GO-SHIP Repeat Hydrography Nutrient Manual:
The precise and accurate determination of dissolved inorganic nutrients in seawater, using Continuous Flow Analysis methods.

Susan Becker[1], Michio Aoyama[2], E. Malcolm S. Woodward[3], Karel Bakker[4], Stephen Coverly[5], Claire Mahaffey[6], Toste Tanhua[7].

1. UC San Diego, Scripps Institution of Oceanography, 9500 Gilman Dr. MC 0236, San Diego, CA 92093, USA.
2. RIGC, JAMSTEC, 2-15 Natsushima, Yokosuka, 237-0061, and CRIED, University of Tsukuba, 305-8572, Japan.
3. Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, PL1 3DH. United Kingdom.
4. NIOZ, Royal Netherlands Institute for Sea Research, Department of Ocean Science, and Utrecht University, PO Box 59 Den Burg 1790 AB, The Netherlands.
5. BLTEC, #1607, 1 Dong, 775, Gyeongin-Ro Yeongdeungpo-Gu, Seoul, 150-972, Korea.
6. Department of Earth, Ocean and Ecological Sciences, SOES, University of Liverpool, Liverpool, L69 3GP. United Kingdom.
7. GEOMAR Helmholtz Centre for Ocean Research Kiel, Duesternbrooker Weg 20, D-24105 Kiel, Germany.

Acknowledgements:
This Nutrient Manual was written and revised by members of WG 147: Towards comparability of global oceanic nutrient data (COMPONUT) of the Scientific Committee on Oceanic Research (SCOR).
Support for this work was provided by the U.S. National Science Foundation (Grants OCE-1546580 and OCE-1840868), national SCOR committees, and other sources.

The authors thank the contributions made by other WG #147 members:
Andrew Dickson, Bernadette Sloyan, Karin Bjorkman, Anne Daniel, Hema Naik, and Raymond Roman.

Thank you also to numerous other scientific colleagues who reviewed the manuscript and provided helpful comments and suggestions for improvements.

Published date 12th August 2019

Table of Contents:

List of Figures and Tables:

GO-SHIP Repeat Hydrography Nutrient Manual:

The precise and accurate determination of dissolved inorganic nutrients in seawater using Continuous Flow Analysis (CFA) methods.

1. Introduction

This GO-SHIP manual is an update to the original version by Hydes et al. (2010), and reviews basic sample collection and storage, aspects of CFA using an Auto-Analyzer, and specific nutrient methods in use by many laboratories doing repeat hydrography. The document also covers laboratory best practices including quality control and quality assurance (QC/QA) procedures to obtain the best results, and suggests protocols for the use of reference materials (RM) and certified reference materials (CRMs).

1.1 Nutrient Data Comparability: GEOSECS to GO-SHIP

The availability of inorganic macronutrients (nitrate (NO_3), phosphate (PO_4), silicic acid ($Si(OH)_4$) commonly referred to as 'silicate', ammonium (NH_4), and nitrite (NO_2)) in upper ocean waters frequently limits and regulates the amount of organic carbon fixed by phytoplankton, thereby constituting a key control mechanism of carbon and biogeochemical cycling. There are a number of biogeographic regions in the open ocean characterized by different macronutrient regimes, either permanently or seasonally limiting the growth of phytoplankton (Moore 2016). Accurately measuring temporal changes in macronutrient concentrations is essential to constraining net biological production and export fluxes, detecting shifts in biogeographic regimes, and for monitoring eutrophication phenomena. For open ocean work an analytical accuracy of 1% should be aimed for by the Global Ocean Ship-based Hydrographic Investigations Program (GO-SHIP) (Talley et al., 2016) to allow reliable quantification of decadal trends in the deep ocean. An internal consistency of nutrient data in the order of 1 - 3% has been achieved through secondary quality control (QC) procedures implemented in the Global Ocean Data Analysis Project (GLODAPv2: Olsen et al., 2016).

The Geochemical Ocean Sections Study (GEOSECS) in the 1970s was one of the first efforts to provide a global survey of chemical, isotopic, and radiochemical tracers in the world's oceans. Since then there have been numerous international collaborations to map and study different chemical, physical, and biological aspects of the oceans. These programs include the Joint Global Ocean Flux Study (JGOFS) in the late 80s, World Ocean Circulation Experiment (WOCE) in the mid to late 90s, and the current global programs including, Climate Variability and predictability (CLIVAR), GEOTRACES, and GO-SHIP. In addition to these large international efforts, there continues to be many other programs led by individual laboratories and countries to study specific areas and processes in the world's oceans, including ocean time-series stations and transects.

All of these efforts have led to large data synthesis studies, including Carbon dioxide in the Atlantic Ocean (CARINA, Key et al. 2010) Pacific Ocean Interior Carbon (PACIFICA, Suzuki et al. 2013), GLODAPv1, and GLODAPv2. These studies include analysis from different international laboratories. It is imperative that the data sets produced by the different laboratories are comparable, and differences in concentrations in time or space are real and not artifacts of differing methods, standards or instrumentation. In an effort to verify the comparability of nutrient data sets there have been a number of inter-laboratory comparability exercises (UNESCO 1965, 1967; ICES 1967, 1977; Kirkwood et al. 1991; Aminot and Kirkwood 1995). There are commercially available nutrient stock standard solutions, e.g. OSIL (http://osil.com/), and other programs supply stock standard solutions that allow laboratories to validate their methods (Topping 1997). However, there was a need for a reference material for nutrients that would allow laboratories to compare and closely monitor data quality.
There have been inter-laboratory comparison studies using reference materials with one of the first being MOOS certified by the National Oceanic and Atmospheric Administration/ National Research Council Canada (NOAA/NRC). The Meteorological Research Institute (MRI) in Japan has led a more recent series of international inter-laboratory comparisons in 2003, 2006, 2008 and 2012 (Aoyama 2006, 2007, 2008, and 2010). The motivation of the exercises led by MRI was the development of reference materials for nutrients in seawater (RMNS). In 2014/2015 and 2017/2018 the International Ocean Carbon Coordination Project (IOCCP) and Japan Agency for Marine-Earth Science and Technology (JAMSTEC) conducted inter-laboratory comparison studies of nutrient CRMs in seawater. These two intercomparison exercises used CRMs as known samples in 2014/2015 (Aoyama et

al. 2016), or as unknown samples in 2017/2018. The availability and use of these CRMs has been instrumental in improving the global comparability of nutrient data sets. These recent exercises were carried out as part of the terms of reference of the International SCOR working group #147: Towards comparability of global oceanic nutrient data (COMPONUT); (http://www.scor-int.org/SCOR_WGs_WG147.htm).

1.2 Methods and Instrumentation

The basic analytical methods and chemistries that are used to determine concentrations of inorganic nutrients in seawater are well established. Strickland and Parsons outlined the manual methods in their book, "A Practical Handbook of Seawater Analysis" (Strickland and Parsons, 1972). The chemical methods have been changed, optimized and automated over the decades by numerous authors, but the basic chemistries remain the same and are based on colorimetric reactions. The exception to this is the newer methods for ammonium/ammonia determination, which are based on fluorometry.

Nitrate is determined using a procedure described by Armstrong et al. (1967), which involves passing a seawater sample through a copper-cadmium reduction column where the nitrate is reduced to nitrite. Nitrite is then diazotized with sulfanilamide and coupled with N-1-naphthyl-ethylenediamine dihydrochloride (N-1-N/NEDD) to form a red azo dye, and the absorbance is measured between 520 and 540nm.

Phosphate is determined by adding acidified ammonium molybdate to the seawater sample to produce phosphomolybdic acid, which is then reduced to a phospho-molybdenum blue complex following the addition of dihydrazine sulfate (Bernhardt and Wilhelms 1967), or ascorbic acid (Murphy and Riley 1962), which was optimized by Zhang et al. (1999). The absorbance is measured between 850 and 880nm.

Silicate is analyzed according to two methods. The method outline in Armstrong (1967) produces a silicomolybdic acid with the addition of ammonium molybdate. A silico-molybdenum complex is then formed following the addition of stannous chloride, and the absorbance is measured at approximately 660nm. Alternatively the method published in Grasshoff et al. (1983) uses ascorbic acid to reduce the silicomolybdic acid to the blue complex, and the absorbance is measured at approximately 820nm.

There are two commonly used ammonium methods, colorimetric and Fluorometric. The colorimetric method uses the Berthelot reaction, and involves the reaction of hypochlorite and phenol with ammonium in an alkaline solution to form an indophenol blue compound. The sample absorbance is measured at approximately 660nm. This method is a modification of the procedure in Grasshoff (1983). The highly sensitive Fluorometric method using ammonia diffusion across a teflon membrane with Fluorometric detection (Jones, 1991) was developed, but obtaining the membrane proved difficult. A simplified technique using fluorometry but without the use of a membrane, was published by Holmes et al. (1999), which was adapted from Kerouel and Aminot (1997). In this method, the seawater sample is combined with a working reagent containing ortho-phthaldialdehyde (OPA), sodium sulfite, and borate buffer, and heated to 75°C. Fluorescence proportional to the ammonium concentration, emission at 460nm following excitation at 370nm is measured.

Laboratories started using CFAs and Auto-Analyzers (AA) in the mid-1970s. The two main forms of CFA are flow injection (FIA) and gas-segmented flow analyzers. While there are some laboratories that are using FIA for nutrient analysis, most global laboratories that carry out 'at-sea' analysis use gas-segmented flow analyzers. This manual focuses primarily on methods for the gas-segmented flow analyzers.

The chapter on nutrient analysis using segmented flow analysis by Aminot et al. (2009) in "Practical Guidelines for the Analysis of Seawater" provides an excellent background on continuous flow analysis. We recommend the reader also review this document as it contains useful information on the technical aspects of the instrument(s), the measurement of nutrients, as well as details on sources of error and contamination.

2. Sample Collection and Storage

2.1 Sample collection

The Data Acquisition Overview section of the GO-SHIP (Swift 2010) manual should be reviewed for details on rosette/Niskin bottle sampling practices. Nutrient samples should be collected from the Conductivity Temperature Depth (CTD) rosette/Niskin bottles immediately after the collection of samples for dissolved gases. This can be challenging if samples for organic properties or biologically sensitive materials are also being taken. Ideally, samples are collected into new, sterile plastic (High Density Polyethylene (HDPE), or Polypropylene (PP) containers that will then fit directly onto the AA auto-sampler, or sub-sampled into smaller containers. Sample containers can be re-used if proper cleaning procedures are followed between stations. Using a new sample container could produce a tremendous amount of plastic waste especially on the long repeat hydrography research cruises and these environmental impacts should be considered. For macro-nutrient analysis (micro-molar (µM) concentrations), rinsing the sample containers with ultrapure water (distilled deionized water or from commercially available systems) followed by a rinse with 10% Hydrochloric Acid (HCl, 1.2M) is sufficient. This stops any biological growth in the sample bottles. These then should be rinsed well with ultrapure water prior to the collection of the next set of samples. Glass sample containers should not be used if measuring silicate. If nanomolar nutrient concentrations are being measured, other cleaning and sample collection procedures will be necessary (see Appendix D).

When taking the seawater samples from the CTD/rosette bottles, rinse the clean sample containers and caps three times before filling. Avoid touching the sampling spigots on the CTD bottles and take care to rinse the spigots as well as the nutrient sample containers. Samples can be collected with the use of a Tygon or silicon sampling tube. If a sampling tube is used, rinse it thoroughly before going out to the rosette to take a series of samples, and make sure to rinse it with each seawater sample prior to collecting the sample. Once rinsed, fill the sample containers two thirds full, and cap immediately. The samples should be analyzed after they have equilibrated to the laboratory room temperature. If analysis will be delayed for longer than a couple of hours (>2), then store the samples in a dark and cool place, for example in a refrigerator, however the samples should be returned to room temperature before analysis. Between CTD sampling events it is important to clean any sampling tubes with clean deionized water and 10% HCl.

N.B. Cigarette smoke can contaminate samples, particularly for ammonium and nitrate/nitrite, so it is imperative that smoking is banned close to the area where samples are collected. Likewise, people who have been recently smoking should stay away from any open samples.

2.2 Filtering and gloves

Some laboratories filter nutrient samples, while many other laboratories do not. In general, filtering is not necessary for samples taken in the (sub) tropical open ocean, where particle loading is low in these oligotrophic environments. The decision to filter or not is dependent on the particulate loading in the water being sampled. For example, samples from near shore or productive environments may require filtering. In these cases, great care must be taken not to contaminate the samples during the sample handling and filtering process. Sample collection tubes, filter holders, and filters should be clean and well rinsed with 10% HCl and ultrapure water prior to sample collection. Types of filters used to filter seawater include cellulose acetate, hydrophilic polypropylene Gelman membrane, and Acrodisc syringe filters (PALL). Glass Fiber filters (GFF) (silicate contamination) or cellulose nitrate filters (nitrate contamination) should NOT be used. Filter size is another consideration, a filter with a pore size of 0.45µm is commonly used, and in the past this was considered the ideal filter size to remove the majority of particles. However, new insight from microscopy and genomics has determined that a 0.45µm filter does not capture all bacteria and phytoplankton. A 0.2µm filter is now the recommended size of filter, and gravity, low pressure, or low vacuum filtration is recommended to avoid cell rupture and sample contamination. It is imperative that tests are performed to check that the method of filtering, filter type, and filter size do not lead to contamination of the samples. Another simple technique to minimize particle interferences is to centrifuge the samples prior to analysis.

Gloves are another source of potential contamination. Neither Neoprene nor colored nitrile gloves should ever be used for the sampling of nutrients; they are a high source of contamination especially for nitrate, nitrite and

ammonium. If care is taken, a clean sample can be collected with bare hands without the use of gloves, however, powder free vinyl gloves are highly recommended for use in the lab and for sample collection at sea.
In general, it is best practice to wear gloves when taking water samples and only experienced scientists who are confident in their techniques should consider sampling without gloves. Likewise, it is important that for any sampling procedures (like gas sampling) being carried out prior to the nutrient sampling from the CTD, then those scientists should also wear non-nutrient contaminating gloves (e.g. powder free vinyl).

2.3 Sample Preservation

The best practice is to analyze the nutrient samples at sea, shortly after they are collected, however there are often instances when nutrient analysis at sea is not possible or is delayed for any number of reasons. If analysis will be delayed by more than 24 hours the samples must be preserved. There are many different types of preservation methods, including poisoning, acidification, pasteurization (Daniel et al. 2012), and freezing. We do not recommend acidification (samples will have to be neutralized before analysis) or poisoning samples with mercuric chloride (environmental hazard). Freezing is the most commonly used method, and there are studies that show that freezing can be a reliable method of sample preservation (Aminot and Kerouel1995; Dore et al. 1996), and this is the recommended procedure.

If freezing samples, it is imperative that there is sufficient headspace in the bottles to allow for expansion of the seawater. Freeze the samples upright and check that the caps are tightened before and after the samples have frozen. Do not freeze samples in a freezer that has had organic material (fish samples or food) stored in it. Analyze frozen samples as soon as possible after returning to the lab.

There is still debate within the nutrient community about the effects of freezing samples on the accuracy and precision of the nutrient concentration, especially for silicate. It is well known that the reactive silica polymerizes when frozen, especially at high concentrations (Burton et al. 1970; MacDonald and McLaughlin 1982; MacDonald et al. 1986). Variables that affect the recovery of silica from frozen samples include salinity, turbidity, bottle size, and the silicate concentration. Much of the current debate centers on the recommended thaw techniques to depolymerize the reactive silica and get complete recovery. Many laboratories have carried out studies of thaw techniques to recover silica, but there are only a few published references. Sakamoto et al. (1990) recommend that samples be thawed overnight, in the dark, at room temperature, or thawed in a water bath for 30 minutes (50˚C) and then cooled back down to room temperature before actual analysis. However, Zhang and Ortner (1998) suggested that it could take up to 4 days to thaw samples at room temperature to get complete recovery of silica. See appendix H for results of recent studies performed at NIOZ and Scripps Institution of Oceanography (SIO). The tests carried out at SIO confirm the 1990 recommendation by Sakamoto of thawing frozen samples in a 50˚C water bath for 30-45 minutes and then allowing the samples to come back to room temperature before analysis. Further systematic tests are needed to determine the effects of long term storage on all of the nutrient concentrations as well as the best thaw techniques for the different types of samples being collected (coastal, estuarine, oligotrophic, etc.)

3. Instrumentation

Aminot et al. (2009) provide a detailed description of the specific AA components, including potential problems of analysis. Most seagoing laboratories are using SEAL, Skalar, Alpkem, or similar analytical systems. Users should refer to the manufacturer's manuals for the specifics on methods, operation, and maintenance. A nutrient auto-analyzer from any manufacturer will consist of the same basic components listed and described here.

3.1 Sampler

The sampler should be robust and able to handle different size sample cups and a reasonable number of samples, plus it should have a wash from which the water is continuously refreshed. A non-metallic or platinum probe should be used, and the internal diameter of the probe should normally be no greater than that of the largest sample pump tube. Having a sampler modified to accept the bottles that you sample straight from the CTD rosette will eliminate possible contamination issues when decanting a sample into another sampling vessel.

3.2 Pump

The peristaltic pump delivers the sample/baseline water, and the reagents to the manifolds for each channel/chemistry and throughout the entire AA system. For precise measurements at low concentrations, a regular bubble pattern and stable baseline are absolutely key, and this is one area that is extremely important to get correct for good analyses.
The composition and quality of pump tubes can vary between manufactures and from batch to batch. Tube wear will also affect the flow rate and method sensitivity, which is why a complete set of standards must be run with every station/set of samples. Replacing a method's pump tubes may then improve the sensitivity and characteristics of the bubble flow. Generally, pump tubes should be changed on a regular basis as the correct delivery of the sample, and particularly for some reagents being pumped through some of the smaller bore pump tubes (e.g. orange/green or orange/yellow), will become a lot less accurate as the tubes wear. For optimum performance, changing tubes after 50-60 hours (depending on the material and manufacturer of the pump tubes in use) will ensure that the liquid delivery remains reliable. The newer phthalate-free pump tubes being sold have a much-reduced reliable life span compared to the original Tygon tubing. It is not good practice to run pump tubes right to the end of their useable life. The analytical results will not be as good or reliable with old tubes as with newer tubes, thus frequent changing of pump tubing is recommended. Some laboratories make a full change of pump tubes and reagents at the same time to co-ordinate machine down time.

3.3 Manifold

The manifold consists of glassware and injection fittings and is the site of the chemical reactions between the seawater samples and reagents. It is imperative that the glass pieces, reaction coils, and connectors are all maintained regularly in order to provide consistent mixing, regular flow patterns and to allow reactions to reach steady state, which ensures full color development.
Introduction of air or nitrogen bubbles allows for complete mixing between segments and again ensures full color development. The bubbles must be large enough to prevent carryover and/or smearing from one segment to another, but if they are too long they will be prone to breaking up in the manifold. It is very important to maintain a regular bubble pattern throughout the system in order to reduce noise and optimize sensitivity. Reference is always made to the segmented gas bubbles as being 'air' bubbles, however ideally these segmenting bubbles should be either nitrogen or another inert gas so as to avoid potential contamination from the air. Some laboratories have gas lines connected directly from cylinders to deliver the gas, but a simpler solution is to use small plastic Tedlar bags (or similar) that contain up to 5 liters of nitrogen. These are particularly useful when working at sea as they can be easily refilled.
There are many factors to consider when building a manifold to ensure consistent flow and bubble pattern. Below is a list of considerations:

1. Match the inner diameter (ID) of the tubing used from the pump to the injection fittings and into the glassware on the manifold as closely as possible.
2. Use the shortest possible length of tubing between connections. Long un-segmented streams cause hydraulic problems, which will manifest in various ways (e.g. smearing or carryover of samples).
3. Make sure there are no gaps/dead spaces between connections. It is important that all glass to glass joints are held close together by plastic sleeving.
4. Add enough wetting agent in each analytical channel to maintain rounded edges at the front and back of each bubble throughout the entire flow stream, including the drain to waste.
5. Segmentation bubbles must completely fill the tubing through which they pass. The length of bubble in contact with the tubing walls should be approximately 1.5 times the tubing diameter.
6. Maintain the cleanliness of the glass coils to ensure smooth flow of the sample and reagent stream. Dirty glass can cause bubbles to stick or break up.
7. Clean the manifolds periodically with a phosphate-free laboratory detergent and consult the manufactures recommendations. A dilute bleach or acidic solution can also be used for nitrate, nitrite and ammonium. Silicate and phosphate channels can be cleaned with a dilute sodium hydroxide plus ethylenediaminetetraacetic acid (EDTA) solution. Many analytical problems will be avoided with a regular cleaning protocol.
8. The segmented flowcell waste line should open to the atmosphere at about bench or flowcell height.
9. Replace any old glass pieces that continue to cause the air bubbles to stick or break up. Glass tubing and coils can become acid etched and will cause irregular peak shapes.

3.4 Detectors

The detectors consist of a light source (e.g. lamp, light emitting diode (LED)), flowcell, photometer, and inlet and outlet tubing (either plastic or glass). As with the manifold, there should be no gaps at the connections, and there should be a regular bubble pattern maintained from the manifold through the detector unit to waste. Depending on the manufacturer, the ability to monitor changes in light output, voltage, and other variables through the software may be available, and should be utilized. In the past the sample flow was always de-bubbled immediately prior to the sample entering the flowcells, but now software developments from some manufactures have allowed the air bubbles to also pass through the cells eliminating the need to de-bubble. The ability to retain the bubble pattern through the flowcell reduces carryover and sample-to-sample smearing. This along with the optical design of the new photometers and flowcells have nearly eliminated the need for refractive index blanks (RIBs), and some other effects that have interfered with peak detection in the past. For more details on these corrections see section 4.6.

3.5 Software

The AA will come installed with software from the manufacturer to control the entire system, program the autosampler, acquire the raw data output from the detectors, display the output real-time, and perform some corrections, and calculate initial concentration values etc.

There are usually different options for the calibration fit to use within the software packages. If using a linear fit or higher order fit, the concentration of nutrients in the matrix and blanks for both the matrix and the samples, must be carefully determined and corrected for. Most software programs will correct for carryover, baseline, and sensitivity drifts but may not have options to make other corrections such as RIBs or non-zero matrix concentrations. Please refer to the software manual for your own type of analyzer to learn the specifics for your instrument.

Calibration fits and blank corrections are discussed in more detail later (Appendix C).

4. Measurement and determination of nutrient concentrations:

The basic steps for sample analysis include:
1) a. Establish a steady baseline with ultrapure water.
   b. Establish a steady baseline with ultrapure water plus reagents.
   c. Check the reagent blank (difference between ultrapure water and ultrapure water plus reagents).
2) Calibration curve determination from standard concentrations and measured peak heights.
3) Measurement of sample peak heights.
4) Corrections for carryover, baseline and sensitivity drift.
5) Determination of initial concentrations of samples based on calibration curve and sample peak heights.
6) Application of other corrections including RIBs, salt effect, etc.

4.1 Baseline determinations

A common baseline solution used throughout the nutrient analysis community is ultrapure fresh water. However, in some cases analysts use low nutrient seawater (LNSW) if they have plentiful supplies. Some labs make their own ‘artificial’ seawater, (ASW) by adding salts to ultrapure water. An example of a recipe for ASW is 41g of sodium chloride plus 168mg of sodium bicarbonate per liter. Here we discuss using ultrapure water as the baseline water as this is a reliable and recommended ‘zero’ for nutrients, and can be obtained easily and quickly within a research laboratory. Determination of the baseline should be straightforward if the correct procedures are followed. The ultrapure water should be at least 18.2 megohm resistance, and be free of organics. Ultraviolet (UV) sterilization is preferred but not strictly necessary. Most commercially available water purification systems will provide ultrapure water that is acceptable for establishing a zero baseline. It should be noted that the wash pot on the sampler and the container that feeds into the wash pot can become contaminated. It is recommended that they be cleaned once per day by rinsing with 10% HCl solution followed by rinsing with ultrapure water. Some manufacturers offer a ‘travelling washpot’, which is a sealed system and hence stays uncontaminated and clean during daily operations so could be an option to consider. In rare cases it is possible that the ultrapure water is not pure, even if the resistivity reading is 18.2 megohm, e.g. silicate can pass through the filtration cartridge but will not affect the megohm reading.

It can be difficult to determine if the ultrapure water is not as pure as required and so analysts should be comparing the difference between the ultrapure baseline and the ultrapure baseline with reagents on a daily basis. Another possible indicator of poor quality baseline water is negative absorbance readings for samples with low nutrient concentrations. This could indicate the filtration cartridges on the ultrapure water system need to be replaced.

The water baseline is determined after the instrument has been running long enough with fresh ultrapure water and the baselines have become stable. This also enables checking for any leaks throughout the system before the reagents are added. It may be necessary in rare cases to add wetting agent to the ultrapure water to establish a good bubble pattern and stable baselines. Once the ultrapure water baseline has stabilized, the reagents can be added and the reagents plus ultrapure water baseline determined. It is often useful to add the reagents one at a time to see if any of them cause a large reagent blank. The reagent baseline is the reference for when the standard curve is determined and the subsequent calculation of sample concentrations. It is good practice to define a regular setting up procedure for the analyzer that can be followed for every day and every run. To minimize the reagent blank, analytical grade (or better) chemicals and fresh ultrapure water should be used.

It is crucial that the nutrient concentrations for LNSW or ASW are calculated if they are being used as a baseline instead of ultrapure water. Aoyama et al. (2015) detailed a procedure which includes analyzing a known value of each standard added to the LNSW, followed by a baseline of LNSW with and without color reagent, and a baseline of ultrapure water with and without reagent. The differences are used to calculate the concentration of each nutrient in LNSW. See Appendix E for details.

There are different ways to obtain LNSW. One option is to collect large batches of surface seawater from oligotrophic waters during a research cruise. It is recommended that the water then be filtered and sterilized to ensure the nutrient levels remain low, e.g. pumped through a 0.45µm filter, past a UV light source, and then through a 0.1µm filter, and re-circulated for approximately16 hours. Alternatively, it is possible to collect surface seawater filtered using a 0.1 or 0.2 µm filter and then allow the seawater to age (stored at room temperature for a period of time (1-2 years)) allowing the already oligotrophic water nutrient concentrations to decrease. The carboys used to store the seawater should allow light penetration (clear or opaque). The surface seawater should be filtered again before use, and the water to be used always analyzed as a sample to ensure it is in fact low in nutrients.

4.2 Calibration

A series of at least four working standards should be analyzed with every set of samples. The standard concentrations should be evenly distributed over the entire concentration range and not skewed toward either end, with the top concentration standard having a slightly higher concentration than the highest sample. Standards are generally analyzed at the beginning of an analytical run with the protocols set up on the analyzer software. Working standards should be prepared fresh at least once a day, or every 8 to 12 hours when the nutrient analyzer is in operation 24 hrs a day, e.g. when working at sea. Working standards are prepared from concentrated secondary or primary standards that are pre-made in ultrapure water (see section 6 for standard preparations). For the working standard curve, the concentrated standards are diluted using water that has a similar matrix to the samples. For example if working in an oligotrophic ocean region aged LNSW or surface seawater should be used as the standard matrix. The standard curve should cover the full range of expected sample concentrations. It is important that LNSW be used for the dilutions. It is strongly recommended that standards and samples should be analyzed from low to high concentrations so as to avoid carryover. Once the peak heights from the standards have been measured, then the calibration curve can be produced. Analytical software from the manufactures of modern analyzers will provide the calibration curve, but read their guidance notes for details. There are many factors that affect the calibration, see appendix C for details on how to determine the best calibration fit.

4.3 Measurement of sample peak heights

Most software uses an algorithm to determine the peak height and will automatically place a peak marker where it considers the correct peak height to be. However, the peak markers should always be checked by the analyst using the system software to ensure the software is reading the peaks accurately, and also to correct for spikes and other anomalies that may affect the validity of the initial peak height. Refer to the software manual for details on how the peaks are measured and how to adjust and save the readings if required.

4.4 Corrections for any baseline drift, sensitivity drift, and carryover

Baseline drift calculations will correct for any linear drift between successive baseline measurements, and these should be placed regularly throughout the run. Sensitivity drift is measured by any change between "drift" samples, which are typically analyzed near the beginning and end of the run, if not more frequently. Carryover is based on the peak height differences between two successive low peaks measured directly following a high peak.

4.5 Determination of initial concentrations of samples

In determining the initial sample concentrations most instrument software will have the option of applying baseline, carryover and drift corrections, and can give both corrected and uncorrected sample concentrations. It is recommended that users review how the calculations are applied to ensure the validity of any post-run corrections. It may be necessary to output the raw data to apply corrections and calculate concentrations in a different software package e.g. Excel.

4.6 Post processing corrections

Refractive index blanks (RIBs) should be determined separately for each channel and if necessary subtracted or added to the sample concentrations. The procedure for determining these values for each channel involves analyzing samples by removing one of the color-forming reagent chemicals (Aminot et al. 2009). For many systems, these values are usually positive, though very small, and should be determined and then any corrections applied to the results before the sample concentrations are finalized. In Fluorometric methods, such as for ammonia, no RIB is produced.

Modern detectors and flowcells minimize the effects of salinity on the analysis of seawater samples with an ultrapure water wash, and a correction may not be necessary, however it should be checked. The optical effect caused by mixing two solutions of different densities, such as ultrapure wash water with a seawater sample, is called the Schlieren effect. This effect is greatly reduced in modern analyzers by flowcells and detectors that allow the inter-sample bubble to pass through them. Use of a debubbler, as fitted before the flowcell on older analyzers, will increase the Schlieren effect leading to tails on peaks.

5. Chemical analytical Methods

Analytical methods, including reagent recipes and coil configurations, are supplied from the manufacturers of all AA instruments. Some laboratories have optimized analytical methods for their own use and specific requirements and these are often passed down over many years through different analysts. One reason to optimize or change methods is for example to allow for greater sensitivity at lower nutrient concentrations if working mostly in oligotrophic waters. See Appendices F and G for detailed methods in use by a couple reference laboratories. These are only supplied as examples to allow comparison with an analysts' own methods and reagent recipes, but are not specifically recommended. Method chemistries are up to the individual analysts to decide.

5.1 Nitrate and Nitrite Analysis

Most laboratories are using an analytical method where N-1-N (NEDD) and sulfanilamide are reacted with the sample to form a red dye, which is measured at an absorbance of 520-540 nm. For nitrate analysis, the nitrate is first reduced to nitrite by the sample being mixed with a buffer solution (e.g. Ammonium Chloride or Imidazole) and passed over a cadmium column that has been treated with copper sulfate, which catalyzes the reduction reaction. The resulting nitrite is then analyzed and the final output for the 'nitrate' channel is a sum of both nitrate and nitrite. It is important therefore to analyze nitrite separately so that nitrate can be determined by subtracting from the total nitrate plus nitrite concentration.

The reduction efficiency of the cadmium column should also be determined and monitored over time. This efficiency is measured by analyzing two separate samples, one for nitrate and the other for nitrite each with the same high concentration (e.g. 25µM). The difference in the measured concentrations will allow the analyst to calculate the column reduction efficiency. If the column reduction efficiency is lower than 95%, the cadmium column should be reconditioned or replaced.

5.2 Phosphate Analysis

There are two commonly used methods for phosphate determination. In both methods, an acidic solution of molybdate is added, followed by the addition of a reducing compound (dihydrazine sulfate or ascorbic acid) to form a phospho-molybdenum blue complex with the absorbance measured at approximately 820 or 880nm, depending on the method and availability of filters.

It is highly recommended that analysts check their phosphate method for any silicate interference. This can be checked by spiking a sample of LNSW with silicate standard to get a high concentration (e.g. 100µM), and analyzing the output on the phosphate channel to ensure that the phosphate concentration does not change due to the addition of the silicate. If there is an influence to the output then the method chemistry should be checked and changed to ensure that silicate does not affect it.

5.3 Silicate Analysis

As with phosphate, there are two commonly used methods for silicate determination. Acidified ammonium molybdate is added to a seawater sample to produce silicomolybdic acid, which is then reduced to a silico-molybdenum blue complex following the addition of stannous chloride or ascorbic acid, and measured at 660nm for stannous chloride or 820nm for ascorbic acid.

NB: It is important to ensure the silicate and phosphate analytical reagents are correctly made up. The phosphate reaction should take place at a pH of <1.0, to ensure there is no competitive reaction from silicate ions. Oxalic or Tartaric acid is used to prevent phosphate interferences in the different silicate methods. Methods with incorrect reagents can cause cross interferences and hence incorrect phosphate and silicate concentrations being reported. See Aoyama et al. (2015) for details on phosphate and silicate interferences.

5.4 Ammonium Analysis:

The two common methods for determining ammonium concentrations are the phenol based colorimetric determination and a fluorometric method.

Colorimetric method
Ammonium is analyzed via the Berthelot reaction in which sodium hypochlorite and phenol react with ammonium in an alkaline solution to form an indophenol blue complex with heating to 55°C . The sample absorbance is measured at 640nm. The method is a modification of the procedure described in Grasshoff (1983).

Fluorometric methods
In the fluorometric method, without using any membrane diffusion, the sample is combined with a working reagent made up of OPA, sodium sulfite, a borate buffer, and then heat to 75°C. Fluorescence proportional to the ammonium concentration is measured at 460nm following excitation at 370nm.

For the membrane diffusion method NH^{4+} ions in the sample are converted to NH_3 gas with subsequent diffusion across a Teflon membrane into a stream of OPA. The product is fluorometrically measured at 460nm following excitation at 370nm. This method is for nanomolar analysis (Jones 1991).

6. Standard Preparation and Standardization

It is not possible to obtain high quality data without proper care and attention to detail when preparing the standard solutions in the laboratory, both at sea and on shore.

Glass Volumetric flasks should be class A quality because their nominal tolerances are 0.05 % or better. Class A flasks are made of borosilicate glass, and the standard solutions should be transferred to plastic bottles as quickly as possible after they are made up to volume and mixed. This is done to prevent excessive dissolution of silicate from the glass. The computation of the volume contained by glass flasks at various temperatures, different from the calibration temperatures, are carried out by using the coefficient of linear expansion of borosilicate glass.

Because of their larger temperature coefficients of expansion, plastic volumetric flasks used should also be gravimetrically calibrated over the temperature range of intended use, e.g. if polymethylpentene (PMP) flasks are used to prepare standard solutions they must be used within 4°C of the temperature of the room when they were calibrated. The ultrapure water used for calibration must also be at room temperature.

It is important to determine the exact concentration of standard solutions by taking into account buoyancy corrections, glassware calibrations, pipette calibrations, and temperature corrections (Appendices A and B).

All pipettes, whether they are manual or electronic, must be regularly calibrated according to the manufacturers recommendations and should be within those tolerances. Calibration can be carried out by the analyst or by commercial companies who will provide certificates. Certainly before going on a research cruise the pipettes should have their calibrations checked and also at regular times during the year. If pipettes are dropped they should be taken out of regular use until their calibration is checked. Pipettes normally have calibration tolerances of 0.1 % or better. These tolerances should be checked with gravimetric calibration.

If using pipettes for preparing working solutions in LNSW or ASW, first pre-rinse the pipette tip at its maximum setting before use.

6.1 Primary Standards

Primary standards should be prepared at a minimum of once every three months, although some laboratories prepare primary standards less frequently if they are confident in their stability. Special care must be taken to ensure that standards kept for these longer periods are not compromised and should be checked regularly. Primary standard solutions are best kept in the dark and at room temperature. If they are stored in a refrigerator they must be brought to room temperature before use. Some labs use chloroform as a preservative (200 µl per liter), but the community is recommending a reduction in the use of toxic and/or poisonous materials.

Primary standard-grade salts for phosphate (anhydrous potassium dihydrogen phosphate, KH_2PO_4), nitrate (potassium nitrate, KNO_3), and nitrite (sodium nitrite, $NaNO_2$), are available with purities of 99.995% or better. No corrections for purity are needed if salts of this quality are used when preparing primary standards. Silicate standards are made with analytical grade sodium hexafluorosilicate or from a silicate standard solution (SiO_2). Ammonium standards are made with analytical grade ammonium sulfate ($(NH_4)_2SO_4$), which is available with a purity of >99.0%. The purity of the salt or solution used for the primary standards in these cases should be adjusted as appropriate and clearly stated in the documentation. Care must be taken to neutralize the silica standard solution if it is provided by the manufacturer in dilute sodium hydroxide.
The standard salts should be dried for 2 to 4 hours at 105°C and cooled to room temperature in a desiccator before weighing. The salts should be weighed out to a precision of 0.1mg and the exact weight recorded. Dissolve the standard salts in ultrapure water and record the temperature of the solution. Calibrated class A glass volumetric flasks should be used (Appendix B).

Adjust the weight of the salt for air buoyancy (Appendix A) when determining the exact final concentration of the primary standard solutions.

The following are examples of primary standard preparations and are supplied here only as a guide. You should record the temperature of the final solutions and calculate the concentration of the primary standard using the volumetric flask volume, temperature, and the true mass of salt. Each solution should be transferred to a clean, dry HDPE bottle and stored ready for use. Silicate standards should never be stored in glass.

Nitrate Standard (approximately 15,000 µmole/L):
In a 1L calibrated class A volumetric flask, dissolve ~1.5xxx g of high purity dried potassium nitrate in ultrapure water to make a 1L final volume solution.

Nitrite Standard (approximately 5,000 µmole/L):
In a 1L calibrated class A volumetric flask, dissolve ~0.34xx g of high purity dried sodium nitrite in ultrapure water to make a 1L final volume solution.

Phosphate Standard (approximately 6,000 µmole/L):
In a 1L calibrated class A volumetric flask, dissolve ~0.81xx g of dried high purity potassium phosphate in ultrapure water to make a 1L final volume solution.

Ammonium Standard (approximately 4,000 µmole/L):
In a 1L calibrated class A volumetric flask, dissolve ~0.26xx g of dried high purity ammonium sulfate in ultrapure water to a 1L final volume solution.

Silicate Standard: (10,000 µmole/L)
In a 1L HDPE plastic volumetric flask, dissolve 1.8806 g of sodium fluorosilicate in about 400 ml of ultrapure water. This will take a minimum of 5 hours to dissolve using ultrasonication, or by stirring. Make the dissolved solution up to 1 L with ultrapure water.

There is an alternative commercially available liquid silicate standard available from the National Institute of Standards and Technology (NIST):
Add 40 ml of a 1g Si/kg solution to 500 ml of ultrapure water for a 2860 µmole/L concentration.
To neutralize the solution add 2.9979 ml of 1N HCl before the solution is diluted to 500 ml.

6.2 Secondary (Sub-primary) Standards

Depending on the desired concentrations for the final working standards, either separate nutrient standards, or a mixed secondary standard can be prepared by diluting the primary standards with ultrapure water. Secondary standard solutions can be made up daily or at the same frequency as the primary standards. The secondary standard for nitrite and ammonium should be made up each time there is the requirement for a set of working standards, ie: every analytical run. The final concentration of the secondary standards should take into account glassware and pipette calibrations (see Appendix B).

6.3 Working standards

Working standards are made up in the same salinity water as the samples. LNSW is the recommended matrix for making up working standard solutions. These are prepared from the secondary, or primary solutions, depending on what the desired final concentrations are. At least four different concentrations of working standards should be analyzed with every set of samples.

7. Quality Control and Quality Assessment (QC/QA):

7.1 Definitions and Determination

Quality control procedures and quality assessment of the data provide a means to determine the accuracy and precision of the measurements.
Definitions are provided as it is important that the analyst understand the difference between quality control, quality assessment, accuracy, and precision. These are taken from Chapter 3 of "Guide to Best Practices for Ocean CO_2 Measurement" (Dickson et al. 2007):

Quality control — The overall system of activities whose purpose is to control the quality of a measurement so that it meets the needs of users. The aim is to ensure that data generated are of known accuracy to some stated, quantitative degree of probability, and thus provides quality that is satisfactory, dependable, and economic.

Quality assessment — The overall system of activities whose purpose is to provide assurance that quality control is being done effectively. It provides a continuing evaluation of the quality of the analyses and of the performance of the analytical system.

Precision - is a measure of how reproducible a particular experimental procedure is. It can refer either to a particular stage of the procedure, e.g. the final analysis, or to the entire procedure including sampling and sample handling. It is estimated by performing replicate measurements and estimating a mean and standard deviation from the results obtained.

Accuracy, however, is a measure of the degree of agreement of a measured value with the "true" value. An accurate method provides unbiased results. It is a much more difficult quantity to estimate and can only be inferred by careful attention to possible sources of systematic error.

7.2 Standard Operating Procedures (SOPs)

Quality control begins with the setup of the instrument and attention to details that are outlined in sections 3.3 pertaining to the assembly of the manifolds and maintenance procedures. Once the instrument is set up and running, a set of SOPs should be put in place and always followed for the analysis of samples.

The SOPs should include:

- Calibration of glassware and pipettes (Appendix B).
- Careful determination of standards and calibration fits (section 6 and Appendix C).
- Daily checks on the system, including visual inspection of bubble patterns, tracking the baseline with and without reagents, and a test sample (usually a high standard) to ensure everything is working properly and to the same settings and sensitivities as previously obtained for that test sample. This is a good standard quality control measure. When using the same test sample concentration, the analyzer sensitivity (gain) settings should stay the same, even after changing reagents or pump tubes. If the sensitivity does change, it is an early indication that there is a problem that needs to be investigated, probably associated with whatever changes have been made (e.g. a reagent has been incorrectly prepared or incorrect pump tubes replaced etc.).
- An established tray protocol in the software should be used, see example in Figure 1 below. This is to ensure standards, samples, and other peaks are included and run in the same order for each analysis, and for every run. It can include carryover, drift, baseline, and other corrections.

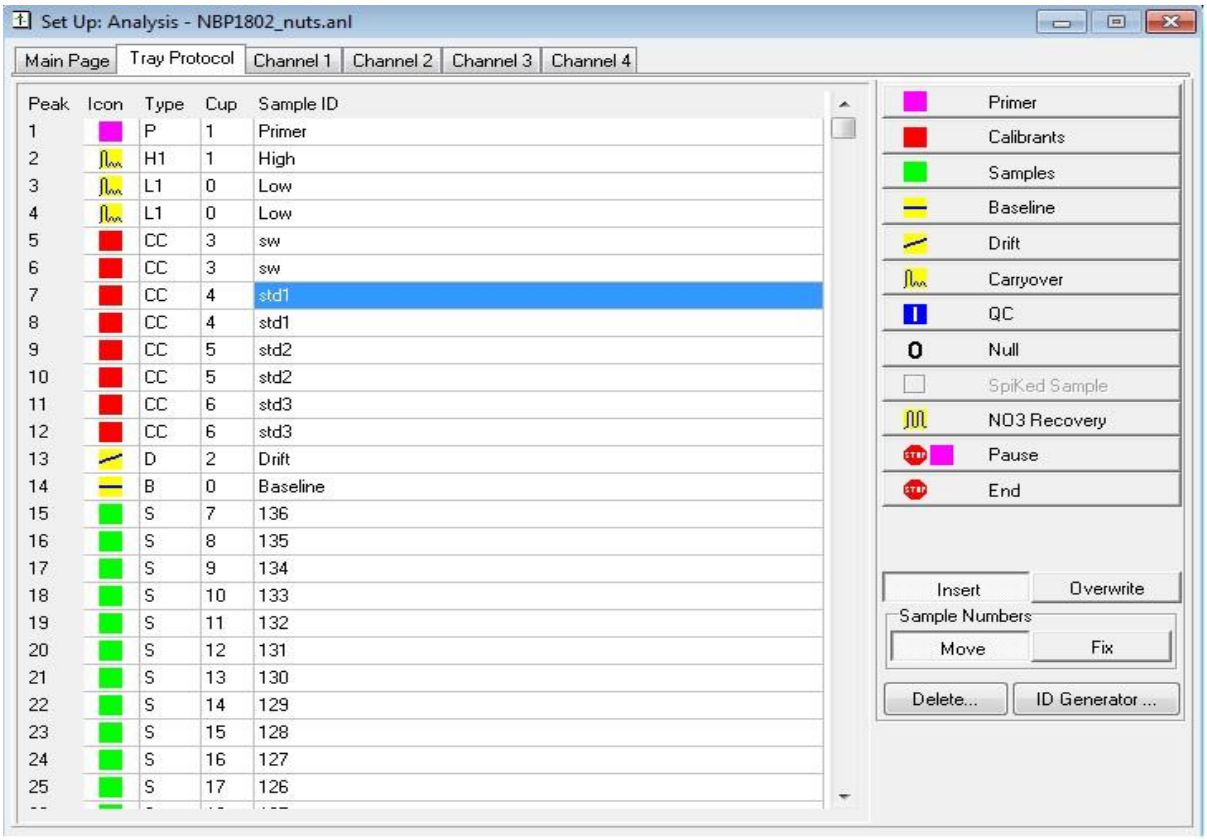


Figure 1. An example of a tray file, in this case from the AACE software used with the SEAL AA3 analyzer. Also note the four standard concentration levels used in each run, as explained in section 4.2.

7.3 Internal Checks:

Internal checks should be used to ensure data quality over the course of a cruise. Different types of internal checks include duplicate sample analysis, use of a check sample (see below), and analysis of an internal standard with each run. Duplicate sample analysis should be carried out on separate sample analysis runs. The deviation of duplicate sample analysis between runs will generally be higher and produce a more accurate measure of the data quality between runs, and over the course of a cruise. The deviation between runs can be reduced by use of a 'check sample' or 'tracking standard' and normalizing the run data and samples to those values.

Check (Tracking) sample:
One option to obtain a check sample is to collect deep water (approximately 1000m) from one of the early cruise CTD casts. The water should have reasonably high (but on-scale) values for all nutrients. This should then be poisoned with a saturated mercuric chloride solution (1ml per 10 L), and then aliquots of this sample analyzed with every analytical run. Running one poisoned sample with every run will not affect the efficiency of the cadmium reduction column. Keeping track of the value of this sample over time can help to alert the operator to any issues with the chemistries and performance of the analyzer. A table should be compiled for the cruise report, showing the average value and standard deviation for each analytical channel. As mentioned, the sample data for a particular run can be normalized if the value of this sample falls outside the desired precision. Individual run values should be within 1% of the overall cruise average value.

The use of an internal standard was further developed at NIOZ. Their procedure calls for preparing a sufficient quantity of mixed concentrated nutrient standard in ultrapure water, which is then preserved by the addition of mercuric chloride. It is prepared independently of the primary and working standards that are used to calibrate the individual analysis runs. This tracking solution is then diluted in LNSW and measured as part of each analysis run. The tracking solution is prepared by a one-step dilution, which means that the reproducibility should be about 0.1%, and variations only due to the inherent pipetting errors. At the end of the cruise, a mean value for the tracking solution or the check sample is calculated and the data for each analysis run can be normalized to that mean value by calculating and applying a factor on a run to run basis. NIOZ has successfully used these internal standard protocols for over 20 years (Hoppema et al 2015).

NB: The use of this tracking solution is only valid if its value is in the same range as the samples being analyzed, and in a range of about 60-80% of full-scale values.

The tracking solution or check sample should be analyzed at least three times within one analysis run to monitor performance within each run as well as between runs, and over the course of the cruise. These internal checks can be used to normalize data for each set of samples. At the end of the cruise a mean value for the internal check is calculated. The data for each run is then normalized by the ratio of the value for the internal check sample for that run against the mean value for the whole cruise. It should be noted that this is an internal quality check and does not substitute for using CRMs.

7.4 External Quality Checks:

External checks help assess the comparability of data from different cruises and different laboratories. Participation in national or international inter-comparison (intercalibration) exercises are one example of an external check and is strongly recommended. Another recommended external check is to include the analysis of CRMs or RMs, within an analytical run. Reference materials are preserved seawater samples with well-defined nutrient concentrations. Certified reference materials have well defined concentrations as well but the values have been verified by comparison to a known standard solution that is traceable to International System of Units (SI) or have been determined by an independent method of analysis. The certified values for most nutrient CRMs are established using traceable standard solutions. It is recommended that CRMs be used over RMs if available. The analyst should be aware of how the values for the materials has been determined and verified. Both RMs and CRMs are used to ensure consistency of measurements within a cruise (i.e. station to station; after a new batch of reagents or standards has been prepared etc.), and between different cruises, most likely executed by different laboratory groups. CRMs can be obtained in various concentrations and with various seawater matrices, representing different ocean conditions/salinities. It is strongly recommended to use nutrient CRMs for all research cruises and for laboratory

analysis, especially for cruises where high quality and accurate data is required, such as for the repeat hydrography programs GO-SHIP (CLIVAR) and GEOTRACES.

KANSO Technos initially developed nutrient reference materials, and in recent years have produced the certified nutrient reference materials. The SCOR Nutrient working group #147 (http://www.scor-int.org/SCOR_WGs_WG147.htm) in association with JAMSTEC, have recently produced a series of 5 sets of nutrient CRMs, with 2 Pacific and 3 Atlantic concentration range solutions. These are sold on a non-profit basis to benefit the global nutrient community and to encourage a wider use of nutrient CRMs. They are available for purchase through JAMSTEC (https://www.jamstec.go.jp/scor/), and have been produced in order to make the use of the CRMs cheaper and hence more accessible to a greater number of global laboratories. These come in 100 ml PP containers and sealed in an airtight aluminum bag. The CRMs should be opened and transferred to clean sample tubes and analyzed with every run, or at least once per day. The nutrient analytical values should be tracked so that any values that deviate from the stated certified concentrations are noted and investigated. There are other reference materials available, e.g. from the Korean Institute of Ocean Science and Technology (KIOST), MOOS-3 (NRC Canada), and Eurofins Scientific.

The certified values of SCOR-JAMSTEC CRMs and KANSO CRMs are traceable to the International System of Units (SI). Standard solutions with stated uncertainties from the Japan Calibration Service System (JCSS) of the Chemicals Evaluation and Research Institute (CERI), and the National Metrology Institute of Japan (NMIJ) are used to certify nitrate, nitrite and phosphate values. A silicon standard solution produced by Merck KGaA, and a silicon standard solution (SRM3150) of the National Institute of Standards and Technology (NIST) are used to certify silicate values. Each solution has a stated uncertainty value.

How to use CRMs/RMs:

CRMs should be run as a sample within each analytical run, similar to the internal check sample or tracking standard described above. A CRM or RM should be run at least once a day and ideally a new bottle of (C)RM should be opened for each new run. Another less desirable use of the CRMs is to utilize multiple lots as the working standards for each analytical run. New bottles should be opened for each run, but this would be prohibitively expensive for most laboratories. The laboratories at SIO and NIOZ have found that a previously opened (C)RM bottle can be used for 1 to 2 days. Care must be taken that the open (C)RM bottles do not get contaminated and should be stored tightly capped at room temperature.

A table should be included with the cruise report showing the true or assigned values of the CRMs, the average value of the CRMs determined during the cruise, and the standard deviations for each analytical channel. Ideally the values obtained for the CRMs agree with the assigned value and thus the data would not need to be normalized.

If the value(s) for the reference materials obtained in the analysis runs do not agree with the assigned value then this must be noted. There is still debate on the best method of normalizing the data to the CRM value. If the recommended use of the CRM (analyzed as an unknown with each run) is followed, then the data set would need to be normalized to the true or assigned value of the material. The analysts running the samples are the most informed about the analytical conditions and any normalization carried out on the data set(s), based on the use of CRMs, should be carried out by that analyst. It is imperative that any normalization made is well documented. The original values of the CRMs should be reported as well as the normalized values obtained. Details on how adjustments were performed should be included in the cruise metadata report.
If the CRMs or RMs are being used for standardization the effect is that the data set is normalized to the values of the material used. This must be specifically and clearly outlined in the metadata and cruise report.

NB: Analysts should be aware that some CRM assigned values are often reported in µmole/kg and the initial nutrient sample concentrations from AAs are calculated in µmole/L. It is therefore important to ensure any normalizations performed on the data are based on the CRM assigned values and are in the same units as the data obtained from the analytical run.

7.5 Data Quality Assessment

Once the initial checks and corrections have been completed, primary and secondary quality assessment (QA) checks should be performed. Primary QA is a process in which data are examined in order to identify outliers and obvious errors. Outliers are either flagged, or the data updated if a correctable error can be identified, e.g. if a sample peak was mis-read and not identified, or adjusted when the analysis run was processed. Secondary QA is a process in which the data are objectively reviewed by the analyst in order to quantify systematic biases in the reported values (e.g. Tanhua et al, 2010).
Primary QA Checks:

Data from each channel/chemistry should be plotted as a function of pressure or depth in order to elucidate any abnormalities that may occur from the CTD bottle tripping incorrectly, leaking, or from contamination issues. This data can then be plotted and compared to other physical and chemical properties of samples analyzed onboard. It is recommended to compare nutrient profiles to salinity, temperature, oxygen, and dissolved inorganic carbon profiles, to see if features or outliers are observed in those parameters also.
Plots of nitrate plus nitrite (and ammonium if analyzed) versus phosphate, and plots of silicate versus oxygen values, also allow for the identification of any problem values. This can be done for each station once all data for the other parameters being measured are available. Values from concurrent stations should also be scrutinized to ensure that any shifts in values are real and not an indication of a sensitivity, analytical, or contamination problem.

Secondary QA Checks:

Comparison of the current data to any historical data can be carried out to detect systematic biases. Records from GO-SHIP (formerly CLIVAR) and WOCE transects covering every global ocean are in the public record and can be accessed via databases such as CCHDO (cchdo.ucsd.edu), although it is recommended to use the bias adjusted data product from GLODAP (https://www.glodap.info/). If a potential bias in the data is detected during the cruise, efforts should be taken to identify any possible issues in the analytical procedure. GLODAP does not recommend a bias correction be applied to the data reported from a cruise, instead a note should be made in the meta-data for any possible bias issues.

8. Documentation

8.1 Cruise reports
The following should be included in the nutrient section of the cruise reports:

- i) Cruise designation (ID) and principle investigator(s).
- ii) If not listed in the cruise report elsewhere, CTD station information including station position, time, sampling depths, bottle numbers etc.
- iii) Names and affiliations of the analysts.
- iv) Numbers of samples analyzed, batches of standards used, pump tube and column changes.
- v) Equipment, methodology, and reagents used.
- vi) Sampling and any storage procedures.
- vii) Calibration standard information, methods, and values.
- viii) Data collection and processing procedures.
- ix) Details of any problems and trouble-shooting that occurred.
- x) QC/QA:
    - stated accuracy and analytical precision
    - detection limits
    - values of check samples and/or tracking standards
    - measured values of the reference materials (including which batch was used, and assigned or certified values)
    - if and how normalizations were made to the data, based on the internal check/tracking samples or the CRM
- xi) Scientific References

8.2 Bottle data files:

Data from nutrient analyses should be merged into files with CTD bottle trip values including depth and CTD bottle number, CTD sensor data, and other chemical parameters that are measured during the cruise/research expedition. Each parameter should include a field for associated quality control flags.

Nutrients will be measured and the initial results reported from the AA will be in µmol/L, so it is imperative to also measure and record the laboratory analytical temperature so it can be used along with the salinity for calculation and final reporting of the results in µmol/kg.
The conversion from volumes (liters) to mass (kg) units should be calculated based on the density of the seawater and the equation of state (Millero, F. J. et al. 1980). The equation of state has been updated in Roquet et al. 2015. Either of these two equations can be used but which one implemented must be clearly stated and referenced in the metadata.

If reference materials were analyzed, the manufacturer, batch number, and given values should be included with the bottle file.

References:

Aminot, A, and Kirkwood, D.S. 1995. Report on the results of the fifth ICES Inter-comparison study for Nutrients in Seawater. ICES Cooperative Research Report No. 213, 79 pp.

Aminot, A., and Kerouel, R. 1995. Reference material for nutrients in seawater: stability of nitrate, nitrite, ammonium and phosphate in autoclaved samples. Mar. Chem., 49, 221–232.

Aminot, A., Kerouel, R., and Coverly, S. 2009. Nutrients in Seawater Using Segmented Flow Analysis. Chapter 8 in Practical Guidelines for the Analysis of Seawater. Oliver Wurl, ed., 143-178.

Aoyama, M. 2006. 2003. Intercomparison Exercise for Reference Material for Nutrients in Seawater in a Seawater Matrix. Technical Reports of the Meteorological Research Institute No. 50, 91 pp.

Aoyama, M. 2010. 2008 Inter-laboratory Comparison Study of a Reference Material for Nutrients in Seawater. Technical Reports of the Meteorological Research Institute No. 60, 134 pp.

Aoyama, M., Abad, M., Anstey, C., Ashraf, M.P., Bakir, A., Becker, S., Bell, S., Berdalet, E., Blum, M., Briggs, R., Caradec, F., Cariou, T., Church, M.J., Coppola, L., Crump, M., Curless, S., Dai, M.H., Daniel, A., Davis, C., de Santis., Braga, E., Solis, M.E., Ekern, L., Faber, D., Fraser, T., Gundersen, K., Jacobsen, S., Knockaert, M., Komada, T., Kralj, M., Kramer, R., Kress, N., Lainela, S., Ledesma, J., Li, X., Lim, J-H., Lohmann, M., Lonborg, C., Lukwichowski, K-U., Mahaffey, C., Malien, F., Margiotta, F., McCormack, T., Murillo, I., Naik, H., Nausch, G., Olafsdottir, S.R., van Ooijen, J., Paranhos, R., Payne, C., Pierre-Duplessix, O., Prove, G., Rabiller, E., Raimbault, P., Reed, L., Rees, C., Rho, T.K., Roman, R., Woodward, E.M.S., Sun, J., Szymczycha, B., Takatani, S., Taylor, A., Thamer, P., Torres-Valdés, S., Trahanovsky, K., Waldron, H.N., Walsham, P., Wang, L., Wang, T., White, L., Yoshimura, T., and Zhang, J.Z. 2016 IOCCP-JAMSTEC 2015 Inter-laboratory Calibration Exercise of a Certified Reference Material for Nutrients in Seawater . Publisher: Yokosuka, 237-0061 Japan, Japan Agency for Marine-Earth Science and Technology, June 2016. ISBN: 978-4-901833-23-3.

Aoyama, M., Bakker, K., van Ooijen, J., Ossebaar, S., Woodward, E.M.S. 2015. Report from an International Nutrient Workshop focusing on Phosphate Analysis. Yang Yang, 78 pp.

Aoyama, M., Barwell-Clarke, J., Becker, S., Blum, M., Braga, E.S., Coverly, S.C., Czobik, E., Dahllof, I., Dai, M.H., Donnell, G.O., Engelke, C., Gong, G.C., Gi-Hoon, Hong., Hydes, D.H., Jin, M.M., Kasai, H., Kerouel, R., Kiyomono, Y., Knockaert, M., Kress, N., Krogslund, K.A., Kumagai, M., Leterme, S., Yarong Li, Masuda, S., Miyao, T., Moutin, T., Murata, A., Nagai, N., Nausch, G., Ngirchechol, M.K., Nybakk, A., Ogawa, H., van Ooijen, J., Ota, H., Pan, J.M., Payne, C., Pierre-Duplessix, O., Pujo-Pay, M., Raabe, T., Saito, K., Sato, K., Schmidt, C., Schuett, M., Shammon, T.M., Sun, J., Tanhua, T., White, L., Woodward, E.M.S., Worsfold, P., Yeats, P., Yoshimura, T., Youenou, A., and Zhang, J.Z. 2008: 2006 Intercomparison Exercise for Reference Material for Nutrients in Seawater in a Seawater Matrix, Technical Reports of the Meteorological Research Institute No. 58, 104 pp.

Aoyama, M., Becker, S., Minhan, D., Hideshi, D., Louis, I. G., Kasai, H., Roger, K., Nurit, K., Doug, M., Murata, A., Nagai, N., Ogawa, H., Ota, H., Saito, H., Saito, K., Shimizu, T., Takano, H., Tsuda, A., Yokouchi, K., and Agnes, Y. 2007. Recent Comparability of Oceanographic Nutrients Data: Results of a 2003 Intercomparison Exercise Using Reference Materials. Analytical Sciences, 23, 1151-1154.

Armstrong, F.A.J., Stearns, C.A., and Strickland, J.D.H. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Research, 14, 381-389.

Bernhardt, H., and Wilhelms, A. 1967. The continuous determination of low level iron, soluble phosphate and total phosphate with the AutoAnalyzer. Technicon Symposia, I, 385-389.

Burton, J.D., Leatherland, T.M., and Liss, P.S. 1970. The reactivity of dissolved silicon in some natural waters. Limnol. Oceanogr., 15, 472-476.

Daniel, A., Kérouel, R., and Aminot, A. 2012. Pasteurization: A reliable method for preservation of nutrient in seawater samples for inter-laboratory and field applications. Marine Chemistry, 128–129, 57–63.

Dickson, A.G., Sabine, C.L., and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 191 pp.

Dore, J.E., Houlihan, T., Hebel, D.V., Tien, G., Tupas, L., and Karl, D.M. 1996. Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. Marine Chemistry, 53, 173-185.

Grasshoff, K., Kremling, K., and Ehrhardt, M. eds. 1983. Determination of nutrients. In Methods of seawater analysis, Wiley-VCH, Weinheim, Germany. 2nd edition.

Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A., and Peterson, B.J. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fisheries and Aquatic Sciences, 56, 1801-1808.

Hoppema, M, Bakker, K.,v.Heuven, S.M.A.C., v,Ooijen, J.C.,. de Baar, H. 2015. Distributions, trends and inter-annual variability of nutrients along a repeat section through the Weddell Sea (1996–2011). Marine Chemistry, 177, 545-553.

Hydes, D. J., M. Aoyama, A. Aminot, K. Bakker, S. Becker, S. Coverly, A. Daniel, A. G. Dickson, O. Grosso, R. Kerouel, J. van Ooijen, K. Sato, T. Tanhua, E. M. S. Woodward, J. Z. Zhang 2010. Determination of dissolved nutrients (N, P, Si) in seawater with high precision and inter-comparability using gas-segmented continuous flow analysers. GO-SHIP Repeat Hydrography Manual: A collection of expert reports and guidelines. IOCCP report #14, ICPO publications series no.134, Version 1.

[ICES] International Council for the Exploration of the Sea. 1967. Report on the analysis of phosphate at the ICES intercalibration trials of chemical methods held at Copenhagen, 1966. ICES CM 1967/C: 20.

[ICES] International Council for the Exploration of the Sea. 1977. The International Intercalibration Exercise for Nutrient Methods. ICES Cooperative Research Report No. 67, 44 pp.

Jones, R, 1991. An improved fluorescence method for the determination of nanomolar concentrations of ammonium in natural waters. Limnol. Oceanogr., 36(4), 1991, 814-819.

Kerouel, R., and Aminot, A. 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Marine Chemistry, 57, 265-275.

Key, R. M., Tanhua, T., Olsen, A., Hoppema, M., Jutterström, S., Schirnick, C., van Heuven, S., Lin, X., Wallace, D. W. R., and Mintrop, L., 2010. The CARINA data synthesis project: introduction and overview, *Earth System Science Data*, 2, 105-121, doi:10.5194/essd-2-105-2010.

Kirkwood, D.S., Aminot, A., and Perttila, M. 1991. Report on the results of the fourth ICES Intercomparison Exercise for Nutrients in Seawater. ICES Cooperative Research Report No. 174, 83 pp.

MacDonald, R.W., and McLaughlin, F.A. 1982. The effect of storage by freezing on dissolved in-organic phosphate, nitrate and reactive silicate for samples from coastal and estuarine waters. Water Res., 1, 95-104.

MacDonald, R.W., McLaughlin, F.A., and Wong, C.S. 1986. Storage of reactive silicate samples by freezing. Limnol. Oceanogr., 31, 1139-1142.

Millero, F.J., Chen, C-T., Bradshaw, A., and Schleicher, K. 1980. A new high pressure equation of state for seawater. Deep Sea Research Part A, Vol. 27, Issue 3-4, pp. 255-264.

Moore, C.M. (2016). Diagnosing oceanic nutrient deficiency. Phil. Trans. Roy. Soc. A. DOI: 10.1098/rsta.2015.0290

Murphy, J., and Riley, J.P. 1962. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Analytica Chimica Acta, 27, 31-36.

Olsen, A., Key, R.M., van Heuven, S., Lauvset, S.K., Velo, A., Lin, X., Schirnick, C., Kozyr, A., Tanhua, T., Hoppema, M., Jutterström, S., Steinfeldt, R., Jeansson, E., Ishii, M., Pérez, F.F., and Suzuki, T. 2016. An internally consistent data product for the world ocean: the Global Ocean Data Analysis Project, version 2 (GLODAPv2), Earth Syst. Sci. Data Discuss., 1-78, 10.5194/essd-2015-42, 2016.

Roquet, F., Madec, G., McDougall, T.J., and Barker, P.M. 2015. Accurate polynominal expressions for the density and specific volume of seawater using the TEOS-10 standard. Ocean Modelling, Vol. 90. pp. 29-43.

Sakamoto, C.M., Friederich, G.E., and Codispoti, L.A. 1990. MBARI procedures for automated nutrient analyses using a modified Alpkem Series 300 Rapid Flow Analyzer. Monterey Bay Aquarium Res. Inst. Tech. Rep. No. 90-2, 84 pp.

Strickland, J.D.H., and Parsons, T.R. 1972. A practical handbook of sea-water analysis (2nd Edition). J. Fish. Res. Bd. Canada, 167, 311 pp.

Suzuki, T., Ishii, M., Aoyama, A., Christian, J. R., Enyo, K., Kawano, T., Key, R. M., Kosugi, N., Kozyr, A., Miller, L. A., Murata, A., Nakano, T., Ono, T., Saino, T., Sasaki, K., Sasano, D., Takatani, Y., Wakita, M., and Sabine, C. L.: PACIFICA Data Synthesis Project, ORNL/CDIAC-159, NDP-092, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U. S. Department of Energy, Oak Ridge, Tennessee, 2013.

Swift, J. H. 2010. Reference-Quality water sample data: Notes on acquisition, record keeping, and evaluation. GO-SHIP Repeat Hydrography Manual: A collection of expert reports and guidelines. IOCCP report #14, ICPO publications series no.134, Version 1.

Talley, L.D., Feely, R.A., Sloyan, B.M., Wanninkhof, R., Baringer, M.O., Bullister, J.L., Carlson, C.A., Doney, S.C., Fine, R.A., Firing, E., Gruber, N., Hansell, D.A., Ishii, M., Johnson, G.C., Katsumata, K., Key, R.M., Kramp, M., Langdon, C., Macdonald, A.M., Mathis, J.T., McDonagh, E.L., Mecking, S., Millero, F.J., Mordy, C.W., Nakano, T., Sabine, C.L., Smethie, W.M., Swift, J.H., Tanhua, T., Thurnherr, A.M., Warner, M.J., and Zhang, J.Z. 2016. Changes in Ocean Heat, Carbon Content, and Ventilation: A Review of the First Decade of GO-SHIP Global Repeat Hydrography, Annual Review of Marine Science, 8, null, doi:10.1146/annurev-marine-052915-100829, 2016.

Tanhua, T., van Heuven, S., Key, R.M., Velo, A., Olsen, A., and Schirnick, C. 2010. Quality control procedures and methods of the CARINA database, Earth Syst. Sci. Data, 2, 35-49, 2010.

Topping, G. 1997. QUASIMEME: quality measurements for marine monitoring. Review of the EU project 1993-1996. Marine Pollution Bulletin, 35, 1-201.

[UNESCO] United Nations Educational, Scientific and Cultural Organization. 1965. Report on the intercalibration measurements. UNESCO Technical Papers in Marine Science No. 3, 14 pp.

[UNESCO] United Nations Educational, Scientific and Cultural Organization. 1967. Report on intercalibration measurements. UNESCO Technical Papers in Marine Science No. 9, 114 pp.

Zhang, J.Z., Fischer, C.J., & Ortner, P.B. 1999. Optimization of performance and minimization of silicate interference in continuous flow phosphate analysis. Talanta, 49(2), 293-304. https://doi.org/10.1016/S0039-9140(98)00377-4

Zhang, J.Z., and Ortner, P.B. 1998. Effect of thawing conditions on the recovery of reactive silicic acid from frozen natural water samples. Water Research, 32, 2553-2555.

Appendix A:
Applying air buoyancy corrections

Taken directly from SOP 21 in Dickson et al. (2007)

1. Scope and field of application

If uncorrected, the effect of air buoyancy is frequently the largest source of error in mass measurements. This procedure provides equations to be used to correct for the buoyant effect of air. An air buoyancy correction should be made in all high accuracy mass determinations.

2. Principle

The upthrust due to air buoyancy acts both on the sample being weighed and on the counter-balancing weights. If these are of different densities and hence of different volumes, it will be necessary to allow for the resulting difference in air buoyancy to obtain an accurate determination of mass.

3. Requirements

3.1 Knowledge of the air density at the time of weighing

For the most accurate measurements, the air density is computed from a knowledge of air pressure, temperature, and relative humidity. Tolerances for the various measurements are given in Table 1.

Table 1: Tolerances for various physical parameters.

Variable	**Uncertainty in computed air density**	
	± 0.1%	**± 1.0%**
Relative humidity (%)	± 11.3%	–
Air temperature (°C)	± 0.29 K	± 2.9 K
Air pressure (kPa)	± 0.10 kPa	± 1.0 kPa

Barometer accurate to ± 0.05 kPa,
Thermometer accurate to ± 0.1°C,
Hygrometer accurate to 10%.
An error of 1% in air density results in an error of approximately 1 part in 10^5 in the mass corrected for air buoyancy. Although meteorological variability can result in variations of up to 3% in air density, the change of pressure (and hence of air density) with altitude can be much more significant. For measurements of moderate accuracy, made at sea level and at normal laboratory temperatures, an assumed air density of 0.0012 g cm^{-3} is often adequate.

3.2 Knowledge of the apparent mass scale used to calibrate the balance

There are two apparent mass scales in common use. The older one is based on the use of brass weights adjusted to a density of 8.4 g cm^{-3}, the more recent one on the use of stainless steel weights adjusted to a density[1] of 8.0 g cm^{-3}.

3.3 Knowledge of the density of the sample

[1] Strictly, these densities apply only at 20°C. The conversion factor from the "apparent mass" obtained by using these values to "true" mass is defined by the expression $Q = \frac{\rho(\text{weights})(D_{20} - 0.0012)}{D_{20}[\rho(\text{weights}) - 0.0012]}$

where D_{20} is the apparent mass scale to which the weights are adjusted. This factor may be considered as unity for most purposes.

The density of the sample being weighed is needed for this calculation.

4. Procedure

4.1 Computation of air density

The density of air in g cm^{-3} can be computed from measurements of pressure, temperature, and relative humidity (Jones 1978):

$$\rho\,(\text{air}) = \frac{3.4848\,(p - 0.0037960U \cdot e_s)}{273.15 + t} \times 10^{-3} \qquad (1)$$

where

p = air pressure (kPa),

U = relative humidity (%),

t = temperature (°C),

e_s = saturation vapor pressure (kPa),

$$e_s = 1.7526 \times 10^8 \exp\left[-5315.56/(t + 273.15)\right]. \qquad (2)$$

4.2 Computation of mass from weight

The mass, m, of a sample of weight, w, and density, ρ(sample), is computed from the expression

$$m = w\left(\frac{1 - \rho\,(\text{air})/\rho\,(\text{weights})}{1 - \rho\,(\text{air})/\rho\,(\text{sample})}\right) \qquad (3)$$

(see Annex for the derivation)

5. Example calculation

The following data were used for this calculation[2]:

weight of sample, w = 100.00000 g,

density of sample, ρ (sample) = 1.0000 g cm^{-3}.

Weighing conditions:

p = 101.325 kPa (1 atm),

U = 30.0%,

t = 20.00°C,

ρ (weights) = 8.0000 g cm^{-3}.

5.1 Computation of air density

e_s = 2.338 kPa,

ρ (air) = 0.0012013 g cm^{-3}.

5.2 Computation of mass

m = 100.10524 g.

[2] The seemingly excessive number of decimal places is provided here so that users of this procedure can check their computation scheme.

Annex: Derivation of the expression for buoyancy correction

An expression for the buoyancy correction can be derived from a consideration of the forces shown in Figure 2. Although the majority of balances nowadays are single-pan, the principles remain the same, the difference being that the forces are compared sequentially using a force sensor rather than simultaneously using a lever. At balance, the opposing forces are equal:

$$m_1 g - V_1 \rho(\text{air}) g = m_2 g - V_2 \rho(\text{air}) g \qquad (4)$$

where g is the acceleration due to gravity and ρ(air) is the density of the air at the temperature, pressure, and humidity of the weighing operation. Note that m_2 is the "weight" of a sample whose true mass is m_1.

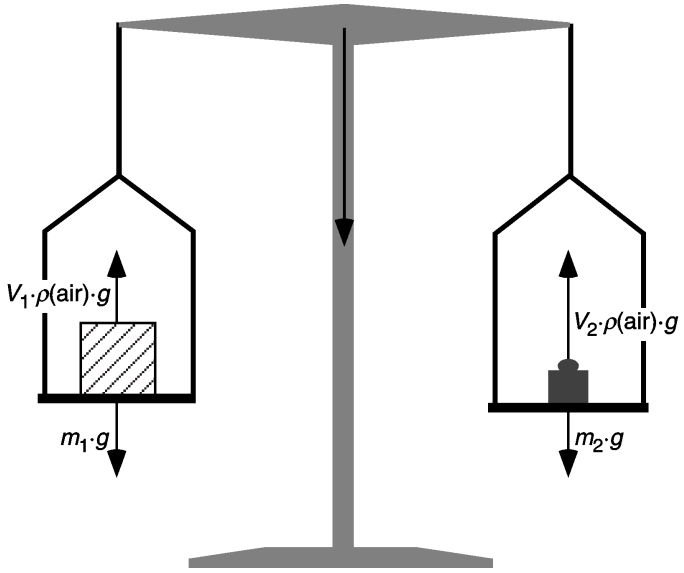


Figure 2. Forces on sample (1) and weights (2) when weighing in air.

As

$$V = m/\rho \,, \qquad (5)$$

we can rewrite equation (4) as

$$m_1 - m_1 \rho(\text{air})/\rho_1 = m_2 - m_2 \rho(\text{air})/\rho_2 \,. \qquad (6)$$

This equation can be rearranged to obtain the expression

$$m_1 = m_2 \frac{1 - \rho(\text{air})/\rho_2}{1 - \rho(\text{air})/\rho_1} \,. \qquad (7)$$

Equation (7) is the basis of the expression used for air buoyancy correction (Schoonover and Jones 1981; Taylor and Oppermann 1986):

$$m = w \frac{1 - \rho(\text{air})/\rho(\text{weights})}{1 - \rho(\text{air})/\rho(\text{sample})} \qquad (8)$$

where w is the "weight" of the sample in air and m is the true mass.

Equation (6) can also be rearranged to give

$$m_1 = m_2 + m_2 \rho(\text{air}) \left(\frac{m_1}{m_2} \frac{1}{\rho_1} - \frac{1}{\rho_2} \right). \qquad (9)$$

As $m_1 \approx m_2$, equation (9) is almost identical to the commonly quoted expression for buoyancy correction,

$$m = w + w\rho(\text{air}) \left[\frac{1}{\rho(\text{sample})} - \frac{1}{\rho(\text{weights})} \right] \qquad (10)$$

(Woodward and Redman 1973; Dean 1985). An approximate value of 0.0012 g cm^{-3} for ρ(air) is often used with this expression; this is appropriate to measurements of moderate accuracy made at sea level pressures and at normal laboratory temperatures.

6. References

Dean, J.A. 1985. Lange's Handbook of Chemistry. McGraw-Hill Book Company, New York, 1792 pp.

Dickson, A.G., Sabine, C.L., and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 191 pp.

Jones, F.E. 1978. The air density equation and the transfer of the mass unit. J. Res. Natl. Bureau Stand., 83, 419–428.

Schoonover, R.M., and Jones, F.E. 1981. Air buoyancy correction in high-accuracy weighing on analytical balances. Anal.Chem., 53, 900–902.

Taylor, J.K., and Oppermann, H.V. 1986. Handbook for the quality assurance of metrological measurements. National Bureau of Standards Handbook, 145.

Woodward, C., and Redman, H.N. 1973. High-precision Titrimetry. The Society for Analytical Chemistry, London, 63 pp.

Appendix B:
Gravimetric calibration of volume contained in volumetric flasks and pipettes using water

Taken directly from SOP 13 (Dickson et al. 2007), with additions from Batista et al. (2006)

1. Scope and field of application

This procedure describes how to calibrate the volume of solution contained by volumetric flasks, pipettes or other containers capable of being filled to a reproducible mark. This is expressed as the volume contained at a standard temperature (usually 20°C). This procedure is capable of achieving a reproducibility of better than 0.01% (1 relative standard deviation).

"Eppendorf" type air displacement pipettes are commonly used along with volumetric flasks for the preparation of calibration solutions. These have a precision of 0.1% if used carefully. The accuracy is expected to be about 0.1% of the stated value when the pipette is new, however, their precision and accuracy should be checked on a regular basis.

2. Principle

The mass of water contained by the flask at a measured calibration temperature is used to compute the volume of water contained at that temperature. The volume that would be contained at the standard temperature (20°C) can be calculated by taking account of the volumetric expansion of the flask. The volume of liquid contained at any desired temperature can be calculated in a similar fashion.

Warning: This requires that the temperature of the calibration solution is known. Taking solutions directly from a refrigerator and preparing a standard solution should be avoided for this reason. Similarly, pipetting a cold solution in an air displacement pipette can cause an increase in the volume by 5% if a pipette calibrated at 20°C is used to pipette a solution at 5°C. Once in the pipette, the cold solution can cause the air above it to contract.

Note: If using micro pipettes for preparing working solutions in LNSW or ASW, first pre-rinse the pipette tip at its maximum setting before use as the displaced volume can differ up to 0.5%.

3. Apparatus

- Analytical balance capable of weighing the quantity of water contained with a sensitivity of 1 part in 10^5 while having the capacity to weigh the water together with the container being calibrated.
- Thermometer accurate to ± 0.1°C.
- Container large enough to retain more than 10 aliquots dispensed by the pipette being calibrated.

4. Ultrapure water in equilibrium with the temperature of the laboratory.

5. Procedure for the calibration of volumetric flasks

- Weigh the clean, dry, empty container together with the associated closure.
- Fill the container being calibrated to the mark with ultrapure water, allowing the temperature of the container and contained water to reach an equilibrium value. Note this temperature.
- Close the container and reweigh it.

6. Calculation and expression of results

6.1 Volume of the water contained at the calibration temperature
Compute the weight of the water contained from the difference between weights of the filled and empty container:

$$w(\mathrm{H_2O}) = w(\text{filled container}) - w(\text{empty container}) \quad (11)$$

Compute the mass of water contained, correcting for air buoyancy (see Appendix A):

$$m(\mathrm{H_2O}) = w(\mathrm{H_2O})\left(\frac{1-\rho\,(\mathrm{air})/\rho\,(\mathrm{weights})}{1-\rho\,(\mathrm{air})/\rho\,(\mathrm{sample})}\right). \qquad (12)$$

The volume contained at the noted temperature (t) is

$$V(t) = m(\mathrm{H_2O})/\rho\,(\mathrm{H_2O},\ t). \qquad (13)$$

The density of air-saturated water in the temperature range 5 to 40°C is given by the expression (Jones and Harris 1992)

$$\begin{aligned}\rho_{\mathrm{W}}/(\mathrm{kg\ m^{-3}}) = {} & 999.84847 + 6.337563\times10^{-2}(t/°\mathrm{C}) \\ & - 8.523829\times10^{-3}(t/°\mathrm{C})^2 + 6.943248\times10^{-5}(t/°\mathrm{C})^3 \\ & - 3.821216\times10^{-7}(t/°\mathrm{C})^4\end{aligned} \qquad (14)$$

where t is the temperature on ITS 90[3]. To achieve an accuracy of 1 part in 10^4, t must be known to within 0.5°C.

6.2 Volume that would be contained at an alternate temperature

To convert the volume contained at one temperature (t_1) to a standard or alternate temperature (t_2), we need to take account of the thermal expansion of the container being used. For Pyrex-like glass (Corning 7740, Kimble KG-33, Schott Duran, Wheaton 200, *etc.*) the coefficient of linear expansion α_l is 32.5 □ 10^{-7} $\mathrm{K^{-1}}$; for glass such as Kimble KG-35, α_l is about 55 □ 10^{-7} $\mathrm{K^{-1}}$.

The coefficient of volumetric expansion,

$$\alpha_V = (1+\alpha_l)^3 - 1 \approx 3\alpha_l, \qquad (15)$$

is used to calculate the corrected volume at the alternate temperature,

$$V(t_2) = V(t_1)\left[1+\alpha_V(t_2-t_1)\right]. \qquad (16)$$

This correction is negligible for all except the most precise work; unless $t_2 - t_1$ exceeds 10°C or if plastic ware is used.

6.3 Example calculation

The following data were used for this calculation:
$w(\mathrm{H_2O})$ = 996.55 g,
calibration temperature = 23.0°C,
ρ ($\mathrm{H_2O}$, 23.0 °C) = 0.997535 g $\mathrm{cm^{-3}}$,
a_l = 32.5 □ 10^{-7} $\mathrm{K^{-1}}$,
Weighing conditions:
ρ (air) = 0.0012 g $\mathrm{cm^{-3}}$,[4]
ρ (weights) = 8.0 g $\mathrm{cm^{-3}}$.
Correct weight of water to mass:

$$\begin{aligned} m(\mathrm{H_2O}) &= 996.55\times\frac{1-0.0012/8.0}{1-0.0012/0.997535} \\ &= 997.60\ \mathrm{g}. \end{aligned}$$

[3] The International Practical Temperature Scale of 1968 (IPTS 68) has been superseded by the International Temperature Scale of 1990 (ITS 90). A simple equation can be used to relate the two over the oceanographic temperature range 0 to 40°C (Jones and Harris 1992):

$$t_{90} = 0.0002 + 0.99975\ t_{68}.$$

The small difference in temperature scales is typically not important to the calibration of glassware for the procedures in this Guide.

[4] This value is appropriate to measurements of moderate accuracy made at sea level pressure (1 atm) and at normal laboratory temperatures (~20°C). For a more accurate value see SOP 12, Equation (1) in Dickson (2007).

Compute volume of water contained at the calibration temperature of 23.0°C:

$$V(23.0\,^{\circ}\mathrm{C}) = 997.60 / 0.997535$$

$$= 1000.07\ \mathrm{cm}^3 .$$

Compute volume that would be contained at the standard temperature of 20.0°C, i.e., the standard calibrated volume:

$$V(20.0\,^{\circ}\mathrm{C}) = 1000.07\left[1 + 3(32.5\times10^{-7})(20.0 - 23.0)\right]$$

$$= 1000.04\ \mathrm{cm}^3 .$$

Compute volume that would be contained at 25°C.

$$V(25.0\,^{\circ}\mathrm{C}) = 1000.04\left[1 + 3(32.5\times10^{-7})(25.0 - 20.0)\right]$$

$$= 1000.09\ \mathrm{cm}^3 .$$

7. Calibration of micro-liter pipettes (Batista et al. 2006)
   Weigh the clean dry empty container.

- Dispense 10 aliquots of deionized water, recording the weight of each aliquot.
- Correct the weight of each aliquot for air buoyancy (see Appendix A).
- Calculate the precision achieved, and record the precision and accuracy of the pipette.

8. References

Batista, E., Pinto, L., Filipe, E., and van der Veen, A.M.H. 2006. Calibration of micropipettes: Test methods and uncertainty analysis. Measurement, 40, 338-342.

Dickson, A.G., Sabine, C.L., and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 191 pp.

Jones, F.E., and Harris, G.L. 1992. ITS-90 density of water formulation for volumetric standards calibration. J. Res. Natl. Inst. Stand. Technol., 97, 335–340.

Appendix C:
Establishing the linearity of standard calibrations

Taken directly from Hydes et al. 2010.

1. Scope and field of application

If insufficient attention is paid to determining the correct calibration fit to AA data, errors of several percent can be generated. The tests suggested here should be carried out whenever a method is set up or modified, in order to establish whether a linear or quadratic equation gives the better slope fit to the data. Some laboratories have run tests on a regular basis during cruises to assess the behavior of their system e.g. when working in high concentration ranges close to the top of the linear range of a method. Changes such as a contaminated reagent could shift the output into a non-linear range.

2. Principle

Non-linearity in the output from an AA can come from two sources:

(1) True non-Beer's Law non-linearity, i.e. when the absorbance of a reacted solution exceeds that for which the particular method is linear. (In this case, the method should become linear if the sample is diluted.)

(2) A non-linear output related to the linearization performed by the electronics of the detector. (In this case the method will not become linear if the sample is diluted.)

The linearity of a method can be tested by running a series of standard solutions over the concentration range of interest and then examining the spread of residual differences between the data, and the best fit linear and quadratic calibration equations when fitted to that data.

The degree of likely error can then be estimated at the mid-point of the calibration range; ideally this offset should be <0.5%.

3. Requirements

- An AA
- System software set to provide raw data output for peak heights
- Standard solutions
- Spreadsheet or statistical software to calculate best fit and residuals

4. Method

1. Set up the AA to run the method of interest over the required concentration range.
2. Load the sample tray with the series of standards at the start of the run and set up the sampling protocol within the software. Measure each sample multiple times to assess the noise of the run, and to take into account variations resulting from peak height carryover e.g. for ten standard solutions numbered 0 to 9 the order might be 0123456789; 9876543210; 9876543210; 0123456789.
3. Run the standards solutions and download the peak heights for the standards at the end of the run.
4. Load the results into Excel or similar software (see Table 2).
5. Plot concentration against peak height (see Figure 3).
6. Calculate the best fit for both linear and quadratic equations (see Figure 3).
7. Calculate the residual difference between the observed and the best fit of the data points (see Table 2).
8. Plot the residual values against the concentration of the standards. For the best fit, the residuals should vary around zero with a spread similar to the precision of the method (see Figure 4).

5. Example results

Std conc.	Peak height	Linear fit	Quadratic fit
Analyser data		Calculated residuals	
0	0	0.0	0.0
1	100	2.2	-1.2
2	200	4.4	-1.4
3	300	6.6	-0.4
4	400	8.7	1.8
5	495	5.9	0.0
6	590	3.1	-0.6
7	685	0.3	-0.1
8	780	-2.5	1.5
9	870	-10.3	-0.8
0	0	0.0	0.0
1	102	4.2	0.8
2	202	6.4	0.6
3	303	9.6	2.6
4	404	12.7	5.8
5	500	10.9	5.0
6	596	9.1	5.4
7	691	6.3	5.9
8	785	2.5	6.5
9	876	-4.3	5.2
0	0	0.0	0.0
1	98	0.2	-3.2
2	198	2.4	-3.4
3	297	3.6	-3.4
4	396	4.7	-2.2
5	490	0.9	-5.0
6	584	-2.9	-6.6
7	679	-5.7	-6.1
8	775	-7.5	-3.5
9	864	-16.3	-6.8
0	0	0.0	0.0
1	101	3.2	-0.2
2	199	3.4	-2.4
3	302	8.6	1.6
4	397	5.7	-1.2
5	498	8.9	3.0
6	587	0.1	-3.6

7	685	0.3	-0.1
8	783	0.5	4.5
9	867	-13.3	-3.8
		Sum of residual differences	
		72.7	-6.2

Table 2: Data for linearity check.

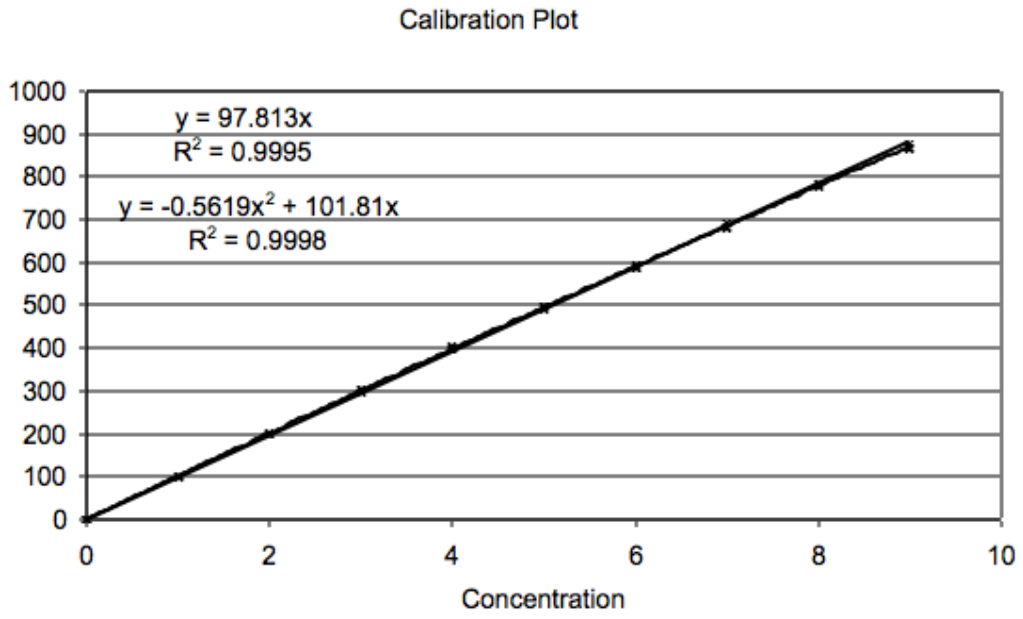


Figure 3: Plot of analyzer data from Table 2. Values for y and R^2 for linear (upper) and quadratic (lower) fits are both shown and close to 1.0.

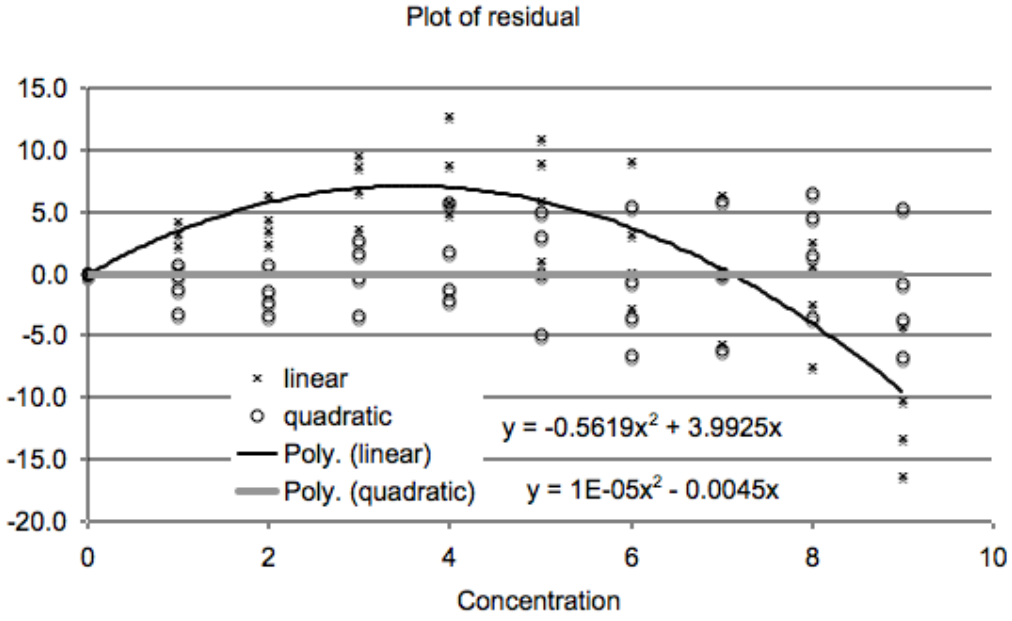


Figure 4: Plot of the residual difference between the measured values at each standard concentration and the best fit value, calculated from equations for a linear and a quadratic fit to the data.
A quadratic fit is then applied to both the linear and quadratic sets of residual data.

6. Discussion

The data from the method used in Table 2 gives a linear response up to the mid-point of the concentration range over which it is being applied. When both linear and quadratic equations are fitted to the data (Figure 3), R^2 values of 0.9995 and 0.9998, respectively, are returned, and the method appears to be close to linear.

Plotting the residual values between the observed data and the best fit value of the peak height gives a magnified view of the differences (Figure 4). Clearly, when a linear fit forced through the origin is applied to the data, the values at intermediate concentrations are overestimated, but at high concentrations are underestimated. The sum of the residuals is 72.7 in this case. Less bias is shown in the residuals estimated with the quadratic equation and the data is scattered around zero and the sum of the residuals is -6.2.

Fitting a quadratic equation to the plotted residuals in Figure 4 suggests that the estimate using a linear fit would be 0.7% high at mid concentrations and 1.0% low at high concentrations.

7. Conclusions

Before sample concentrations are calculated using a specific calibration fit, the calibration fit should be verified. This is best carried out by comparing the residual differences between the calibration fits being considered. The most appropriate equation (linear or quadratic) for calibrating the data can then be selected.

8. References

Hydes, D. J., M. Aoyama, A. Aminot, K. Bakker, S. Becker, S. Coverly, A. Daniel, A. G. Dickson, O. Grosso, R. Kerouel, J. van Ooijen, K. Sato, T. Tanhua, E. M. S. Woodward, J. Z. Zhang 2010. Determination of dissolved nutrients (N, P, Si) in seawater with high precision and inter-comparability using gas-segmented continuous flow analysers. GO-SHIP Repeat Hydrography Manual: A collection of expert reports and guidelines. IOCCP report #14, ICPO publications series no.134, Version 1.

Appendix D:
Low-level (nanomolar) nutrients

More sensitive analytical techniques are required to measure nutrient concentrations at the nanomolar level that are typically found in oligotrophic waters. These nanomolar techniques either use the standard colorimetric analytical methods with a more sensitive detection flow cell like a liquid waveguide capillary cell (LWCC) e.g. nitrate, nitrite, and phosphate. For nanomolar ammonium a fluorometric method is used.
The limits of detection for standard autoanalytical techniques is typically 20 nmoles/L for phosphate and nitrate and 10 nmoles/L for nitrite. Although the modern autoanalyser outputs generate data to 5 decimal points, this does not reflect the sensitivity of the instrument and it is not possible to achieve nanomolar detection on a standard AA. Comparison of phosphate concentrations in the ultra-oligotrophic eastern Mediterranean using a conventional CFA system versus a LWCC revealed that results were comparable between 20 and 100 nmoles/L. However, below 20 nmoles/L, results from the CFA system were deemed unreliable (Krom et al 2005).

Sampling and Storage:
It is imperative that sampling of low nutrient concentration seawater is carried out in as clean conditions as possible. Samples should be collected in clean, either new or 'aged' HDPE sample bottles that are cleaned with 10% HCl and rinsed with fresh ultrapure water. Bottles should be stored dry between cruises. In order to avoid the build-up of microbial films while at sea it is recommended to add a small spray of 10% HCl to the bottles between any sampling. Bottles should be rinsed with ultrapure water before sample collection. To prevent contamination during sample collection, nutrient free gloves must be worn and sampling with bare hands is not recommended. Vinyl powder-free gloves are recommended and colored nitrile or neoprene gloves should be avoided due to significant risk of contamination.
Collection of samples for nanomolar nutrient analysis should occur immediately after the collection of samples for oxygen and trace gases, especially if samples are to be analysed for nanomolar ammonium. Persons sampling before nanomolar nutrient samples are collected should wear vinyl gloves.
Seawater can be taken directly from the spigot of the Niskin bottle. However, it is possible to contaminate samples from water running off the outside of the Niskin bottle and/or CTD frame. For consistency, it is recommended to collect samples using an 'aged' silicon tube attached to the Niskin bottle spigot. This tube must be kept clean by soaking in 10% HCL solution between sampling events and rinsing with ultrapure water and sample seawater before sample collection. Sample bottles should be rinsed three times with sample seawater prior to sample collection.
Analysis of seawater samples for nanomolar nutrient concentrations should be carried out as soon as possible after sample collection. We do not recommend freezing samples for nanomolar nutrient analysis as this can lead to errors of up to 300%.
Atmospheric contamination is an important consideration especially for ammonium. All outside air sources like ships emissions, air vents and smoking should be avoided. People who have been recently smoking should not be involved in nutrient sampling because the smoke lingers in the lungs for a number of minutes and this will cause contamination, particularly of ammonium.

Filtration:
As stated previously, filtering is not necessary for samples taken in the oligotrophic open ocean as oligotrophic environments contain a very small number of particles in the water column. If filtering is deemed necessary, then great care must be taken not to contaminate the samples during the sample handling and filtering process. All considerations relating to this are discussed in Section 2.2. It is preferable if filtration is not carried out to avoid any possible contamination.

Analytical Procedures:

a) Nitrate, nitrite and phosphate

A review of the methods used for analysis of nanomolar nutrients has been published by Ma et al., 2014.
The chemistry used for nanomolar detection of nutrients is the same for micromolar detection. The most commonly used sea going analytical detection technique uses LWCCs, which can vary in size from 50 cm to 2 m in length. The longer cells are more sensitive but can be more troublesome due to micro bubbles and analytical noise. Air bubbles need to be removed prior to the flow entering the LWCC. This is especially problematic for cold water samples that have not reached room temperature as these degas. Cleaning the LWCC is essential and a consistent cleaning protocol is required before and after the analytical run. The regular sequential use of Methanol, 10% HCL, and then finally ultrapure water will keep the LWCC clean.

b) Ammonium

A summary of the more commonly used analytical methods for nanomolar ammonium using colorimetric and Fluorometric analytical methods are detailed above in Section 5.4. The Fluorometric methods are the most sensitive for nanomolar use and they all use the OPA Fluorometric reagent. The most commonly used analytical method is based on Kerouel and Aminot (1997) mainly as this is a simpler continuous flow method, does not require the use of Teflon membranes, and is recommended by some of the major analyzer manufacturers. The alternate Jones (1991) method makes the sample alkaline, and then the ammonia transfers by differential pH, across a hydrophobic 5µm Teflon membrane into a Fluorometric OPA reagent flow, which is then detected. This method also uses a 1m long Teflon tube immersed in a 10% sulphuric acid solution which removes ammonia in the alkali reagent, which lowers the baseline and increases sensitivity.

The OPA reagent is prepared and bubbled with nitrogen gas for 30 minutes and then allowed to stand for 24 hours to reduce background fluorescence before use. The reagent bottles should be fitted with a 3-way valve so the headspace removed during reagent consumption is replaced by clean nitrogen gas from a Tedlar bag or a fixed gas line. When making standards or reagents glass flasks should not be used so as to avoid ammonium contamination (Kodama et al. 2015). It is recommended to use acid washed, followed by ultrapure water rinsed PP flasks for all preparations.

Reference Materials:

There is a low level nutrient CRM (Lot CE) available through JAMSTEC/SCOR, (https://www.jamstec.go.jp/scor/available.html). However, the certified concentration values for nitrate and phosphate as stated are below the quantifiable detection limit of the analysis techniques. There are no certified nanomolar level CRMs available because the number of laboratories performing nanomolar analysis is limited. One method for checking the validity of the nanomolar results is to compare the results of oceanic samples that are between 20 and 100 nmoles/L between the CFA and LWCC analyzers. As long as the CFA analyzer output calibrates correctly with a CRM of detectable concentrations and the slope of the nanomolar analyzer standard output from 5 nmoles/L is linear then it can be concluded that the results from the LWCC analyzer are comparable with those generated using the CFA system. Hence the low-level nutrient concentrations less than 20 nmoles/L can be accepted as reliable.

References

Kerouel, R., and Aminot, A. 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Marine Chemistry, 57, 265-275

Kodama, T., Ichikawa, T., Hidaka, K., and Furuya, K. 2015. A highly sensitive and large concentration range colorimetric continuous flow analysis for ammonium concentration. Journal of Oceanography vol. 71, issue 1, pp. 65-75.

Krom, M., Woodward, E.M.S., Herut, B., Kress, N., Carbo, P., Mantoura, R.F.C., Spyres, G., Thingstad, T.F., Wassmann, P., Wexels Riser, C., Kitidis, V., Law, C., Zodiatis, G., and Zohary, T. 2005. Nutrient cycling in the south east Levantine basin of the eastern Mediterranean - results from a P starved system. Deep-Sea Research II, Issue 22-23, 2879-2896.

Ma, Jian., Adornato, Lori., Byrne, H., Yuan, Dongxing. 2014. Determination of nanomolar levels of nutrients in seawater. Trends in Analytical Chemistry, Volume 60, September 2014, Pages 1-15. doi.org/10.1016/j.trac.2014.04.013

Jones, R, (1991). An improved fluorescence method for the determination of nanomolar concentrations of ammonium in natural waters. Limnol. Oceanogr., 36(4), 1991, 814-819.

Appendix E:
Determination of nutrient concentrations in LNSW or ASW

If LNSW or ASW is used as the baseline matrix, then it is imperative to determine the background concentration of nutrients in that LNSW or ASW. If ultrapure water is being used then the background nutrient concentrations do not need to be determined but a salt correction, due to the refractive index (see section 4.6), will need to be quantified. To determine the background nutrient concentrations of LNSW or ASW, this water should be online along with all reagents. The sensitivity setting of the colorimeter should be set at the maximum value. A LNSW/ASW solution with a known addition of a nutrient standard (e.g. 0.2 µM) is run and its peak height recorded. Once a steady state baseline is reached, the color forming reagent (e.g. ascorbic acid or dihydrazine for phosphate) is removed from the flow and replaced by ultrapure water. After establishing a steady state baseline again, the color reagent is added back into the flow. This procedure, with and without the color reagent, is then repeated with fresh ultrapure water being used as the baseline, (See Figure 5). Therefore, by using the peak height from the added standard in LNSW/ASW, and the difference between the baselines with and without the color reagent, measured with LNSW/ASW and ultrapure water baselines, the nutrient concentration in the LNSW/ASW can be quantified (see Aoyama et al. 2015).

Example for Phosphate chemistry:

Concentration of PO_4 in LNSW = ([PO_4] peak/y) x (ΔLNSW-Δ ultrapure water)
Where:
[PO_4] peak: the added concentration of phosphate (µM) into the LNSW
y: peak height of the low PO_4 standard solution
ΔLNSW: difference in baseline height that is seen with and without the color reagent for LNSW baseline
Δ ultrapure water: difference in baseline height that is seen with and without the color reagent for the ultrapure baseline

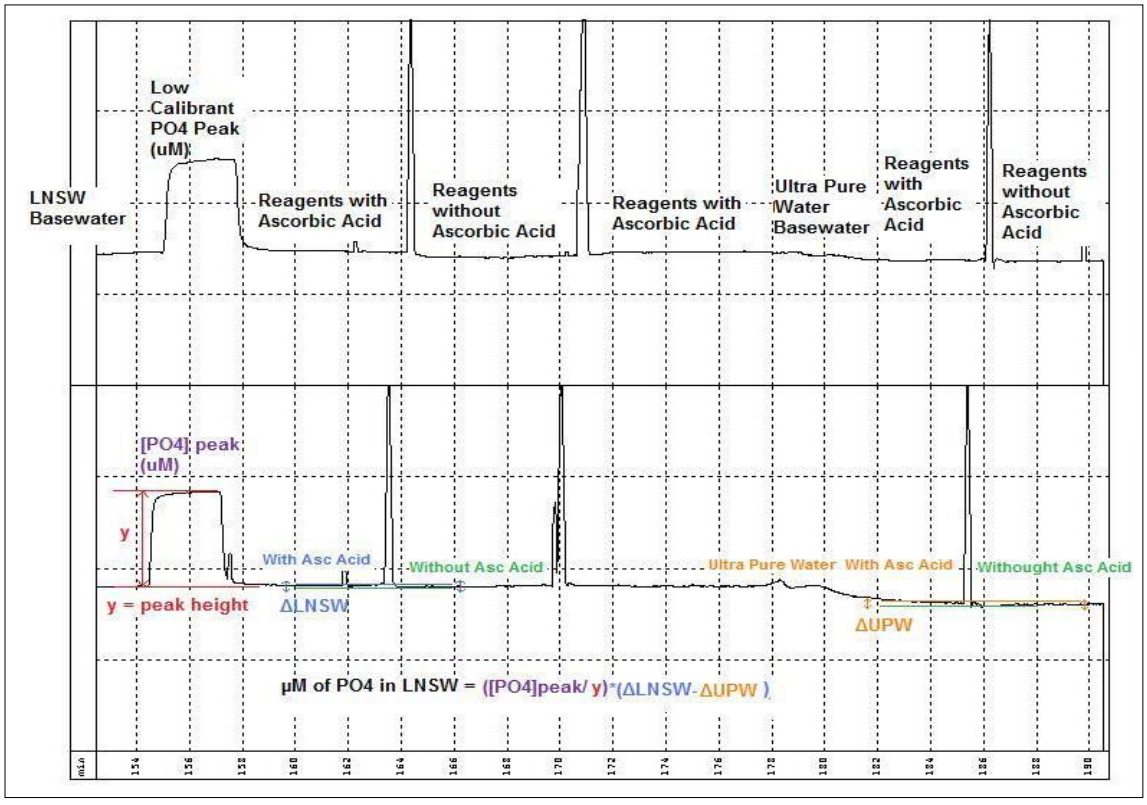


Figure 5: Determination of Phosphate concentration in LNSW using ascorbic acid as the color-forming reagent.

Reference:
M. Aoyama, K. Bakker, J. van Ooijen, S. Ossebaar, E.M.S. Woodward (2015). Report from an International Nutrient Workshop focusing on Phosphate Analysis, 2015, Yang Yang Publisher, Fukushima, Japan. ISBN 978 -4-908583-01-8

Appendix F:
Detailed methods utilized at SIO for AA3

Please note that the analytical methods that follow in Appendices F and G are just examples of the methods used by the Oceanographic Data Facility (ODF) at Scripps Institution of Oceanography (SIO) and at JAMSTEC. They are not prescriptive as there are many methods available in the literature and supplied by the analytical instrument manufacturers. Choice of an analytical method is up to the analysts and individual laboratories themselves.

Nitrate plus Nitrite Analysis
A modification of the Armstrong et al. (1967) procedure is used for the analysis of nitrate and nitrite. For nitrate analysis, a seawater sample is passed through a cadmium column where the nitrate is reduced to nitrite. Nitrite is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine to form a red dye. The sample is then passed through a 10mm flowcell and absorbance measured at 540nm. The procedure is the same for the nitrite analysis but without the cadmium column.
Nitrate concentrations are calculated by subtraction of the nitrite from the nitrate plus nitrite concentration.
The efficiency of the cadmium column should be determined and tracked over time. Two standards are prepared, one with a high concentration of nitrate and the other with the same concentration of nitrite (25µM). The difference in these values gives the column efficiency. If the column efficiency is lower than 95%, the cadmium column should be reconditioned or replaced.

Reagents
Sulfanilamide:
Dissolve 10g sulfanilamide in 1.2N HCl and make to 1 L volume. Add 2 drops of 40% surfynol 465/485 surfactant. Store at room temperature in a dark PP bottle.
Note: 40% Surfynol 465/485 is 20% 465, plus 20% 485 in ultrapure water. Brij 35 can aldo be used for the surfactant.

N-(1-Naphthyl)-ethylenediamine dihydrochloride (N-1-N: NEDD):
Dissolve 1g N-1-N in ultrapure water and make to 1 L volume. Add 2 drops 40% surfynol 465/485 surfactant. Store at room temperature in a dark PP bottle. Discard if the solution turns dark reddish brown.

Imidazole Buffer:
Dissolve 13.6g imidazole in ~3.8 L ultrapure water in a 4 L PP container. Stir for at least 30 minutes to completely dissolve, then add 60 ml of $CuSO_4$ + NH_4Cl mix (see below). Add 4 drops 40% Surfynol 465/485 surfactant. Let this sit overnight before proceeding.
Using a calibrated pH meter adjust to a pH of 7.83-7.85 using 10% (1.2N) HCl (about 20-30 ml of acid is required, depending on exact strength). Bring the final solution to 4 L with ultrapure water.
Store at room temperature.

NH_4Cl + CuSO4 mix:
Dissolve 2g cupric sulfate in ultrapure water, make to 100 ml volume (2%)
Dissolve 250g ammonium chloride in ultrapure water, make to l L volume.
For the mixed solution add 5ml of 2% $CuSO_4$ solution to the NH_4Cl. This should last many months.

Nitrate Standard:
In a 1 L calibrated glass volumetric "A" flask, dissolve ~1.5xxgm of high purity dried KNO_3 in ultrapure water to make a 1 L final volume solution. Record the temperature of the final solution. Calculate the concentration of this primary nitrate standard using the volumetric flask volume, temperature and exact weight of powder as described in Appendix A.

Nitrite Standard:
In a 1 L calibrated glass volumetric "A" flask, dissolve ~0.34xxgm of high purity dried $NaNO_2$ in ultrapure water to make a 1 L final volume solution. Record the temperature of the final solution. Calculate concentration of this primary standard.

Dilute secondary standards as necessary. Prepare a secondary nitrite standard daily.

Preparation of Packed columns
Cadmium columns are typically prepared on land and shipped ready for use at sea. They can be stored in a HDPE container filled with ultrapure water. Before using, the column should be primed (see instructions below). The columns should be topped off with the loose, processed cadmium also stored in the HDPE container when necessary. Remember that cadmium is a toxic substance and it should not be exposed to air once it is processed.

Items needed for Preparation of cadmium columns:

Cadmium granules
Buffer
Concentrated HCl
1.2 N (10%) HCl
Glass beaker
Glass stir rod
2% $CuSO_4$

Preparing the cadmium:
Use cadmium granules of approximately 2mm in size. Consolidate all of the cadmium in an oversized glass beaker. Rinse glass column tubes and end caps with 10% HCl and then ultrapure water. Rinse the cadmium granules with ultrapure water approximately 10 times. Rinses should be collected as hazardous waste and disposed of appropriately. Stir vigorously between rinses. The rinse water may become cloudy. Then rinse the cadmium with 1.2N HCl, agitate with a stirring rod for a few minutes, and decant the excess. Do this at least twice and until the solution is clear and the cadmium is shiny. Rinse with ultrapure water 5 to 10 times, being careful to completely rinse away all traces of the HCl. Rinse the cadmium granules with a small amount of concentrated HCl. This pits the surface of the cadmium to increase the surface area and may make the solution cloudy. Do NOT let this solution sit. Rinse with ultrapure water several times (20 or more) being sure to rinse away all of the HCl. (until the solution is clear again). Add enough ultrapure water to the cadmium to cover the granules, and then begin adding 2% $CuSO_4$, a little at a time. From this point on, stir gently to protect the copper coating. Stir with a glass rod in between each addition, but do not decant. Keep adding the copper sulfate slowly in small aliquots until the solution stays slightly bluish in tone (approximately 3 x 20 ml additions). The cadmium is now treated. Do not let this solution sit or be exposed to air, decant almost all of the solution from the cadmium, minimizing air exposure. Rinse and decant many (20) times with ultrapure water, stirring gently between the rinses. During this entire procedure, do not stir too vigorously with the glass rod to avoid breaking up the cadmium granules. To remove the fine particles, stir the liquid above the cadmium to make them rise to the surface and decant them. Continue rinsing until the rinse water is no longer cloudy, and the cadmium appears dark, spotty, and grayish. Store the granules in the imidazole buffer solution.

Packing the columns:
Use an approximately 12mm long glass rod with a 6mm outer diameter (OD) and 4mm internal diameter (ID). Soak approximately 2 cm pieces of dental floss in ultrapure water to prevent bubbles and make them easier to work with. Pack the bottom with a small ball of dental floss, being sure that it is not too tight and allows flow. Attach the bottom nipple with silicone tubing (ID: 1/8" OD: 1/4"). Attach a funnel to the top of the empty column and secure it. Fill a large (60 ml) syringe with imidazole buffer. Attach the syringe to the bottom of the column with tubing (ID:1/16"). Make sure the tubing is long enough to tie off when finished. Using the syringe, fill the column with buffer and load the cadmium through the funnel on the top end being sure not to expose the cadmium to the air. Tap the column with a pencil to pack down the cadmium and fill until there is no dead space. Remove the funnel, and insert another ball of dental floss at the top end. Cap off with the appropriate sized nipple and silicon tubing. Cap the top end with tied off tygon tubing and tie off the tygon tubing attached to the bottom end. When packing the column, avoid loading small particulates by scooping heavier and larger particles of cadmium from the bottom of the beaker.

Topping off the column with Cadmium:
It is very important to keep the column full of cadmium to minimize dead space. As samples are run, the cadmium volume will be reduced (through use and settling). This dead space WILL AFFECT THE DATA. To top off the column, turn the AA on and the column off. Remove the tubing cap and the floss from the top of the column. Attach the funnel to the top of the column and fill the funnel with buffer. With a spatula, transfer prepared column granules to the column on the AA, tap with a pencil and continue filling. Leave enough head space for the dental floss. Remove the excess buffer with the syringe, remove the funnel, reinsert the dental floss ball, and reconnect the tubing cap to the top of the column. Turn the column on to flush the cadmium and then prime the column.

Activating the column:
The column needs to be primed whenever it is new, or has been topped off with new granules. If the column is not activated, the nitrate sensitivity and response will not be stable. For a new column, activate by running approximately 200 mls of 50µM nitrate standard through the system with the column turned on. Flush the column afterwards by running imidazole and ultrapure water through the system for 20-25 minutes.

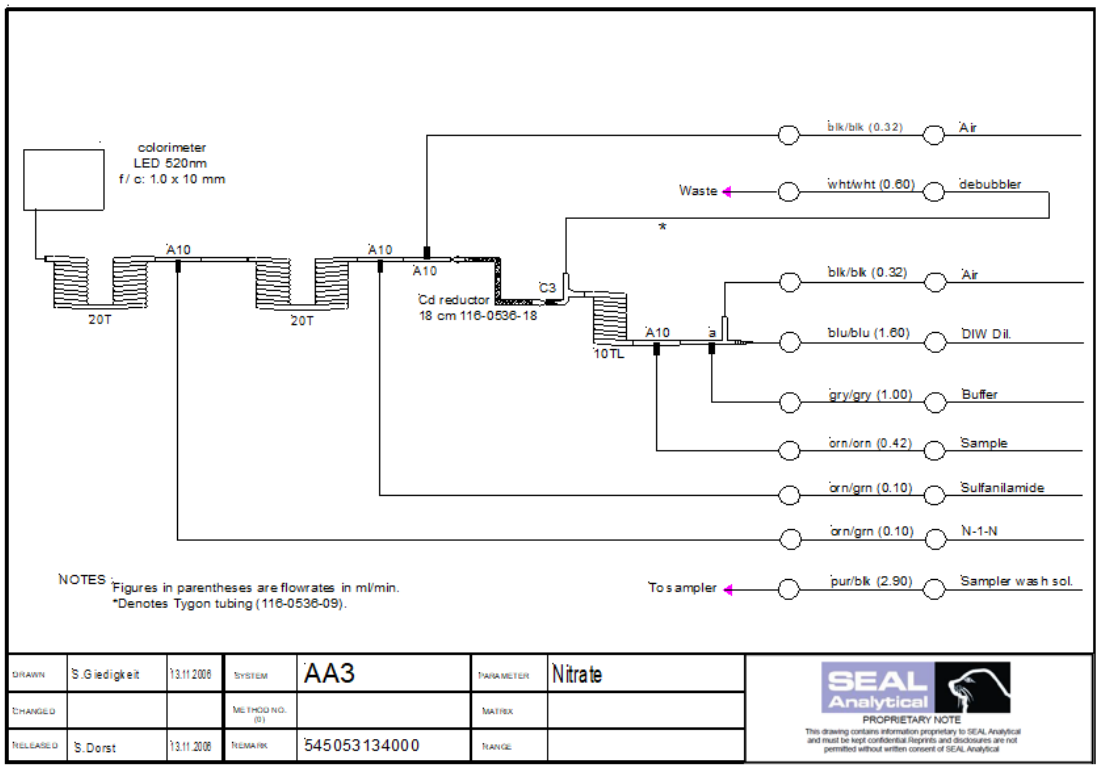


Figure 6: SIO Nitrate flow diagram

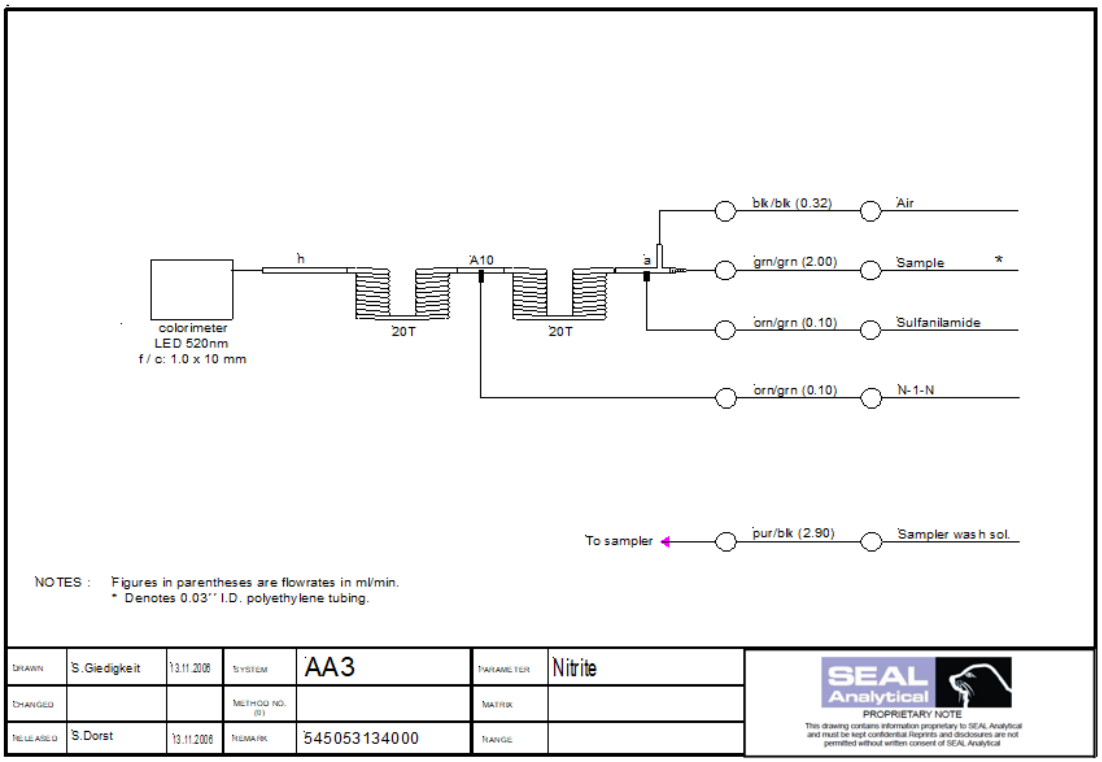


Figure 7: SIO Nitrite flow diagram

Phosphate Analysis:
Ortho-phosphate is analyzed using a modification of the Bernhardt and Wilhelms (1967) method. Acidified ammonium molybdate is added to a seawater sample to produce phosphomolybdic acid, which is then reduced to a blue phosphomolybdenum complex following the addition of dihydrazine sulfate. The sample is passed through a 10mm flowcell and absorbance measured at 820nm.

Reagents:
Ammonium Molybdate
Sulfuric acid solution:
Pour 420 ml of ultrapure water into a 2 L Ehrlenmeyer flask or beaker, place this flask or beaker into an ice bath. SLOWLY add 330 ml of concentrated sulfuric acid.
This solution gets VERY HOT!! Cool in the ice bath. Make up as much as necessary in the above proportions.

Dissolve 27g ammonium molybdate in 250 ml of ultrapure water. Bring to 1 L volume with the cooled sulfuric acid solution. Add 3 drops of 15% Sodium dodecyl sulfate (SDS) surfactant. Store in a dark PP bottle.

Dihydrazine Sulfate
Dissolve 6.4g dihydazine sulfate in ultrapure water, bring to 1 L volume and refrigerate.

Phosphate Standard:
In a 1 L calibrated glass volumetric "A" flask, dissolve ~0.81xxgm of dried high purity potassium phosphate in ultrapure water. Record the temperature. Dilute to the mark with ultrapure water. Calculate the concentration of this primary phosphate standard using the volumetric flask volume, temperature and exact weight of powder (see Appendix A).

Dilute the secondary and working standards as necessary.

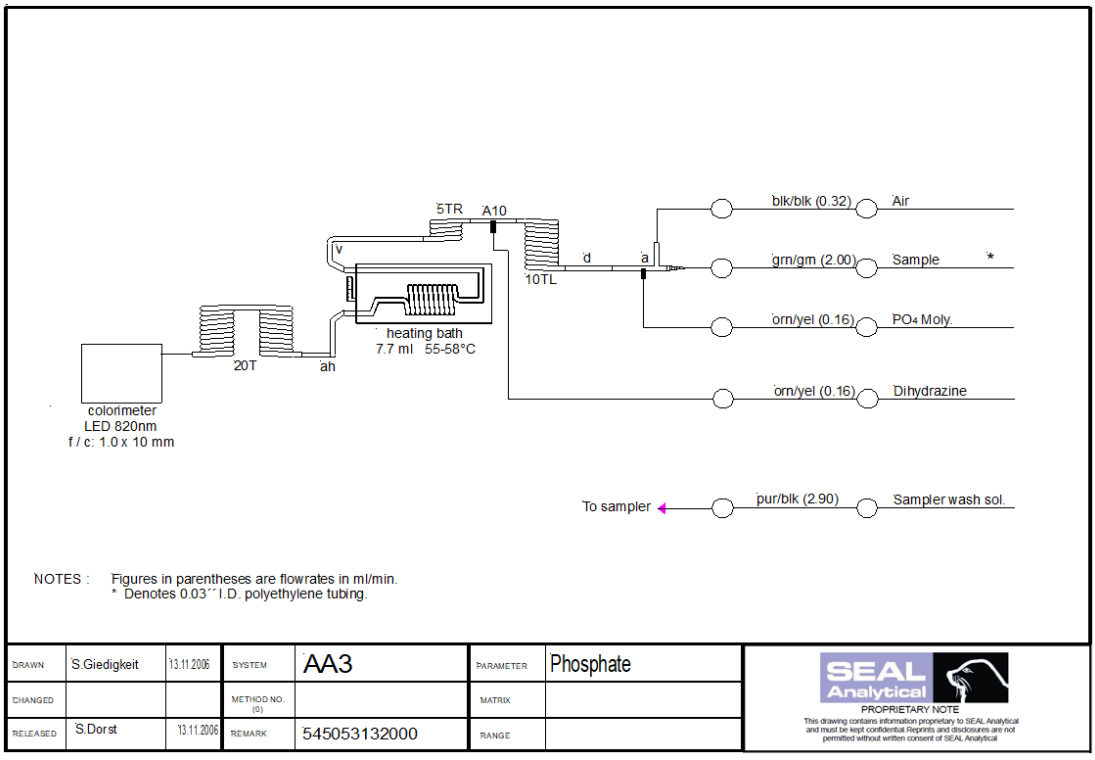


Figure 8: SIO Phosphate flow diagram

Silicate Analysis:
Silicate is analyzed using the basic method of Armstrong et al. (1967). Acidified ammonium molybdate is added to a seawater sample to produce silicomolybdic acid which is then reduced to a silicomolybdenum blue complex following the addition of stannous chloride. The sample is passed through a 10mm flowcell and measured at 660nm.

Reagents
Tartaric Acid
Dissolve 200g tartaric acid in ultrapure water and bring to 1 L volume. Store at room temperature in a PP bottle.

Ammonium Molybdate
Dissolve 10.8g Ammonium Molybdate Tetrahydrate in 800 ml of ultrapure water, add 2.8 ml concentrated sulfuric acid and dilute to1 L with ultrapure water
Add 3-5 drops 15% SDS surfactant per liter of solution.

Stannous Chloride Stock solution:
Dissolve 40g of stannous chloride in 100 ml 5N HCl. Refrigerate in a PP bottle.

Note:
Minimize oxygen introduction by swirling rather than shaking the solution. Discard if a white solution (oxychloride) forms.

Working solution: (prepared every 24 hours)
Bring 5 ml of stannous chloride stock to 200 ml final volume with 1.2N HCl. Make up daily - refrigerate when not in use in a dark PP bottle.

Silicate Standard:
In a plastic flask, dissolve 0.5642g dried high purity sodium hexafluorosilicate in about 300 ml ultrapure water. This solution will take 4 to 6 hrs to dissolve. Using this 300 ml solution, make up a mixed secondary standard (nitrate, phosphate, silicate) according to oceanic nutrient ranges.

At 1 L, the silicate concentration is 3000 µM. At 2 L, the silicate concentration is 1500 µM.

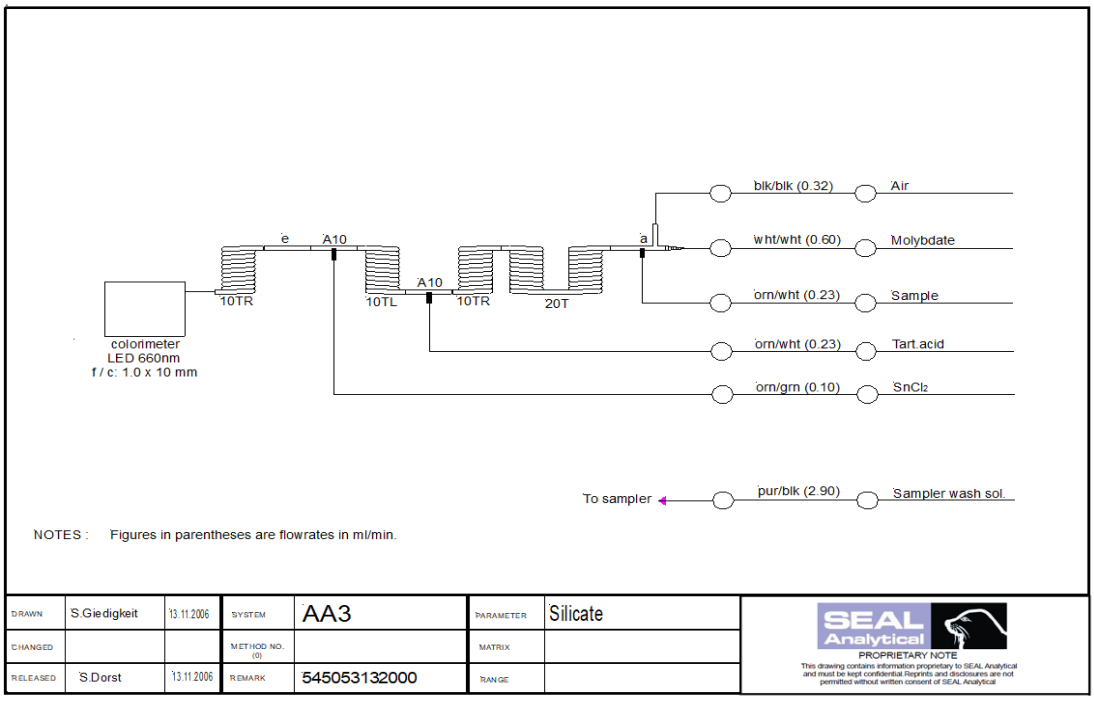


Figure 9: SIO Silicate flow diagram

Ammonium Analysis:
Fluorometric method
Ammonia is analyzed using the method described by Kerouel and Aminot (1997). The sample is combined with a working reagent made up of OPA, sodium sulfite and borate buffer and heated to 75°C. Fluorescence proportional to the ammonia concentration is emitted at 460nm following excitation at 370nm.

Reagents:
Ortho-phthaldialdehyde stock (OPA):
Dissolve 8g of OPA in 200ml ethanol and mix thoroughly. Store in a dark glass bottle and keep refrigerated.

Sodium sulfite stock:
Dissolve 0.8g sodium sulfite in ultrapure water and dilute up to 100ml. Store in a glass bottle, replace weekly.

Borate buffer
Dissolve 120g disodium tetraborate in ultrapure water and bring up to 4 L volume.

Working reagent:
In the following order and proportions combine:
1 L borate buffer
20ml stock OPA,
2 ml stock sodium sulfite,
4 drops 40% Surfynol 465/485 surfactant and mix.
Store in a glass bottle and protect from light. Replace weekly.
Make this up at least one day prior to use. Store in dark bottle and protect from outside air/NH_4 contamination.

Ammonium Standard:
In a 1 L calibrated glass volumetric "A" flask, dissolve ~0.26xxgm of dried high purity ammonium sulfate in ultrapure water. Record the temperature. Dilute to the mark with ultrapure water. Calculate the concentration of this primary ammonium standard using the volumetric flask volume, temperature and exact weight of powder (see Appendix A).
Dilute for secondary and working standards as necessary.

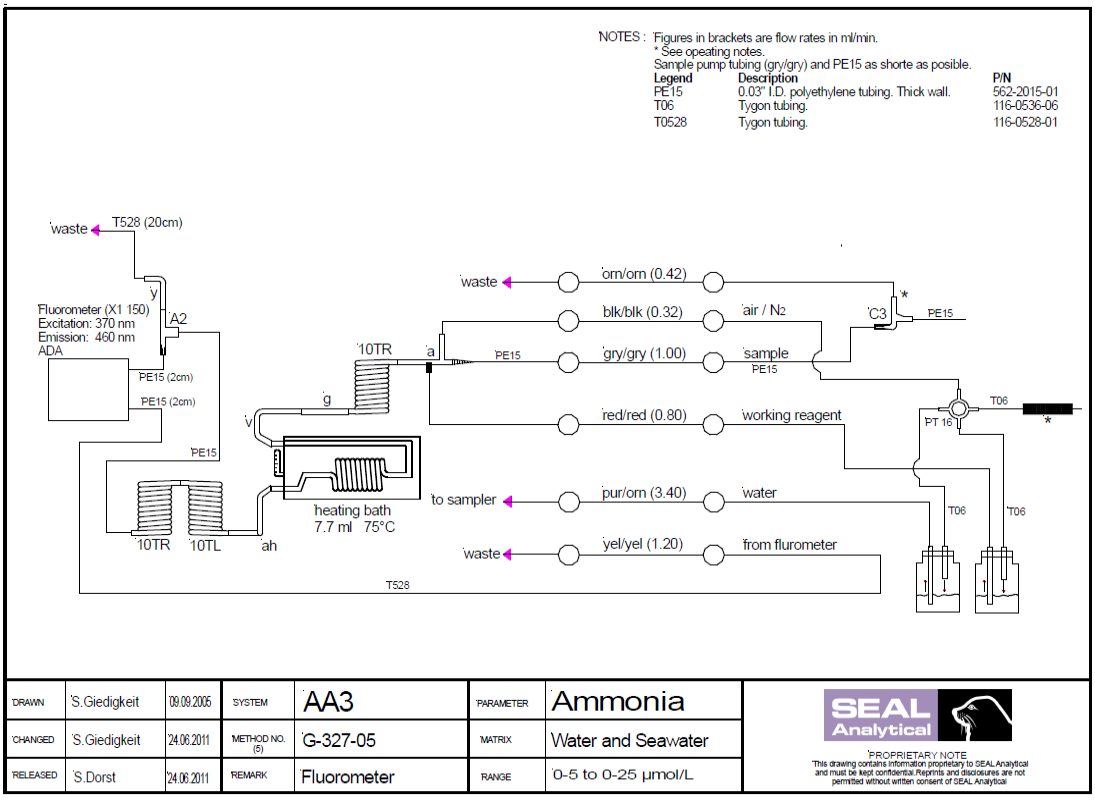


Figure 10: SIO Ammonium flow diagram

References

Armstrong, F.A.J., Stearns, C.A., and Strickland, J.D.H. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Research, 14, 381-389.

Bernhardt, H., and Wilhelms, A. 1967. The continuous determination of low level iron, soluble phosphate and total phosphate with the AutoAnalyzer. Technicon Symposia, I, 385-389.

Kerouel, R., and Aminot, A. 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Marine Chemistry, 57, 265-275.

Appendix G:
Detailed methods for QuAAtro 2-HR utilized at JAMSTEC

Nitrate plus Nitrite Analysis
Nitrate plus nitrite and nitrite alone are analyzed following a modification of the method of Grasshoff (1976). The nitrate is reduced to nitrite in a cadmium tube, the inside of which is coated with metallic copper. The sample stream after reduction is treated with an acidic, sulfanilamide reagent to produce a diazonium ion. Then NEDD (N-1-N) is added to the sample stream to produce a red azo dye. With the reduction of the nitrate to nitrite, they are measured in total, without reduction, only nitrite reacts. Thus, for the nitrite analysis, no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by the difference.

Reagents for Nitrate plus Nitrite
50% Triton solution
Mix 50 ml Triton™ X-100 provided by Sigma-Aldrich Japan G. K. (CAS No. 9002-93-1) with 50ml Ethanol (99.5%).

Imidazole (buffer), 0.06 M (0.4% w/v)
Dissolve 4 g Imidazole (CAS No. 288-32-4), in 1 L ultrapure water, add 2 ml Hydrochloric Acid (CAS No. 7647-01-0). After mixing, 1 ml 50% Triton solution is added.

Sulfanilamide, 0.06 M (1% w/v) in 1.2 M HCl
Dissolve 10 g 4-Aminobenzenesulfonamide (CAS No. 63-74-1), in 900 ml of ultrapure water, add 100 ml Hydrochloric Acid (CAS No. 7647-01-0). After mixing, 2 ml 50% Triton solution is added.

NEDD, 0.004 M (0.1% w/v)
Dissolve 1 g N-(1-Naphthalenyl)-1,2-ethanediamine, dihydrochloride (CAS No. 1465-25-4), in 1 L of ultrapure water and add 10 ml Hydrochloric Acid (CAS No. 7647-01-0). After mixing, 1 ml 50% Triton solution is added. This reagent is stored in a dark bottle.

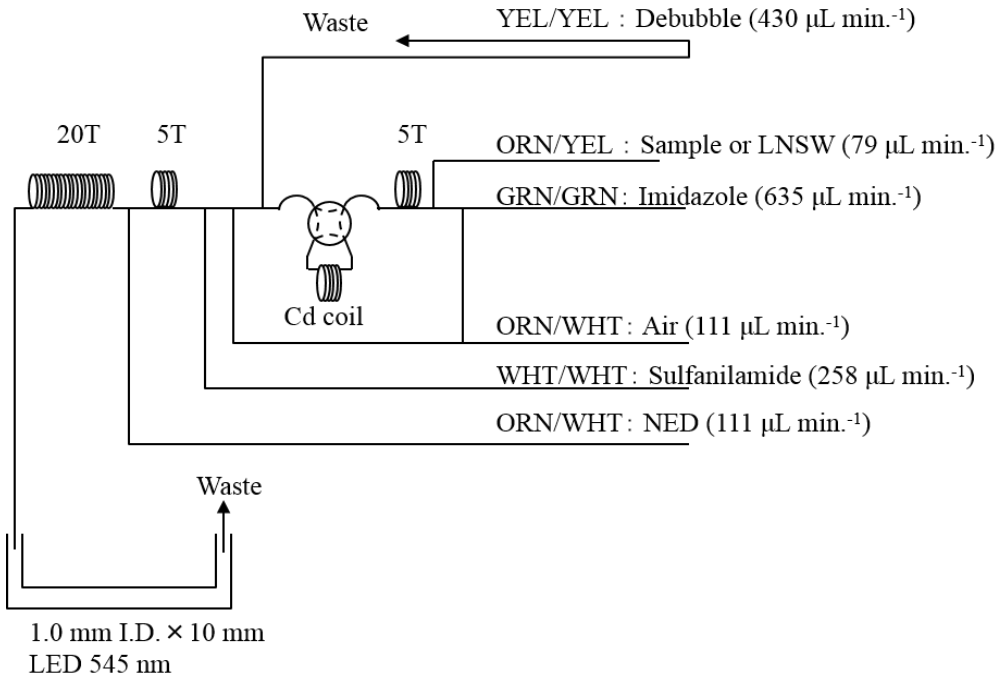


Figure 11: JAMSTEC Nitrate plus Nitrite flow diagram.

Reagents for Nitrite:
50% Triton solution
Mix 50 ml Triton™ X-100 provided by Sigma-Aldrich Japan G. K. (CAS No. 9002-93-1) with 50 ml Ethanol (99.5%).

Sulfanilamide, 0.06 M (1% w/v) in 1.2 M HCl
Dissolve 10 g 4-Aminobenzenesulfonamide (CAS No. 63-74-1), in 900 ml of ultrapure water, add 100 ml Hydrochloric Acid (CAS No. 7647-01-0). After mixing, 2 ml 50% Triton solution is added.

NEDD, 0.004 M (0.1% w/v)
Dissolve 1 g N-(1-Naphthalenyl)-1,2-ethanediamine, dihydrochloride (CAS No. 1465-25-4), in 1 L of ultrapure water and add 10 ml Hydrochloric Acid (CAS No. 7647-01-0). After mixing, 1 ml 50% Triton solution is added. This reagent is stored in a dark bottle.

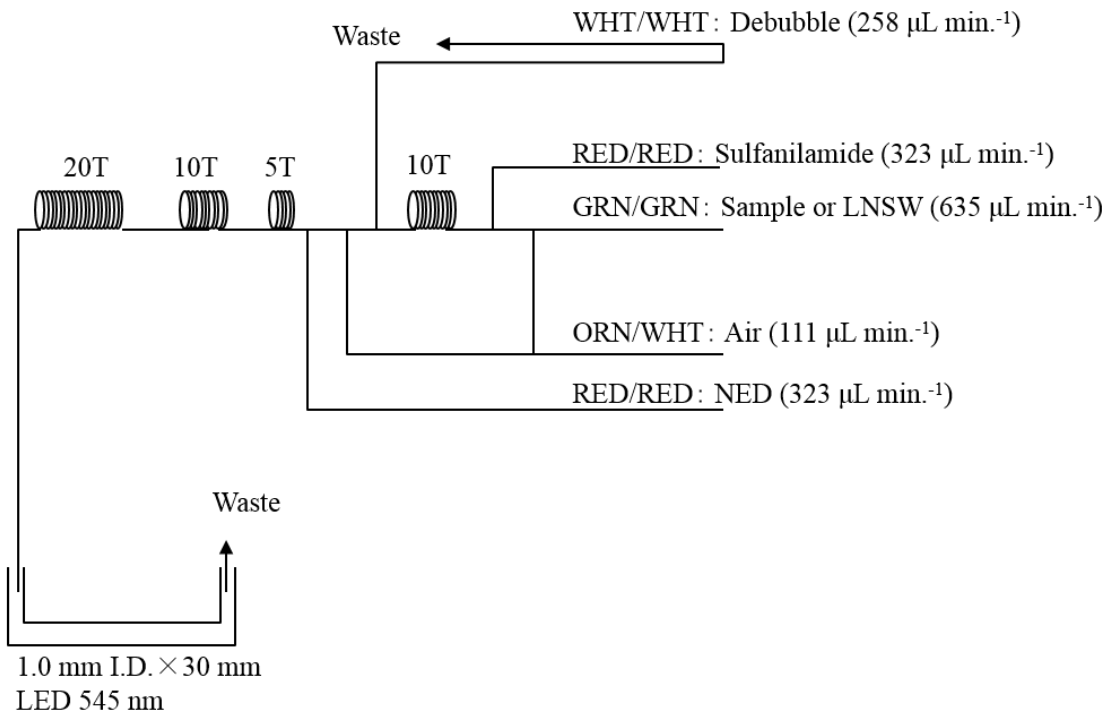


Figure 12: JAMSTEC Nitrite flow diagram

Silicate Analysis
The silicate method is analogous to that described for phosphate. The method used is essentially that of Grasshoff et al. (1999). Silicomolybdic acid is first formed from the silicate in the sample and molybdic acid. The silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue," using ascorbic acid.

Reagents:
15% Sodium dodecyl sulfate (SDS) solution
Dissolve 75 g SDS (CAS No. 151-21-3) in 425 ml ultrapure water.

Molybdic acid, 0.06 M (2% w/v)
Dissolve 15 g Sodium molybdate dihydrate (CAS No. 10102-40-6), in 980 ml ultrapure water, add 6 ml Sulfuric acid (CAS No. 7664-93-9). After mixing, 20 ml 15% SDS solution is added.

Oxalic acid, 0.6 M (5% w/v)
Dissolve 50 g Oxalic acid (CAS No. 144-62-7), in 950 ml of ultrapure water, make up to 1 L.

Ascorbic acid, 0.01 M (3% w/v)
Dissolve 2.5 g L-Ascorbic acid (CAS No. 50-81-7), in 100 ml of ultrapure water. Prepare this solution daily.

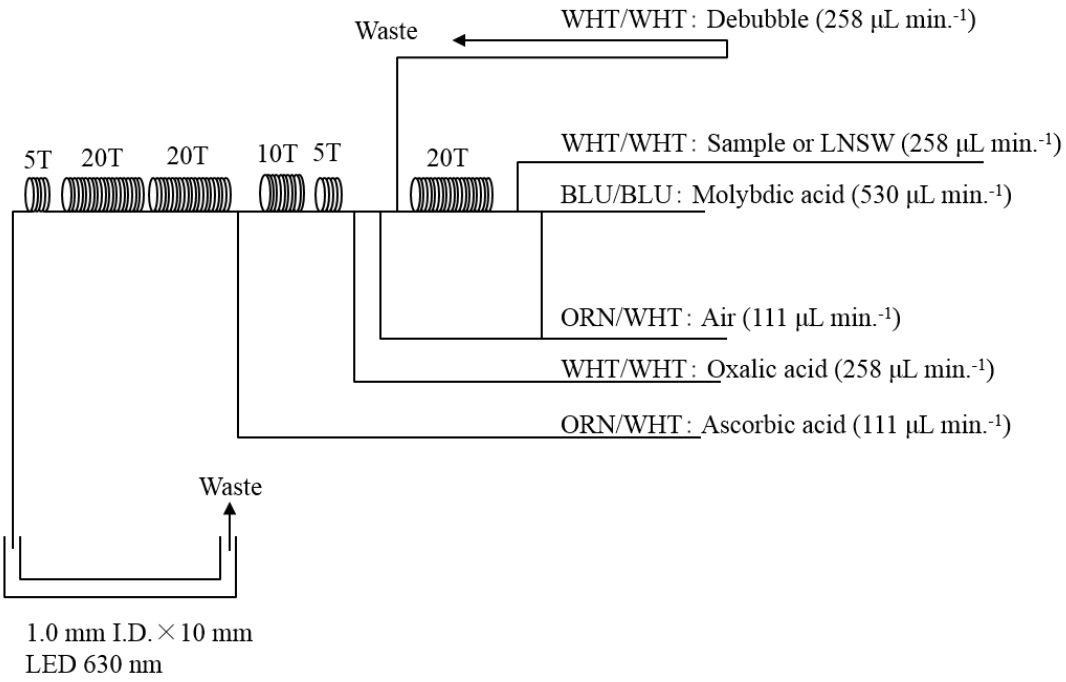


Figure 13: JAMSTEC Silicate flow diagram.

Phosphate Analysis
The phosphate analysis is a modification of the procedure of Murphy and Riley (1962). Molybdic acid is added to the seawater sample to form phosphomolybdic acid, which is in turn reduced to phosphomolybdous acid using L-ascorbic acid as the reductant.

Reagents:
15% SDS solution
Dissolve 75 g SDS (CAS No. 151-21-3) in 425 ml ultrapure water.

Stock molybdate solution, 0.03 M (0.8% w/v)
Dissolve 8 g Sodium molybdate dihydrate (CAS No. 10102-40-6), and 0.17 g Antimony potassium tartrate trihydrate (CAS No. 28300-74-5), in 950 ml of ultrapure water and add 50 ml Sulfuric acid (CAS No. 7664-93-9).

Phosphate color reagent
Dissolve 1.2 g L-Ascorbic acid (CAS No. 50-81-7), in 150 ml of stock molybdate solution. After mixing, 3 ml 15% SDS solution is added. This reagent should be prepared before every analytical run.

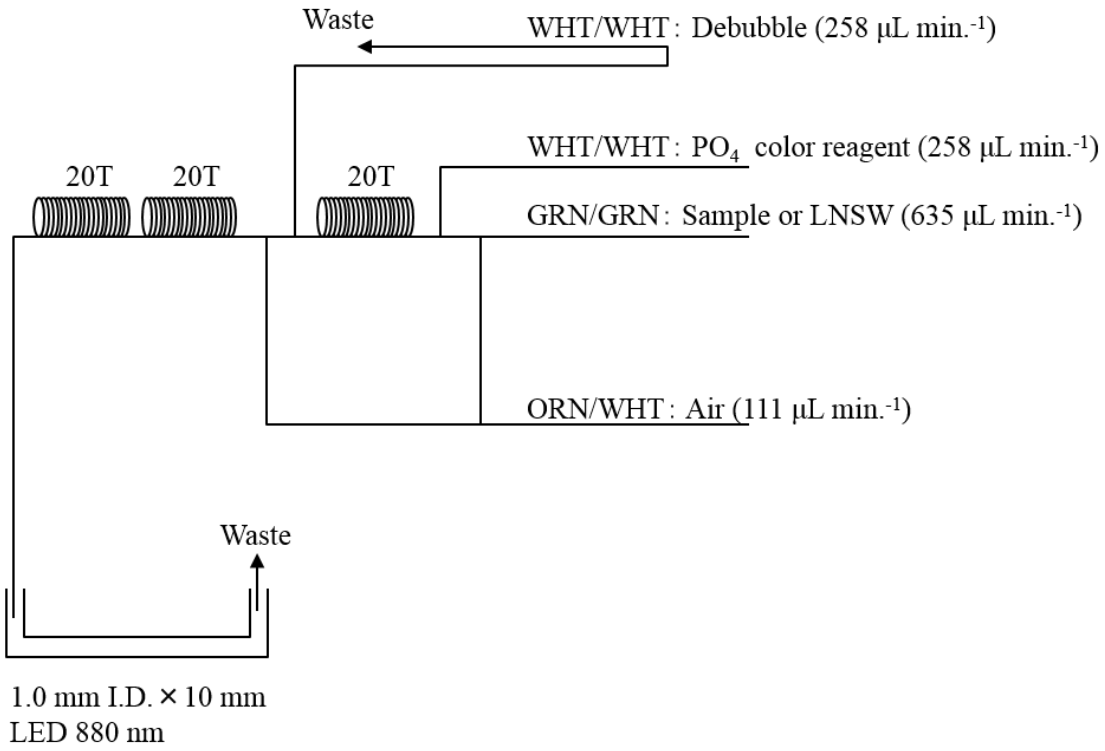


Figure 14: JAMSTEC Phosphate flow diagram.

Ammonium Analysis
Ammonium in seawater is mixed with an alkaline solution containing EDTA, ammonia as gas state is formed from seawater. The ammonia (gas) is absorbed into sulfuric acid through a 0.5 μm pore size membrane filter (ADVANTEC PTFE) in a dialyzer attached to the analytical system. The ammonia absorbed in sulfuric acid is determined by coupling with phenol and hypochlorite to form indophenol blue. The wavelength for ammonia analysis is 630 nm.

Reagents:
30% Triton solution
Mix 30 ml Triton™ X-100 provided by Sigma-Aldrich Japan G. K. (CAS No. 9002-93-1) with 70 ml ultrapure water.

EDTA
Dissolve 41 g Ethylenediaminetetraacetic acid (CAS No. 13235-36-4), and 2 g Boric acid (CAS No. 10043-35-3), in 200 ml of ultrapure water. After mixing, 1ml 30% Triton solution is added. Prepare this solution weekly.

Sodium hydroxide solution
Dissolve 5 g Sodium hydroxide (CAS No. 1310-73-2), and 16 g EDTA (CAS No. 13235-36-4) in 100 ml of ultrapure water. Prepare this solution weekly.

Stock nitroprusside
Dissolve 0.25 g Sodium nitroprusside (CAS No. 13755-38-9) in 100 ml of ultrapure water and add 0.2 ml 1M Sulfuric acid. Store in a dark bottle and prepare monthly.

Working nitroprusside solution
Mix 4 ml stock nitroprusside and 5 ml 1M Sulfuric acid in 500 ml of ultrapure water. After mixing, add 2 ml 30% Triton solution. This reagent is stored in a dark bottle and prepared every 2 or 3 days.

Alkaline phenol solution
Dissolve 10 g Phenol (CAS No. 108-95-2), 5 g Sodium hydroxide (CAS No. 1310-73-2) and 2 g Sodium citrate dihydrate (CAS No. 6132-04-3), in 200 ml ultrapure water. Stored in a dark bottle and prepare weekly.

Sodium hypochlorite solution
Mix 3 ml Sodium hypochlorite (CAS No. 7681-52-9) in 47 ml ultrapure water. Store in a dark bottle and prepare before every analytical run.

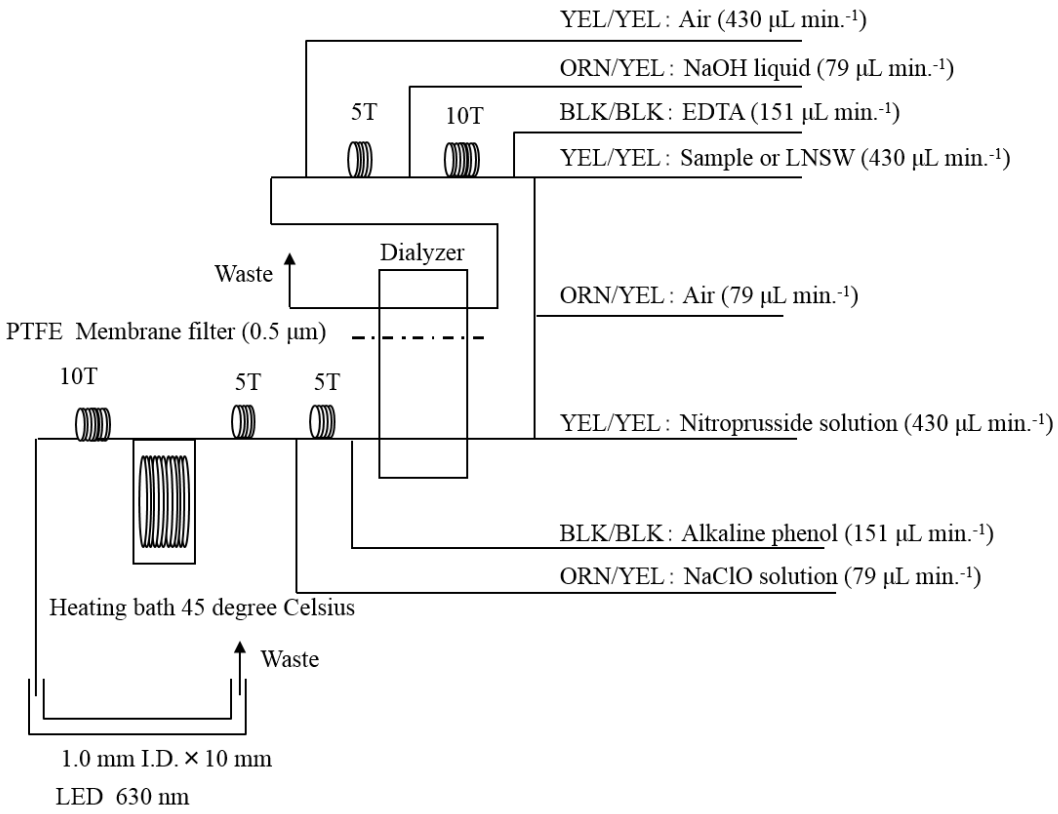


Figure 15: JAMSTEC Ammonium flow diagram.

References

Grasshoff, K. (ED). 1976. Methods of seawater analysis, Verlag Chemie, Weinheim and New York.

Grasshoff, K., Kremling, K., and Ehrhardt, M. G. eds. 1999. Methods of seawater analysis (3rd edition), VCH Publishers.

Murphy, J., and Riley, J.P. 1962. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Analytica Chimica Acta, 27, 31-36.

Appendix H:
Experimental results: NIOZ and SIO sample freezing and thawing on silicate results

As was stated earlier in this manual in section 2.2.
"It is well known that the reactive silica polymerizes when frozen, especially at high concentrations (Burton et al. 1970; MacDonald and McLaughlin 1982; MacDonald et al. 1986). Variables that affect the recovery of silica from frozen samples include salinity, turbidity, bottle size, and the silicate concentration. Much of the current debate centers on the recommended thaw techniques to depolymerize the reactive silica and get complete recovery."

Systematic tests have been performed at SIO and NIOZ to determine the best thawing techniques for nutrient samples prior to analysis. A variety of seawater types were used and include samples from a coastal environment (NIOZ), an estuary (NIOZ) and the open ocean (NIOZ and SIO). NIOZ also tested different bottle types and sizes. Here we report on the initial results, conclusions and recommendations.

SIO:
Different thawing techniques were performed on samples that had been frozen for one month, two months and three months. To do this approximately 10 L of water was collected from four different depths on a local cruise. Approximately 100 samples were drawn from each depth into 30 ml PP centrifuge tubes. One sample at each depth was analyzed "fresh" to get the initial nutrient concentrations. The other samples were then frozen in a -20°C freezer. The thaw techniques included: a) 24 hour thaw at room temperature, b) 24 hour thaw in the refrigerator, (4°C), c) 48 hour thaw at room temperature, and d) thaw in a water bath of warm water drawn from the sink tap. The graph below (Figure 16) shows the results of the different thaw techniques after one month in the freezer. The results indicate that thawing using the warm water bath produced the best recovery of silicate. But at higher concentrations there is incomplete recovery with any the thaw techniques. Results from the two month and three month freeze times also showed similar results.

Based on these initial results, more tests were carried out at sea to see if thawing frozen samples in a constant temperature water bath at 50°C would result in more complete recovery of the reactive silicate that had been polymerized. Duplicate samples were taken at four different stations on the GO-SHIP cruise P06 on the R/V Palmer in 2017. The initial samples were analyzed as normal on the ship. The second set of samples was frozen for a period of approximately one week before being thawed and analyzed. The frozen samples were placed in a 50°C water bath between 30 to 45 minutes and then allowed to cool down to room temperature before analysis (between 30-45 minutes). The recovery of reactive silicate utilizing this thaw technique was much improved (Figure 17) compared to the initial experiments. These results are consistent with Sakamoto et al. (1990).

In the thaw experiment at sea analysis for nitrate plus nitrite and phosphate (Figures 18 and 19), as well as for silicate (Figure 17), was carried out. These results indicate that there was no effect to the concentrations of nitrate plus nitrite, phosphate, or silicate by freezing and thawing. Further tests should be performed with this thaw technique to test the effect of longer freeze times.

Care must be taken during all steps of sampling, freezing and thawing to avoid compromising the samples.

In light of these results, the recommendation to the community is to thaw frozen seawater samples prior to nutrient analysis using a water bath at 50°C for 40 minutes. The samples must return to room temperature before analysis (45 minutes).

References

Sakamoto, C.M., Friederich, G.E., and Codispoti, L.A. 1990. MBARI procedures for automated nutrient analyses using a modified Alpkem Series 300 Rapid Flow Analyzer. Monterey Bay Aquarium Res. Inst. Tech. Rep. No. 90-2, 84 pp.

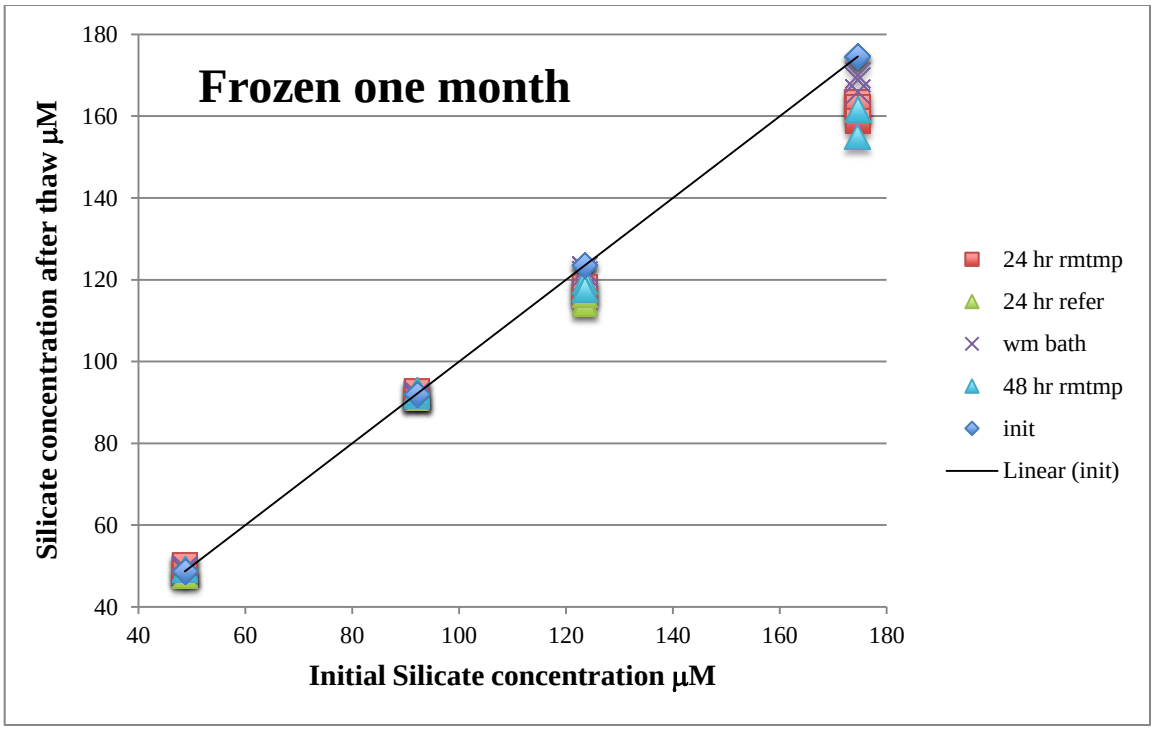


Figure 16: Results of different thaw techniques on silicate concentrations on frozen nutrient samples (24 hour room temperature (24 hr rmtmp); 24 hour in refrigerator (24 hr refer); water tap water bath (wm bath); 48 hour room temperature (48 hr rmtmp); initial concentration (init)).

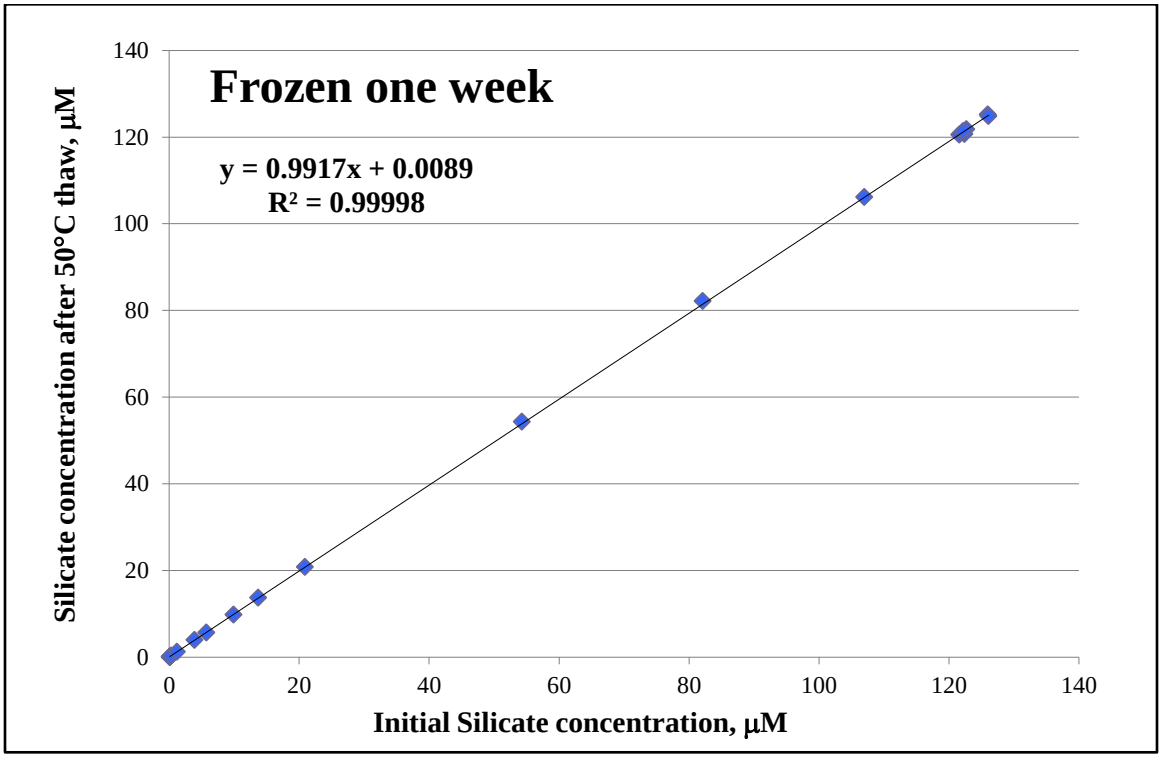


Figure 17: Silicate results from thawing at 50°C.

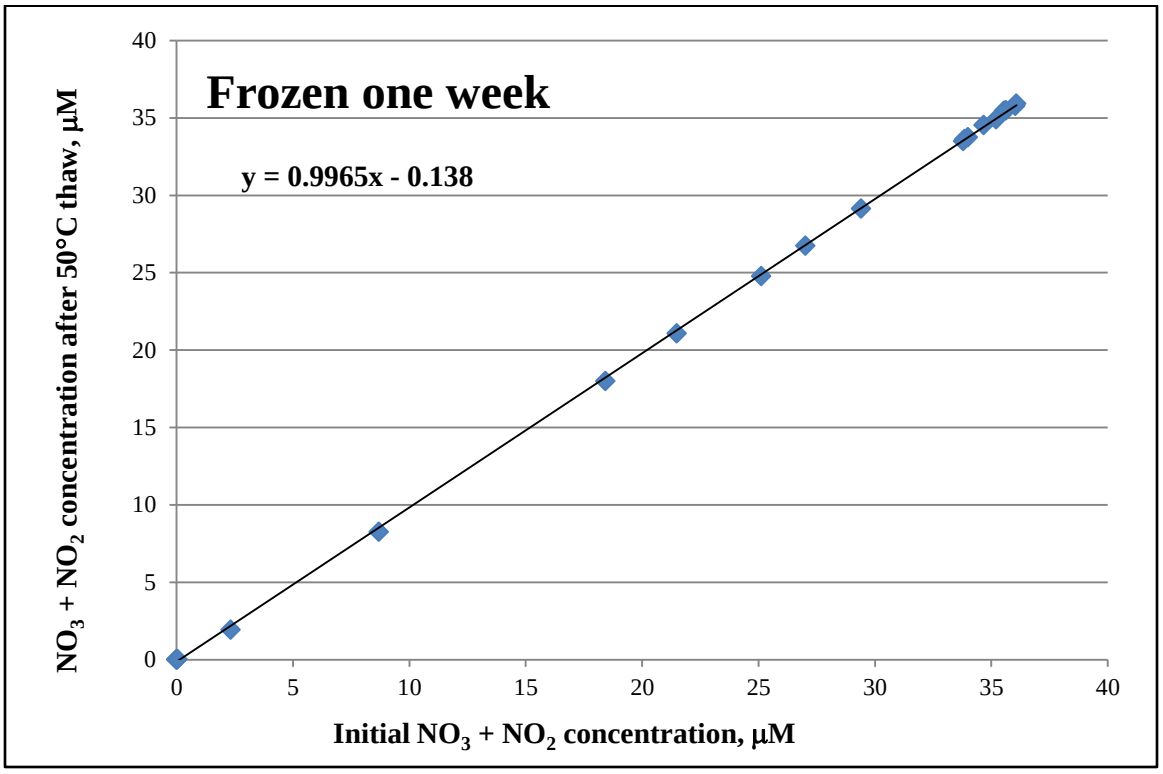


Figure 18: Nitrate plus Nitrite results from thawing at 50°C.

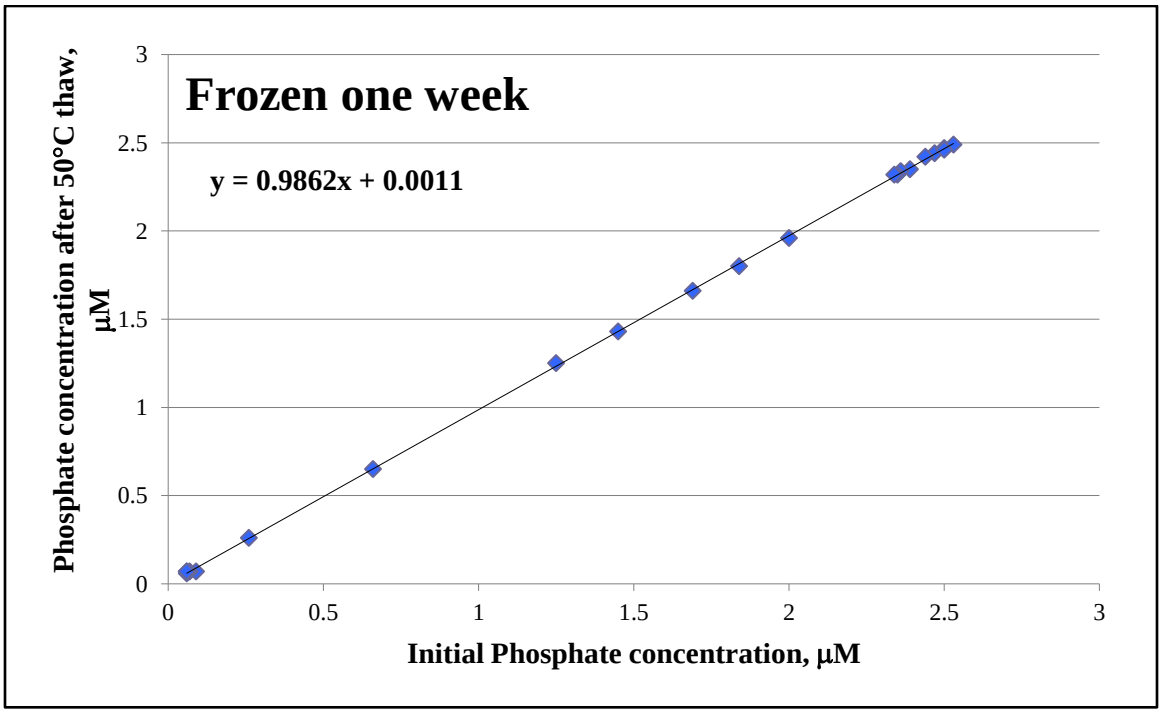


Figure 19: Phosphate results from thawing at 50°C.

NIOZ:
A Poster Presentation made at the 2018 Ocean Sciences Meeting held in Portland, Oregon, is included here for further details on the Silicate freezing/thawing issues.

Biogeochemistry and Nutrients in open ocean waters: Sustainable Ocean Observations and Time Series Efforts.

Storage of silicate samples, implications for Oceanic nutrient observations

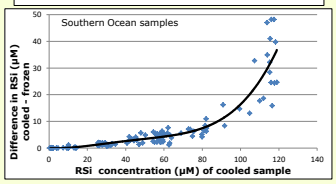
Karel Bakker, Sharyn Ossebaar, Jan van Ooijen

NIOZ Royal Netherlands Institute for Sea Research;
(SCOR Working Group #147: Towards comparability of oceanic nutrients)

Introduction

Historically it has been stated that the freezing of oceanic water samples for silicate with concentrations below 40µM, is not a problem if the samples are allowed to thaw for at least 24 hours *(Strickland and Parsons 1968; Grasshoff 1976).*
However, we observed for both long and short term freezing times that there is a significant loss effect on the final Reactive Silicate (RSi) concentration, due to the silicate being partly polymerized even after an extended thawing time of up to 42 hours. Resolving this issue is extremely important when considering results from analysis of ocean observations and data that are submitted to global data time series.

Measured reactive silicate differences



Statements from Literature

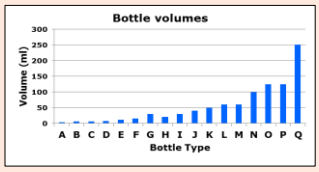
i. Freezing of samples can convert RSi to a non-reactive fraction referred to as **polymerisation** *(Alexander 1953, Kobayashi 1967, Burton 1970).*
ii. Si maximal **depolymerisation** is observed at pH>10.5 and higher temperatures >40ºC *(Greenberg 1957).*
iii. Si **depolymerisation** at 25ºC is faster in seawater than in river water *(Burton.J.D., et All, 1970).*
iv. Long frozen storage time or only frozen for a few days is very significant *(MacDonald and McLaughlin 1982).*

Experimental set-up with coastal water

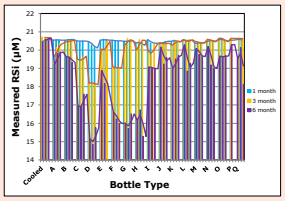
In 2016, coastal North Sea water of 32 salinity, was collected and filtered (0.7µm) into bottles ranging in volume from 4 to 250ml and frozen at -20ºC (see image below). The samples were thawed after 1, 3 and 6 months and the results were compared with an initial unfrozen sample stored at 4 – 7 ºC . Effects of thawing time on depolymerisation were studied performing analysis after 18, 42 hours and 10 days at 20ºC . A certified reference material of nutrients, **CRM-BT**, was used with every silicate analysis.

Different size and material of bottles used for storage during freezing at -20ºC container

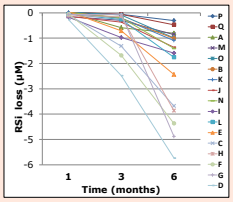
Bottle I.D.	Bottle brand	Bottle Volume ml	Bottle Material	Description
A	NALGENE	4	PP	
B	PONYVIAL	6	HDPE	SNAPTWIST SCINTILLATION VIAL
C	PONYVIAL	6.5	HDPE	SNAPTWIST SCINTILLATION VIAL
D	NALGENE	8	HDPE	
E	KARTELL	12	PP	KOREA RMNS VIAL
F	FALCON	15	PP	
G	NALGENE	30	PC	
H		20	HDPE	SCINTILLATION VIAL
I	NALGENE	30	PP	
J	CUSTOM	40	TEFLON	TEFLON BOMMEN
K	FALCON	50	PP	
L	NALGENE	60	PETG	
M	NALGENE	60	HDPE	
N	AS ONE	100	PP	JAPAN RMNS BOTTLE
O	NALGENE	125	PP	
P	NALGENE	125	HDPE	CTD TAP BOTTLES
Q	NALGENE	250	HDPE	



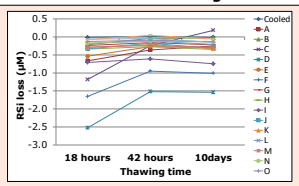
RSi concentrations after 1, 3, 6 months frozen storage at -20ºC



Loss of RSi after 1, 3, 6 months frozen storage at -20ºC and thawed for 18 hours at 20ºC



Extended thawing time of 18, 42 hours and 10 days at 20ºC after 3 months storage at -20ºC



Discussion

Experimental results show that even at low concentrations of 20µM Si, loss of the reactive silicate by freezing is observed dependent on the type of storage bottle used.

- In general, the smaller the sample bottle, the more silicate loses are observed.
- The type of plastic material used seems to have no effect.
- Depolymerisation effect; after 42 hours of thawing, reactive silicate concentrations stay constant, however they are still lower in respect to the initial unfrozen sample.
- Samples frozen up to 1 month have a minor reactive silicate loss of up to 1%, after 3 months up to 10%, and after 6 months up to 25% loss of its initial value, dependent on the type of bottle used.

Freezing causes more or less losses of reactive silicate irrespective of the source of seawater as observed in further experiments performed during this study.

- "Deep Blue" Atlantic Ocean waters has minor losses of reactive silicate between 0.5 to 1%.
- Near shore Antarctic samples can have losses of up to 5% of reactive silicate.
- Coastal or estuarine waters show up to 20% loss of reactive silicate.

Recommendations

Do not freeze seawater samples for silicate unless a thorough recovery study has been performed on the reactive silicate loss after frozen storage.
However, if it is absolutely necessary to freeze silicate samples, only use large 125 or 250ml HDPE bottles and a maximum freezing time of 1 month with silicate analysis being performed after 42 hours of thawing.

NIOZ is an institute of NWO in cooperation with Utrecht University