Sampling

Ideally, samples are collected into new, sterile plastic (HDPE, PP) vials that will then fit directly onto the AA sampler carousel. However, using new vials can be costly and produces a tremendous amount of plastic waste. Sample containers can be re-used if proper cleaning procedures are followed between stations. For macro-nutrient analysis (micro-molar concentrations), rinsing the sample containers with distilled deionized water followed by a rinse with 10% HCl (hydrochloric acid) is sufficient. This stops any biological growth in bottles. The bottles should be rinsed well with deionized water prior to the collection of the next samples. Glass sample containers should not be used due to silicate contamination.

When taking the seawater samples from the rosette, rinse the clean sample containers and caps three times before filling. Avoid touching the sampling spigots on the Niskin bottles and take care to rinse the spigots as well as the nutrient sample containers. Samples can be collected without 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. Between CTD sampling events it is important to clean any sampling tube with clean deionized water and 10% HCl. Once rinsed then fill the sample containers two thirds full, and cap immediately.

The samples should be analyzed as soon after sample collection as possible. If analysis will be delayed for longer than a couple hours (1-2 hrs), 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. ***We recommend for short sample storage to use a refrigerator at 4°C.***
When analysis is not possible within 24 h of sample collection, the samples need to be prepared for storage (for details see below).

Sample filtration

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 mostly 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 prior to sample collection. Types of filters often used to filter seawater include cellulose acetate, hydrophilic polypropylene Gelman membrane, and Acrodisc syringe filters. Glass Fiber filters (GFF) (silicate contamination) or cellulose nitrate filters (nitrate contamination) should NOT be used. Filter size is another consideration. A pore size of 0.45 µm filter 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 received that a 0.45 µm filter does not capture all bacteria and phytoplankton. Instead a 0.2 µm filter is now the preferred size of filter to be used. The flow rate through these filters is low and if filtration is done under pressure or high vacuum, there is a risk of cell rupture and sample contamination. Gravity, low pressure, or low vacuum filtration is therefore recommended. It is imperative that tests are performed to check that the method of filtering, filter type, and size do not lead to contamination of the samples. Gloves are another source of debate regarding possible contamination. Neither Neoprene nor colored nitrile gloves should ever be used in the lab or for sampling for 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, vinyl, powder-free, gloves are recommended for use in the lab and for sample collection. In general, it is good 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 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.

We recommend to use pre-cleaned cellulose acetate filters (pore size 0.45 µm, acid and MilliQ washed) to process nutrient samples. During sampling, it is recommended to use gloves made from vinyl and powder-free. Do not use nitril lab gloves.

Sample preservation

There are many 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 poisoning samples with mercuric chloride or by acidification. Freezing is the most commonly used method, and there are studies that show that freezing can be a reliable method of sample preservation (Aminot 1995; Dore et al. 1996).

It is imperative that frozen seawater samples have sufficient head space in the bottles to allow for expansion during freezing. 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. Store samples vials in zip-lock plastic bags in the freezer.

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 et al. 1982; MacDonald et al. 1986). Much of the current debate centers on the proper thaw techniques to depolymerize the reactive silica to get complete recovery. Many laboratories have done 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 and then cooled back down to room temperature before actual analysis. Zhang and Ortner (1998) suggest that it can take up to 4 days to thaw samples at room temperature to get complete recovery of silica. Recent tests done at the Royal Netherlands Institute for Sea Research and Scripps Institution of Oceanography 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.

Variables, which affect the recovery of silica from frozen samples, include salinity, turbidity, and silica concentration. The nutrient community and authors of this manual are carrying out systematic tests to determine the best thaw techniques for the types of samples being collected (coastal, estuarine, oligotrophic, etc)

For long time storage, please put 10 mL of sample into each 14 mL sample tube; cap the tube and freeze immediately in up-right position at -20°C. Please, take always one back-up sample each. Samples should be thawed overnight in the dark at room temperature before analysis.

General comment

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.

High bacterial activity may rapidly produce nitrite in the sample. The determination of nitrite should, therefore, be carried out without delay, i.e., the reagents should be added to the sample within 30 min of subsampling.

Reference: *"GO-SHIP Repeat Hydrography Nutrient Manual, 2019: The precise and accurate determination of dissolved inorganic nutrients in seawater; Continuous Flow Analysis methods and laboratory practices."*