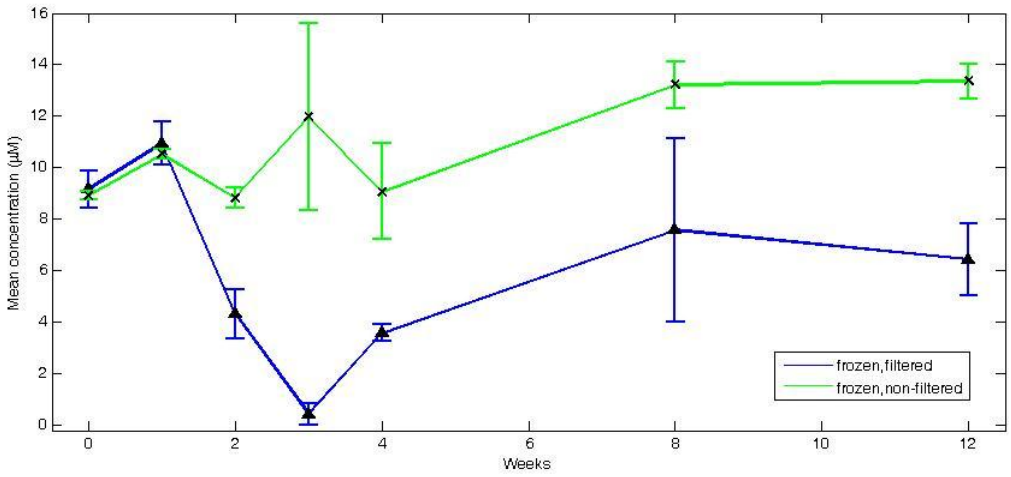


Figure 3.4 Mean dissolved inorganic silicate concentration in stored samples of coastal water collected at Muizenberg beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

At week 0 the concentration of dissolved inorganic silicate in filtered samples (μ=9.173 μmol $L^{-1}$) was lower than in non-filtered samples (μ=10.524μmol $L^{-1}$). Between week 0 and week 1 the concentration of dissolved inorganic silicate increased in the filtered and in the non-filtered samples. Between weeks 1 and 3 the dissolved inorganic silicate concentration in the filtered frozen samples decreased significantly ($p<0.001$) but increased thereafter and stabilised between weeks 8 and 12. The dissolved inorganic silicate concentration in the

non-filtered frozen sample fluctuated widely but an overall trend of an increase in concentration of the solute over the 12 week storage period was noted.

At the time of sampling (week 0) the inorganic silicate concentration in both the filtered and non-filtered seawater samples was not significantly different with mean values of 9.173 and 10.524 µmol $L^{-1}$ being recorded in the filtered and non-filtered samples respectively (Figure 3.4). In samples analysed after 12 weeks of storage the mean dissolved inorganic silicate concentration in both samples had not deviated significantly from the mean concentration determined at week 0. Because of the large standard deviation recorded for the filtered frozen sample at week 12 the mean value at this point may not be representative. Using Holm-Sidak analysis for the multiple comparisons at this point, no significant difference between the mean inorganic silicate concentrations in the seawater stored under the different storage regimes was noted.

## 3.1.3 Dissolved nitrite and nitrate

The mean nitrite concentrations in stored samples of seawater collected at Llandudno beach as a function of time are presented in Figure 3.5. The standard error within the triplicate samples for each storage strategy is indicated. Results for the sample collected at Muizenberg beach are shown in Figure 3.6.

### 3.1.3.1 Llandudno beach

No valid results were obtained for nitrate determinations (see discussion).

The general trend noted was that the mean nitrite concentrations in stored samples remained fairly constant over the first two (non-filtered) to three (filtered) weeks of storage before decreasing (Figure 3.5). At the time of sampling (week 0) the mean nitrite concentration in filtered samples was marginally higher (µ=0.619 µmol $L^{-1}$) than in non-filtered samples (µ=0.596 µmol $L^{-1}$). This difference was not statistically significant.

The mean concentration of nitrite in the frozen filtered samples analysed between weeks 0 and 3 increased slightly. Between weeks 3 and 4 a dramatic decrease (>50%) in the mean nitrite concentration of the samples was observed. This decrease was statistically significant

(p<0.001 when comparing values for weeks 0, 1, 2 and 3 to the value for week 4, Appendix 1). Between weeks 4 and 12 the mean nitrite concentration in the frozen filtered samples stabilized. The mean nitrite concentration at week 12 in the frozen filtered sample was 0.341µmol $L^{-1}$. This value was not statistically significantly different to the mean value determined on collection of the sample.

Mean nitrite concentrations in the frozen non-filtered samples followed a similar trend with the nitrite concentration in samples increasing slightly between week 0 and 2 before the statistically significant decrease between weeks 2 and 3 (p<0.001). A stabilisation in the mean nitrite concentration was noted between weeks 3 and 8 but thereafter a decrease in the recorded concentration to 0.171 µM was recorded. The difference between the results of non-filtered samples taken at week 0 and week 12 was statistically significant (p <0.001).

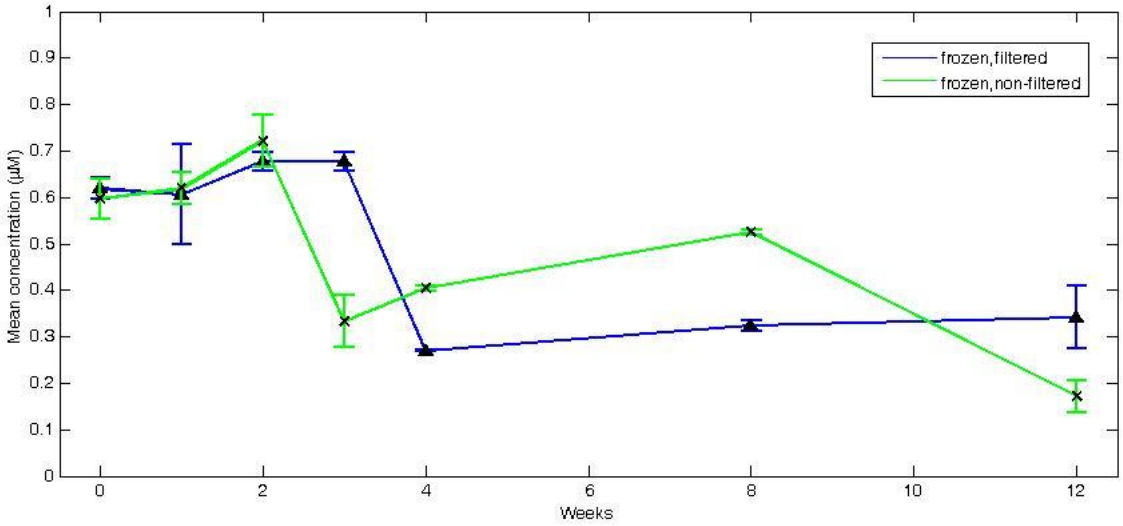


Figure 3.5 Mean dissolved nitrite concentration in stored samples of coastal water collected at Llandudno beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

In summary, despite the fluctuations in the mean nitrite concentrations determined in both the filtered and non-filtered frozen samples, no significant difference in the mean nitrite concentration between filtered and non-filtered samples was noted when the values determined at the time of sampling (week 0) were compared and at 12 weeks of storage at -20$^0$C (Appendix 1).

### 3.1.3.2 Muizenberg beach

No valid results were obtained for nitrate determinations (see discussion).

The differences in the mean nitrite concentration in filtered and non-filtered seawater collected at Muizenberg beach on sampling (week 0) were not statistically significant (Figure 3.6). The non-filtered sample contained a marginally higher mean concentration of nitrite ($\mu$=0.413 µmol $L^{-1}$) than the filtered sample ($\mu$=0.373 µmol $L^{-1}$) .

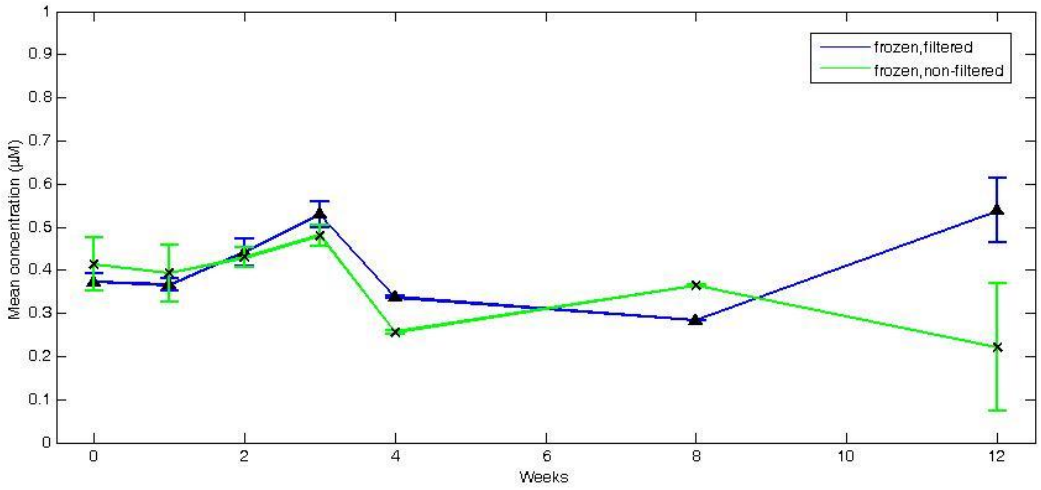


Figure 3.6 Mean dissolved nitrite concentration in stored samples of coastal water collected at Muizenberg beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

Initially, between week 0 and week 3, the concentration of nitrite increased slightly in both filtered and non filtered samples. Between week 3 and week 4 the nitrite concentration in both filtered and non filtered samples decreased rapidly. Between week 4 and week 8 the nitrite concentration in the filtered frozen samples continued to decrease after which an increase at week 12 was noted.

A different trend of an increase between weeks 4 and 8 followed by a decrease at week 12 was noted in the mean nitrite concentration of the non-filtered frozen samples. At week 12 no statistically significant difference in nitrite concentration was noted between the two storage treatments. Over the twelve week storage period (i.e. when comparing only the

values recorded for weeks 0 and 12) no significant difference in the mean nitrite concentrations for the filtered and the non-filtered frozen samples was evident.

## 3.1.4 Dissolved urea

The mean urea concentrations in stored samples of seawater collected at Llandudno beach as a function of time are presented in Figure 3.7. The standard error within the triplicate samples for each storage strategy is indicated. Results for the sample collected at Muizenberg beach are shown in Figure 3.8.

### 3.1.4.1 Llandudno beach

The mean urea concentrations in stored samples of coastal water sampled at Llandudno beach fluctuated little over the first eight weeks of storage (Figure 3.7).

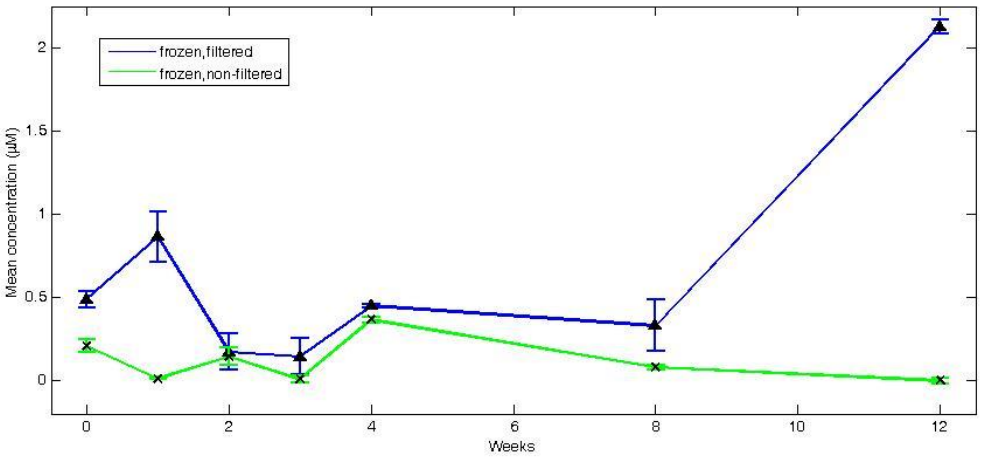


Figure 3.7 Mean urea concentration in stored samples of coastal water collected at Llandudno beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

At week 0 the mean concentration of urea in the filtered samples was higher (µ=0.486 µmol $L^{-1}$) than in the non-filtered samples (µ=0.209 µmol $L^{-1}$). A spike in the concentration of urea in filtered frozen samples was evident at week 1 but the value decreased at week 2.

Between week 2 and 8 a gradual increase in concentration was noted while a large increase at week 12 was evident. Starting at week 0 with a value of 0.209 µmol $L^{-1}$, the concentration of urea in the non-filtered frozen samples increased slightly to week 4 following which the values decreased until a near zero value at week 12 was recorded. In both the filtered and the non-filtered frozen samples analysed after 12 weeks of storage the mean urea concentration in all samples had deviated significantly from the mean concentration determined at week 0 ($p<0.001$). Using Holm-Sidak analysis for the multiple comparisons at these points, no significant difference between the mean urea concentrations in the seawater stored under the different storage regimes was noted at week 0 however at week 12 a significant difference between the two treatments was noted ($p<0.001$).

### 3.1.4.2 Muizenberg beach

The difference in the mean urea concentration in filtered and non-filtered seawater collected at Muizenberg beach on sampling (week 0) is shown in Figure 3.8 with the non-filtered sample having a higher concentration of urea (µ=1.520 µmol $L^{-1}$) than the filtered sample (µ=0.0333 µmol $L^{-1}$).

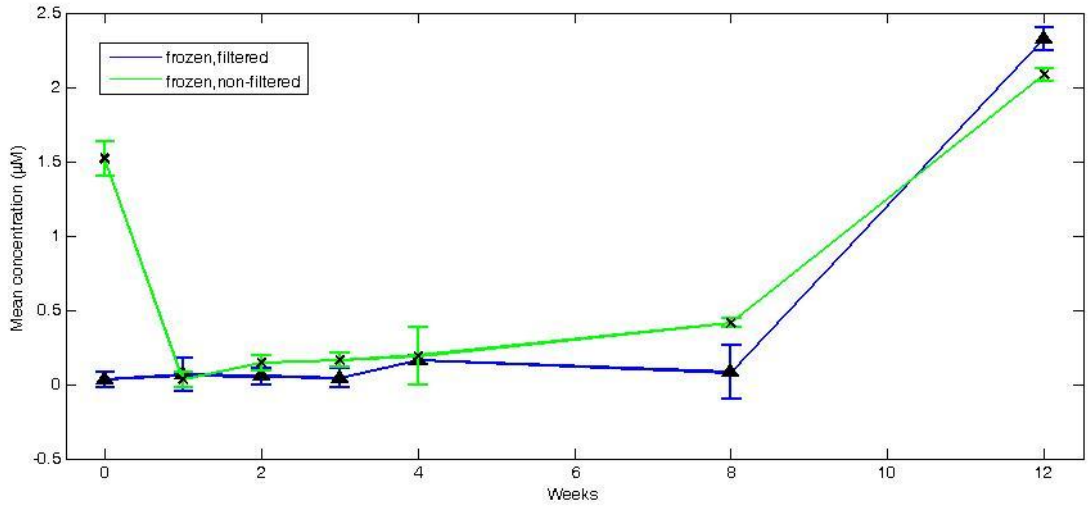


Figure 3.8 Mean urea concentration in stored samples of coastal water collected at Muizenberg beach over time. The standard error within the triplicate samples for each storage strategy is indicated. Storage procedures used were filtered and frozen and non-filtered and frozen. Samples were analysed after 1, 2, 3, 4, 8, and 12 weeks.

The initially high values in these samples decreased rapidly (within one week) in the filtered sample. Concentrations of urea in the filtered frozen samples remained low and stable. After week 8, the concentrations of urea in all samples across both of the storage strategies increased and at week 12 concentrations were >2 µmol $L^{-1}$. At this stage no statistically significant differences in urea concentrations were detectable between the two treatments. Over the twelve week storage period (i.e. when comparing only the values recorded for weeks 0 and 12) no significant difference in the mean urea concentrations for both the filtered samples and the non-filtered frozen samples was noted. Statistically the differences in the median values among treatment groups were not great enough to exclude the chance that the differences were due to random sampling variability.

## 3.2 Comparison of methodologies for the analysis of nutrients in seawater

Ten Atlantic Ocean surface water samples were collected in plastic containers from sites along the False Bay coastline and the Atlantic seaboard of Cape Town. Water samples were analysed in triplicate and the mean nutrient concentration was used to compare methodologies for the analysis of dissolved inorganic silicate (Figure 3.9) and dissolved nitrate (Figures 3.10 and 3.11). Seawater samples were collected from the following beaches: Llandudno, Houtbay, Muizenberg, Sea Point, Kalk Bay, and Glen Beach.

### 3.2.1 Determination of dissolved inorganic silicate

The mean concentration of silicate determined using the manual and the FIA method for ten beach samples is displayed in Figure 3.9. The methods used were a modification of the manual method of Grasshoff (1976) (section 2.5.2) (termed the manual method) and automated flow injection analysis (FIA method). The mean concentration of silicate in 70% of the water samples determined using the manual and the FIA methods was significantly different ($p<0.001$) (Figure 3.9). In these samples the mean concentration of silicate determined in the FIA method was higher than the mean concentration determined using

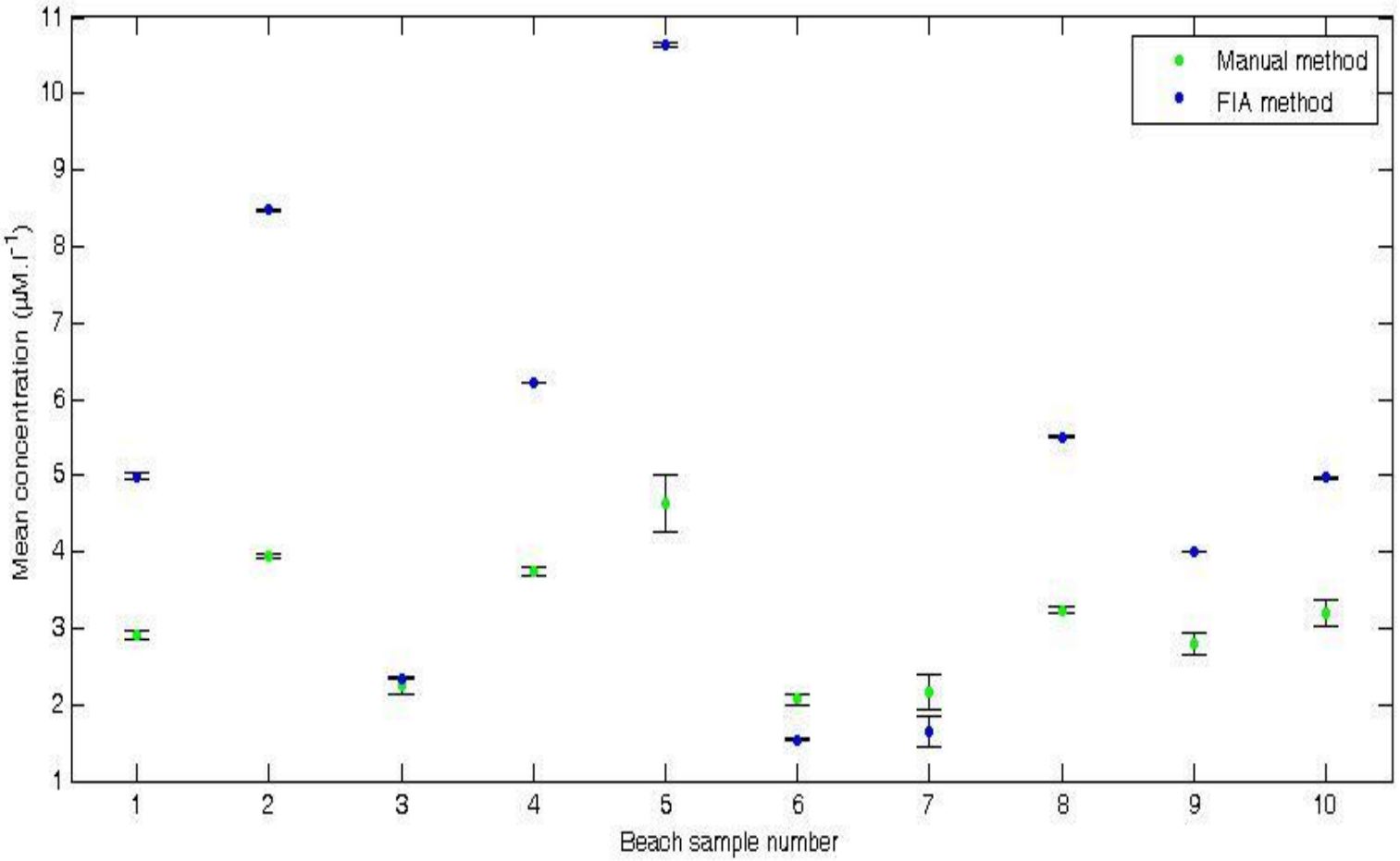


Figure 3.9 The mean concentration of dissolved inorganic silicate in seawater samples as determined using a modification of the manual method of Grasshoff (1976) (manual method) and automated flow injection analysis (FIA method). The beach sample numbers refer to samples collected from the following beaches: 1) Llandudno1, 2) Houtbay1, 3) Muizenberg1, 4) Sea Point1, 5) Kalk Bay, 6) Muizenberg2, 7) Sea Point2, 8) Glen Beach, 9) Houtbay2 and 10) Llandudno2.

the manual method. For samples 3 and 7 no significant difference in the silicate concentration was determined.

In samples 6 and 7 the mean concentration of silicate determined by the FIA method was lower than the mean concentration determined using the manual method. With the exception of beach sample 5 analysed using the manual method and sample 7 analysed using both the manual and FIA method, the standard deviation from the mean value was low.

### 3.2.2 Determination of dissolved nitrate

Two manual and one automated methods employed for the determination of dissolved nitrate concentrations in sea water were compared using the ten seawater samples collected along the False Bay and Atlantic Ocean coastline. The three analytical methods performed were the manual method (the shaking method) described in section 2.5.3, the manual method using a column to contain the cadmium granules (the cadmium column method) (section 2.6.3.2) and automated flow injection analysis (FIA) described in section 2.6.3.3. In addition the effect of substituting Tris buffer for ammonium chloride ($NH_4Cl$) buffer was investigated. Two analytical methods were used for this comparison: the shaking method and the cadmium column method. Samples were analysed in triplicate.

The mean concentration of dissolved nitrate in seawater samples as determined using the five analytical techniques for eight of the ten seawater samples is shown in Figure 3.10. For the sake of clarity the mean nitrate concentration of seawater in two samples (samples 9 and 10) using the five analytical techniques, which produced outliers with very high concentrations, are shown in Figure 3.11.

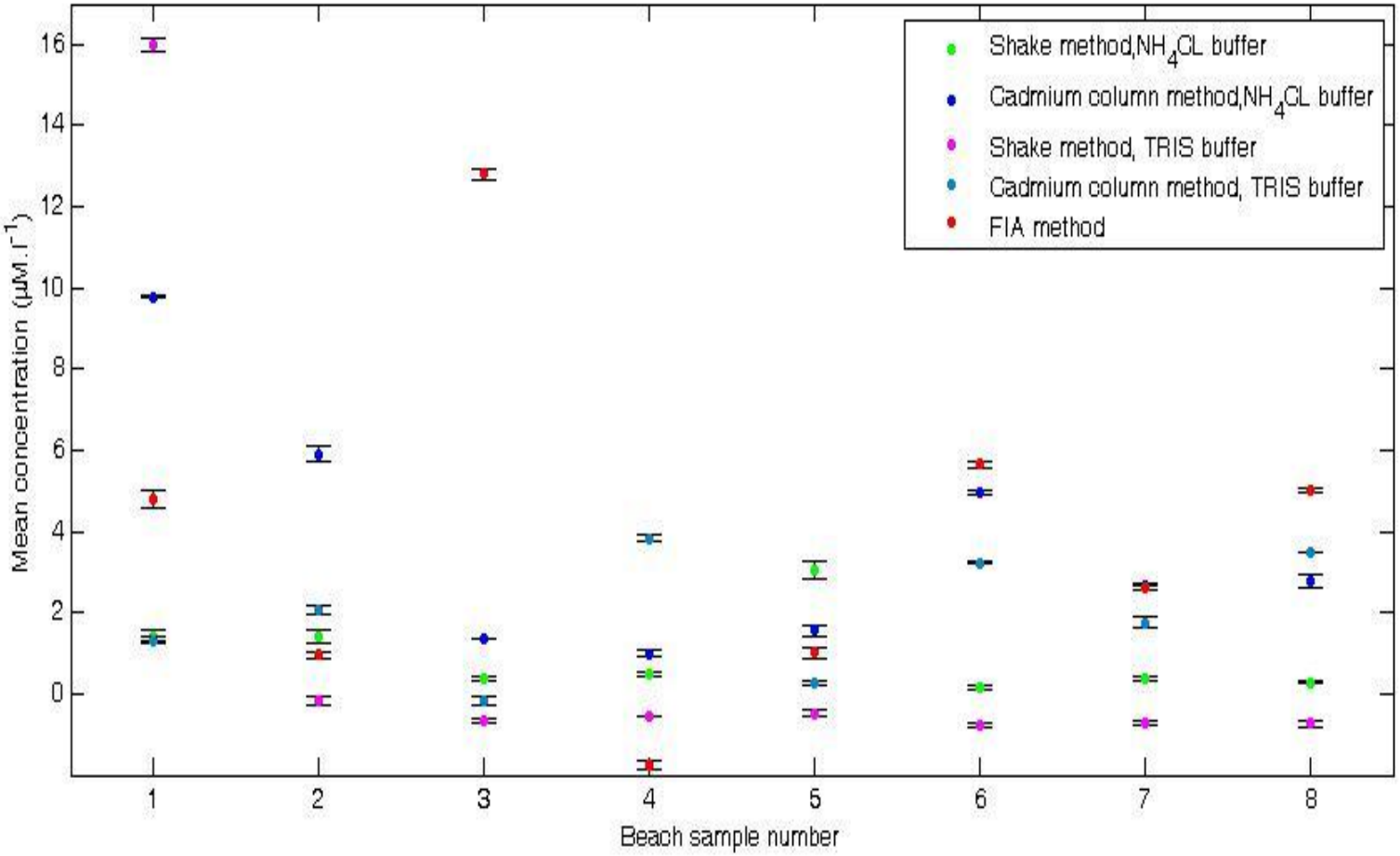


Figure 3.10 The mean concentration of dissolved nitrate in seawater samples as determined using the shaking method ($NH_4Cl$ buffer), the cadmium column method ($NH_4Cl$ buffer), the shaking method (TRIS buffer), the cadmium column method (TRIS buffer) and the FIA method. The beach sample numbers refer to samples collected from the following beaches: 1) Llandudno1, 2) Houtbay1, 3) Muizenberg1, 4) Kalk Bay, 5) Muizenberg2, 6) Sea Point2, 7) Houtbay2 and 8) Llandudno2.

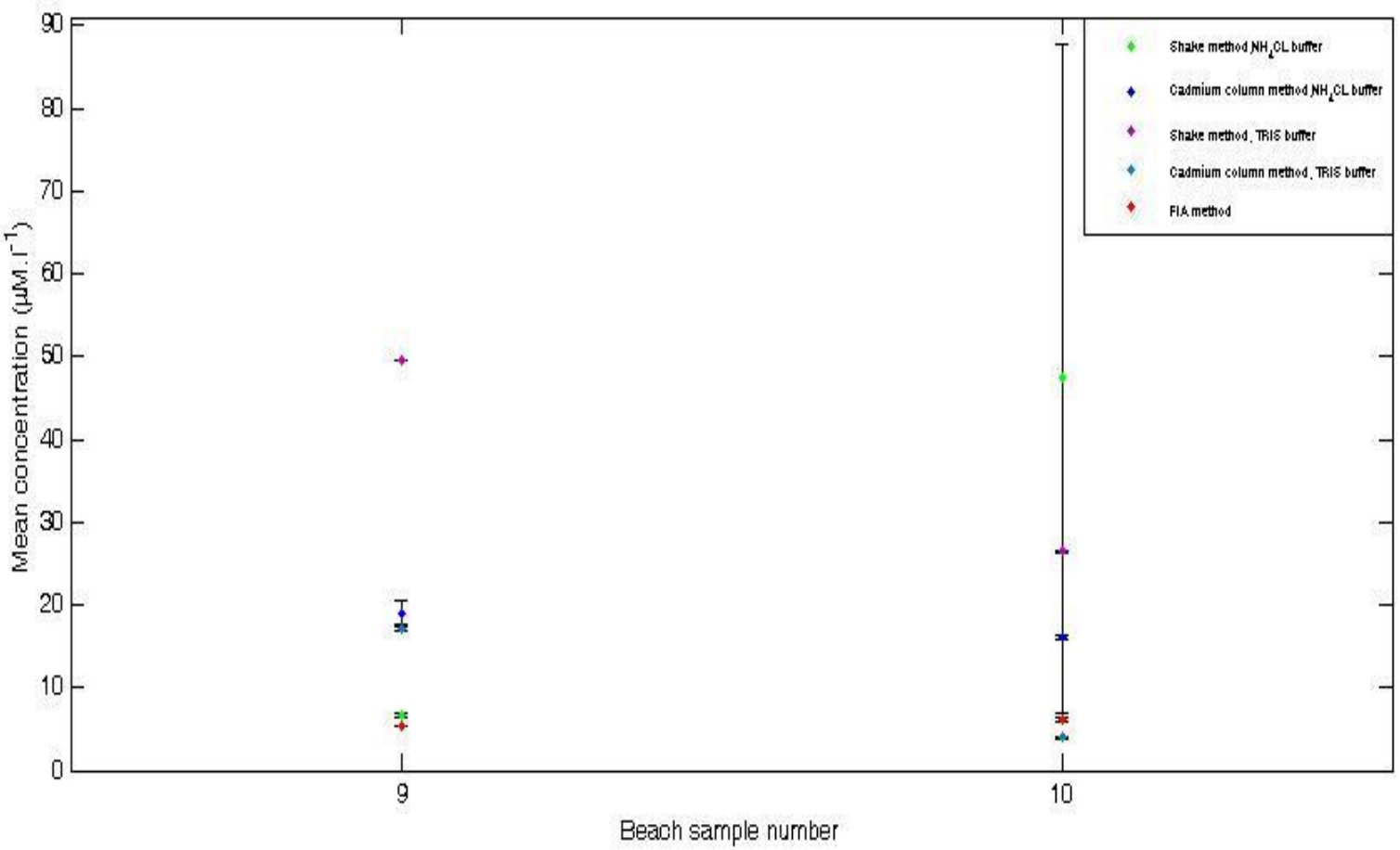


Figure 3.11 The mean concentration of dissolved nitrate in seawater samples as determined using the shaking method (NH4CL buffer), the cadmium column method (NH4CL buffer), the shaking method (TRIS buffer), the cadmium column method (TRIS buffer) and the FIA method. The beach sample numbers refer to samples collected from 9) Sea Point 1 and 10) Glen Beach.

The study produced results which were difficult to interpret. The following observations were made:

- with the exception of one instance using the shaking method ($NH_4Cl$ buffer) the standard deviation from the mean between the triplicate samples analysed using all five techniques was low (Figures 3.10 and 3.11)
- results generated using the shaking method where the reaction buffer was Tris produced negative values in seven instances. This may be attributed to the inadvertent use of a 0.087 mol $L^{-1}$ buffer as opposed to the intended 0.2 mol $L^{-1}$ buffer. Negative values were also recorded using the shaking method with $NH_4Cl$ buffer (one instance) and the FIA method (one instance) (Figure 3.10)
- analysis of the results of the mean dissolved nitrate concentrations determined in the ten seawater samples using the five techniques revealed that in most instances the methods yielded results that were statistically different
- Results of the analysis of seawater collected from Glen Beach (sample 10) were non-normally distributed. The differences between the median values of the treatments groups were not great enough to exclude the possibility that the noted differences were due to random sampling variability

  The results in Figures 3.10 and 3.11 illustrate that the dissolved nitrate concentrations in the water samples determined by FIA and the cadmium column method (using both Tris and $NH_4Cl$ buffers) differed significantly. Those determined by the shaking method (using both Tris and $NH_4Cl$ buffers) were frequently low (in the case of Tris buffer negative values were obtained) and significantly different to the values determined using FIA and the cadmium column.

# Chapter 4: Discussion and conclusion

No chain is stronger than its weakest link. This axiom applies also to the efforts of chemical oceanographers to quantitatively analyse the nutrients present in samples of seawater. To achieve reliable and accurate results requires the input of skilled analysts using reproducible techniques and sensitive instrumentation.

## 4.1 Filtration of samples

Seawater is not a homogeneous fluid, with the distribution of constituents being influenced by chemical, biological and physical processes (Grasshoff, 1976). In the study of ocean processes chemical oceanographers differentiate between dissolved matter and particulate matter. Size-exclusion filtration is an inexpensive and practical method to separate the two fractions in the field. The fraction of water that passes through a filter with a pore size of 0.45 µM is commonly known as the dissolved fraction (Grasshoff, 1976). A variety of biological and mineral particles pass through filters with a pore size of 0.45 µM. A filtered seawater sample would continue to be a biologically active system since the picoplankton (bacteria, archaea and some flagellates) and femtoplankton (viruses) continue to metabolise (Ikner *et al*., 2012).

Grasshoff (1976) and Hansen and Koroleff (1999) do not recommend the filtration of seawater samples prior to storage and analysis as they consider that the additional procedures required during the filtration process to be an avoidable source of contamination. The disadvantages of filtration (adsorption from the sample by the filter material and elution of nutrient containing intracellular fluids from mechanically lysed

micro-, nano- and picoplankton into the filtrate) outweigh the advantages of filtration (removal of solids that may interfere with the analysis and a decreased likelihood of biological activity in the filtrate) (Martin, 1968).

In this study chemical analysis of filtered and non-filtered water samples on the day of collection demonstrated that the filtering procedure did affect the concentrations of nutrients in the samples. In three of the eight filtered seawater samples the filtrate contained a lower concentration of nutrients than that of the unfiltered seawater sample (Table 4.1). Sample t-tests indicated that the differences in nutrient concentrations between filtered and non-filtered samples were not significant. No single strategy can be recommended for use for the determination of all stored nutrients. The concentration of dissolved nitrite in filtered samples was higher than in non-filtered water samples from both locations. Results for silicate, phosphate and urea concentrations in the filtered and non-filtered samples were not consistent.

Table 4.1 The effect of filtration on the concentration of nutrients in seawater samples analysed on the day of collection.

| **Nutrient** | **Sample site** | **Sample with higher nutrient concentration** |
|---|---|---|
| **Inorganic phosphate** | Llandudno | Filtered |
| **Inorganic phosphate** | Muizenberg | non-filtered |
| **Dissolved inorganic silicate** | Llandudno | Filtered |
| **Dissolved inorganic silicate** | Muizenberg | non-filtered |
| **Dissolved nitrite** | Llandudno | Filtered |
| **Dissolved nitrite** | Muizenberg | Filtered |
| **Dissolved urea** | Llandudno | Filtered |
| **Dissolved urea** | Muizenberg | non-filtered |

## 4.2 Storage of seawater samples for the analysis of nutrients

Analysis of the nutrients in seawater samples within a few hours of sampling is often not possible. Grasshoff (1976) cautions that no general procedure for the storage of seawaters can be recommended and that suitable storage strategies for each constituent should be determined. For many nutrients rapid freezing (i.e. freezing to -20$^0$C within 20 minutes using a glycol bath) is the best option. In this study one storage strategy for the filtered and non-filtered samples was considered. Freezing was effected by storing samples in a domestic freezer where the time taken for freezing of the entire sample was longer than 20 minutes.

### 4.2.1 Inorganic phosphate

The mean inorganic phosphate concentrations present on sampling at Llandudno and Muizenberg beach ranged from approximately 0.4 µM to 0.7 µmol $L^{-1}$ (Figures 3.1 and 3.2). Filtration of the samples did not affect the means significantly. Despite the spike in the mean value recorded for the filtered sample of water from Muizenberg beach stored at -20$^0$C for one week, filtration and storage of samples at -20°C showed smaller variability over the twelve week trial and appears to be the better storage strategy for the preservation of inorganic phosphate. This storage strategy was recommended by Heron (1962) who reported that the significant changes in the inorganic phosphate content that occur within an hour after collection are due to the release of phosphate from plankton, bacterial metabolism and by adsorption to mineralogenous compounds. Ideally samples should be analysed within the first 2 weeks of storage (Figures 3.1 and 3.2). Thereafter a gradual increase in the inorganic phosphate concentration was observed.

### 4.2.2 Dissolved inorganic silicate

The mean dissolved silicate concentrations present on sampling at Llandudno and Muizenberg beach ranged from approximately 9 µM to 14 µmol $L^{-1}$ (Figures 3.3 and 3.4). Filtration of the samples did not affect the means determined on sampling significantly. Neither of the storage strategies explored during this study proved ideal as fluctuations in the levels of nutrients were noted using both storage regimes. Polymerization of dissolved inorganic silicate in low-salinity frozen samples has been reported (Venrick and Hayward, 1985). To counteract this samples may be stored under acidic conditions (pH=2.5) and allowed to stand for a period of three hours following thawing. The better of the two tested strategies, which provided the least variability, was storage of non-filtered samples at -20°C. Grasshoff (1976) determined that storage and preservation of samples for silicate analysis was best achieved by acidifying the sample with sulphuric acid to a pH of 2.5 and storage at $4^0$C. Filtration of samples prior to preservation was not recommended. In this study filtered samples stored at -$20^0$C produced erratic results with values decreasing with storage time (Figures 3.3 and 3.4).

### 4.2.3 Dissolved nitrite and nitrate

Due to the unavailability of a cadmium column the shaking method was used to determine nitrate and nitrite in the twelve week trial. In this method the copperized cadmium granules used as a reductor are exposed to the atmosphere and rapidly lose the ability to reduce the nitrates. This method yielded unsatisfactory results (data not shown) and cannot be recommended for routine use. The method used to determine the concentration of nitrite

in this study is valid as the problematic reduction step used in the nitrate determinations is not necessary for the determination of nitrite concentrations. The mean nitrite concentrations present on sampling at Llandudno and Muizenberg beach ranged from approximately 0.4 µM to 0.7 µmol $L^{-1}$ (Figures 3.5 and 3.6). Filtration of the samples did not affect the means significantly. Despite the rapid decrease in the mean values recorded for the filtered and non-filtered frozen samples between weeks two to four both storage strategies showed a low variability over the first two weeks. From previous studies (Grasshoff, 1976; Kremling *et al*., 2007; Segura-Noguera *et al*., 2011) and data collected during this study the following sampling and storage strategies are recommended. Filtration using polycarbonate filters is advised for samples collected from turbid water regions but appears to be unnecessary for other water samples. Immediate analysis of samples is recommended (Grasshoff, 1976) for seawater samples with high nitrite concentrations, signifying high bacterial activity. Ideally samples (filtered and non-filtered) should be analysed within the first 2 weeks of storage (Figures 3.5 and 3.6). Further delays in analysis result in a sharp decrease in the nitrite concentration.

### 4.2.4 Urea

The mean dissolved urea concentration present on sampling at Llandudno was 0.2 µmol $L^{-1}$ in the non-filtered sample and 0.5 µmol $L^{-1}$ in the filtered sample (Figure 3.7). Values for the Muizenberg beach sample were 1.5 µmol $L^{-1}$ in the non-filtered sample with no detectable level of urea being present in the filtered sample (Figure 3.8). Filtration clearly affected the urea levels in the samples. No systemic studies on preservation techniques for the storage of seawater for urea analysis have been conducted but Grasshoff (1976) specifically states

that samples should not be filtered as many filters contain traces of urea. The non-detection of urea in the filtered samples from Muizenberg beach is difficult to explain. The urea concentrations in the non-filtered sample were not below the detection limit (0.1 µmol $L^{-1}$, Grasshoff, 1976). Compounds which are known to interfere with the analytical technique include citrulline, allantoine and thiourea (Goeyens *et al*., 1998). These compounds which are found in waters having a high animal production (Grasshoff, 1976) may be present in the more impacted waters off Muizenberg beach.

The outstanding feature of the preservation study on variations in the concentration of urea in stored water over time is the major increase in concentration (between 2.5- and 6- fold) after 12 weeks of storage in three of the four trials (Figures 3.7 and 3.8). In addition when comparing the urea concentration in non-filtered water sampled at Muizenberg a sharp decrease is noted between weeks 0 and 1 (Figure 3.8). Grasshoff (1976) recommends rapid freezing of non-filtered samples for urea analysis. In this study samples for storage were frozen in a domestic freezer, a process which requires time and the effects of which may not be comparable to rapid freezing in liquid nitrogen. Nevertheless freezing of non-filtered samples proved to be the strategy which provided the least variability for the sample taken at Llandudno beach (Figure 3.7). The two storage strategies used on samples taken at Llandudno provided fairly unchanged results for urea content up until eight weeks after collection (Figure 3.7).

## 4.3 Comparison of methodologies for the analysis of nutrients in seawater

### 4.3.1 Dissolved inorganic silicate

The results of this study are inconclusive. Ten seawater samples were analysed to determining the dissolved inorganic silicate concentration in triplicate using a manual method and an automated FIA method. The results show a statistically significant difference in the mean dissolved silicate concentrations determined using the two methods in eight out of ten samples while results for the remaining two were not statistically significant (Figure 3.7). Standard deviation within the triplicates of each sample was low for both methods with those for the FIA method being less variable and showing more precision. For seven out of the ten seawater samples the FIA method yielded dissolved silicate concentrations that were on average two times higher than the manual method. Human error, calibration of the spectrophotometer and scratches on plastic test tubes may have played a role in the lower concentration values determined using the manual method in the seawater samples.

### 4.3.2 Dissolved nitrate

In many cases three of the five methods (FIA, cadmium column using Tris buffer and cadmium column using $NH_4Cl$ buffer) used to determine the dissolved nitrate concentration in ten seawater samples yielded similar results (Figures 3.8 and 3.9) yet they were found to be statistically significantly different. The total variability of individual samples (i.e. the standard deviation) using each of the three techniques was low and the values yielded by

these methods are deemed to be close to the true concentration of dissolved nitrate present in the water samples. In nine out of the ten samples analysed the dissolved nitrate concentrations determined using the shaking method using both the Tris buffer and the $NH_4Cl$ buffer did not agree with those determined using the other methods. In general the concentrations of dissolved nitrate determined using the shaking method with an $NH_4Cl$ buffer were low while those determined using a Tris buffer produced negative results.

## 4.4 Conclusion and future studies

This study on the storage of filtered and unfiltered samples of coastal seawater from the Atlantic Ocean showed that filtration affected the concentration of nutrients in the samples. Minor variations in the concentration of phosphate, silicate and nitrite were detected whereas filtered samples for the analysis of urea showed significant variations. No single storage strategy for the determination of all nutrients can be recommended. Filtered samples for inorganic phosphate determinations stored well at -20°C for eight weeks. No strategy for the preservation of samples for silicate determination was ideal and immediate analysis is recommended. Analysis within the first two weeks is recommended for the determination of nitrite and no extended storage strategy is recommended. Samples which were non-filtered and frozen provided the least amount of variability in the concentration of urea for one of the two trials with concentrations remaining relatively stable for eight weeks after collection.

A study on coastal water samples comparing two analytical techniques used to determine silicate concentrations in seawater showed that the FIA method yielded higher concentrations in seven out of the ten analyses than the manual method of Grasshoff (1976). The results of the study comparing five analytical techniques used to determine dissolved nitrate concentrations in seawater were less convincing. Although the standard deviation from the mean between the triplicate samples analysed using the five techniques was low in 98% of the cases, no clear trends emerged. The five methods generated different results. In addition it could not be concluded that any two methods routinely produced comparable results.

The outcomes of this research may be considered to be a preliminary study as numerous obstacles were encountered which in this study were dealt with in the best available manner. Analyses in marine analytical chemistry depend on a combination of the skill of the analyst, the use of appropriate sampling, storage and analytical techniques, sensitive instrumentation and the use of analytical grade reagents for analyses. Since the dissolved nutrients in seawater may be present in parts per million concentrations the latter point is of critical importance.

Filtration and rapid freezing equipment were not always available during the course of the study. The norm in oceanography is for water samples to be filtered through Whatman GFF filters (H.Waldron, personal communication) or through filters with a pore size of 0.45µM (Grasshoff, 1976; Munn, 2011). Bulk seawater samples were filtered using filters with a pore size of 0.7 µm permitting many nano- and phytoplankton species to remain in the filtrate where they would remain biologically active. Rapid freezing in a glycol bath is frequently

recommended (Grasshoff, 1976) and liquid nitrogen or dry ice freezing prior to storage at -20°C may be considered in future studies.

The tools of the trade for many analytical chemists determining the concentration of nutrients in seawater are a range of micropipettes and a spectrophotometer. Servicing and calibration of these instruments is essential. Although a glass test tube adaptor is routinely employed during field and cruise analysis the use of optical quality cuvettes in a high-sensitivity spectrophotometer is recommended. The use of low nutrient seawater or artificial seawater in spectrophotometric assays will aid in overcoming any interferences due to the seawater matrix or the salt-effects of seawater (Wurl, 2009; Daniel et al., 2012). Primary standard solutions prepared using chemicals of certified purity may be validated against standards obtained from reputable sources e.g. MOOS-2: Seawater Certified Reference Material for Nutrients (NRC Canada Institute for National Measurement Standards) or the CentriPUR range of standards (Merck, Darmstadt).

Two modifications to the techniques used in this study are suggested:

1. Grasshoff (1976) recommends thawing for 2-3 hours when analysing frozen samples for silicate. The effect of extending the period for thawing to 24 hours should be investigated. When analysing for nitrates using a cadmium column and Tris buffer, the use of higher concentrations of Tris buffer should be investigated. In this study the final concentration of buffer in the reaction was 0.087M – an error in calculation as it was intended to use a final concentration of 0.2M.

2. The study comparing analytical techniques used to determine dissolved nitrate concentrations in seawater resulted in a dataset that was difficult to interpret. Although the

standard deviation from the mean between triplicate samples was low, the five methods generated values which differed. It is suggested that the study should be redesigned focussing on fewer methods (the shake method yielded low or negative results for nitrate concentrations in six of the ten samples), decreasing the sampling stations initially to a single site and attempting a large number of replicate analyses with this water sample. These measures would allow conclusions to be drawn about the reliability of the technique and the equipment used and would give an indication of the accuracy and precision of the results generated. The exercise would benefit from the inclusion of an externally sourced validated standard.

Once a reliable analytical technique is available, it would be valuable to determine whether the apparent increase in nutrient concentrations noted in the bulk water samples is due to regeneration events takinf place in the sample.

Finally, pasteurization has been shown to be a reliable method for the preservation of nutrients in seawater samples (Daniel et al., 2012) and its use should be considered in future studies. The process is simple and does not require sophisticated equipment. Samples are pasteurized in high density polyethylene bottles (except for ammonium samples which are treated in glass bottles) for two hours at $80^0C \pm 3^0C$. Pasteurised samples are stored at room temperature. Except for ammonium, detectable concentration changes in nutrient concentrations were not produced (Daniel et al., 2012).

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# Appendix 1

Statistics for different storage strategies for Inorganic phosphate, silicate and urea and nitrite at Llandudno and Muizenberg Beach

Ff =Samples stored at -20°C and filtered
FNF = Samples stored at -20°C non filtered
Numbers = weeks
i.e. FF1 = A sample that was stored at -20°C and was filtered for one week

## 1.0 Inorganic Phosphate

### 1.1 Llandudno Beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.347)

**Equal Variance Test:** Passed (P = 0.649)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 0.598 | 0.190 | 0.110 |
| ff1 | 3 | 0 | 0.630 | 0.0760 | 0.0439 |
| ff2 | 3 | 0 | 0.488 | 0.0685 | 0.0395 |
| ff3 | 3 | 0 | 0.565 | 0.362 | 0.209 |
| ff4 | 3 | 0 | 0.587 | 0.209 | 0.121 |
| ff8 | 3 | 0 | 0.598 | 0.219 | 0.126 |
| ff12 | 3 | 0 | 0.751 | 0.0190 | 0.0110 |
| fnf0 | 3 | 0 | 0.389 | 0.100 | 0.0580 |
| fnf1 | 3 | 0 | 0.411 | 0.119 | 0.0685 |
| fnf2 | 3 | 0 | 0.510 | 0.0658 | 0.0380 |
| fnf3 | 3 | 0 | 0.981 | 0.169 | 0.0975 |
| fnf4 | 3 | 0 | 0.610 | 0.172 | 0.0996 |
| fnf8 | 3 | 0 | 0.751 | 0.116 | 0.0667 |
| fnf12 | 3 | 0 | 1.212 | 0.0380 | 0.0219 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 2 | 0.0470 | 0.0235 | | |
| Between Treatments | 13 | 1.906 | 0.147 | 5.480 | <0.001 |
| Residual | 26 | 0.696 | 0.0268 | | |
| Total | 41 | 2.649 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 0.996

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| fnf12 vs. fnf0 | 0.822 | 6.158 | <0.001 | Yes |
| fnf12 vs. fnf1 | 0.800 | 5.994 | <0.001 | Yes |
| fnf12 vs. ff2 | 0.724 | 5.419 | <0.001 | Yes |
| fnf12 vs. fnf2 | 0.702 | 5.255 | 0.002 | Yes |
| fnf12 vs. ff3 | 0.647 | 4.844 | 0.004 | Yes |
| fnf12 vs. ff4 | 0.625 | 4.680 | 0.007 | Yes |
| fnf12 vs. ff0 | 0.614 | 4.598 | 0.008 | Yes |
| fnf12 vs. ff8 | 0.614 | 4.598 | 0.008 | Yes |
| fnf12 vs. fnf4 | 0.601 | 4.503 | 0.010 | Yes |
| fnf3 vs. fnf0 | 0.592 | 4.434 | 0.012 | Yes |
| fnf12 vs. ff1 | 0.581 | 4.351 | 0.015 | Yes |
| fnf3 vs. fnf1 | 0.570 | 4.269 | 0.018 | Yes |
| fnf3 vs. ff2 | 0.493 | 3.695 | 0.078 | No |
| fnf3 vs. fnf2 | 0.471 | 3.530 | 0.115 | No |
| fnf12 vs. ff12 | 0.461 | 3.448 | 0.138 | No |
| fnf12 vs. fnf8 | 0.461 | 3.448 | 0.137 | No |
| fnf3 vs. ff3 | 0.417 | 3.120 | 0.281 | No |
| fnf3 vs. ff4 | 0.395 | 2.956 | 0.385 | No |
| fnf3 vs. ff0 | 0.384 | 2.874 | 0.443 | No |
| fnf3 vs. ff8 | 0.384 | 2.874 | 0.438 | No |
| fnf3 vs. fnf4 | 0.371 | 2.778 | 0.510 | No |
| fnf8 vs. fnf0 | 0.362 | 2.709 | 0.563 | No |
| ff12 vs. fnf0 | 0.362 | 2.709 | 0.558 | No |
| fnf3 vs. ff1 | 0.351 | 2.627 | 0.623 | No |
| fnf8 vs. fnf1 | 0.340 | 2.545 | 0.687 | No |
| ff12 vs. fnf1 | 0.340 | 2.545 | 0.682 | No |
| fnf8 vs. ff2 | 0.263 | 1.970 | 0.981 | No |
| ff12 vs. ff2 | 0.263 | 1.970 | 0.980 | No |
| ff1 vs. fnf0 | 0.241 | 1.806 | 0.996 | No |
| fnf8 vs. fnf2 | 0.241 | 1.806 | 0.995 | No |
| ff12 vs. fnf2 | 0.241 | 1.806 | 0.995 | No |
| fnf12 vs. fnf3 | 0.230 | 1.724 | 0.998 | No |
| fnf3 vs. ff12 | 0.230 | 1.724 | 0.997 | No |
| fnf3 vs. fnf8 | 0.230 | 1.724 | 0.997 | No |
| fnf4 vs. fnf0 | 0.221 | 1.655 | 0.999 | No |
| ff1 vs. fnf1 | 0.219 | 1.642 | 0.999 | No |
| ff8 vs. fnf0 | 0.208 | 1.560 | 1.000 | No |
| ff0 vs. fnf0 | 0.208 | 1.560 | 0.999 | No |
| fnf4 vs. fnf1 | 0.199 | 1.491 | 1.000 | No |
| ff4 vs. fnf0 | 0.197 | 1.478 | 1.000 | No |
| fnf8 vs. ff3 | 0.186 | 1.396 | 1.000 | No |
| ff8 vs. fnf1 | 0.186 | 1.396 | 1.000 | No |
| ff12 vs. ff3 | 0.186 | 1.396 | 1.000 | No |
| ff0 vs. fnf1 | 0.186 | 1.396 | 1.000 | No |
| ff3 vs. fnf0 | 0.175 | 1.314 | 1.000 | No |
| ff4 vs. fnf1 | 0.175 | 1.314 | 1.000 | No |
| fnf8 vs. ff4 | 0.164 | 1.232 | 1.000 | No |
| ff12 vs. ff4 | 0.164 | 1.232 | 1.000 | No |
| fnf8 vs. ff0 | 0.154 | 1.149 | 1.000 | No |
| ff12 vs. ff0 | 0.154 | 1.149 | 1.000 | No |
| fnf8 vs. ff8 | 0.154 | 1.149 | 1.000 | No |
| ff12 vs. ff8 | 0.154 | 1.149 | 1.000 | No |
| ff3 vs. fnf1 | 0.154 | 1.149 | 1.000 | No |
| ff1 vs. ff2 | 0.143 | 1.067 | 1.000 | No |
| fnf8 vs. fnf4 | 0.141 | 1.054 | 1.000 | No |
| ff12 vs. fnf4 | 0.141 | 1.054 | 1.000 | No |

| | | | | |
|---|---|---|---|---|
| fnf4 vs. ff2 | 0.122 | 0.916 | 1.000 | No |
| fnf2 vs. fnf0 | 0.121 | 0.903 | 1.000 | No |
| fnf8 vs. ff1 | 0.121 | 0.903 | 1.000 | No |
| ff12 vs. ff1 | 0.121 | 0.903 | 1.000 | No |
| ff1 vs. fnf2 | 0.121 | 0.903 | 1.000 | No |
| ff8 vs. ff2 | 0.110 | 0.821 | 1.000 | No |
| ff0 vs. ff2 | 0.110 | 0.821 | 1.000 | No |
| fnf4 vs. fnf2 | 0.100 | 0.752 | 1.000 | No |
| ff2 vs. fnf0 | 0.0987 | 0.739 | 1.000 | No |
| fnf2 vs. fnf1 | 0.0987 | 0.739 | 1.000 | No |
| ff4 vs. ff2 | 0.0987 | 0.739 | 1.000 | No |
| ff8 vs. fnf2 | 0.0877 | 0.657 | 1.000 | No |
| ff0 vs. fnf2 | 0.0877 | 0.657 | 1.000 | No |
| ff2 vs. fnf1 | 0.0768 | 0.575 | 1.000 | No |
| ff3 vs. ff2 | 0.0768 | 0.575 | 1.000 | No |
| ff4 vs. fnf2 | 0.0768 | 0.575 | 1.000 | No |
| ff1 vs. ff3 | 0.0658 | 0.493 | 1.000 | No |
| ff3 vs. fnf2 | 0.0548 | 0.411 | 1.000 | No |
| fnf4 vs. ff3 | 0.0456 | 0.342 | 1.000 | No |
| ff1 vs. ff4 | 0.0439 | 0.328 | 1.000 | No |
| ff1 vs. ff8 | 0.0329 | 0.246 | 1.000 | No |
| ff8 vs. ff3 | 0.0329 | 0.246 | 1.000 | No |
| ff0 vs. ff3 | 0.0329 | 0.246 | 1.000 | No |
| ff1 vs. ff0 | 0.0329 | 0.246 | 1.000 | No |
| fnf4 vs. ff4 | 0.0237 | 0.177 | 1.000 | No |
| fnf2 vs. ff2 | 0.0219 | 0.164 | 1.000 | No |
| fnf1 vs. fnf0 | 0.0219 | 0.164 | 1.000 | No |
| ff4 vs. ff3 | 0.0219 | 0.164 | 1.000 | No |
| ff1 vs. fnf4 | 0.0202 | 0.151 | 1.000 | No |
| fnf4 vs. ff0 | 0.0127 | 0.0952 | 1.000 | No |
| fnf4 vs. ff8 | 0.0127 | 0.0952 | 1.000 | No |
| ff0 vs. ff4 | 0.0110 | 0.0821 | 1.000 | No |
| ff8 vs. ff4 | 0.0110 | 0.0821 | 1.000 | No |
| fnf8 vs. ff12 | 2.220E-016 | 1.663E-015 | 1.000 | No |
| ff8 vs. ff0 | 2.220E-016 | 1.663E-015 | 1.000 | No |

## 1.2 Muizenberg Beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.883)

**Equal Variance Test:** Passed (P = 0.234)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 0.400 | 0.0828 | 0.0478 |
| ff1 | 3 | 0 | 0.839 | 0.197 | 0.114 |
| ff2 | 3 | 0 | 0.367 | 0.0760 | 0.0439 |
| ff3 | 3 | 0 | 0.291 | 0.125 | 0.0719 |
| ff4 | 3 | 0 | 0.181 | 0.0870 | 0.0502 |
| ff8 | 3 | 0 | 0.554 | 0.0950 | 0.0548 |
| ff12 | 3 | 0 | 0.751 | 0.0685 | 0.0395 |
| fnf0 | 3 | 0 | 0.674 | 0.119 | 0.0685 |
| fnf1 | 3 | 0 | 0.291 | 0.116 | 0.0667 |
| fnf2 | 3 | 0 | 0.510 | 0.0570 | 0.0329 |
| fnf3 | 3 | 0 | 0.469 | 0.0119 | 0.00685 |
| fnf4 | 3 | 0 | 0.367 | 0.0685 | 0.0395 |

| | | | | | |
|---|---|---|---|---|---|
| fnf8 | 3 | 0 | 0.981 | 0.166 | 0.0956 |
| fnf12 | 3 | 0 | 1.113 | 0.298 | 0.172 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Subjects | 2 | 0.0139 | 0.00696 | | |
| Between Treatments | 13 | 3.030 | 0.233 | 12.982 | <0.001 |
| Residual | 26 | 0.467 | 0.0180 | | |
| Total | 41 | 3.511 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| **Comparison** | **Diff of Means** | **t** | **P** | **P<0.050** |
|---|---|---|---|---|
| fnf12 vs. ff4 | 0.932 | 8.519 | <0.001 | Yes |
| fnf12 vs. ff3 | 0.822 | 7.517 | <0.001 | Yes |
| fnf12 vs. fnf1 | 0.822 | 7.517 | <0.001 | Yes |
| fnf8 vs. ff4 | 0.800 | 7.316 | <0.001 | Yes |
| fnf12 vs. ff2 | 0.746 | 6.815 | <0.001 | Yes |
| fnf12 vs. fnf4 | 0.746 | 6.815 | <0.001 | Yes |
| fnf12 vs. ff0 | 0.713 | 6.515 | <0.001 | Yes |
| fnf8 vs. ff3 | 0.691 | 6.314 | <0.001 | Yes |
| fnf8 vs. fnf1 | 0.691 | 6.314 | <0.001 | Yes |
| ff1 vs. ff4 | 0.658 | 6.013 | <0.001 | Yes |
| fnf12 vs. fnf3 | 0.644 | 5.888 | <0.001 | Yes |
| fnf8 vs. fnf4 | 0.614 | 5.613 | <0.001 | Yes |
| fnf8 vs. ff2 | 0.614 | 5.613 | <0.001 | Yes |
| fnf12 vs. fnf2 | 0.603 | 5.512 | <0.001 | Yes |
| fnf8 vs. ff0 | 0.581 | 5.312 | 0.001 | Yes |
| ff12 vs. ff4 | 0.570 | 5.212 | 0.001 | Yes |
| fnf12 vs. ff8 | 0.559 | 5.111 | 0.002 | Yes |
| ff1 vs. ff3 | 0.548 | 5.011 | 0.002 | Yes |
| ff1 vs. fnf1 | 0.548 | 5.011 | 0.002 | Yes |
| fnf8 vs. fnf3 | 0.513 | 4.685 | 0.006 | Yes |
| fnf0 vs. ff4 | 0.493 | 4.510 | 0.009 | Yes |
| ff1 vs. ff2 | 0.471 | 4.310 | 0.014 | Yes |
| ff1 vs. fnf4 | 0.471 | 4.310 | 0.014 | Yes |
| fnf8 vs. fnf2 | 0.471 | 4.310 | 0.014 | Yes |
| ff12 vs. ff3 | 0.461 | 4.209 | 0.018 | Yes |
| ff12 vs. fnf1 | 0.461 | 4.209 | 0.018 | Yes |
| ff1 vs. ff0 | 0.439 | 4.009 | 0.029 | Yes |
| fnf12 vs. fnf0 | 0.439 | 4.009 | 0.029 | Yes |
| fnf8 vs. ff8 | 0.428 | 3.909 | 0.037 | Yes |
| fnf0 vs. ff3 | 0.384 | 3.508 | 0.098 | No |
| ff12 vs. fnf4 | 0.384 | 3.508 | 0.097 | No |
| ff12 vs. ff2 | 0.384 | 3.508 | 0.095 | No |
| fnf0 vs. fnf1 | 0.384 | 3.508 | 0.094 | No |
| ff8 vs. ff4 | 0.373 | 3.408 | 0.117 | No |
| ff1 vs. fnf3 | 0.370 | 3.383 | 0.122 | No |
| fnf12 vs. ff12 | 0.362 | 3.307 | 0.143 | No |
| ff12 vs. ff0 | 0.351 | 3.207 | 0.177 | No |

| | | | | |
|---|---|---|---|---|
| ff1 vs. fnf2 | 0.329 | 3.007 | 0.269 | No |
| fnf2 vs. ff4 | 0.329 | 3.007 | 0.265 | No |
| fnf0 vs. fnf4 | 0.307 | 2.806 | 0.387 | No |
| fnf0 vs. ff2 | 0.307 | 2.806 | 0.381 | No |
| fnf8 vs. fnf0 | 0.307 | 2.806 | 0.375 | No |
| fnf3 vs. ff4 | 0.288 | 2.631 | 0.502 | No |
| ff1 vs. ff8 | 0.285 | 2.606 | 0.515 | No |
| ff12 vs. fnf3 | 0.282 | 2.581 | 0.528 | No |
| fnf0 vs. ff0 | 0.274 | 2.506 | 0.583 | No |
| fnf12 vs. ff1 | 0.274 | 2.506 | 0.575 | No |
| ff8 vs. ff3 | 0.263 | 2.405 | 0.650 | No |
| ff8 vs. fnf1 | 0.263 | 2.405 | 0.641 | No |
| ff12 vs. fnf2 | 0.241 | 2.205 | 0.790 | No |
| fnf8 vs. ff12 | 0.230 | 2.105 | 0.849 | No |
| fnf2 vs. ff3 | 0.219 | 2.004 | 0.898 | No |
| ff0 vs. ff4 | 0.219 | 2.004 | 0.892 | No |
| fnf2 vs. fnf1 | 0.219 | 2.004 | 0.886 | No |
| fnf0 vs. fnf3 | 0.206 | 1.879 | 0.936 | No |
| ff12 vs. ff8 | 0.197 | 1.804 | 0.956 | No |
| fnf4 vs. ff4 | 0.186 | 1.704 | 0.975 | No |
| ff2 vs. ff4 | 0.186 | 1.704 | 0.973 | No |
| ff8 vs. fnf4 | 0.186 | 1.704 | 0.969 | No |
| ff8 vs. ff2 | 0.186 | 1.704 | 0.966 | No |
| fnf3 vs. fnf1 | 0.178 | 1.629 | 0.978 | No |
| fnf3 vs. ff3 | 0.178 | 1.629 | 0.975 | No |
| ff1 vs. fnf0 | 0.164 | 1.503 | 0.989 | No |
| fnf0 vs. fnf2 | 0.164 | 1.503 | 0.987 | No |
| ff8 vs. ff0 | 0.154 | 1.403 | 0.994 | No |
| fnf2 vs. ff2 | 0.143 | 1.303 | 0.997 | No |
| fnf2 vs. fnf4 | 0.143 | 1.303 | 0.997 | No |
| fnf8 vs. ff1 | 0.143 | 1.303 | 0.996 | No |
| fnf12 vs. fnf8 | 0.132 | 1.203 | 0.998 | No |
| fnf0 vs. ff8 | 0.121 | 1.102 | 0.999 | No |
| ff0 vs. ff3 | 0.110 | 1.002 | 1.000 | No |
| fnf1 vs. ff4 | 0.110 | 1.002 | 1.000 | No |
| ff0 vs. fnf1 | 0.110 | 1.002 | 0.999 | No |
| fnf2 vs. ff0 | 0.110 | 1.002 | 0.999 | No |
| ff3 vs. ff4 | 0.110 | 1.002 | 0.999 | No |
| fnf3 vs. fnf4 | 0.101 | 0.927 | 0.999 | No |
| fnf3 vs. ff2 | 0.101 | 0.927 | 0.999 | No |
| ff1 vs. ff12 | 0.0877 | 0.802 | 1.000 | No |
| ff8 vs. fnf3 | 0.0850 | 0.777 | 1.000 | No |
| ff2 vs. ff3 | 0.0768 | 0.702 | 1.000 | No |
| fnf4 vs. ff3 | 0.0768 | 0.702 | 0.999 | No |
| ff2 vs. fnf1 | 0.0768 | 0.702 | 0.999 | No |
| fnf4 vs. fnf1 | 0.0768 | 0.702 | 0.998 | No |
| ff12 vs. fnf0 | 0.0768 | 0.702 | 0.995 | No |
| fnf3 vs. ff0 | 0.0685 | 0.626 | 0.995 | No |
| ff8 vs. fnf2 | 0.0439 | 0.401 | 0.999 | No |
| fnf2 vs. fnf3 | 0.0411 | 0.376 | 0.998 | No |
| ff0 vs. ff2 | 0.0329 | 0.301 | 0.997 | No |
| ff0 vs. fnf4 | 0.0329 | 0.301 | 0.987 | No |
| fnf4 vs. ff2 | 0.000 | 0.000 | 1.000 | No |
| fnf1 vs. ff3 | 3.886E-016 | 3.552E-015 | 1.000 | No |

## 2.0 Dissolved silicates

## 2.1 Llandudno Beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.083)

**Equal Variance Test:** Passed (P = 0.030)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 14.019 | 0.977 | 0.564 |
| ff1 | 3 | 0 | 9.390 | 2.092 | 1.208 |
| ff2 | 3 | 0 | 8.450 | 3.261 | 1.883 |
| ff3 | 3 | 0 | 4.062 | 0.292 | 0.169 |
| ff4 | 3 | 0 | 5.922 | 0.182 | 0.105 |
| ff8 | 3 | 0 | 6.222 | 0.100 | 0.0577 |
| ff12 | 3 | 0 | 7.944 | 0.985 | 0.568 |
| fnf0 | 3 | 0 | 13.296 | 1.469 | 0.848 |
| fnf1 | 3 | 0 | 10.909 | 3.150 | 1.818 |
| fnf2 | 3 | 0 | 9.656 | 2.968 | 1.714 |
| fnf3 | 3 | 0 | 7.389 | 0.301 | 0.174 |
| fnf4 | 3 | 0 | 10.596 | 4.125 | 2.381 |
| fnf8 | 3 | 0 | 13.789 | 1.106 | 0.639 |
| fnf12 | 3 | 0 | 12.664 | 0.543 | 0.313 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 2 | 7.504 | 3.752 | | |
| Between Treatments | 13 | 382.312 | 29.409 | 7.239 | <0.001 |
| Residual | 26 | 105.627 | 4.063 | | |
| Total | 41 | 495.443 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| ff0 vs. ff3 | 9.957 | 6.050 | <0.001 | Yes |
| fnf8 vs. ff3 | 9.726 | 5.910 | <0.001 | Yes |
| fnf0 vs. ff3 | 9.234 | 5.611 | <0.001 | Yes |
| fnf12 vs. ff3 | 8.602 | 5.227 | 0.002 | Yes |
| ff0 vs. ff4 | 8.097 | 4.920 | 0.004 | Yes |
| fnf8 vs. ff4 | 7.867 | 4.780 | 0.005 | Yes |
| ff0 vs. ff8 | 7.798 | 4.738 | 0.006 | Yes |
| fnf8 vs. ff8 | 7.567 | 4.598 | 0.008 | Yes |
| fnf0 vs. ff4 | 7.374 | 4.481 | 0.011 | Yes |
| fnf0 vs. ff8 | 7.074 | 4.299 | 0.017 | Yes |
| fnf1 vs. ff3 | 6.847 | 4.160 | 0.025 | Yes |
| fnf12 vs. ff4 | 6.742 | 4.097 | 0.029 | Yes |
| ff0 vs. fnf3 | 6.630 | 4.029 | 0.034 | Yes |

| | | | | |
|---|---|---|---|---|
| fnf4 vs. ff3 | 6.534 | 3.970 | 0.039 | Yes |
| fnf12 vs. ff8 | 6.442 | 3.915 | 0.044 | Yes |
| fnf8 vs. fnf3 | 6.399 | 3.888 | 0.046 | Yes |
| ff0 vs. ff12 | 6.075 | 3.692 | 0.075 | No |
| fnf0 vs. fnf3 | 5.907 | 3.589 | 0.095 | No |
| fnf8 vs. ff12 | 5.845 | 3.552 | 0.103 | No |
| fnf2 vs. ff3 | 5.593 | 3.399 | 0.146 | No |
| ff0 vs. ff2 | 5.569 | 3.384 | 0.149 | No |
| fnf0 vs. ff12 | 5.352 | 3.252 | 0.199 | No |
| fnf8 vs. ff2 | 5.339 | 3.244 | 0.200 | No |
| ff1 vs. ff3 | 5.328 | 3.238 | 0.200 | No |
| fnf12 vs. fnf3 | 5.275 | 3.205 | 0.212 | No |
| fnf1 vs. ff4 | 4.987 | 3.031 | 0.303 | No |
| fnf0 vs. ff2 | 4.846 | 2.945 | 0.355 | No |
| fnf12 vs. ff12 | 4.720 | 2.868 | 0.405 | No |
| fnf1 vs. ff8 | 4.688 | 2.848 | 0.415 | No |
| fnf4 vs. ff4 | 4.674 | 2.840 | 0.416 | No |
| ff0 vs. ff1 | 4.629 | 2.813 | 0.432 | No |
| fnf8 vs. ff1 | 4.398 | 2.673 | 0.539 | No |
| ff2 vs. ff3 | 4.388 | 2.666 | 0.538 | No |
| fnf4 vs. ff8 | 4.374 | 2.658 | 0.539 | No |
| ff0 vs. fnf2 | 4.364 | 2.652 | 0.538 | No |
| fnf12 vs. ff2 | 4.214 | 2.560 | 0.609 | No |
| fnf8 vs. fnf2 | 4.133 | 2.511 | 0.643 | No |
| fnf0 vs. ff1 | 3.906 | 2.373 | 0.750 | No |
| ff12 vs. ff3 | 3.882 | 2.359 | 0.754 | No |
| fnf2 vs. ff4 | 3.734 | 2.269 | 0.814 | No |
| fnf0 vs. fnf2 | 3.640 | 2.212 | 0.845 | No |
| fnf1 vs. fnf3 | 3.520 | 2.139 | 0.883 | No |
| ff1 vs. ff4 | 3.469 | 2.108 | 0.894 | No |
| fnf2 vs. ff8 | 3.434 | 2.087 | 0.900 | No |
| ff0 vs. fnf4 | 3.423 | 2.080 | 0.898 | No |
| fnf3 vs. ff3 | 3.327 | 2.022 | 0.921 | No |
| fnf12 vs. ff1 | 3.274 | 1.989 | 0.930 | No |
| fnf4 vs. fnf3 | 3.206 | 1.948 | 0.941 | No |
| fnf8 vs. fnf4 | 3.193 | 1.940 | 0.940 | No |
| ff1 vs. ff8 | 3.169 | 1.925 | 0.941 | No |
| ff0 vs. fnf1 | 3.110 | 1.890 | 0.949 | No |
| fnf12 vs. fnf2 | 3.008 | 1.828 | 0.963 | No |
| fnf1 vs. ff12 | 2.965 | 1.802 | 0.966 | No |
| fnf8 vs. fnf1 | 2.879 | 1.750 | 0.974 | No |
| fnf0 vs. fnf4 | 2.700 | 1.641 | 0.988 | No |
| fnf4 vs. ff12 | 2.652 | 1.611 | 0.990 | No |
| ff2 vs. ff4 | 2.528 | 1.536 | 0.994 | No |
| fnf1 vs. ff2 | 2.459 | 1.494 | 0.996 | No |
| fnf0 vs. fnf1 | 2.387 | 1.450 | 0.997 | No |
| fnf2 vs. fnf3 | 2.266 | 1.377 | 0.998 | No |
| ff2 vs. ff8 | 2.229 | 1.354 | 0.998 | No |
| ff8 vs. ff3 | 2.159 | 1.312 | 0.999 | No |
| fnf4 vs. ff2 | 2.146 | 1.304 | 0.999 | No |
| fnf12 vs. fnf4 | 2.068 | 1.257 | 0.999 | No |
| ff12 vs. ff4 | 2.022 | 1.229 | 0.999 | No |
| ff1 vs. fnf3 | 2.001 | 1.216 | 0.999 | No |
| ff4 vs. ff3 | 1.860 | 1.130 | 1.000 | No |
| fnf12 vs. fnf1 | 1.755 | 1.066 | 1.000 | No |
| ff12 vs. ff8 | 1.722 | 1.046 | 1.000 | No |
| fnf2 vs. ff12 | 1.712 | 1.040 | 1.000 | No |
| fnf1 vs. ff1 | 1.519 | 0.923 | 1.000 | No |

| | | | | |
|---|---|---|---|---|
| fnf3 vs. ff4 | 1.468 | 0.892 | 1.000 | No |
| ff1 vs. ff12 | 1.447 | 0.879 | 1.000 | No |
| ff0 vs. fnf12 | 1.355 | 0.824 | 1.000 | No |
| fnf1 vs. fnf2 | 1.254 | 0.762 | 1.000 | No |
| fnf4 vs. ff1 | 1.205 | 0.732 | 1.000 | No |
| fnf2 vs. ff2 | 1.205 | 0.732 | 1.000 | No |
| fnf3 vs. ff8 | 1.168 | 0.710 | 1.000 | No |
| fnf8 vs. fnf12 | 1.125 | 0.683 | 1.000 | No |
| ff2 vs. fnf3 | 1.061 | 0.645 | 1.000 | No |
| fnf4 vs. fnf2 | 0.940 | 0.571 | 1.000 | No |
| ff1 vs. ff2 | 0.940 | 0.571 | 1.000 | No |
| ff0 vs. fnf0 | 0.723 | 0.439 | 1.000 | No |
| fnf0 vs. fnf12 | 0.632 | 0.384 | 1.000 | No |
| ff12 vs. fnf3 | 0.555 | 0.337 | 1.000 | No |
| ff2 vs. ff12 | 0.506 | 0.308 | 1.000 | No |
| fnf8 vs. fnf0 | 0.493 | 0.299 | 0.999 | No |
| fnf1 vs. fnf4 | 0.313 | 0.190 | 0.999 | No |
| ff8 vs. ff4 | 0.300 | 0.182 | 0.997 | No |
| fnf2 vs. ff1 | 0.265 | 0.161 | 0.984 | No |
| ff0 vs. fnf8 | 0.231 | 0.140 | 0.890 | No |

## 2.2 Muizenberg beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.207)

**Equal Variance Test:** Passed (P = 0.263)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 9.173 | 1.241 | 0.716 |
| ff1 | 3 | 0 | 10.933 | 1.449 | 0.837 |
| ff2 | 3 | 0 | 4.328 | 1.656 | 0.956 |
| ff3 | 3 | 0 | 0.446 | 0.728 | 0.420 |
| ff4 | 3 | 0 | 3.585 | 0.585 | 0.338 |
| ff8 | 3 | 0 | 7.588 | 6.185 | 3.571 |
| ff12 | 3 | 0 | 6.449 | 2.425 | 1.400 |
| fnf0 | 3 | 0 | 8.908 | 0.274 | 0.158 |
| fnf1 | 3 | 0 | 10.524 | 0.315 | 0.182 |
| fnf2 | 3 | 0 | 8.836 | 0.656 | 0.379 |
| fnf3 | 3 | 0 | 11.970 | 6.259 | 3.613 |
| fnf4 | 3 | 0 | 9.077 | 3.243 | 1.873 |
| fnf8 | 3 | 0 | 13.224 | 1.572 | 0.908 |
| fnf12 | 3 | 0 | 13.363 | 1.164 | 0.672 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 2 | 54.281 | 27.140 | | |
| Between Treatments | 13 | 541.594 | 41.661 | 6.911 | <0.001 |
| Residual | 26 | 156.739 | 6.028 | | |
| Total | 41 | 752.614 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| fnf12 vs. ff3 | 12.917 | 6.443 | <0.001 | Yes |
| fnf8 vs. ff3 | 12.778 | 6.374 | <0.001 | Yes |
| fnf3 vs. ff3 | 11.524 | 5.748 | <0.001 | Yes |
| ff1 vs. ff3 | 10.487 | 5.231 | 0.002 | Yes |
| fnf1 vs. ff3 | 10.078 | 5.027 | 0.003 | Yes |
| fnf12 vs. ff4 | 9.778 | 4.877 | 0.004 | Yes |
| fnf8 vs. ff4 | 9.638 | 4.808 | 0.005 | Yes |
| fnf12 vs. ff2 | 9.036 | 4.507 | 0.010 | Yes |
| fnf8 vs. ff2 | 8.896 | 4.438 | 0.012 | Yes |
| ff0 vs. ff3 | 8.727 | 4.353 | 0.015 | Yes |
| fnf4 vs. ff3 | 8.631 | 4.305 | 0.017 | Yes |
| fnf0 vs. ff3 | 8.462 | 4.221 | 0.021 | Yes |
| fnf2 vs. ff3 | 8.390 | 4.185 | 0.023 | Yes |
| fnf3 vs. ff4 | 8.385 | 4.182 | 0.022 | Yes |
| fnf3 vs. ff2 | 7.643 | 3.812 | 0.057 | No |
| ff1 vs. ff4 | 7.348 | 3.665 | 0.081 | No |
| ff8 vs. ff3 | 7.142 | 3.562 | 0.103 | No |
| fnf1 vs. ff4 | 6.938 | 3.461 | 0.130 | No |
| fnf12 vs. ff12 | 6.914 | 3.449 | 0.132 | No |
| fnf8 vs. ff12 | 6.775 | 3.379 | 0.153 | No |
| ff1 vs. ff2 | 6.606 | 3.295 | 0.183 | No |
| fnf1 vs. ff2 | 6.196 | 3.091 | 0.282 | No |
| ff12 vs. ff3 | 6.003 | 2.994 | 0.338 | No |
| fnf12 vs. ff8 | 5.776 | 2.881 | 0.414 | No |
| fnf8 vs. ff8 | 5.636 | 2.811 | 0.464 | No |
| ff0 vs. ff4 | 5.588 | 2.787 | 0.478 | No |
| fnf3 vs. ff12 | 5.521 | 2.754 | 0.500 | No |
| fnf4 vs. ff4 | 5.492 | 2.739 | 0.506 | No |
| fnf0 vs. ff4 | 5.323 | 2.655 | 0.571 | No |
| fnf2 vs. ff4 | 5.251 | 2.619 | 0.596 | No |
| ff0 vs. ff2 | 4.846 | 2.417 | 0.757 | No |
| fnf4 vs. ff2 | 4.749 | 2.369 | 0.788 | No |
| fnf0 vs. ff2 | 4.581 | 2.285 | 0.841 | No |
| fnf12 vs. fnf2 | 4.527 | 2.258 | 0.853 | No |
| fnf2 vs. ff2 | 4.508 | 2.249 | 0.854 | No |
| ff1 vs. ff12 | 4.484 | 2.237 | 0.857 | No |
| fnf12 vs. fnf0 | 4.455 | 2.222 | 0.861 | No |
| fnf8 vs. fnf2 | 4.388 | 2.189 | 0.875 | No |
| fnf3 vs. ff8 | 4.383 | 2.186 | 0.872 | No |
| fnf8 vs. fnf0 | 4.316 | 2.153 | 0.885 | No |
| fnf12 vs. fnf4 | 4.286 | 2.138 | 0.888 | No |
| fnf12 vs. ff0 | 4.190 | 2.090 | 0.908 | No |
| fnf8 vs. fnf4 | 4.147 | 2.068 | 0.913 | No |
| fnf1 vs. ff12 | 4.074 | 2.032 | 0.925 | No |
| fnf8 vs. ff0 | 4.050 | 2.020 | 0.926 | No |
| ff8 vs. ff4 | 4.002 | 1.996 | 0.931 | No |
| ff2 vs. ff3 | 3.882 | 1.936 | 0.948 | No |
| ff1 vs. ff8 | 3.346 | 1.669 | 0.993 | No |
| ff8 vs. ff2 | 3.260 | 1.626 | 0.995 | No |
| ff4 vs. ff3 | 3.139 | 1.566 | 0.997 | No |
| fnf3 vs. fnf2 | 3.134 | 1.563 | 0.997 | No |
| fnf3 vs. fnf0 | 3.062 | 1.527 | 0.997 | No |

| | | | | |
|---|---|---|---|---|
| fnf1 vs. ff8 | 2.936 | 1.465 | 0.999 | No |
| fnf3 vs. fnf4 | 2.893 | 1.443 | 0.999 | No |
| ff12 vs. ff4 | 2.864 | 1.428 | 0.999 | No |
| fnf12 vs. fnf1 | 2.840 | 1.416 | 0.999 | No |
| fnf3 vs. ff0 | 2.797 | 1.395 | 0.999 | No |
| ff0 vs. ff12 | 2.724 | 1.359 | 0.999 | No |
| fnf8 vs. fnf1 | 2.700 | 1.347 | 0.999 | No |
| fnf4 vs. ff12 | 2.628 | 1.311 | 0.999 | No |
| fnf0 vs. ff12 | 2.459 | 1.227 | 1.000 | No |
| fnf12 vs. ff1 | 2.430 | 1.212 | 1.000 | No |
| fnf2 vs. ff12 | 2.387 | 1.191 | 1.000 | No |
| fnf8 vs. ff1 | 2.290 | 1.142 | 1.000 | No |
| ff12 vs. ff2 | 2.122 | 1.058 | 1.000 | No |
| ff1 vs. fnf2 | 2.097 | 1.046 | 1.000 | No |
| ff1 vs. fnf0 | 2.025 | 1.010 | 1.000 | No |
| ff1 vs. fnf4 | 1.856 | 0.926 | 1.000 | No |
| ff1 vs. ff0 | 1.760 | 0.878 | 1.000 | No |
| fnf1 vs. fnf2 | 1.688 | 0.842 | 1.000 | No |
| fnf1 vs. fnf0 | 1.615 | 0.806 | 1.000 | No |
| ff0 vs. ff8 | 1.586 | 0.791 | 1.000 | No |
| fnf4 vs. ff8 | 1.490 | 0.743 | 1.000 | No |
| fnf1 vs. fnf4 | 1.447 | 0.722 | 1.000 | No |
| fnf3 vs. fnf1 | 1.447 | 0.722 | 1.000 | No |
| fnf12 vs. fnf3 | 1.393 | 0.695 | 1.000 | No |
| fnf1 vs. ff0 | 1.350 | 0.673 | 1.000 | No |
| fnf0 vs. ff8 | 1.321 | 0.659 | 1.000 | No |
| fnf8 vs. fnf3 | 1.254 | 0.625 | 1.000 | No |
| fnf2 vs. ff8 | 1.248 | 0.623 | 1.000 | No |
| ff8 vs. ff12 | 1.138 | 0.568 | 1.000 | No |
| fnf3 vs. ff1 | 1.037 | 0.517 | 1.000 | No |
| ff2 vs. ff4 | 0.742 | 0.370 | 1.000 | No |
| ff1 vs. fnf1 | 0.410 | 0.204 | 1.000 | No |
| ff0 vs. fnf2 | 0.338 | 0.168 | 1.000 | No |
| ff0 vs. fnf0 | 0.265 | 0.132 | 1.000 | No |
| fnf4 vs. fnf2 | 0.241 | 0.120 | 1.000 | No |
| fnf4 vs. fnf0 | 0.169 | 0.0842 | 1.000 | No |
| fnf12 vs. fnf8 | 0.139 | 0.0695 | 1.000 | No |
| ff0 vs. fnf4 | 0.0964 | 0.0481 | 0.999 | No |
| fnf0 vs. fnf2 | 0.0723 | 0.0361 | 0.971 | No |

## 3.0 Urea

## 3.1 Llandudno Beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.021)

**Equal Variance Test:** Passed (P = 0.164)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 0.486 | 0.0890 | 0.0514 |
| ff1 | 3 | 0 | 0.864 | 0.262 | 0.151 |
| ff2 | 3 | 0 | 0.172 | 0.189 | 0.109 |
| ff3 | 3 | 0 | 0.144 | 0.184 | 0.106 |
| ff4 | 3 | 0 | 0.449 | 0.0160 | 0.00923 |
| ff8 | 3 | 0 | 0.329 | 0.267 | 0.154 |

| | | | | | |
|---|---|---|---|---|---|
| ff12 | 3 | 0 | 2.129 | 0.0733 | 0.0423 |
| fnf0 | 3 | 0 | 0.209 | 0.0697 | 0.0402 |
| fnf1 | 3 | 0 | 0.0130 | 0.00999 | 0.00577 |
| fnf2 | 3 | 0 | 0.144 | 0.0890 | 0.0514 |
| fnf3 | 3 | 0 | 0.00748 | 0.0418 | 0.0241 |
| fnf4 | 3 | 0 | 0.366 | 0.0320 | 0.0185 |
| fnf8 | 3 | 0 | 0.0795 | 0.0277 | 0.0160 |
| fnf12 | 3 | 0 | -0.613 | 0.000 | 0.000 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Subjects | 2 | 0.0378 | 0.0189 | | |
| Between Treatments | 13 | 14.479 | 1.114 | 65.641 | <0.001 |
| Residual | 26 | 0.441 | 0.0170 | | |
| Total | 41 | 14.958 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| **Comparison** | **Diff of Means** | **t** | **P** | **P<0.050** |
|---|---|---|---|---|
| ff12 vs. fnf12 | 2.742 | 25.784 | <0.001 | Yes |
| ff12 vs. fnf3 | 2.122 | 19.950 | <0.001 | Yes |
| ff12 vs. fnf1 | 2.116 | 19.898 | <0.001 | Yes |
| ff12 vs. fnf8 | 2.050 | 19.273 | <0.001 | Yes |
| ff12 vs. ff3 | 1.985 | 18.666 | <0.001 | Yes |
| ff12 vs. fnf2 | 1.985 | 18.666 | <0.001 | Yes |
| ff12 vs. ff2 | 1.958 | 18.405 | <0.001 | Yes |
| ff12 vs. fnf0 | 1.921 | 18.058 | <0.001 | Yes |
| ff12 vs. ff8 | 1.801 | 16.929 | <0.001 | Yes |
| ff12 vs. fnf4 | 1.764 | 16.582 | <0.001 | Yes |
| ff12 vs. ff4 | 1.681 | 15.801 | <0.001 | Yes |
| ff12 vs. ff0 | 1.644 | 15.453 | <0.001 | Yes |
| ff1 vs. fnf12 | 1.477 | 13.891 | <0.001 | Yes |
| ff12 vs. ff1 | 1.265 | 11.894 | <0.001 | Yes |
| ff0 vs. fnf12 | 1.099 | 10.331 | <0.001 | Yes |
| ff4 vs. fnf12 | 1.062 | 9.984 | <0.001 | Yes |
| fnf4 vs. fnf12 | 0.979 | 9.203 | <0.001 | Yes |
| ff8 vs. fnf12 | 0.942 | 8.855 | <0.001 | Yes |
| ff1 vs. fnf3 | 0.857 | 8.057 | <0.001 | Yes |
| ff1 vs. fnf1 | 0.851 | 8.004 | <0.001 | Yes |
| fnf0 vs. fnf12 | 0.822 | 7.727 | <0.001 | Yes |
| ff2 vs. fnf12 | 0.785 | 7.379 | <0.001 | Yes |
| ff1 vs. fnf8 | 0.785 | 7.379 | <0.001 | Yes |
| fnf2 vs. fnf12 | 0.757 | 7.119 | <0.001 | Yes |
| ff3 vs. fnf12 | 0.757 | 7.119 | <0.001 | Yes |
| ff1 vs. fnf2 | 0.720 | 6.772 | <0.001 | Yes |
| ff1 vs. ff3 | 0.720 | 6.772 | <0.001 | Yes |
| fnf8 vs. fnf12 | 0.693 | 6.511 | <0.001 | Yes |
| ff1 vs. ff2 | 0.693 | 6.511 | <0.001 | Yes |
| ff1 vs. fnf0 | 0.656 | 6.164 | <0.001 | Yes |
| fnf1 vs. fnf12 | 0.626 | 5.886 | <0.001 | Yes |

| | | | | |
|---|---|---|---|---|
| fnf3 vs. fnf12 | 0.620 | 5.834 | <0.001 | Yes |
| ff1 vs. ff8 | 0.536 | 5.035 | 0.002 | Yes |
| ff1 vs. fnf4 | 0.499 | 4.688 | 0.004 | Yes |
| ff0 vs. fnf3 | 0.478 | 4.497 | 0.007 | Yes |
| ff0 vs. fnf1 | 0.473 | 4.445 | 0.008 | Yes |
| ff4 vs. fnf3 | 0.441 | 4.150 | 0.017 | Yes |
| ff4 vs. fnf1 | 0.436 | 4.098 | 0.019 | Yes |
| ff1 vs. ff4 | 0.416 | 3.907 | 0.031 | Yes |
| ff0 vs. fnf8 | 0.406 | 3.820 | 0.038 | Yes |
| ff1 vs. ff0 | 0.379 | 3.559 | 0.072 | No |
| ff4 vs. fnf8 | 0.369 | 3.473 | 0.087 | No |
| fnf4 vs. fnf3 | 0.358 | 3.368 | 0.110 | No |
| fnf4 vs. fnf1 | 0.353 | 3.316 | 0.122 | No |
| ff0 vs. fnf2 | 0.342 | 3.212 | 0.152 | No |
| ff0 vs. ff3 | 0.342 | 3.212 | 0.149 | No |
| ff8 vs. fnf3 | 0.321 | 3.021 | 0.223 | No |
| ff8 vs. fnf1 | 0.316 | 2.969 | 0.244 | No |
| ff0 vs. ff2 | 0.314 | 2.952 | 0.248 | No |
| ff4 vs. ff3 | 0.305 | 2.865 | 0.291 | No |
| ff4 vs. fnf2 | 0.305 | 2.865 | 0.285 | No |
| fnf4 vs. fnf8 | 0.286 | 2.691 | 0.390 | No |
| ff4 vs. ff2 | 0.277 | 2.604 | 0.446 | No |
| ff0 vs. fnf0 | 0.277 | 2.604 | 0.437 | No |
| ff8 vs. fnf8 | 0.249 | 2.344 | 0.637 | No |
| ff4 vs. fnf0 | 0.240 | 2.257 | 0.697 | No |
| fnf4 vs. ff3 | 0.222 | 2.084 | 0.816 | No |
| fnf4 vs. fnf2 | 0.222 | 2.084 | 0.807 | No |
| fnf0 vs. fnf3 | 0.201 | 1.893 | 0.907 | No |
| fnf0 vs. fnf1 | 0.196 | 1.841 | 0.923 | No |
| fnf4 vs. ff2 | 0.194 | 1.823 | 0.924 | No |
| ff8 vs. ff3 | 0.185 | 1.736 | 0.949 | No |
| ff8 vs. fnf2 | 0.185 | 1.736 | 0.944 | No |
| ff2 vs. fnf3 | 0.164 | 1.545 | 0.982 | No |
| ff2 vs. fnf1 | 0.159 | 1.493 | 0.987 | No |
| ff8 vs. ff2 | 0.157 | 1.476 | 0.986 | No |
| ff0 vs. ff8 | 0.157 | 1.476 | 0.984 | No |
| fnf4 vs. fnf0 | 0.157 | 1.476 | 0.981 | No |
| ff3 vs. fnf3 | 0.137 | 1.285 | 0.996 | No |
| fnf2 vs. fnf3 | 0.137 | 1.285 | 0.994 | No |
| fnf2 vs. fnf1 | 0.131 | 1.233 | 0.996 | No |
| ff3 vs. fnf1 | 0.131 | 1.233 | 0.994 | No |
| fnf0 vs. fnf8 | 0.129 | 1.215 | 0.994 | No |
| ff0 vs. fnf4 | 0.120 | 1.129 | 0.996 | No |
| ff4 vs. ff8 | 0.120 | 1.129 | 0.995 | No |
| ff8 vs. fnf0 | 0.120 | 1.129 | 0.993 | No |
| ff2 vs. fnf8 | 0.0923 | 0.868 | 0.999 | No |
| ff4 vs. fnf4 | 0.0831 | 0.781 | 1.000 | No |
| fnf8 vs. fnf3 | 0.0720 | 0.677 | 1.000 | No |
| fnf8 vs. fnf1 | 0.0665 | 0.625 | 1.000 | No |
| fnf0 vs. ff3 | 0.0646 | 0.608 | 1.000 | No |
| fnf2 vs. fnf8 | 0.0646 | 0.608 | 1.000 | No |
| fnf0 vs. fnf2 | 0.0646 | 0.608 | 0.999 | No |
| ff3 vs. fnf8 | 0.0646 | 0.608 | 0.998 | No |
| fnf0 vs. ff2 | 0.0369 | 0.347 | 1.000 | No |
| ff0 vs. ff4 | 0.0369 | 0.347 | 1.000 | No |
| fnf4 vs. ff8 | 0.0369 | 0.347 | 0.999 | No |
| ff2 vs. ff3 | 0.0277 | 0.260 | 0.998 | No |
| ff2 vs. fnf2 | 0.0277 | 0.260 | 0.992 | No |

| | | | | |
|---|---|---|---|---|
| fnf1 vs. fnf3 | 0.00554 | 0.0521 | 0.998 | No |
| fnf2 vs. ff3 | 5.551E-017 | 5.219E-016 | 1.000 | No |

### 3.2 Muizenberg Beach

**Normality Test (Shapiro-Wilk)** Failed (P < 0.010)

**Friedman Repeated Measures Analysis of Variance on Ranks**

| Group | N | Missing | Median | 25% | 75% |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | -0.00360 | -0.0313 | 0.135 |
| ff1 | 3 | 0 | 0.0795 | -0.142 | 0.246 |
| ff2 | 3 | 0 | 0.0795 | -0.0590 | 0.135 |
| ff3 | 3 | 0 | 0.107 | -0.0867 | 0.107 |
| ff4 | 3 | 0 | 0.163 | 0.107 | 0.218 |
| ff8 | 3 | 0 | -0.0590 | -0.142 | 0.440 |
| ff12 | 3 | 0 | 2.296 | 2.212 | 2.462 |
| fnf0 | 3 | 0 | 2.212 | -0.198 | 2.545 |
| fnf1 | 3 | 0 | -0.00360 | -0.0313 | 0.135 |
| fnf2 | 3 | 0 | 0.107 | 0.0795 | 0.190 |
| fnf3 | 3 | 0 | 0.163 | 0.0795 | 0.246 |
| fnf4 | 3 | 0 | 0.0518 | -0.0590 | 0.578 |
| fnf8 | 3 | 0 | 0.412 | 0.357 | 0.467 |
| fnf12 | 3 | 0 | 2.074 | 2.019 | 2.157 |

Chi-square= 19.788 with 13 degrees of freedom. (P = 0.101)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.101)

## 4.0 Nitrite

## 4.1 Llandudno Beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.384)

**Equal Variance Test:** Passed (P = 0.318)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 0.619 | 0.0232 | 0.0134 |
| ff1 | 3 | 0 | 0.606 | 0.108 | 0.0622 |
| ff2 | 3 | 0 | 0.677 | 0.0205 | 0.0118 |
| ff3 | 3 | 0 | 0.677 | 0.206 | 0.119 |
| ff4 | 3 | 0 | 0.238 | 0.0279 | 0.0161 |
| ff8 | 3 | 0 | 0.323 | 0.1000 | 0.0577 |
| ff12 | 3 | 0 | 0.341 | 0.0676 | 0.0390 |
| fnf0 | 3 | 0 | 0.596 | 0.0431 | 0.0249 |
| fnf1 | 3 | 0 | 0.619 | 0.0338 | 0.0195 |
| fnf2 | 3 | 0 | 0.722 | 0.0559 | 0.0323 |
| fnf3 | 3 | 0 | 0.332 | 0.00775 | 0.00447 |
| fnf4 | 3 | 0 | 0.404 | 0.1000 | 0.0577 |
| fnf8 | 3 | 0 | 0.525 | 0.200 | 0.115 |

| | | | | | |
|---|---|---|---|---|---|
| fnf12 | 3 | 0 | 0.171 | 0.0338 | 0.0195 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Subjects | 2 | 0.0336 | 0.0168 | | |
| Between Treatments | 13 | 1.289 | 0.0991 | 11.611 | <0.001 |
| Residual | 26 | 0.222 | 0.00854 | | |
| Total | 41 | 1.544 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| **Comparison** | **Diff of Means** | **t** | **P** | **P<0.050** |
|---|---|---|---|---|
| fnf2 vs. fnf12 | 0.550 | 7.295 | <0.001 | Yes |
| ff2 vs. fnf12 | 0.506 | 6.702 | <0.001 | Yes |
| ff3 vs. fnf12 | 0.506 | 6.702 | <0.001 | Yes |
| fnf2 vs. ff4 | 0.483 | 6.405 | <0.001 | Yes |
| fnf1 vs. fnf12 | 0.448 | 5.934 | <0.001 | Yes |
| ff0 vs. fnf12 | 0.447 | 5.931 | <0.001 | Yes |
| ff2 vs. ff4 | 0.438 | 5.812 | <0.001 | Yes |
| ff3 vs. ff4 | 0.438 | 5.812 | <0.001 | Yes |
| ff1 vs. fnf12 | 0.434 | 5.756 | <0.001 | Yes |
| fnf0 vs. fnf12 | 0.425 | 5.634 | <0.001 | Yes |
| fnf2 vs. ff8 | 0.398 | 5.278 | 0.001 | Yes |
| fnf2 vs. fnf3 | 0.389 | 5.160 | 0.002 | Yes |
| fnf1 vs. ff4 | 0.381 | 5.044 | 0.002 | Yes |
| fnf2 vs. ff12 | 0.380 | 5.041 | 0.002 | Yes |
| ff0 vs. ff4 | 0.380 | 5.041 | 0.002 | Yes |
| ff1 vs. ff4 | 0.367 | 4.866 | 0.004 | Yes |
| fnf0 vs. ff4 | 0.358 | 4.745 | 0.005 | Yes |
| ff2 vs. ff8 | 0.353 | 4.685 | 0.006 | Yes |
| ff3 vs. ff8 | 0.353 | 4.685 | 0.006 | Yes |
| fnf8 vs. fnf12 | 0.353 | 4.685 | 0.006 | Yes |
| ff2 vs. fnf3 | 0.345 | 4.567 | 0.007 | Yes |
| ff3 vs. fnf3 | 0.345 | 4.567 | 0.007 | Yes |
| ff2 vs. ff12 | 0.336 | 4.448 | 0.010 | Yes |
| ff3 vs. ff12 | 0.336 | 4.448 | 0.010 | Yes |
| fnf2 vs. fnf4 | 0.318 | 4.211 | 0.018 | Yes |
| fnf1 vs. ff8 | 0.296 | 3.917 | 0.038 | Yes |
| ff0 vs. ff8 | 0.295 | 3.914 | 0.037 | Yes |
| fnf1 vs. fnf3 | 0.287 | 3.799 | 0.049 | Yes |
| fnf8 vs. ff4 | 0.286 | 3.796 | 0.049 | Yes |
| ff0 vs. fnf3 | 0.286 | 3.796 | 0.048 | Yes |
| ff1 vs. ff8 | 0.282 | 3.739 | 0.055 | No |
| fnf1 vs. ff12 | 0.278 | 3.680 | 0.062 | No |
| ff0 vs. ff12 | 0.277 | 3.677 | 0.062 | No |
| ff1 vs. fnf3 | 0.273 | 3.621 | 0.070 | No |
| ff3 vs. fnf4 | 0.273 | 3.618 | 0.069 | No |
| ff2 vs. fnf4 | 0.273 | 3.618 | 0.068 | No |
| fnf0 vs. ff8 | 0.273 | 3.618 | 0.067 | No |
| ff1 vs. ff12 | 0.264 | 3.502 | 0.087 | No |

| | | | | |
|---|---|---|---|---|
| fnf0 vs. fnf3 | 0.264 | 3.499 | 0.086 | No |
| fnf0 vs. ff12 | 0.255 | 3.381 | 0.113 | No |
| fnf4 vs. fnf12 | 0.233 | 3.084 | 0.217 | No |
| fnf1 vs. fnf4 | 0.215 | 2.850 | 0.346 | No |
| ff0 vs. fnf4 | 0.215 | 2.847 | 0.342 | No |
| ff1 vs. fnf4 | 0.202 | 2.672 | 0.462 | No |
| fnf8 vs. ff8 | 0.201 | 2.669 | 0.458 | No |
| fnf2 vs. fnf8 | 0.197 | 2.610 | 0.497 | No |
| fnf8 vs. fnf3 | 0.192 | 2.550 | 0.538 | No |
| fnf0 vs. fnf4 | 0.192 | 2.550 | 0.530 | No |
| fnf8 vs. ff12 | 0.183 | 2.432 | 0.620 | No |
| ff12 vs. fnf12 | 0.170 | 2.254 | 0.754 | No |
| fnf4 vs. ff4 | 0.166 | 2.194 | 0.790 | No |
| fnf3 vs. fnf12 | 0.161 | 2.135 | 0.823 | No |
| ff3 vs. fnf8 | 0.152 | 2.016 | 0.886 | No |
| ff8 vs. fnf12 | 0.152 | 2.016 | 0.880 | No |
| ff2 vs. fnf8 | 0.152 | 2.016 | 0.873 | No |
| fnf2 vs. fnf0 | 0.125 | 1.661 | 0.984 | No |
| fnf8 vs. fnf4 | 0.121 | 1.601 | 0.989 | No |
| fnf2 vs. ff1 | 0.116 | 1.539 | 0.993 | No |
| fnf2 vs. ff0 | 0.103 | 1.364 | 0.999 | No |
| ff12 vs. ff4 | 0.103 | 1.364 | 0.999 | No |
| fnf2 vs. fnf1 | 0.103 | 1.361 | 0.998 | No |
| fnf1 vs. fnf8 | 0.0942 | 1.248 | 0.999 | No |
| ff0 vs. fnf8 | 0.0940 | 1.245 | 0.999 | No |
| fnf3 vs. ff4 | 0.0940 | 1.245 | 0.999 | No |
| ff8 vs. ff4 | 0.0850 | 1.127 | 1.000 | No |
| ff1 vs. fnf8 | 0.0808 | 1.070 | 1.000 | No |
| ff3 vs. fnf0 | 0.0805 | 1.068 | 1.000 | No |
| fnf4 vs. ff8 | 0.0805 | 1.068 | 1.000 | No |
| ff2 vs. fnf0 | 0.0805 | 1.068 | 1.000 | No |
| fnf4 vs. fnf3 | 0.0716 | 0.949 | 1.000 | No |
| fnf0 vs. fnf8 | 0.0716 | 0.949 | 1.000 | No |
| ff2 vs. ff1 | 0.0714 | 0.946 | 1.000 | No |
| ff3 vs. ff1 | 0.0714 | 0.946 | 1.000 | No |
| ff4 vs. fnf12 | 0.0671 | 0.890 | 1.000 | No |
| fnf4 vs. ff12 | 0.0626 | 0.830 | 1.000 | No |
| ff3 vs. ff0 | 0.0582 | 0.771 | 1.000 | No |
| ff2 vs. ff0 | 0.0582 | 0.771 | 1.000 | No |
| ff3 vs. fnf1 | 0.0579 | 0.768 | 1.000 | No |
| ff2 vs. fnf1 | 0.0579 | 0.768 | 1.000 | No |
| fnf2 vs. ff2 | 0.0447 | 0.593 | 1.000 | No |
| fnf2 vs. ff3 | 0.0447 | 0.593 | 1.000 | No |
| fnf1 vs. fnf0 | 0.0226 | 0.300 | 1.000 | No |
| ff0 vs. fnf0 | 0.0224 | 0.297 | 1.000 | No |
| ff12 vs. ff8 | 0.0179 | 0.237 | 1.000 | No |
| fnf1 vs. ff1 | 0.0134 | 0.178 | 1.000 | No |
| ff0 vs. ff1 | 0.0132 | 0.175 | 1.000 | No |
| ff1 vs. fnf0 | 0.00917 | 0.122 | 1.000 | No |
| ff12 vs. fnf3 | 0.00895 | 0.119 | 1.000 | No |
| fnf3 vs. ff8 | 0.00895 | 0.119 | 0.999 | No |
| fnf1 vs. ff0 | 0.000224 | 0.00297 | 1.000 | No |
| ff3 vs. ff2 | 0.000 | 0.000 | 1.000 | No |

## 4.2 Muizenberg Beach

**One Way Repeated Measures Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.076)

**Equal Variance Test:** Passed (P = 0.788)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| ff0 | 3 | 0 | 0.373 | 0.0205 | 0.0118 |
| ff1 | 3 | 0 | 0.366 | 0.00891 | 0.00515 |
| ff2 | 3 | 0 | 0.441 | 0.0310 | 0.0179 |
| ff3 | 3 | 0 | 0.529 | 0.287 | 0.166 |
| ff4 | 3 | 0 | 0.337 | 0.1000 | 0.0577 |
| ff8 | 3 | 0 | 0.283 | 0.1000 | 0.0577 |
| ff12 | 3 | 0 | 0.564 | 0.101 | 0.0581 |
| fnf0 | 3 | 0 | 0.413 | 0.0620 | 0.0358 |
| fnf1 | 3 | 0 | 0.392 | 0.1000 | 0.0577 |
| fnf2 | 3 | 0 | 0.430 | 0.232 | 0.134 |
| fnf3 | 3 | 0 | 0.480 | 0.0205 | 0.0118 |
| fnf4 | 3 | 0 | 0.256 | 0.1000 | 0.0577 |
| fnf8 | 3 | 0 | 0.364 | 0.1000 | 0.0577 |
| fnf12 | 3 | 0 | 0.221 | 0.148 | 0.0857 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 2 | 0.116 | 0.0582 | | |
| Between Treatments | 13 | 0.375 | 0.0288 | 2.258 | 0.037 |
| Residual | 26 | 0.332 | 0.0128 | | |
| Total | 41 | 0.823 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.037). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050: 0.549

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| ff12 vs. fnf12 | 0.344 | 3.724 | 0.083 | No |
| ff3 vs. fnf12 | 0.309 | 3.346 | 0.202 | No |
| ff12 vs. fnf4 | 0.308 | 3.336 | 0.204 | No |
| ff12 vs. ff8 | 0.281 | 3.045 | 0.372 | No |
| ff3 vs. fnf4 | 0.273 | 2.958 | 0.434 | No |
| fnf3 vs. fnf12 | 0.260 | 2.813 | 0.549 | No |
| ff3 vs. ff8 | 0.246 | 2.667 | 0.671 | No |
| ff12 vs. ff4 | 0.227 | 2.464 | 0.827 | No |
| fnf3 vs. fnf4 | 0.224 | 2.425 | 0.850 | No |
| ff2 vs. fnf12 | 0.221 | 2.393 | 0.866 | No |
| fnf2 vs. fnf12 | 0.209 | 2.265 | 0.929 | No |
| ff12 vs. fnf8 | 0.200 | 2.173 | 0.959 | No |
| ff12 vs. ff1 | 0.198 | 2.146 | 0.965 | No |
| fnf3 vs. ff8 | 0.197 | 2.134 | 0.966 | No |
| ff3 vs. ff4 | 0.192 | 2.085 | 0.975 | No |
| fnf0 vs. fnf12 | 0.192 | 2.085 | 0.974 | No |

| | | | | |
|---|---|---|---|---|
| ff12 vs. ff0 | 0.191 | 2.076 | 0.975 | No |
| ff2 vs. fnf4 | 0.185 | 2.005 | 0.985 | No |
| fnf2 vs. fnf4 | 0.173 | 1.877 | 0.996 | No |
| fnf1 vs. fnf12 | 0.172 | 1.862 | 0.996 | No |
| ff12 vs. fnf1 | 0.172 | 1.862 | 0.996 | No |
| ff3 vs. fnf8 | 0.166 | 1.794 | 0.998 | No |
| ff3 vs. ff1 | 0.163 | 1.768 | 0.998 | No |
| ff2 vs. ff8 | 0.158 | 1.714 | 0.999 | No |
| fnf0 vs. fnf4 | 0.157 | 1.697 | 0.999 | No |
| ff3 vs. ff0 | 0.157 | 1.697 | 0.999 | No |
| ff0 vs. fnf12 | 0.152 | 1.649 | 1.000 | No |
| ff12 vs. fnf0 | 0.151 | 1.639 | 1.000 | No |
| fnf2 vs. ff8 | 0.146 | 1.586 | 1.000 | No |
| ff1 vs. fnf12 | 0.146 | 1.579 | 1.000 | No |
| fnf3 vs. ff4 | 0.143 | 1.552 | 1.000 | No |
| fnf8 vs. fnf12 | 0.143 | 1.552 | 1.000 | No |
| ff3 vs. fnf1 | 0.137 | 1.484 | 1.000 | No |
| fnf1 vs. fnf4 | 0.136 | 1.474 | 1.000 | No |
| ff12 vs. fnf2 | 0.135 | 1.460 | 1.000 | No |
| fnf0 vs. ff8 | 0.130 | 1.406 | 1.000 | No |
| ff12 vs. ff2 | 0.123 | 1.331 | 1.000 | No |
| ff0 vs. fnf4 | 0.116 | 1.261 | 1.000 | No |
| fnf3 vs. fnf8 | 0.116 | 1.261 | 1.000 | No |
| ff3 vs. fnf0 | 0.116 | 1.261 | 1.000 | No |
| ff4 vs. fnf12 | 0.116 | 1.261 | 1.000 | No |
| fnf3 vs. ff1 | 0.114 | 1.234 | 1.000 | No |
| ff1 vs. fnf4 | 0.110 | 1.191 | 1.000 | No |
| fnf1 vs. ff8 | 0.109 | 1.183 | 1.000 | No |
| fnf8 vs. fnf4 | 0.107 | 1.164 | 1.000 | No |
| fnf3 vs. ff0 | 0.107 | 1.164 | 1.000 | No |
| ff2 vs. ff4 | 0.104 | 1.132 | 1.000 | No |
| ff3 vs. fnf2 | 0.0998 | 1.081 | 1.000 | No |
| fnf2 vs. ff4 | 0.0926 | 1.004 | 1.000 | No |
| ff0 vs. ff8 | 0.0895 | 0.970 | 1.000 | No |
| ff3 vs. ff2 | 0.0879 | 0.953 | 1.000 | No |
| fnf3 vs. fnf1 | 0.0877 | 0.951 | 1.000 | No |
| ff12 vs. fnf3 | 0.0841 | 0.912 | 1.000 | No |
| ff1 vs. ff8 | 0.0830 | 0.900 | 1.000 | No |
| fnf8 vs. ff8 | 0.0805 | 0.873 | 1.000 | No |
| ff4 vs. fnf4 | 0.0805 | 0.873 | 1.000 | No |
| ff2 vs. fnf8 | 0.0776 | 0.841 | 1.000 | No |
| fnf0 vs. ff4 | 0.0761 | 0.824 | 1.000 | No |
| ff2 vs. ff1 | 0.0752 | 0.815 | 1.000 | No |
| ff2 vs. ff0 | 0.0687 | 0.744 | 1.000 | No |
| fnf3 vs. fnf0 | 0.0671 | 0.727 | 1.000 | No |
| fnf2 vs. fnf8 | 0.0658 | 0.713 | 1.000 | No |
| fnf2 vs. ff1 | 0.0633 | 0.686 | 1.000 | No |
| ff8 vs. fnf12 | 0.0626 | 0.679 | 1.000 | No |
| fnf2 vs. ff0 | 0.0568 | 0.616 | 1.000 | No |
| fnf1 vs. ff4 | 0.0555 | 0.601 | 1.000 | No |
| ff4 vs. ff8 | 0.0537 | 0.582 | 1.000 | No |
| fnf3 vs. fnf2 | 0.0506 | 0.548 | 1.000 | No |
| ff3 vs. fnf3 | 0.0492 | 0.533 | 1.000 | No |
| fnf0 vs. fnf8 | 0.0492 | 0.533 | 1.000 | No |
| ff2 vs. fnf1 | 0.0490 | 0.531 | 1.000 | No |
| fnf0 vs. ff1 | 0.0468 | 0.507 | 1.000 | No |
| fnf0 vs. ff0 | 0.0403 | 0.436 | 1.000 | No |
| fnf3 vs. ff2 | 0.0387 | 0.419 | 1.000 | No |

| | | | | |
|---|---|---|---|---|
| fnf2 vs. fnf1 | 0.0371 | 0.403 | 1.000 | No |
| ff0 vs. ff4 | 0.0358 | 0.388 | 1.000 | No |
| fnf4 vs. fnf12 | 0.0358 | 0.388 | 1.000 | No |
| ff12 vs. ff3 | 0.0349 | 0.378 | 1.000 | No |
| ff1 vs. ff4 | 0.0293 | 0.318 | 1.000 | No |
| fnf1 vs. fnf8 | 0.0286 | 0.310 | 1.000 | No |
| ff2 vs. fnf0 | 0.0284 | 0.308 | 1.000 | No |
| ff8 vs. fnf4 | 0.0268 | 0.291 | 1.000 | No |
| fnf8 vs. ff4 | 0.0268 | 0.291 | 1.000 | No |
| fnf1 vs. ff1 | 0.0262 | 0.284 | 1.000 | No |
| fnf0 vs. fnf1 | 0.0206 | 0.223 | 1.000 | No |
| fnf1 vs. ff0 | 0.0197 | 0.213 | 1.000 | No |
| fnf2 vs. fnf0 | 0.0166 | 0.179 | 1.000 | No |
| ff2 vs. fnf2 | 0.0119 | 0.129 | 1.000 | No |
| ff0 vs. fnf8 | 0.00895 | 0.0970 | 1.000 | No |
| ff0 vs. ff1 | 0.00649 | 0.0703 | 0.997 | No |
| ff1 vs. fnf8 | 0.00246 | 0.0267 | 0.979 | No |

# Appendix 2

The concentration values determined using the shaking method for nitrate using a tris buffer and nitrate using and $NH_4Cl$ buffer.

ff = samples that were filtered and stored at -20°C

fnf = samples that were not filtered and were stored at -20°C

| week | Storage | Llandudno Beach | | Muizenberg Beach | |
|---|---|---|---|---|---|
| | | Nitrate Tris buffer | Nitrate $NH_4Cl$ buffer | Nitrate Tris buffer | Nitrate $NH_4Cl$ buffer |
| **0** | **Ff** | 5.357399 | 0.515806 | 21.3858 | -0.08725 |
| **0** | **Fnf** | 4.903579 | 0.773046 | 4.338725 | -0.11849 |
| **1** | **Ff** | 1.486924 | 0.422501 | 1.283664 | -0.44278 |
| **1** | **Fnf** | 1.201393 | -0.15099 | 2.011373 | -0.46549 |
| **2** | **Ff** | 9.108757 | 1.053846 | 2.403511 | -0.5618 |
| **2** | **Fnf** | 3.111633 | -0.82468 | 2.74004 | -0.40917 |
| **3** | **Ff** | 0 | 9.834334 | -0.18792 | -0.56002 |
| **3** | **Fnf** | 0.389262 | 0.061419 | -0.23714 | 0.338339 |
| **4** | **Ff** | 187.7812 | 2.066245 | 3.326353 | 1.646827 |
| **4** | **Fnf** | 9.483728 | 2.934727 | 2.794645 | 0.507852 |
| **8** | **Ff** | 133.0337 | 4.885182 | 2.461676 | 0.101602 |
| **8** | **Fnf** | 2.424147 | 13.84373 | 1.870935 | 0.183667 |
| **12** | **Ff** | 40.35375 | 6.782456 | 36.16473 | 2.555832 |
| **12** | **Fnf** | 36.06529 | 5.086779 | 18.59221 | 1.76916 |

# Appendix 3

### 3.0 Comparison of methods used to determine dissolved silicate and nitrate

### 3.1 Comparison of methods for dissolved silicate

a=Manual method

b=Flow injection analysis method

Number = Beach sample number

The beach sample numbers refer to samples collected from the following beaches:
1) Llandudno1, 2) Houtbay1, 3) Muizenberg1, 4) Sea Point 1 5) Kalk Bay, 6) Muizenberg2, 7) Sea Point2, 8) Glen Beach 9) Houtbay2 and 10) Llandudno2.

i.e. a1= A sample from Llandudno1 that has been analysed for silicate using the manual method.

**Sample 1**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.382)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A1 | 3 | 0 | 2.794 | 0.0612 | 0.0353 |
| B1 | 3 | 0 | 4.990 | 0.0513 | 0.0296 |
| Difference | 3 | 0 | -2.195 | 0.105 | 0.0609 |

t = -36.058 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.457 to -1.933

Two-tailed P-value = 0.000768

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.000384

The sample mean of treatment B1 exceeds the sample mean of treatment A1 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A1 is greater than or equal to the population mean of treatment B1. (P = <0.001)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

**Sample 2**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.370)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A2 | 3 | 0 | 3.834 | 0.0265 | 0.0153 |
| B2 | 3 | 0 | 8.477 | 0.00968 | 0.00559 |
| Difference | 3 | 0 | -4.642 | 0.0342 | 0.0198 |

t = -234.972 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -4.727 to -4.557

Two-tailed P-value = 0.0000181

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.00000906

The sample mean of treatment B2 exceeds the sample mean of treatment A2 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A2 is greater than or equal to the population mean of treatment B2. (P = <0.001)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

**Sample 3**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.920)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A3 | 3 | 0 | 2.137 | 0.107 | 0.0618 |
| B3 | 3 | 0 | 2.340 | 0.00573 | 0.00331 |
| Difference | 3 | 0 | -0.203 | 0.111 | 0.0638 |

t = -3.189 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.478 to 0.0711

Two-tailed P-value = 0.0859

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.086)

One-tailed P-value = 0.0429

The sample mean of treatment B3 exceeds the sample mean of treatment A3 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A3 is greater than or equal to the population mean of treatment B3. (P = 0.086)

Power of performed two-tailed test with alpha = 0.050: 0.421

Power of performed one-tailed test with alpha = 0.050: 0.657

**Sample 4**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.504)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A4 | 3 | 0 | 3.625 | 0.0467 | 0.0270 |
| B4 | 3 | 0 | 6.212 | 0.00338 | 0.00195 |
| Difference | 3 | 0 | -2.587 | 0.0456 | 0.0263 |

t = -98.191 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.700 to -2.473

Two-tailed P-value = 0.000104

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.0000519

The sample mean of treatment B4 exceeds the sample mean of treatment A4 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A4 is greater than or equal to the population mean of treatment B4. (P = <0.001)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

**Sample 5**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.464)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A5 | 3 | 0 | 4.334 | 0.373 | 0.216 |
| B5 | 3 | 0 | 10.649 | 0.0319 | 0.0184 |
| Difference | 3 | 0 | -6.315 | 0.342 | 0.197 |

t = -32.019 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -7.163 to -5.466

Two-tailed P-value = 0.000974

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.000487

The sample mean of treatment B5 exceeds the sample mean of treatment A5 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A5 is greater than or equal to the population mean of treatment B5. (P = <0.001)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

## Sample 6

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.264)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A6 | 3 | 0 | 1.775 | 0.0723 | 0.0417 |
| B6 | 3 | 0 | 1.543 | 0.00963 | 0.00556 |
| Difference | 3 | 0 | 0.232 | 0.0800 | 0.0462 |

t = 5.017 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: 0.0330 to 0.431

Two-tailed P-value = 0.0375

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.038)

One-tailed P-value = 0.0188

The sample mean of treatment A6 exceeds the sample mean of treatment B6 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment B6 is greater than or equal to the population mean of treatment A6. (P = 0.038)

Power of performed two-tailed test with alpha = 0.050: 0.721

Power of performed one-tailed test with alpha = 0.050: 0.918

**Sample 7**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.022)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A7 | 3 | 0 | 1.862 | 0.239 | 0.138 |
| B7 | 3 | 0 | 1.643 | 0.191 | 0.110 |
| Difference | 3 | 0 | 0.218 | 0.428 | 0.247 |

t = 0.883 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -0.846 to 1.283

Two-tailed P-value = 0.470

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.470)

One-tailed P-value = 0.235

The sample mean of treatment A7 does not exceed the sample mean of the treatment B7 by an amount great enough to exclude the possibility that the difference is due to random sampling variability. The hypothesis that the population mean of treatment B7 is greater than or equal to the population mean of treatment A7 cannot be rejected. (P = 0.470)

**Sample 8**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.751)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A8 | 3 | 0 | 2.937 | 0.0492 | 0.0284 |
| B8 | 3 | 0 | 5.509 | 0.0235 | 0.0136 |
| Difference | 3 | 0 | -2.572 | 0.0436 | 0.0252 |

t = -102.224 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.680 to -2.464

Two-tailed P-value = 0.0000957

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = <0.001)

One-tailed P-value = 0.0000478

The sample mean of treatment B8 exceeds the sample mean of treatment A8 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A8 is greater than or equal to the population mean of treatment B8. (P = <0.001)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

**Sample 9:**

**Paired t-test:**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.909)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A9 | 3 | 0 | 2.494 | 0.138 | 0.0796 |
| B9 | 3 | 0 | 4.010 | 0.00331 | 0.00191 |
| Difference | 3 | 0 | -1.516 | 0.135 | 0.0782 |

t = -19.382 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -1.852 to -1.179

Two-tailed P-value = 0.00265

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.003)

One-tailed P-value = 0.00133

The sample mean of treatment B9 exceeds the sample mean of treatment A9 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A9 is greater than or equal to the population mean of treatment B9. (P = 0.003)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

**Sample 10**

**Paired t-test**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.109)

| Treatment Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A10 | 3 | 0 | 2.886 | 0.174 | 0.100 |
| B10 | 3 | 0 | 4.966 | 0.0148 | 0.00855 |
| Difference | 3 | 0 | -2.079 | 0.180 | 0.104 |

t = -19.989 with 2 degrees of freedom.

95 percent two-tailed confidence interval for difference of means: -2.527 to -1.632

Two-tailed P-value = 0.00249

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.002)

One-tailed P-value = 0.00125

The sample mean of treatment B10 exceeds the sample mean of treatment A10 by an amount that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment A10 is greater than or equal to the population mean of treatment B10. (P = 0.002)

Power of performed two-tailed test with alpha = 0.050: 1.000

Power of performed one-tailed test with alpha = 0.050: 1.000

## 3.2 Comparisons of methods for nitrate analysis

a=Shake method using $NH_4CL$ buffer
b=Cadmium column method using $NH_4CL$ buffer
c= Shake method using Tris buffer
d= Cadmium column method using Tris buffer

e=Flow Injection Analysis method
Number = Beach sample number
The beach sample numbers refer to samples collected from the following beaches:
1) Llandudno1, 2) Houtbay1, 3) Muizenberg1, 4) Sea Point 1 5) Kalk Bay, 6) Muizenberg2, 7) Sea Point2, 8) Glen Beach 9) Houtbay2 and 10) Llandudno2.

i.e. a1= A sample from Llandudno1 that has been analysed for nitrate using the Shake method with an $NH_4CL$ buffer

**SAMPLE 1**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.924)

**Equal Variance Test:** Passed (P = 0.451)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A1 | 3 | 0 | 1.436 | 0.142 | 0.0822 |
| B1 | 3 | 0 | 9.784 | 0.0315 | 0.0182 |
| C1 | 3 | 0 | 15.992 | 0.156 | 0.0898 |
| D1 | 3 | 0 | 1.324 | 0.0978 | 0.0565 |
| E1 | 3 | 0 | 4.786 | 0.205 | 0.118 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 4 | 468.368 | 117.092 | 6044.118 | <0.001 |
| Residual | 10 | 0.194 | 0.0194 | | |
| Total | 14 | 468.562 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| C1 vs. D1 | 14.668 | 129.066 | <0.001 | Yes |
| C1 vs. A1 | 14.556 | 128.085 | <0.001 | Yes |
| C1 vs. E1 | 11.206 | 98.604 | <0.001 | Yes |
| B1 vs. D1 | 8.460 | 74.444 | <0.001 | Yes |
| B1 vs. A1 | 8.349 | 73.463 | <0.001 | Yes |
| C1 vs. B1 | 6.208 | 54.622 | <0.001 | Yes |
| B1 vs. E1 | 4.998 | 43.982 | <0.001 | Yes |
| E1 vs. D1 | 3.462 | 30.462 | <0.001 | Yes |
| E1 vs. A1 | 3.350 | 29.482 | <0.001 | Yes |
| A1 vs. D1 | 0.111 | 0.980 | 0.350 | No |

**SAMPLE 2**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.315)

**Equal Variance Test:** Passed (P = 0.840)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A2 | 3 | 0 | 1.431 | 0.160 | 0.0925 |
| B2 | 3 | 0 | 5.914 | 0.198 | 0.114 |
| C2 | 3 | 0 | -0.153 | 0.109 | 0.0632 |
| D2 | 3 | 0 | 2.072 | 0.107 | 0.0617 |
| E2 | 3 | 0 | 0.950 | 0.101 | 0.0581 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 4 | 64.146 | 16.037 | 814.552 | <0.001 |
| Residual | 10 | 0.197 | 0.0197 | | |
| Total | 14 | 64.343 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| B2 vs. C2 | 6.068 | 52.964 | <0.001 | Yes |
| B2 vs. E2 | 4.965 | 43.335 | <0.001 | Yes |
| B2 vs. A2 | 4.483 | 39.131 | <0.001 | Yes |
| B2 vs. D2 | 3.842 | 33.537 | <0.001 | Yes |
| D2 vs. C2 | 2.226 | 19.427 | <0.001 | Yes |
| A2 vs. C2 | 1.585 | 13.833 | <0.001 | Yes |
| D2 vs. E2 | 1.123 | 9.798 | <0.001 | Yes |
| E2 vs. C2 | 1.103 | 9.629 | <0.001 | Yes |
| D2 vs. A2 | 0.641 | 5.594 | <0.001 | Yes |
| A2 vs. E2 | 0.482 | 4.204 | 0.002 | Yes |

**SAMPLE 3**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.422)

**Equal Variance Test:** Passed (P = 0.212)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A3 | 3 | 0 | 0.377 | 0.0358 | 0.0207 |

| | | | | | |
|---|---|---|---|---|---|
| B3 | 3 | 0 | 1.356 | 0.0143 | 0.00826 |
| C3 | 3 | 0 | -0.659 | 0.0437 | 0.0252 |
| D3 | 3 | 0 | -0.170 | 0.0973 | 0.0562 |
| E3 | 3 | 0 | 12.797 | 0.148 | 0.0855 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 4 | 385.983 | 96.496 | 13867.277 | <0.001 |
| Residual | 10 | 0.0696 | 0.00696 | | |
| Total | 14 | 386.053 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| **Comparison** | **Diff of Means** | **t** | **P** | **P<0.050** |
|---|---|---|---|---|
| E3 vs. C3 | 13.456 | 197.561 | <0.001 | Yes |
| E3 vs. D3 | 12.966 | 190.375 | <0.001 | Yes |
| E3 vs. A3 | 12.420 | 182.358 | <0.001 | Yes |
| E3 vs. B3 | 11.441 | 167.970 | <0.001 | Yes |
| B3 vs. C3 | 2.015 | 29.591 | <0.001 | Yes |
| B3 vs. D3 | 1.526 | 22.405 | <0.001 | Yes |
| A3 vs. C3 | 1.035 | 15.203 | <0.001 | Yes |
| B3 vs. A3 | 0.980 | 14.388 | <0.001 | Yes |
| A3 vs. D3 | 0.546 | 8.017 | <0.001 | Yes |
| D3 vs. C3 | 0.489 | 7.186 | <0.001 | Yes |

**SAMPLE 4**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.823)

**Equal Variance Test:** Passed (P = 0.675)

| **Group Name** | **N** | **Missing** | **Mean** | **Std Dev** | **SEM** |
|---|---|---|---|---|---|
| A4 | 3 | 0 | 0.483 | 0.0511 | 0.0295 |
| B4 | 3 | 0 | 1.000 | 0.0713 | 0.0412 |
| C4 | 3 | 0 | -0.557 | 0.0168 | 0.00970 |
| D4 | 3 | 0 | 3.837 | 0.104 | 0.0601 |
| E4 | 3 | 0 | -1.749 | 0.0911 | 0.0526 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 4 | 52.528 | 13.132 | 2422.337 | <0.001 |
| Residual | 10 | 0.0542 | 0.00542 | | |
| Total | 14 | 52.583 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| D4 vs. E4 | 5.586 | 92.917 | <0.001 | Yes |
| D4 vs. C4 | 4.395 | 73.100 | <0.001 | Yes |
| D4 vs. A4 | 3.354 | 55.795 | <0.001 | Yes |
| D4 vs. B4 | 2.837 | 47.196 | <0.001 | Yes |
| B4 vs. E4 | 2.749 | 45.721 | <0.001 | Yes |
| A4 vs. E4 | 2.232 | 37.122 | <0.001 | Yes |
| B4 vs. C4 | 1.557 | 25.904 | <0.001 | Yes |
| C4 vs. E4 | 1.191 | 19.817 | <0.001 | Yes |
| A4 vs. C4 | 1.040 | 17.305 | <0.001 | Yes |
| B4 vs. A4 | 0.517 | 8.599 | <0.001 | Yes |

**SAMPLE 5**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.758)

**Equal Variance Test:** Passed (P = 0.534)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A5 | 3 | 0 | 3.055 | 0.202 | 0.117 |
| B5 | 3 | 0 | 1.566 | 0.139 | 0.0805 |
| C5 | 3 | 0 | -0.480 | 0.0636 | 0.0367 |
| D5 | 3 | 0 | 0.262 | 0.0535 | 0.0309 |
| E5 | 3 | 0 | 1.015 | 0.131 | 0.0754 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 4 | 21.732 | 5.433 | 322.388 | <0.001 |
| Residual | 10 | 0.169 | 0.0169 | | |
| Total | 14 | 21.901 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| A5 vs. C5 | 3.535 | 33.351 | <0.001 | Yes |
| A5 vs. D5 | 2.793 | 26.352 | <0.001 | Yes |
| B5 vs. C5 | 2.046 | 19.301 | <0.001 | Yes |

| | | | | |
|---|---|---|---|---|
| A5 vs. E5 | 2.041 | 19.253 | <0.001 | Yes |
| E5 vs. C5 | 1.494 | 14.099 | <0.001 | Yes |
| A5 vs. B5 | 1.489 | 14.050 | <0.001 | Yes |
| B5 vs. D5 | 1.304 | 12.302 | <0.001 | Yes |
| E5 vs. D5 | 0.752 | 7.099 | <0.001 | Yes |
| D5 vs. C5 | 0.742 | 6.999 | <0.001 | Yes |
| B5 vs. E5 | 0.551 | 5.203 | <0.001 | Yes |

**SAMPLE 6**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.388)

**Equal Variance Test:** Passed (P = 0.980)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A6 | 3 | 0 | 0.174 | 0.0612 | 0.0353 |
| B6 | 3 | 0 | 4.950 | 0.0523 | 0.0302 |
| C6 | 3 | 0 | -0.753 | 0.0608 | 0.0351 |
| D6 | 3 | 0 | 3.235 | 0.0357 | 0.0206 |
| E6 | 3 | 0 | 5.644 | 0.0690 | 0.0399 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 4 | 96.915 | 24.229 | 7468.937 | <0.001 |
| Residual | 10 | 0.0324 | 0.00324 | | |
| Total | 14 | 96.948 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| E6 vs. C6 | 6.397 | 137.548 | <0.001 | Yes |
| B6 vs. C6 | 5.703 | 122.637 | <0.001 | Yes |
| E6 vs. A6 | 5.470 | 117.617 | <0.001 | Yes |
| B6 vs. A6 | 4.776 | 102.706 | <0.001 | Yes |
| D6 vs. C6 | 3.988 | 85.748 | <0.001 | Yes |
| D6 vs. A6 | 3.061 | 65.817 | <0.001 | Yes |
| E6 vs. D6 | 2.409 | 51.799 | <0.001 | Yes |
| B6 vs. D6 | 1.715 | 36.889 | <0.001 | Yes |
| A6 vs. C6 | 0.927 | 19.931 | <0.001 | Yes |
| E6 vs. B6 | 0.693 | 14.911 | <0.001 | Yes |

**SAMPLE 7**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.770)

**Equal Variance Test:** Passed (P = 0.094)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A7 | 3 | 0 | 0.378 | 0.0610 | 0.0352 |
| B7 | 3 | 0 | 2.685 | 0.0254 | 0.0147 |
| C7 | 3 | 0 | -0.706 | 0.0576 | 0.0332 |
| D7 | 3 | 0 | 1.756 | 0.146 | 0.0843 |
| E7 | 3 | 0 | 2.640 | 0.0566 | 0.0327 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 4 | 26.356 | 6.589 | 1022.940 | <0.001 |
| Residual | 10 | 0.0644 | 0.00644 | | |
| Total | 14 | 26.421 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| B7 vs. C7 | 3.391 | 51.750 | <0.001 | Yes |
| E7 vs. C7 | 3.347 | 51.069 | <0.001 | Yes |
| D7 vs. C7 | 2.463 | 37.580 | <0.001 | Yes |
| B7 vs. A7 | 2.307 | 35.207 | <0.001 | Yes |
| E7 vs. A7 | 2.262 | 34.525 | <0.001 | Yes |
| D7 vs. A7 | 1.379 | 21.037 | <0.001 | Yes |
| A7 vs. C7 | 1.084 | 16.544 | <0.001 | Yes |
| B7 vs. D7 | 0.929 | 14.170 | <0.001 | Yes |
| E7 vs. D7 | 0.884 | 13.489 | <0.001 | Yes |
| B7 vs. E7 | 0.0446 | 0.681 | 0.511 | No |

**SAMPLE 8**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.129)

**Equal Variance Test:** Passed (P = 0.467)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| A8 | 3 | 0 | 0.289 | 0.0166 | 0.00958 |

| | | | | | |
|---|---|---|---|---|---|
| B8 | 3 | 0 | 2.789 | 0.170 | 0.0982 |
| C8 | 3 | 0 | -0.744 | 0.0932 | 0.0538 |
| D8 | 3 | 0 | 3.502 | 0.00857 | 0.00495 |
| E8 | 3 | 0 | 5.011 | 0.0417 | 0.0241 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 4 | 66.764 | 16.691 | 2100.885 | <0.001 |
| Residual | 10 | 0.0794 | 0.00794 | | |
| Total | 14 | 66.843 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| **Comparison** | **Diff of Means** | **t** | **P** | **P<0.050** |
|---|---|---|---|---|
| E8 vs. C8 | 5.755 | 79.071 | <0.001 | Yes |
| E8 vs. A8 | 4.722 | 64.882 | <0.001 | Yes |
| D8 vs. C8 | 4.245 | 58.332 | <0.001 | Yes |
| B8 vs. C8 | 3.533 | 48.541 | <0.001 | Yes |
| D8 vs. A8 | 3.213 | 44.143 | <0.001 | Yes |
| B8 vs. A8 | 2.500 | 34.352 | <0.001 | Yes |
| E8 vs. B8 | 2.222 | 30.530 | <0.001 | Yes |
| E8 vs. D8 | 1.509 | 20.739 | <0.001 | Yes |
| A8 vs. C8 | 1.033 | 14.190 | <0.001 | Yes |
| D8 vs. B8 | 0.713 | 9.791 | <0.001 | Yes |

**SAMPLE 9**

**One Way Analysis of Variance**

**Normality Test (Shapiro-Wilk)** Passed (P = 0.002)

**Equal Variance Test:** Passed (P = 0.143)

| **Group Name** | **N** | **Missing** | **Mean** | **Std Dev** | **SEM** |
|---|---|---|---|---|---|
| A9 | 3 | 0 | 6.566 | 0.250 | 0.145 |
| B9 | 3 | 0 | 19.058 | 1.459 | 0.842 |
| C9 | 3 | 0 | 49.544 | 0.0294 | 0.0170 |
| D9 | 3 | 0 | 17.133 | 0.185 | 0.107 |
| E9 | 3 | 0 | 5.385 | 0.0897 | 0.0518 |

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Between Groups | 4 | 3824.889 | 956.222 | 2139.766 | <0.001 |

| | | | |
|---|---|---|---|
| Residual | 10 | 4.469 | 0.447 |
| Total | 14 | 3829.358 | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

| **Comparison** | **Diff of Means** | **t** | **P** | **P<0.050** |
|---|---|---|---|---|
| C9 vs. E9 | 44.159 | 80.904 | <0.001 | Yes |
| C9 vs. A9 | 42.978 | 78.741 | <0.001 | Yes |
| C9 vs. D9 | 32.411 | 59.380 | <0.001 | Yes |
| C9 vs. B9 | 30.486 | 55.854 | <0.001 | Yes |
| B9 vs. E9 | 13.673 | 25.050 | <0.001 | Yes |
| B9 vs. A9 | 12.492 | 22.887 | <0.001 | Yes |
| D9 vs. E9 | 11.748 | 21.523 | <0.001 | Yes |
| D9 vs. A9 | 10.567 | 19.360 | <0.001 | Yes |
| B9 vs. D9 | 1.925 | 3.527 | 0.011 | Yes |
| A9 vs. E9 | 1.181 | 2.163 | 0.056 | No |

**SAMPLE 10**

**Normality Test (Shapiro-Wilk)** Failed (P < 0.001)

**Kruskal-Wallis One Way Analysis of Variance on Ranks** Wednesday, January 09, 2013, 05:04:32 PM

**Data source:** Data 1 in Notebook1

| **Group** | **N** | **Missing** | **Median** | **25%** | **75%** |
|---|---|---|---|---|---|
| A10 | 3 | 0 | 70.381 | 0.774 | 71.158 |
| B10 | 3 | 0 | 15.983 | 15.903 | 16.376 |
| C10 | 3 | 0 | 26.355 | 26.278 | 26.670 |
| D10 | 3 | 0 | 3.934 | 3.913 | 4.037 |
| E10 | 3 | 0 | 5.941 | 5.874 | 6.531 |

H = 7.500 with 4 degrees of freedom. (P = 0.112)

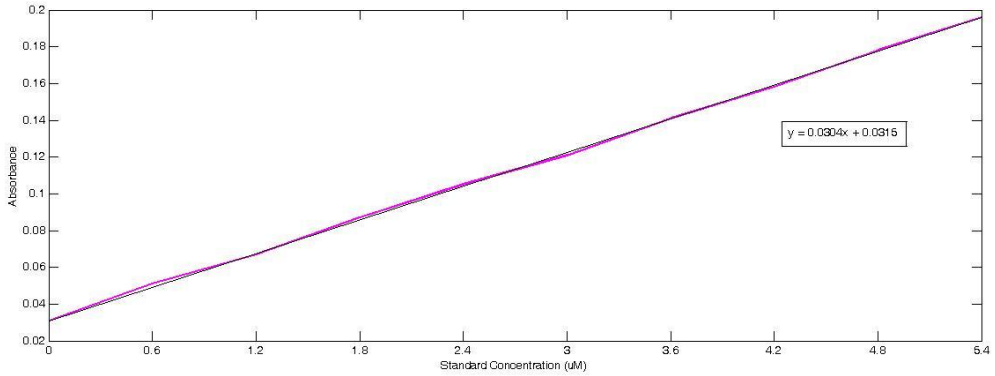
The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.112)

# Appendix 4

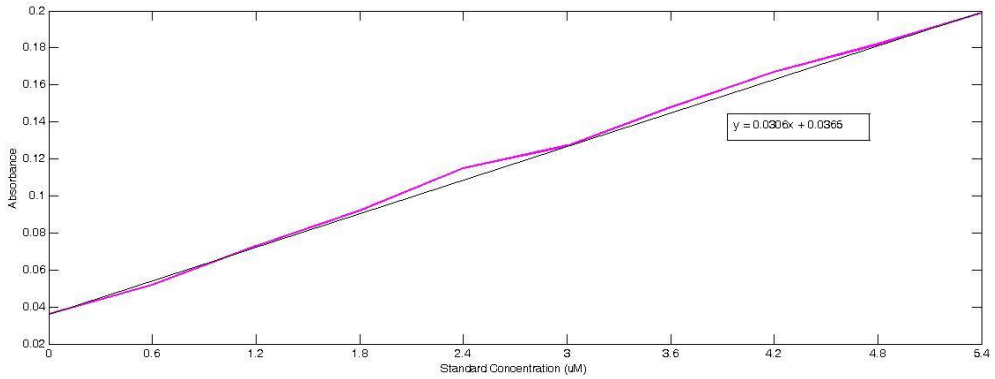
## 4.1 Standard curves used to determine the concentration of nutrients in stored seawater

### 4.1.1 Standard curves used to determine the concentration of inorganic phosphate in seawater samples (absorbance at 880 nm versus concentration)

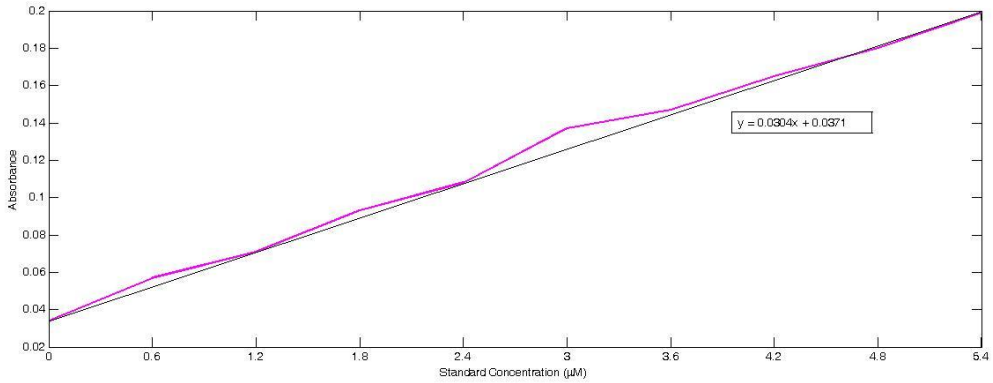
#### 4.1.1.1 Week 0



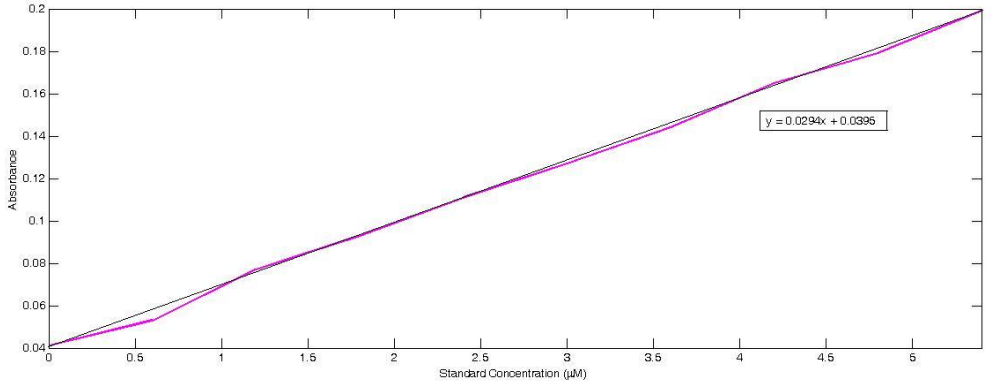

#### 4.1.1.2 Week 1



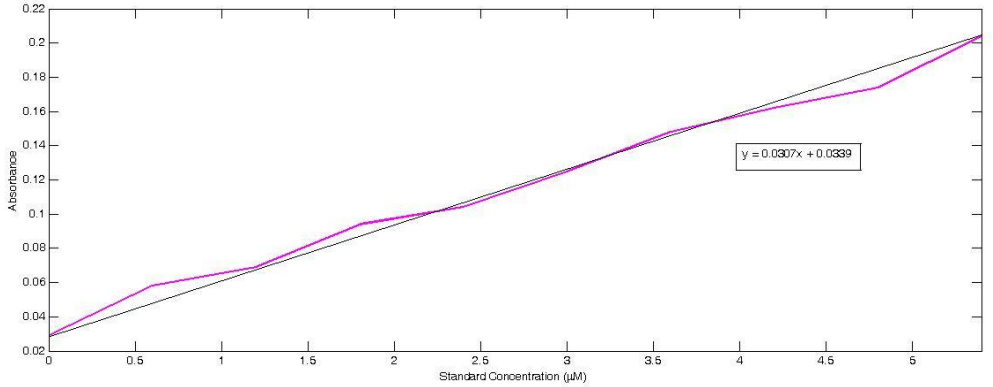
### 4.1.1.3 Week 2



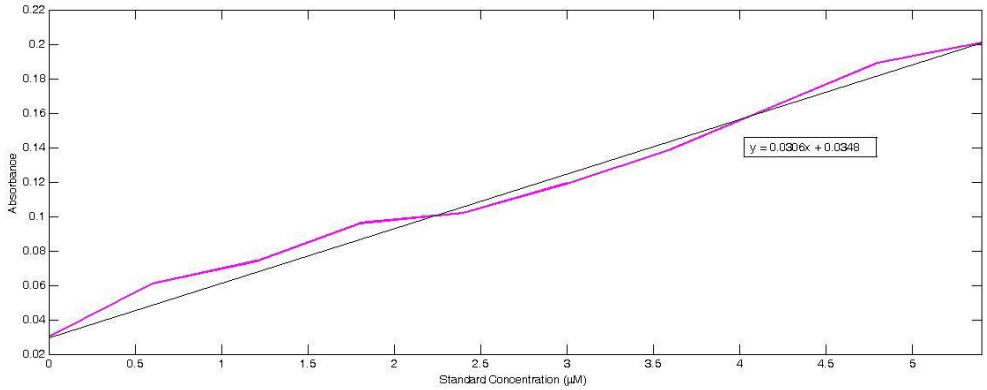

### 4.1.1.4 Week 3



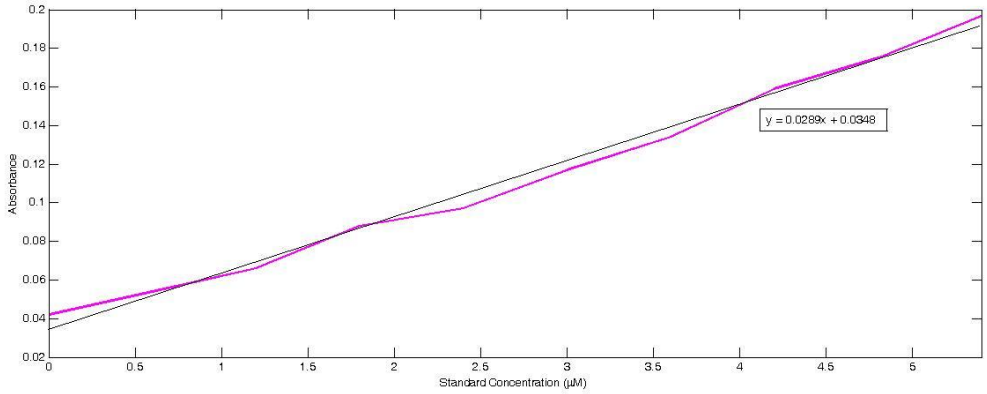

### 4.1.1.5 Week 4



#### 4.1.1.6 Week 8

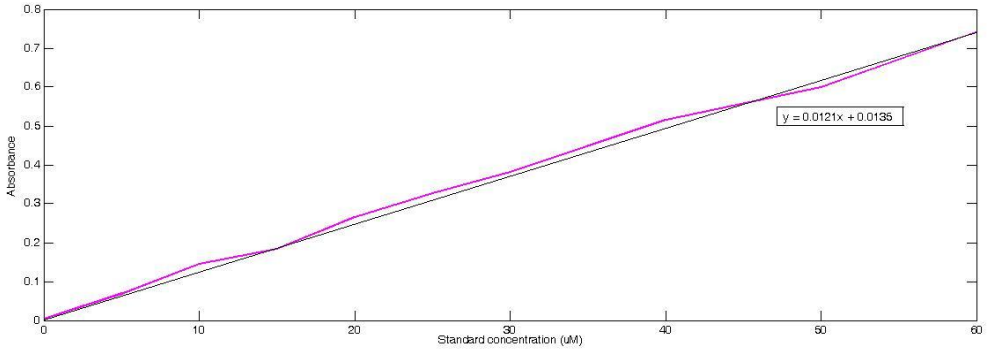



#### 4.1.1.7 Week 12

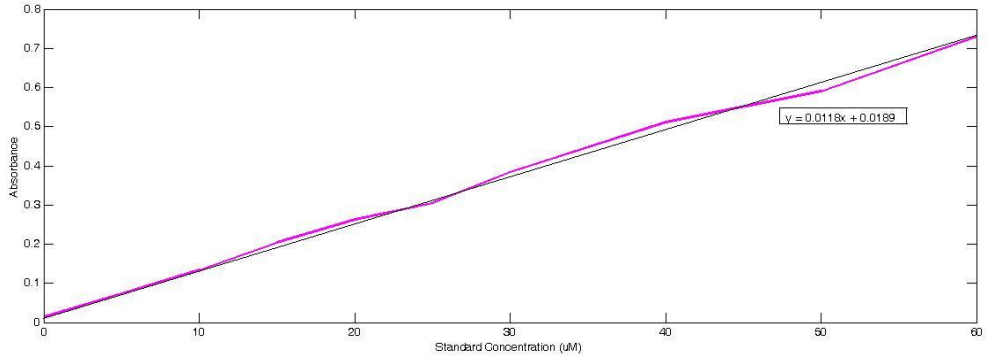



### 4.1.2 Standard curves used to determine the concentration of dissolved inorganic silicate in seawater samples (absorbance at 810 nm versus concentration)

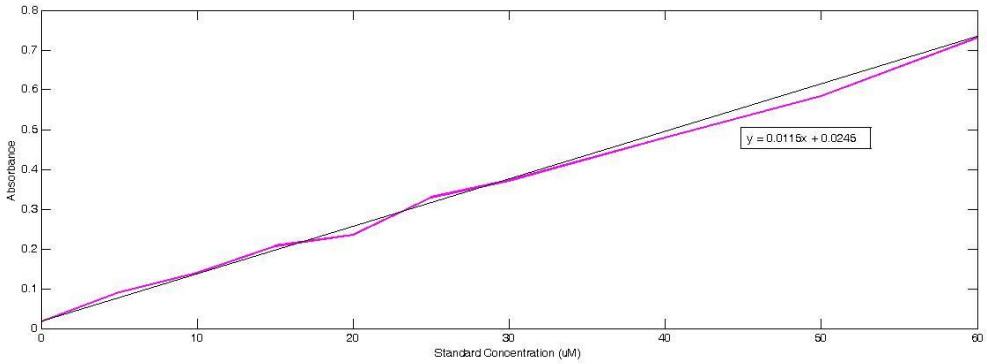
#### 4.1.2.1 Week 0



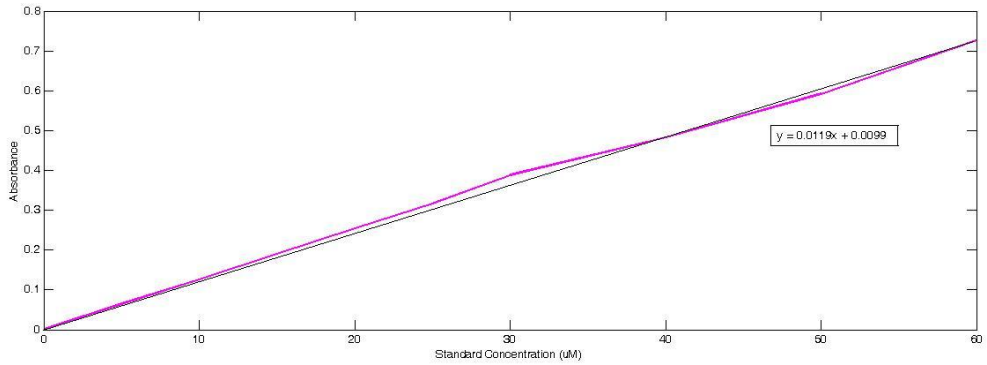
### 4.1.2.2 Week 1



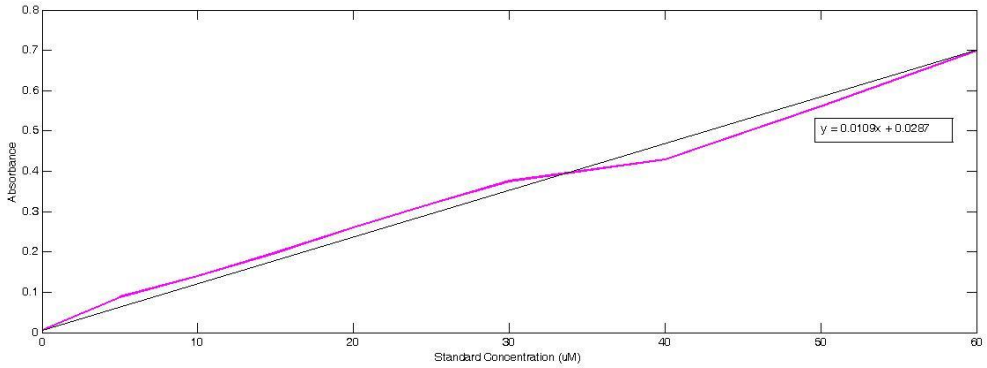

### 4.1.2.3 Week 2



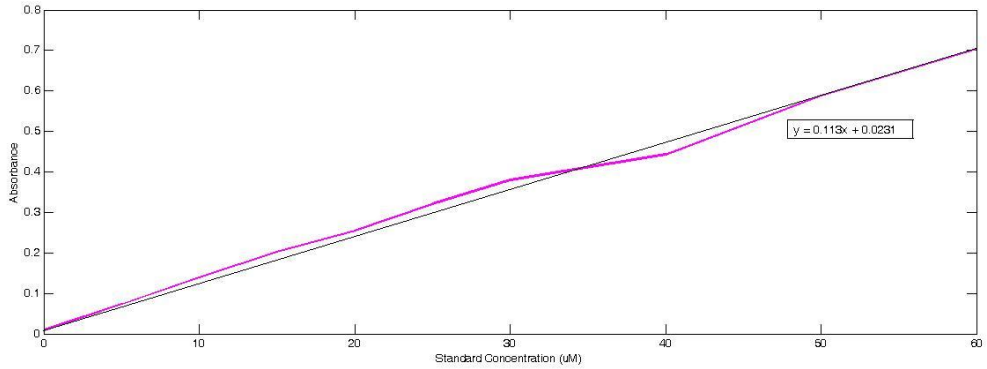

### 4.1.2.4 Week 3



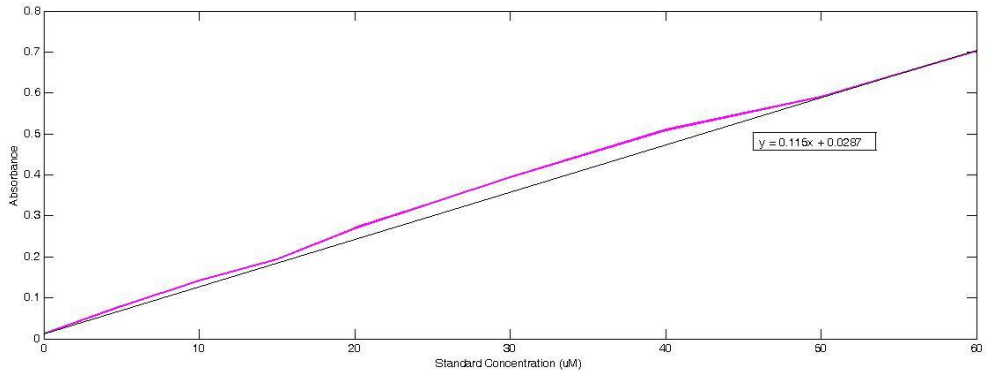
### 4.1.2.5 Week 4




### 4.1.2.6 Week 8

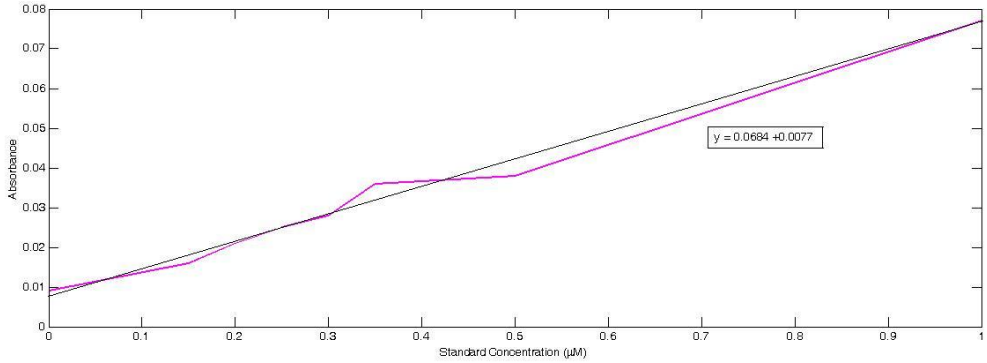



### 4.1.2.7 Week 12

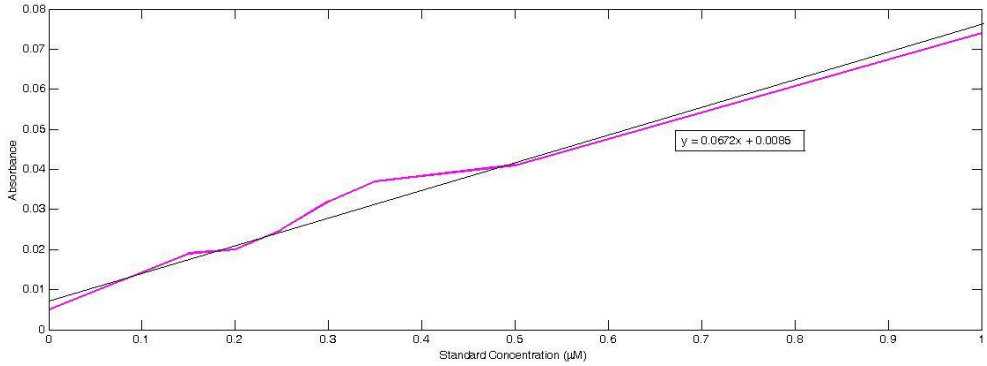


### 4.1.3 Standard curves used to determine the concentration of dissolved nitrites in seawater samples (absorbance at 540 nm versus concentration)

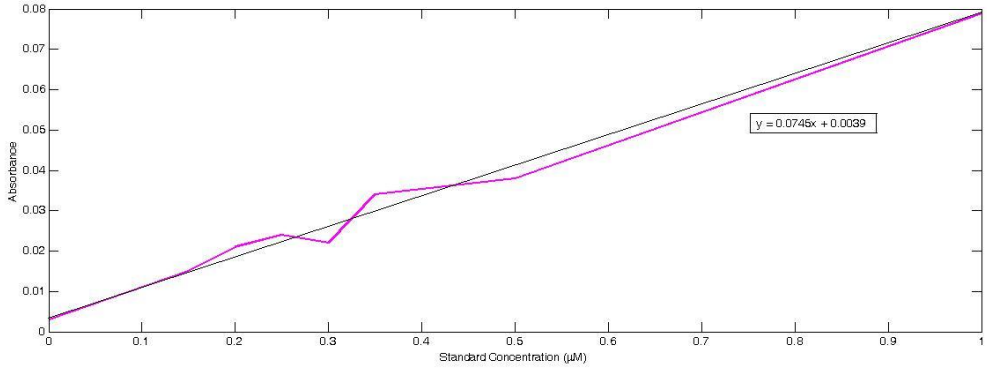
#### 4.1.3.1 Week 0



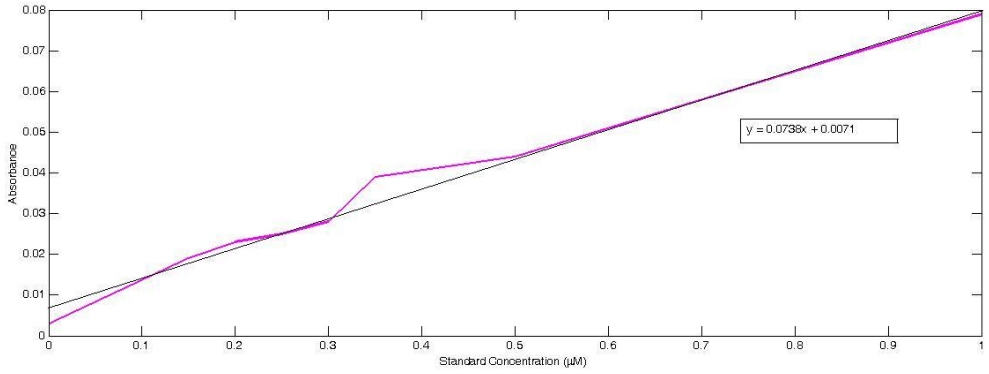

#### 4.1.3.2 Week 1



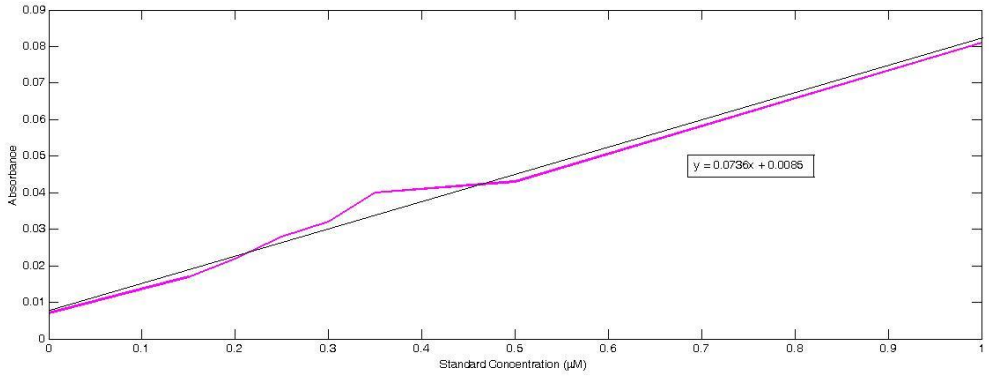

#### 4.1.3.3 Week 2



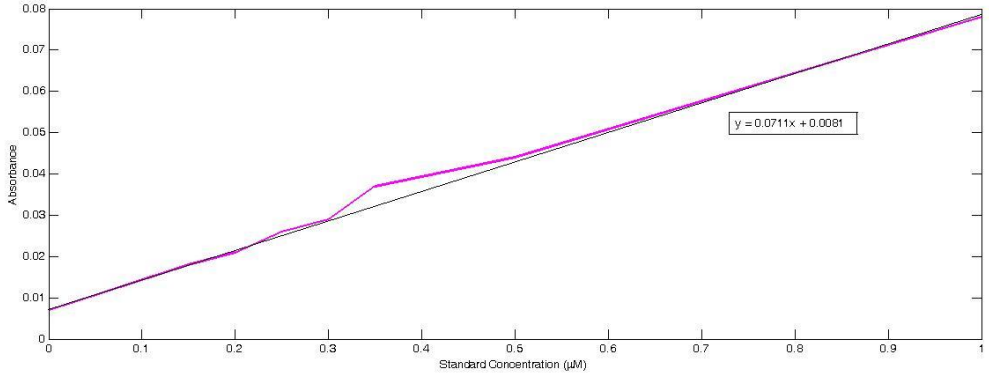
### 4.1.3.4 Week 3



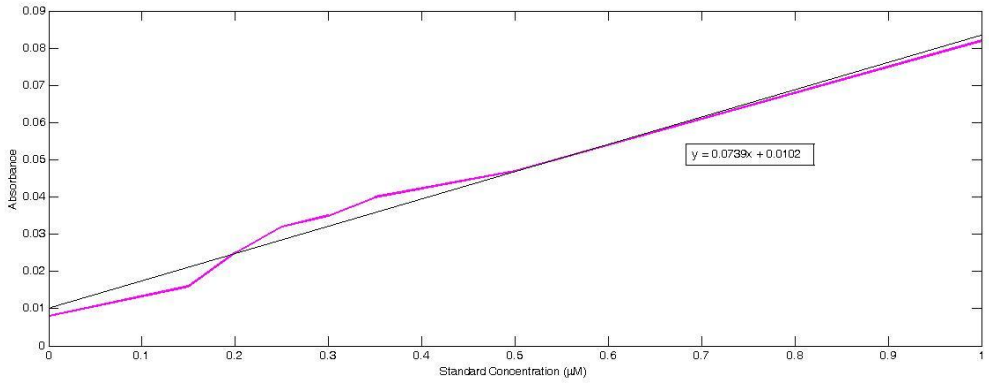

### 4.1.3.5 Week 4




### 4.1.3.6 Week 8

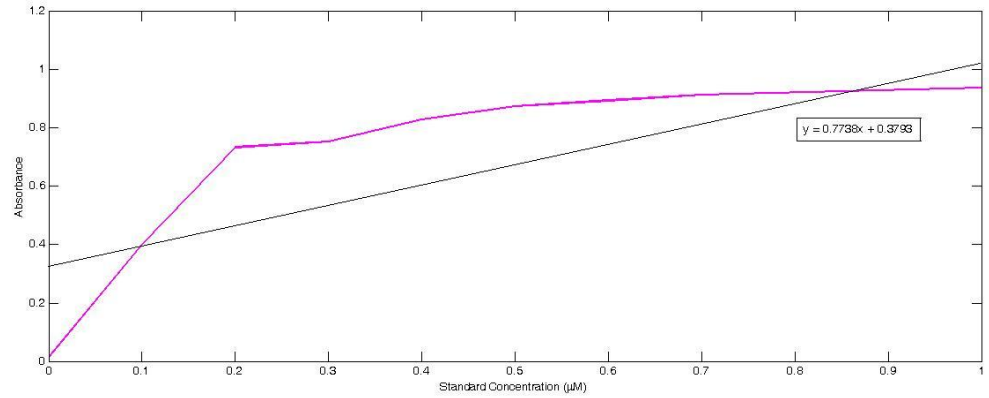


#### 4.1.3.7 Week 12

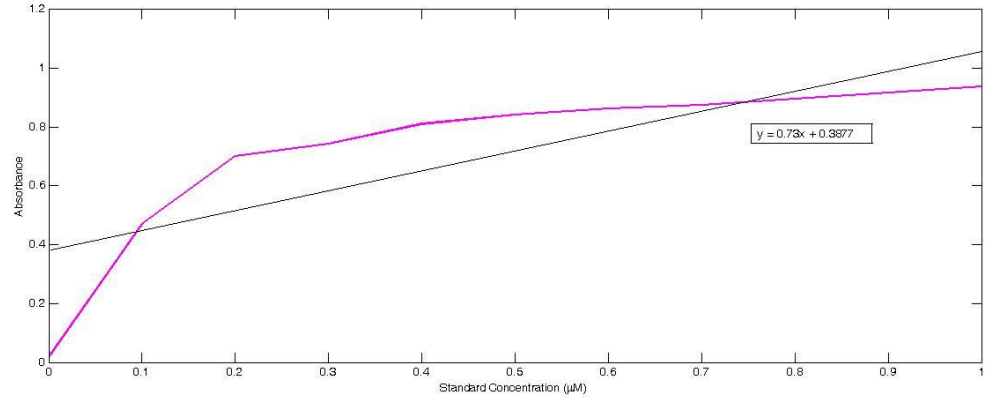



### 4.1.4 Standard curves used to determine the concentration of dissolved urea in seawater samples (absorbance at 520 nm versus concentration)

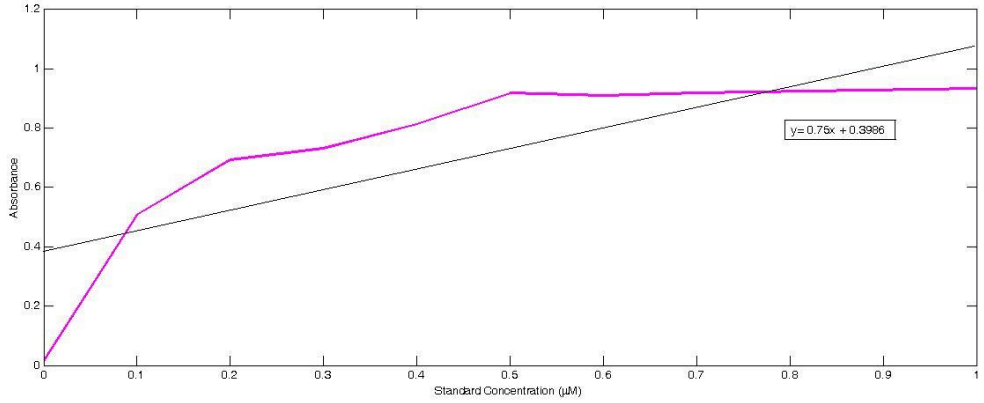
#### 4.1.4.1 Week 0



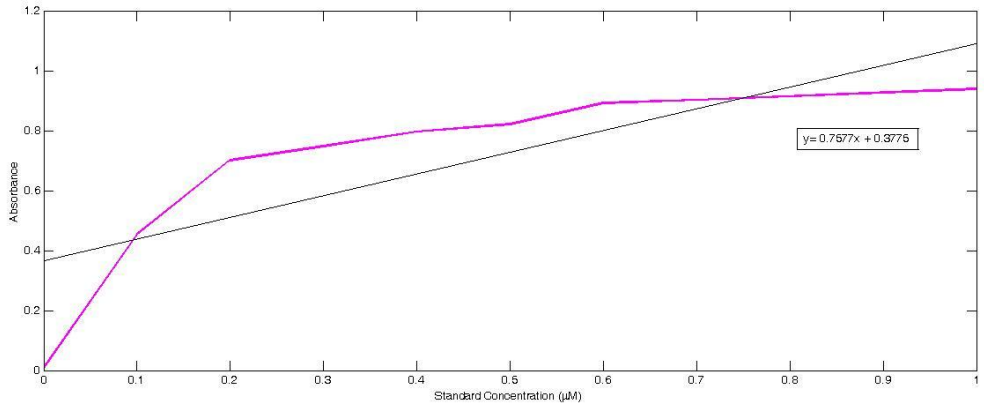

#### 4.1.4.2 Week 1



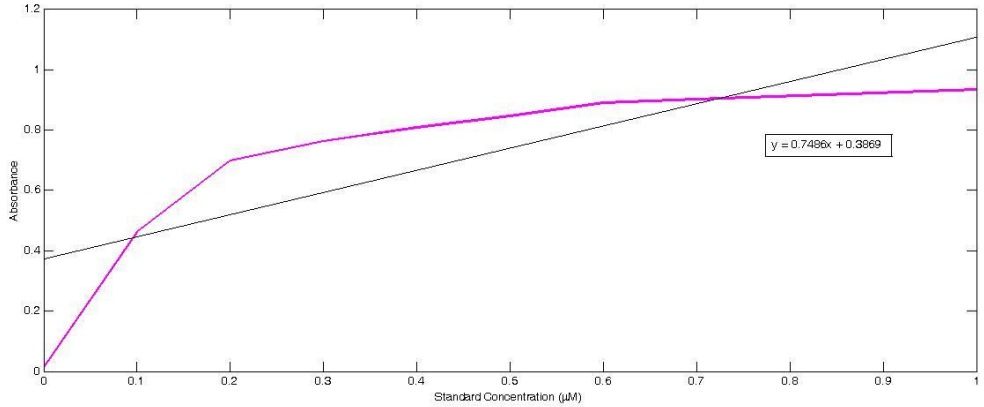
### 4.1.4.3 Week 2



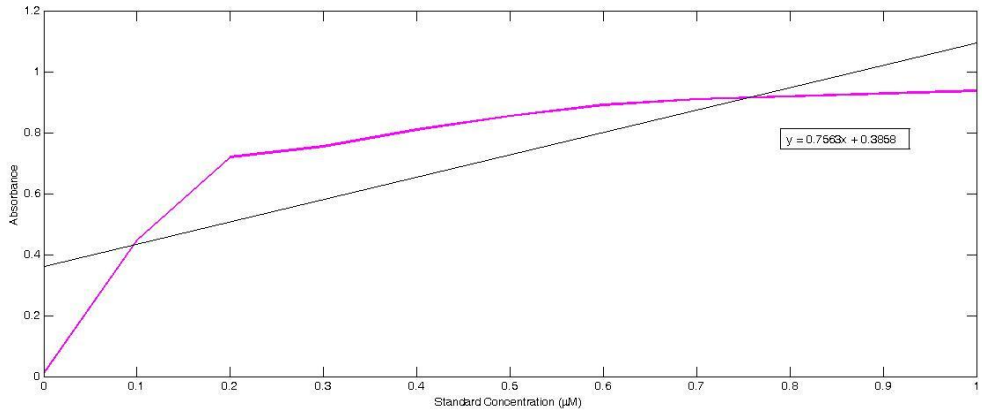

### 4.1.4.4 Week 3



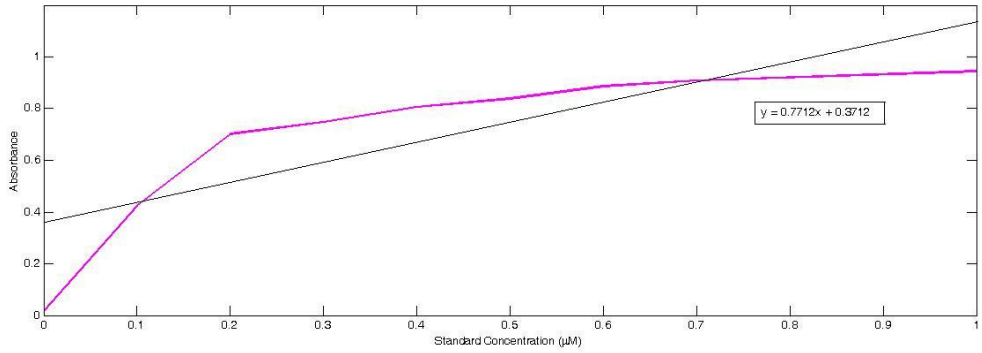

### 4.1.4.5 Week 4



#### 4.1.4.6 Week 8

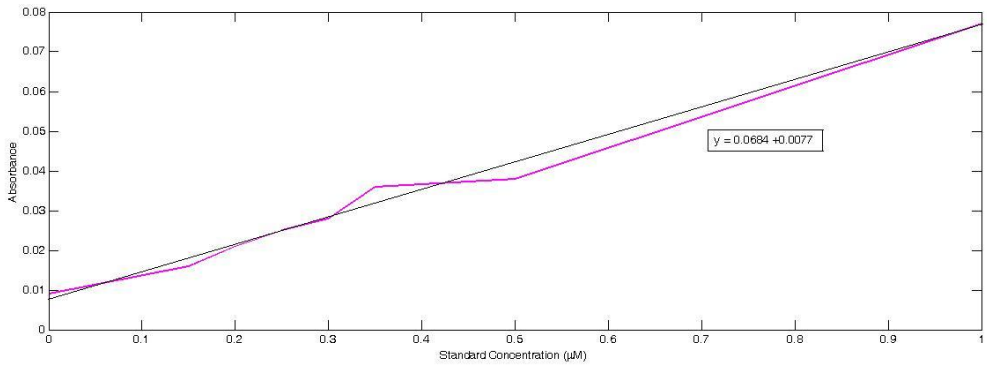



#### 4.1.4.7 Week 12

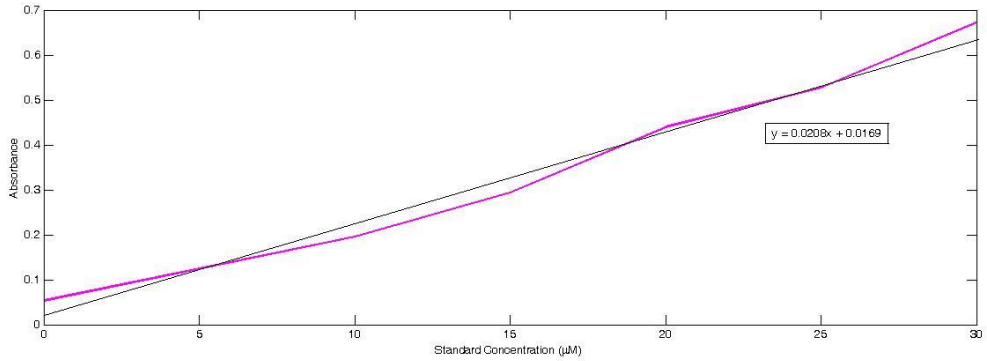



## 4.2 Standard curves used when comparing methodologies for the analysis of nutrients in seawater

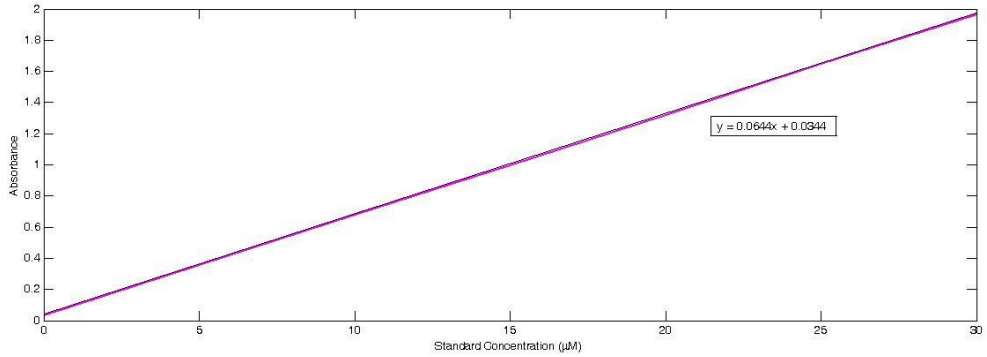
### 4.2.1 Standard curves used to determine the concentration of dissolved inorganic silicate in seawater samples (absorbance at 810 nm versus concentration)



**4.2.2 Standard curves used to determine the concentration of dissolved nitrite/nitrate in seawater samples using the cadmium column and shaking methods (absorbance at 540 nm versus concentration)**




**4.2.3 Standard curves used to determine the concentration of dissolved nitrite/nitrate in seawater samples using $NH_4Cl$ buffer (absorbance at 540 nm versus concentration)**




**4.2.2 Standard curves used to determine the concentration of dissolved nitrite/nitrate in seawater samples using Trisl buffer (absorbance at 540 nm versus concentration)**