Sampling and Sample-handling Protocols for GEOTRACES Cruises

Edited by the 2013 GEOTRACES Standards and Intercalibration Committee:

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I. Introduction

The GEOTRACES Standards and Intercalibration (S&I) Committee is charged with ensuring that the data generated during GEOTRACES are as precise and accurate as possible, which includes all the steps from sampling to analysis. Thus, sampling methods for dissolved and particulate constituents must take a representative (of the water depth/water mass) and uncontaminated sample, the samples must be stored (or immediately analyzed) in a fashion that preserves the concentrations (activities) and chemical speciation, and the analyses of these samples must yield accurate data (concentration, activity, isotopic composition, and chemical speciation). To this end, experiences from the 2008-2010 GEOTRACES Intercalibration Program, actual GEOTRACES cruises in 2010-2013, and other related intercalibration efforts, helped to create the protocols in this document. However, methods continually evolve and the GEOTRACES S&I Committee will monitor these advances as validated by intercalibrations and modify the methods as warranted. The protocols here are divided into trace element and isotope groups: Hydrography and Ancillary Parameters, Radioactive Isotopes, Radiogenic Isotopes, Trace Elements, and Nutrient Isotopes. Those who contributed to preparing these protocols are listed in Appendix 1 and are sincerely thanked for their efforts in helping GEOTRACES and the worldwide TEI community.

II. General Considerations

The following items must be included as a part of a standard intercalibration effort during all GEOTRACES cruises:

A. Every cruise must occupy at least one GEOTRACES Baseline Station (where previous intercalibration cruises have established the concentrations, activities, and/or speciation of at least the key GEOTRACES TEIs), or an overlap/cross-over station with a previous GEOTRACES cruise, to affect an intercalibration for sampling through analyses.

B. If there are no GEOTRACES Baseline Stations or crossover stations to occupy, an intercalibration must be conducted via replicate sampling during each cruise. In particular, a minimum of 3 depths (e.g., near surface, mid-water, and deep) at 2 stations should be sampled with duplicate hydrocasts, and samples from these replicates sent to several labs for the determination of trace elements and isotopes. The results from this effort should be examined later for data integrity and coherence.

C. Nutrient and salinity samples should be taken along with all trace element samples in order to verify proper bottle and rosette operation and sampling depths (i.e., compare to the hydrography established with the conventional CTD/rosette). Experience to date indicates that routine nutrient samples and salinity samples should not be filtered. If samples are filtered this should be noted in the metadata. Experience has also shown that hydrographic rosette and “clean rosette” nutrient data sometimes do not agree because of the long waits before drawing nutrient samples from the “clean rosette” (or other type of clean sampling devices). Investigators are urged to compare the two types of nutrient data as soon as possible during a cruise to see if such problems exist.

D. We will not recommend specific analytical methods for most variables (except for the ancillary parameters and several methods for some TEIs are suggested in the sections to follow). However, during analyses (at sea or in a shore-based lab) appropriate certified reference materials (See IX. Glossary of Terms), or SAFe or GEOTRACES Consensus Intercalibration samples as described in the Trace Element Section (VI), must be processed to assess analytical accuracy. The results of certified reference materials or Consensus sample analyses must be reported in the labs'/cruise's metadata.

E. All aspects of metadata (e.g., sampling devices, analytical methods used, data processing techniques, analytical figures of merit) related to sampling, sample logging, and resulting data should follow the guidelines found on the International GEOTRACES Data Assembly Centre (http://www.bodc.ac.uk/geotraces/) web site. Except where activities are reported (e.g., radionuclides), we recommend concentration units be in fractions of a mole per unit mass (kilogram) or volume (liter; most appropriately when shipboard analyses are used) - μmol l^{-1} or nmol kg^{-1} as examples. Use of capital "M" to indicate moles l^{-1} should not be used because this causes confusion in the GEOTRACES data base.

III. Hydrography and Ancillary Parameters

Although GEOTRACES is focused on trace elements and their isotopes (TEIs), to achieve the overarching goal of understanding the biogeochemical processes controlling them, the suite of TEIs must be examined in the context of the oceans' hydrography, including nutrient (C, N, P, Si) cycling. Therefore, the same care in sampling and sample processing of ancillary parameters must be included in GEOTRACES protocols to ensure the best possible precision and accuracy. The Global Ocean Ship-Based Hydrographic Investigations Program (GO-SHIP) has a hydrography manual with detailed procedures for sampling, analyses, and data processing of water column hydrography (salinity, temperature, depth/pressure via CTD), dissolved oxygen (CTD sensor and bottle), and nutrients that should be followed to insure accurate and precise hydrographic data (http://www.go-ship.org/HydroMan.html; cited as Hood et al., 2010). In addition to the basic water column hydrographic parameters of salinity, temperature, and depth, as well as in situ measurements of fluorescence, transmissometry (See Optics Section VIII), and oxygen concentrations, Table 1 lists GEOTRACES ancillary parameters (and suggested methods of determination) for discrete (depth profile) samples that should be determined on all cruises. It should be noted that these protocols assume the use of "rosette" sampling devices, but if contamination-prone TEIs are sampled with single sampling bottle methods (e.g., GO-FLO bottle hung on Kevlar cable and triggered with a plastic messenger), special care must be taken with determining its depth. In addition to the use of wire out and angle measurements, and salinity and nutrient data compared to that from the conventional CTD/rosette, the use of depth/pressure recorders mounted on the bottles should be considered.

The JGOFS Report 19 sections that include pigments and POC/PON (Appendix 2) and PICES Report 34, DOC/DON section (Appendix 3) are included at the end of this

document. Modified Report 19, Report 34, and the publications by Hood et al. (2010) and Parsons et al. (1984) cover all recommended procedures for sampling, sample processing/storage, and analyses for hydrography and ancillary data for GEOTRACES cruises. The GO-SHIP collection is particularly relevant to GEOTRACES in that it contains all the recommended procedures used in the CLIVAR Repeat Hydrography Program. However, more accurate and precise determinations of ancillary parameters are encouraged; the methods in Table 1 are capable of the best performance at the time of writing (2014).

Table 1. Ancillary Parameters and Recommended Methods for GEOTRACES Cruises

Parameter	Method	Detection Limit	Reference
Salinity	Conductivity	NA (not applicable)	Hood et al., 2010
Oxygen	Manual or automated Winkler	1 μmol l^{-1}	Hood et al., 2010
Ammonium	Automated colorimetric	0.1 μmol l^{-1}	Parsons et al., 1984 Hood et al., 2010
Nitrite	Automated colorimetric	0.1 μmol l^{-1}	Hood et al., 2010
Nitrate	Automated colorimetric	0.1 μmol l^{-1}	Hood et al., 2010
Phosphate	Automated colorimetric	0.03 μmol l^{-1}	Hood et al., 2010
Silicate	Automated colorimetric	0.4 μmol l^{-1}	Hood et al., 2010
Pigments	Fluorometry and HPLC	NA	JGOFS Report 19
DOC/DON	Oxidative Combustion	NA	PICES Report 34
POC/PON	Oxidative Combustion	NA	JGOFS Report 19

Hood, E.M., C.L. Sabine, and B.M. Sloyan, eds. 2010. *The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines*. IOCCP Report Number 14, ICPO Publication Series Number 134. Available online at http://www.go-ship.org/HydroMan.html

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon, Oxford, 173 pp.

IV. Radioactive Isotopes

A. Protocols for ^{230}Th and ^{231}Pa

There is not a unique sampling and analytical procedure that can be recommended, so a range of qualified options is presented.

1. Analytical instrument

The most widely used instruments for seawater analysis are sector-field ICP-MS (multi or single collector; Choi et al., 2001; Shen et al., 2002) and TIMS (Shen et al., 2003). ICP-MS is increasingly the instrument of choice because of higher sample throughput.

2. Volumes required

The volume required for analysis of dissolved ^{230}Th and ^{231}Pa range from a few liters (Shen et al., 2003) to 15-20 liters (Choi et al., 2001). As a rule of thumb, the volume required to analyze suspended particles is 5 times larger for ^{230}Th (10-100L) and 20x larger for ^{231}Pa (40-400L). The volume required for analysis bears significantly on sampling methods (for particles) and sample processing (for dissolved).

There are several options at each step of the procedure. This provides flexibility, but will necessitate careful intercalibrations.

3. Sampling

3.1 Dissolved

3.1.1 Sampling
Niskin bottles with epoxy-coated stainless steel springs are applicable for radioisotopes (Th and Pa). If the volume required is 10-20 L, dedicated radionuclide hydrocasts may be necessary.

3.1.2 Sample Filtration
Samples for operationally-defined dissolved Th and Pa should be filtered. Filtration using capsule filters, preferably 0.8 µm/0.45 µm Acropak® 500 filters, is most feasible for large-volume samples. Different groups use different pre-cleaning methods for these capsules and there are a variety of protocols available. The capsules can be cleaned with HCl, 1.2 M, and rinsed with and stored in Milli-Q water. In the field it is recommended that the capsules be flushed with 1 L seawater prior to first use, and then 10 capsule volumes between casts. This is experience derived from the Intercalibration Cruises 1 and 2. In general, all seawater samples should be processed as quickly as possible to avoid loss of dissolved Th and Pa by absorption on sampling bottle (e.g., Niskin) walls. If membrane filtration (i.e., to keep the particles) is being used, at the time this document was written there is no evidence that one type of membrane filter is preferable to another.

However, quartz/glass fiber filters are not recommended as dissolved Th and Pa are likely to adsorb to these materials.

3.1.3 Sample container rinses

There is no evidence that dissolved Th and Pa concentrations are compromised by filling acid-cleaned sample containers directly, without rinsing. Nevertheless, rinsing of each sample bottle with sample water is preferable.

3.2 Particles

Results from the GEOTRACES Intercalibration exercise indicate that most labs are unable to measure particulate ^{230}Th and ^{231}Pa concentrations in particles filtered from standard sample bottles (e.g., volumes of 10 to 20 liters). Analytical sensitivity of current instrumentation is such that larger samples are generally required, thus necessitating the use of in situ pumps to collect samples for particulate ^{230}Th and ^{231}Pa concentrations (see Section IV.B.1). Ideally, membrane filters used with in situ pumps to collect samples for particulate Th and Pa will be matched with the membrane filters used to collect samples for analysis of dissolved Th and Pa.

4. Sample Processing

Filtered seawater samples must be stored in acid-cleaned high/low density polyethylene (HDPE or LDPE) or polycarbonate containers. The GEOTRACES Intercalibration exercise showed that bottle blanks can be a problem for Th and Pa, and these blanks must be quantified for each isotope. In previous studies, filtered seawater samples have either been acidified, spiked and pre-concentrated at sea, or acidified and shipped to the home laboratory for spiking and pre-concentration. For larger volumes, "at sea" processing is often the method of choice. Smaller samples can more easily be shipped to home institutions. The advantages of "at sea" processing are: (1) lower risk of ^{230}Th and ^{231}Pa loss by absorption on the walls of the storage container, and (2) avoids shipping of large quantity of seawater. The advantages of "on land" processing are: (1) avoids shipping and handling of radioisotopes at sea; (2) requires less space and personnel on-board; (3) allows more accurate determination of the sample volume; and (4) loss of ^{233}Pa spike by decay during the cruise/shipping and storing the samples prior to measurement is not a problem.

4.1 Acidification

As soon as possible after collection, samples for dissolved Th and Pa should be acidified with HCl to a pH < 2.0 (target 1.7 to 2.0). It is recommended that 6M Hydrochloric Acid is used for sample acidification. It is much easier to commercially transport seawater acidified with Hydrochloric Acid than Nitric Acid. Seawater acidified with Hydrochloric Acid to pH~2 is not considered "hazardous materials", while the same samples acidified with Nitric Acid are considered "hazardous materials". Dilution of the Hydrochloric Acid to 6M reduces irritating fumes from the reagent bottle, which, in turn, allows sample acidification without the need for a fume hood. Following acidification, sample integrity

should be protected by covering the cap and thread with Parafilm® or similar plastic wrap. Double plastic bags around each bottle/container are recommended. Labeling of samples should be made with a specific GEOTRACES # for each sample and depth.

4.2 Sample volume or weight

A variety of approaches have been used to record sample weight or volume, and the literature should be consulted for the best one to use in a particular cruise (e.g., open water vs. in the ice). Some labs use an electronic balance to weigh samples at sea, using a simple computer algorithm to average weights on the moving ship until a stable reading is obtained. Other labs weigh samples after they are returned to the home institution.

4.3 Spiking

If spiking is done on board it should be done by pre-weighed spikes and thorough careful rinsing of the spike vial, disposing multiple rinses into the sample container.

4.3.1 ^{233}Pa spike preparation

There are two ways for producing ^{233}Pa: (1) by milking ^{237}Np (2) by neutron activation of ^{232}Th.

^{237}Np milking: the ^{233}Pa spike must be checked for ^{237}Np bleeding. Preferentially by Mass spectrometry (2nd cleaning step may be needed). Advantages: Lower ^{231}Pa blank; Lower ^{232}Th contamination

^{232}Th irradiation: Advantages: Large quantities (1mCi) can be easily produced Disadvantages: ^{232}Th contamination precludes its measurement in the same sample. ^{231}Pa is produced by neutron activation of ^{230}Th traces in the ^{232}Th target. ^{231}Pa contamination can be kept low by preparing a new spike before the cruise to minimize the ^{231}Pa/^{233}Pa in the spike. It can also be precisely quantified by measuring ^{231}Pa/^{233}Pa in the spike before ^{233}Pa decay. Typically, ^{231}Pa blanks range from ~10% in surface water to ~1% in deep water

4.4 Pre-concentration

Pre-concentration of ^{230}Th and ^{231}Pa is done by adsorption on a precipitate formed in seawater (scavenging), which is then recovered by decantation and centrifugation and returned to the home laboratory for ^{230}Th and ^{231}Pa purification by ion-exchange. Several scavenging methods have been used: (1) Fe hydroxide; (2) Mg hydroxide; (3) MnO_2.

Fe hydroxide: 0.05 ml $FeCl_3$ (50 mg Fe/ml; cleaned by extraction in isopropyl ether) is added per liter of acidified seawater with the ^{229}Th and ^{233}Pa spikes. The spiked seawater is left to equilibrate for at least 24 hours. Thereafter, ammonium hydroxide (ultraclean) is added to bring the pH to 8.5-9 and precipitate $Fe(OH)_3$. After 12-24 hours of settling, most of the supernatant is removed and the precipitate is centrifuged.

Mg hydroxide: Seawater is acidified, spiked and left to equilibrate for 24 hours. Thereafter, concentrated NH_4OH (ultraclean) is added to precipitate $Mg(OH)_2$. The precipitate is decanted and transferred into 250ml polyethylene bottles. 7M HNO_3 is then slowly added to reduce the volume of precipitate.

Mn dioxide: Seawater is spiked and left to equilibrate for 12 hours. Thereafter, a few drops of ultraclean, concentrated ammonium hydroxide are added, with 0.75 mg/L $KMnO_4$ and 2mg/L $MnCl_2$ (Rutgers van der Loeff and Moore, 1999). After 24 hours, the MnO_2 is filtered on 1μm polycarbonate filter.

Sample storage: We are not yet sure how long we can store filtered acidified samples for subsequent spiking, pre-concentration and analysis without losing ^{230}Th or ^{231}Pa on the walls of the containers. Samples collected during the first GEOTRACES intercalibration cruise (July, 2008), acidified to pH 1.7, and analyzed over a period of 1.5 years showed no drift in concentrations of dissolved Th or Pa. NOTE: For samples stored this long it is necessary to make corrections for ingrowth of dissolved ^{230}Th and ^{231}Pa due to radioactive decay of dissolved uranium. The different scavenging methods ($Fe(OH)_3$ vs. $Mg(OH)_2$ vs. MnO_2) still have to be compared.

5. Spike calibrations

GEOTRACES should agree on a primary Th standard (e.g. NIST SRM 3159) to calibrate the ^{229}Th spikes used by different laboratories. In the meantime, ^{229}Th spikes used in GEOTRACES cruises should be archived for future intercalibrations.

Calibration of ^{233}Pa is best done by measuring the ingrowth of ^{233}U by isotope dilution with a ^{236}U standard. GEOTRACES should agree on a primary U standard (e.g. NIST CRM-145) to calibrate the ^{236}U standards used by different laboratories. In the meantime, the ^{236}U standards used to calibrate ^{233}Pa spikes for GEOTRACES cruises should be archived for future intercalibrations.

6. Precision of measurements

Precision of measurements conducted on each cruise are best documented by analyzing a set of replicate seawater samples (3 to 6) in the mid-concentration range during each cruise (see Section IIA. above).

7. References

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Shen, C.-C., Cheng, H., Edwards, R. L., Moran, S. B., Edmonds, H. N., Hoff, J. A., Thomas, R. B. 2003. Measurement of attogram quantities of ^{231}Pa in dissolved and particulate fractions of seawater by isotope dilution thermal ionization mass spectroscopy. Anal. Chem., 75, 1075-1079.

B. Protocols for ^{234}Th

1. Particulate ^{234}Th Sampling

In-situ filtration allows the collection of large volume size-fractionated marine particles from the water column. Commercially available battery-operated in-situ pumping systems (e.g., McLane, Challenger) can be deployed simultaneously at multiple depths to collect particulate ^{234}Th samples.

1.1 Filter Type

No single filter type can accommodate all the different measurements needed during GEOTRACES. Quartz fiber filters (Whatman QMA) and polyethersulfone (Pall Supor) filters were extensively tested during the Intercalibration Cruises. QMA filters have a nominal pore size of 1μm, have a long track record of use in in-situ filtration, have the best flow characteristics, and result in even particle distribution. QMA filters can be pre-combusted for particulate organic carbon (POC) analyses. Paired filters (two back to back filters) can be used so that the bottom filter can act as a flow-through blank. QMA filters are found to have significant flow-through blanks due to adsorption especially when low sample volumes are filtered.

If sampling constraints makes it necessary to use a plastic filter, then hydrophilic polyethersulfone (PES) membrane filters (e.g., Pall Supor) have the best blank and flow characteristics of the available plastic filters, and are thus currently the plastic filter of choice. The biggest drawbacks for this type of filter is the poor (heterogeneous) particle distribution observed on deep (>500 m) samples. The particle distribution on the filter worsens with depth. However the ^{234}Th absorption blanks for this filter type is negligible.

For large (>51 μm) particle collection, 51μm polyester mesh (e.g., 07-51/33 from Sefar Filtration) is a good option. For ^{234}Th analysis of this size fraction, we recommend rinsing the prefilter onto a 25 mm silver membrane filter using filtered seawater.

1.2 Pump deployment and handling

The preliminary results from the US GEOTRACES intercalibration cruises indicate particle loss from the >51 μm size fraction with increasing flow-rate. We recommend using an initial flow rate of around 0.04 L/cm^2/min (equivalent to 6 L/min on a McLane pump) so as to strike a balance between deployment time and particle loss. However if other pumping systems do not allow user to control the initial flow rate, care should be taken to maintain the same initial flow rate during all their deployments.

During recovery the pumps should be kept vertical as much as possible. Once the pump is on board, disconnect the filter holders from the pump and attach vacuum lines to filter holders to evacuate residual seawater in the filter holder headspace.

2. Total ^{234}Th sampling

Comparison of small volume ^{234}Th method between 12 different labs produced consistent results. The total sample volume used varied between 2L to 8L depending on individual labs. All the labs followed their own version of the analytical method similar to those outlined in Pike et al. (2005) and Rutgers van der Loeff et al. (2006). The addition of a thorium spike to each sample makes it easier to quantity ^{234}Th loss due to leakage, filter breakage or bad precipitation chemistry. So, it is important to add a recovery spike to each sample, however care should be taken to add a precise amount using a well calibrated pipette (we recommend an electronic repeater pipette) and giving the samples adequate time to equilibrate with the spike. No comparison was made between large volume MnO_2 impregnated cartridge method and small volume technique, but given the fact that the majority of the labs worldwide have adopted the small volume technique with great success, we would recommend this method.

3. General Considerations for ^{234}Th

The method of choice for sampling and analysis of ^{234}Th will depend on the environment and on the questions to be answered. We refer to the recent review of Rutgers van der Loeff et al. (2006) and the methodological papers on which this is based (Buesseler et al., 2001; Buesseler et al., 1992; Cai et al., 2006; Pike et al., 2005; Rutgers van der Loeff and Moore, 1999). For direction in choosing the appropriate ^{234}Th procedure, a decision flow chart was developed by Rutgers van der Loeff et al. (2006). Here are some additional recommendations from that paper for the measurement of dissolved, particulate, and total ^{234}Th:

1. The validity of the U–Salinity relationship is only appropriate for estimating dissolved ^{238}U in the open ocean, where waters are well oxygenated and removed from freshwater input. In other regimes, i.e. continental shelves, estuaries, marginal or semi-closed seas, and suboxic/anoxic basins, the U concentration must be measured.

2. Beta counting of filters can be well calibrated only if a) the loading is small enough that self-absorption of ^{234m}Pa is absent or b) the loading is constant and can be reproduced

with a standard or c) the filter can be prepared to form a homogeneous source of radiation (as in the case of a multiply folded filter) which allows the correction technique described in Section 3.2 of Rutgers van der Loeff (2006). In other cases there is no way to correct for self-absorption of the sample and non-destructive beta counting is not a viable option.

3. Calibration of detectors for various sample types remains a complex issue. In order to standardize the use of "home-made" standards (such as the examples described in section 3.5 of the paper), it would be extremely useful to provide the scientific community with a standard operational procedure. A relatively easy method that can be followed by any lab is to process a natural sample of aged acidified filtered (sea)water in which ^{234}Th and ^{238}U have reached secular equilibrium and ^{238}U activity has been determined (by alpha spectrometry or ICP-MS). Alternatively, one of the best standards for the inter-calibration of ^{234}Th techniques is to use filtered aged deep-ocean water where the activity of ^{238}U is precisely known and the colloidal ^{234}Th significantly lower than that found in surface waters. Care must be taken in storing that water, e.g. by acidifying it immediately after collection, to prevent Th absorption onto container walls. Aliquots of this water would then be neutralized to seawater pH prior to use.

4. References

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Rutgers van der Loeff, M.M. and Moore, W.S., 1999. Determination of natural radioactive tracers. Chapter 13. In: K. Grasshoff, M. Ehrhardt and K. Kremling (Editors), Methods of Seawater Analysis, third Edition. Verlag Chemie, Weinheim, pp. 365-397.

C. Protocols for ^{226}Ra and ^{228}Ra Measurements in Sea Water

Because of the wide range of activities present in the ocean and the different uses that will be made of the data, each procedure should be researched adequately before its adoption. The procedures we report are not rigid, but are intended as a guide to the methods that are available. In most cases the procedure adopted may be somewhat modified from the specific procedures outlined here.

Historically, ^{226}Ra in seawater has been measured by capturing its decay product, ^{222}Rn, and measuring this by alpha scintillation (Broecker, 1965). On GEOSECS (1971-1976) 20 L water samples were returned to shore labs, where ^{222}Rn was allowed to partially equilibrate with ^{226}Ra in a glass bottle. The ^{222}Rn was extracted and measured. This technique was plagued by variable "bottle blanks" which varied with the type or lot of glass bottles used for the extraction and caused inconsistent results among labs. On TTO (Transient Tracers in the Ocean, 1981-1989), ^{226}Ra was extracted from 20 L water samples at sea by passing the water through a column containing MnO_2-coated fiber (Mn-fiber; Moore 1976). This eliminated shipping large volumes of water and considerably reduced the bottle blank (Moore et al., 1985).

During the Atlantic GEOSECS cruise ^{228}Ra was measured by extracting radium from large volume (200-600 L) sea water samples by $Ba(Ra)SO_4$ precipitation followed by sample cleanup and extraction of partially equilibrated ^{228}Th using alpha spectrometry (Li et al., 1980). This large volume sample was used to measure the ^{228}Ra/^{226}Ra activity ratio. This ratio was multiplied by the ^{226}Ra activity to determine ^{228}Ra activity. On Pacific and Indian Ocean GEOSECS cruises, large volume samples were extracted onto Mn-fiber either on deck or in situ followed by sample cleanup and measurement of partially equilibrated ^{228}Th (Moore 1976). On TTO water samples (270 L) were first stripped of CO_2 for ^{14}C measurements and after pH adjustment, radium was extracted onto Mn-fiber (Moore et al., 1985). More recently workers have demonstrated that radium may be recovered essentially quantitatively (97±3%) from 200 – 800 L sea water samples by passing the water through a column of Mn-fiber at a flow rate of <1 L/min (Moore, 2007), so a single sample can be used for both isotopes.

Recently, there have been efforts to measure ^{226}Ra and ^{228}Ra by ICP-MS and TIMS (Foster et al., 2004; Olivier et al., 2008). These techniques offer the promise of smaller sample size and increased precision. Currently only a few labs are working with open ocean samples. We encourage additional labs to take the challenge and develop reliable techniques.

There is a fundamental trade-off in selecting a method for the analysis of radium in seawater: sample volume vs. time (i.e., the larger the sample volume, the less time is required for an analysis). The procedure requiring the smallest volume (2-5 L) samples is alpha spectrometry, but considerable time for sample preparation and counting is required. Alpha scintillation counting of 20 L samples is the standard procedure for ^{226}Ra measurement in seawater, but other Ra isotopes cannot be measured by this technique. Larger volume samples (100-1000 L) and patience are required to measure ^{228}Ra in open

ocean samples via ^{228}Th in-growth. For high activity estuarine or coastal samples, gamma spectrometry offers an easy method of measuring ^{226}Ra and ^{228}Ra and delayed coincidence scintillation counting can be used to measure ^{223}Ra and ^{224}Ra in the same sample.

1. Alpha scintillation measurement of ^{226}Ra and ^{222}Rn

The most commonly used method for measuring ^{226}Ra and ^{222}Rn in seawater was first developed by Broecker (1965). This procedure begins with a 15-20 L sample collected in a 30 L Niskin bottle. If ^{222}Rn is to be measured, the water is drawn into an evacuated 20 L glass bottle (wrapped with tape or enclosed in an appropriate container in case of breakage). Containers made from 20 cm diameter plastic pipe are also used (Key et al., 1979). Helium is used to transfer the Rn from the sample to a glass or stainless steel trap cooled with liquid nitrogen or a charcoal-filled trap cooled with dry ice (Broecker, 1965; Key et al., 1979; Mathieu et al., 1988). The helium may be repeatedly circulated through the sample and trap using a diaphragm pump, or passed through once and vented. Traps to remove water vapor and CO_2 are usually incorporated into the system. The Rn is transferred from the trap to a scintillation cell by warming the glass trap to room temperature or warming the charcoal-filled trap to 450°C.

The scintillation or Lucas cell (Lucas 1957) is made by coating the inside of a Plexiglas, quartz or metal cell with silver-activated zinc sulfide (ZnS[Ag]). After transferring the Rn to the cell, it is stored for 1-2 hours to allow ^{222}Rn daughters, ^{218}Po, ^{214}Pb, ^{214}Bi, and ^{214}Po to partially equilibrate. Alpha decays from ^{222}Rn, ^{218}Po, and ^{214}Po cause emissions of photons from the ZnS[Ag]. These are converted to electrical signals using a photomultiplier tube (PMT) attached to the cell and routed to a counter.

After the ^{222}Rn measurement, the sample in the same container may be used for ^{226}Ra measurement by ^{222}Rn emanation. In this case the container is sealed for several days to several weeks to allow ^{226}Ra to generate a known activity of ^{222}Rn. Then ^{222}Rn is again stripped from the sample and measured using the procedure outlined above. In addition to the factors considered in the excess ^{222}Rn calculation, the fraction of equilibrium between ^{222}Rn and ^{226}Ra must be included to calculate the ^{226}Ra activity.

Schlosser et al. (1984) modified this technique to make high precision measurements of ^{226}Ra in seawater. They degassed the sample by boiling 14 L for 45 minutes and transferred the ^{222}Rn to an activated charcoal trap at -78°C. The charcoal trap was warmed to 450°C and the ^{222}Rn transferred to a proportional counter with a mixture of 90% argon and 10% methane. Details of the proportional counter and associated electronics are given in Schlosser et al. (1983).

The calculation of the excess Rn activity of the sample must include (1) a decay correction from the time the sample was collected until the mid-point of the counting time, (2) the fraction of equilibrium attained with the Rn daughters before counting, (3) the efficiency of the detector, (4) the background of the detector, (5) the blank associated with the sample container and extraction system. These calculations and the errors

associated with the measurements have been discussed by Lucas and Woodward (1964), Sarmiento et al. (1976), and Key et al. (1979). The best precision obtained for the scintillation counting procedures is approximately ±3%. Schlosser et al. (1984) claim a precision of ±1% for the proportional counting technique.

In some cases it is more practical to concentrate ^{226}Ra from the sample at sea to reduce the blank and avoid the problem of shipping large samples of water. In this case ^{226}Ra may be quantitatively removed using a small column (2 cm diameter x 10 cm long) containing a few grams of Mn-fiber (Moore 1976). If the pH of the sample was lowered for other purposes, e. g. ^{14}C extraction, it must first be readjusted to ~7. The sample is passed through the fiber at a flow rate of 0.1-0.3 L/min and discarded after the volume is recorded. In the lab the ^{226}Ra may be removed from the Mn-fiber using HCl, or the ^{222}Rn may be determined by direct emanation from the Mn-fiber. In either case a gas system is used to transfer the Rn to a scintillation cell as described above. Moore et al. (1985) determined that the precision of the Mn-fiber extraction technique followed by alpha scintillation counting of ^{222}Rn is ±3%.

A variation on the scintillation technique for ^{226}Ra measurement was suggested by Butts et al. (1988). After concentrating the ^{226}Ra on Mn-fiber, the fiber was partially dried, placed in a glass equilibrator, flushed with nitrogen and sealed to allow ^{222}Rn to partially equilibrate. The equilibrator was connected directly to an evacuated Lucas cell to transfer a fraction of the ^{222}Rn to the cell. The fraction of ^{222}Rn transferred was calculated by measuring the volumes of the equilibrator and Lucas cell and applying the gas law. Butts et al. (1988) demonstrated that this passive technique was much simpler and faster than quantitatively transferring the ^{222}Rn, and gave comparable results for samples containing 8-75 dpm ^{226}Ra.

Alternatively, ^{226}Ra collected on Mn-fiber can be measured via its daughters, ^{222}Rn and ^{218}Po by a radon-in-air monitor, RAD7 (Kim et al., 2001). The Mn-fiber is sealed in a column for several days to weeks and then connected to a closed loop with the RAD7. The circulating air carries ^{222}Rn and ^{220}Rn to the detector chamber where their polonium daughters are measured by alpha-spectrometry.

Obviously, great care must be taken to assess the blank associated with any Ra measurement. Glass containers are a source of Rn contamination that can be difficult to assess accurately when low levels of ^{226}Ra are being determined by ^{222}Rn in-growth. Ba salts used to precipitate Ra from solution (discussed later) can contribute significant ^{226}Ra and ^{228}Ra blanks. We suggest screening kg lots of Ba salts by gamma-ray spectrometry to help select the ones with lowest Ra contamination.

2. Measurements of ^{226}Ra and ^{228}Ra by Ba(Ra)SO_4 precipitation from small volume (20 – 40 L) samples

The precipitation of radium as Ba(Ra)SO_4 is a quantitative method for the determination of ^{226}Ra and ^{228}Ra by gamma-spectrometry. Prerequisite to this is the slow and complete precipitation of radium in the presence of a barium carrier solution from a known volume

of water, thereby making use of the natural sulfate content. $BaCl_2$ solutions are prepared prior to a cruise/campaign as pre-weighed 100ml aliquots, following the method described by Rutgers van der Loeff and Moore (1999). This method takes advantage of the low solubility product of $BaSO_4$ and the chemical similarity of barium and radium. Efficiency is determined gravimetrically through $BaSO_4$ recovery.

2.1 Sampling procedures

- Use a pre-weighed container, note empty weight in log sheet to work out sample volume
- Rinse container twice with sample water
- Fill 20-40 L of sea water in container
- Weigh the container, note total weight in log sheet
- Place a magnetic stirring bar (about 5 cm in length) on the bottom of the container and put container on magnetic stirrer
- Place a syringe or small column, equipped with a tip at the end, over the container, fill with deionised water and check dripping velocity; adjust by squeezing tip more or less; 100 ml should roughly take 20 min to percolate through
- Fill one pre-weighed $BaCl_2$ aliquot in syringe and let drip into sample
- Rinse bottle of aliquot, including lid, several times and add to syringe
- Rinse syringe several times after aliquot has passed through
- Let the sample on the stirrer for another 60-90 min; white clouds of $BaSO_4$ should start forming after 15 min
- Stop magnetic stirrer, remove and rinse magnetic stirring bar
- Close container and set aside for 2-3 days to allow $BaSO_4$ crystals to settle; knock on container walls after about a day to remove air bubbles
- Concentrate crystals by repeated decantation and transfer to smaller containers (20 L -> 5 L, maybe 1 L), allow time for crystals to settle in-between, remove air bubbles from container walls; finally concentrate crystals in falcon tube by centrifugation
- Clean containers, syringe and magnetic stirring bar mechanically with sponge or paper; take especially care of corners and taps, give rinse with diluted HCl and deionised water
- Store syringe in plastic bag between precipitations
- To be done in the home lab:
    - Wash precipitate with deionised water and centrifuge; repeat this step 3-5 times until all interfering ions are washed out
    - Dry crystals in glass beakers
    - Weigh crystals into vials or plastic tubes suitable for gamma spectrometry; samples should be sealed with for example Parafilm.

2.2 Additional remarks

- The use of clear containers (polycarbonate) facilitates recovery of the white crystals and subsequent cleaning.
- Empty weight of the containers should be known and marked on lid before the cruise.
- Weighing on a moving ship can introduce an error; yet even under rough conditions it rarely exceeds 100 g for 20 L when carefully carried out.
- Surface water should be pre-filtered before precipitation as the particulate matter will alter the recovery which is determined gravimetrically.
- Sampling can be done either on station or on a sailing ship. In the latter case, it is recommended to split the sampling in 3 x 7 L, evenly distributed over the sampling transect. Note sample points in log sheet.
- Addition of extra SO_4^{2-} ions might become necessary for samples of lower salinity (Baltic Sea, estuaries). Use e.g. diluted sulphuric acid.
- Water profiles: three 12 L Niskin bottles are necessary for one depth. If station time is restricted, less water can be used (which must be compensated by longer gamma-counting times). Add extra SO_4^{2-} ions when using only 12 L of water.
- If samples cannot be precipitated straight after sampling, immediately acidify sample to pH <2 with 6M HCl.
- When filling the dried precipitates into counting tubes, care should be taken to apply the same pressure for all samples. Similarity in density and geometry is one prerequisite for the successful calibration of the samples.
- Sealing of the dried $BaSO_4$ precipitates is more important to prevent the loss of sample material than the escape of Radon. Radium is tightly bound in the crystal lattice of $BaSO_4$. If any, only a small fraction of ^{222}Rn will be able to leave the sample within its short half-life (<2%; Michel et al., 1981).
- Care should be applied to the preparation of a calibration source with a certified ^{226}Ra and ^{228}Ra activity. This is best done by precipitation of a spike solution of known activity with a $BaCl_2$ aliquot. This will result in a calibration source of same matrix, geometry and density as the samples (Reyss et al.. 1995). Ideally, three to five sources are prepared and the samples calibrated against the mean of them.

3. Measurement of ^{228}Ra via ^{228}Th in-growth

Open ocean waters have low activities of ^{228}Ra (<2 dpm/100 L). To measure ^{228}Ra in these waters, large volume samples and sensitive counting techniques are required. Most measurements are made by concentrating the Ra from 100-400 L samples, separating and purifying the Ra, allowing ^{228}Th to partially equilibrate with ^{228}Ra, extracting the ^{228}Th, and measuring its activity in an alpha spectrometer using ^{230}Th as a yield tracer. A separate sample of the same water is measured for ^{226}Ra activity using the ^{222}Rn emanation technique.

Water samples are obtained from a large volume collector such as a 270 L Gerard barrel, by tripping multiple Niskin bottles per depth on a CTD rosette, by pumping the sample

into a processing tank on the ship, or by concentrating Ra in situ on Mn-fiber or Mn-cartridges. The in situ extraction may utilize a submersible pumping system to force water through an extraction column containing the Mn-coated media, or by sealing Mn-fiber in a mesh bag and exposing it to water at a certain depth (Moore, 1976; Bourquin et al., 2008). This large volume sample is used to determine the $^{228}Ra/^{226}Ra$ AR of the water.

Radium is removed from Mn-fiber by leaching with a mixture of hot hydroxylamine hydrochloride and HCl. This may be done in a suitable beaker on a hotplate followed by vacuum filtration of the solution and thorough washing of the fiber. Leaching may also be accomplished in a Soxhlet extraction apparatus. The Mn-fiber is packed into a glass thimble in the extraction vessel and covered with concentrated HCl for several hours. The HCl reduces Mn^{4+} to Mn^{2+} and releases the adsorbed Ra. Dilute (6M) HCl is added to the extraction vessel to induce siphoning to the boiling flask and the system is refluxed until the fiber in the extraction vessel is clear (2-4 hours). During the extraction the solution should stabilize at close to 20% HCl at 108°C.

The extract containing Ra and Mn is filtered and mixed with 10 mL of saturated $Ba(NO_3)_2$ followed by 25 mL of 7M H_2SO_4 to coprecipitate Ra with $BaSO_4$. Warming the extract to near boiling produces larger particles of the precipitate and facilitates its separation.

After precipitating $Ba(Ra)SO_4$, the precipitant is washed with 3M HCl and water to remove all remaining Mn and dried. The $Ba(Ra)SO_4$ is converted to $Ba(Ra)CO_3$ by fusing it with a mixture of K_2CO_3 and Na_2CO_3. The solid is washed with water to remove all traces of sulfate and dissolved in HCl. Fe carrier is added and precipitated with ammonia to remove Th. After removing all traces of $Fe(OH)_3$ from the solution, Ba and Ra are coprecipitated with K_2CO_3 solution and the precipitate stored for 5-20 months to allow ^{228}Th to partially equilibrate. Approximately 30% equilibration is attained in 1 year. The $Ba(Ra)CO_3$ precipitate is dissolved in HCl and the solution is spiked with ^{230}Th. After adjusting the pH to 1.5, Th is extracted into a TTA-benzene solution and this solution is mounted on a stainless steel disk. The $^{228}Th/^{230}Th$ AR is determined by alpha spectrometry and ^{228}Th is calculated from the activity of the spike. The initial ^{228}Ra activity of the sample is calculated by multiplying the measured ^{228}Th activity by the reciprocal of the fraction of $^{228}Th/^{228}Ra$ equilibrium and this result is decay corrected for the time elapsed from sample collection to the initial purification and precipitation of $Ba(Ra)CO_3$. The solution containing the Ra is measured for ^{226}Ra using the ^{222}Rn scintillation technique to calculate the $^{228}Ra/^{226}Ra$ AR of the water sample. The activity of ^{228}Ra in the water is obtained by multiplying this AR by the ^{226}Ra activity determined from a separate sample of the same water. The overall precision of this technique, which includes a ±3% error on the ^{226}Ra measurement is ±5% (Moore et al., 1985).

Orr (1988) evaluated various methods of measuring ^{228}Ra in open ocean samples and concluded that results could probably be obtained more quickly and with equal precision using beta-gamma coincidence spectrometry (McCurdy and Mellor 1981) or liquid

scintillation alpha spectrometry (McKlveen and McDowell 1984). However, these techniques have not been applied to open ocean samples.

Procedures for preparing Mn-fiber are detailed in Moore (1976) and Rutgers van der Loeff and Moore (1999). Currently several groups are exploring new media for extracting Ra from seawater. These include wound acrylic and cellulose cartridges with coatings of MnO_2. The aim is to provide a larger surface area for Ra adsorption, thus allowing higher flow rates. After tests of these media are complete, the results will be added to the protocols.

4. Gamma spectrometry measurement of ^{226}Ra and ^{228}Ra

This technique is applicable to samples containing relatively high activities of ^{226}Ra and ^{228}Ra (>5 dpm) due to the low detection efficiency of most germanium detectors (Moore 1984). Generally, 100 L samples are required for ^{226}Ra measurements. However, recent advancements in the production of large, high efficiency detectors has extended the technique to 20 L open ocean samples (Reyss et al., 1995; Schmidt and Reyss, 1996). ^{228}Ra in estuarine, coastal and large volume surface ocean samples is also measured using this technique; however, it is not applicable to ^{228}Ra measurements in the ocean interior unless a high efficiency detector is available or Ra is preconcentrated from a suitably large (>500 L) volume of seawater.

The Ra may be quantitatively extracted from a known sample volume on Mn-fiber or simply concentrated on Mn-fiber from an unknown volume. In the latter case the gamma technique is used to establish the ^{228}Ra/^{226}Ra AR and a separate small volume sample is processed to quantitatively measure ^{226}Ra. Alternatively, the Ra may be coprecipitated with $BaSO_4$. In this case the recovery may be determined gravimetrically (Reyss et al., 1995).

If the Mn-fiber sample is to be used to quantitatively determine Ra activity, all extractions and purification must be quantitative. This can be accomplished by extracting the Ra on a column of Mn-fiber at a flow rate of 1 L min-1 followed by the Soxhlet extraction apparatus described above. This procedure ensures the complete removal of the radium from the fiber into a relatively small volume of acid. After precipitating the $Ba(Ra)SO_4$, the precipitant is washed and concentrated into a small vial. The vial is stored for 3-4 weeks to allow ^{228}Ac to equilibrate with ^{228}Ra and ^{222}Rn and daughters to equilibrate with ^{226}Ra.

An alternative to leaching is ashing the sample to provide a sufficiently small amount of ash to be counted in a bore-hole gamma detector. Ashing is done at 820° C for 16 hours in a covered 250 mL ceramic crucible (Charette et al., 2001). Thirty grams (dry wt.) fiber is reduced to ~3-4 g of ash. The ash is then homogenized with a spatula, placed in a counting vial, and sealed with epoxy for >3 weeks prior to counting to allow for in-growth of the ^{214}Pb daughter. Alternatively, the ashing can be accomplished in a crucible of stainless steel foil. After ashing the foil is compressed into a small pellet to seal against ^{222}Rn loss (Dulaiova and Burnett, 2004).

The ^{226}Ra and ^{228}Ra activities of the sample are measured using a germanium gamma ray spectrometer. The detector actually measures gamma ray emissions that accompany the decay of ^{214}Bi and ^{214}Pb (^{226}Ra daughters) and ^{228}Ac (^{228}Ra daughter). There are three prominent gamma emissions commonly used for each Ra isotope. For ^{214}Pb emissions occur at 295 and 352 keV; ^{214}Bi has an emission at 609 keV. For ^{228}Ac emissions at 338, 911 and 968 keV are commonly used. These are not the only peaks that can be used for measurement of these isotopes, but they are the most prominent for most detectors. However if a planar or low energy detector is being used, the 209 keV peak from ^{228}Ac and the 186 keV emission from ^{226}Ra may be more useful than the higher energy peaks, but note that the 186 keV peak overlaps a ^{235}U peak. A problem often encountered in samples with relatively high ^{226}Ra but low ^{228}Ra activities is the shielding of the ^{228}Ra peaks by the increased Compton scattering.

To quantify the signal from the gamma detector, the detector must be calibrated with respect to its efficiency (E) for detecting each gamma emission and the intensity (I) or probability of gamma emission for each decay must be known. In laboratories that measure a variety of gamma-emitting radionuclides, detectors are usually calibrated for detection efficiency with respect to energy using a set of standards of known activity. This E vs. energy calibration curve can be used to determine the E at each energy of interest. The intensity of gamma emission for each peak can be ascertained from the literature. However there are problems with this method for radium measurements. The literature values for I may include a component derived from coincidence summations. The fraction of the summation component measured by the detector is a function of the counting geometry. Differences are observed when the sample is placed near or far from the detector. When germanium crystals with wells are used to measure samples, the literature values for some emission intensities are considerably different from measured values (Moore 1984). Also, the lower energy gamma rays are preferentially absorbed by the sample matrix. The $BaSO_4$ is a strong gamma ray absorber. Therefore, the best way to calibrate a germanium detector for Ra measurement is to prepare standards containing ^{228}Ra and ^{226}Ra in the same matrix and geometry as will be used for samples (including the ashing method described above). For each gamma emission that will be used to calculate the Ra activity, determine a factor that converts counts per minute (cpm) to decays per minute (dpm) or Bq (60 dpm = 1 Bq). This factor is the reciprocal of E x I for each peak of interest.

Peaks of interest in the signal from the germanium detector must be separated from (1) other peaks in the spectrum, (2) background due to impurities in the detector housing and shielding, and (3) scattering of higher energy emissions (Compton scattering). There are a number of computer programs that perform these functions, but they are often not flexible enough to allow the operator to enter individual factors for each peak. For Ra measurement it is best to use two programs, one that only identifies and quantifies the peaks by separating them from other peaks and Compton scattering and another that converts the peaks to Ra activities using the factors and detector backgrounds for each peak. If activities are determined for each of three peaks, a weighted means assessment

can be used to obtain a final result. An excellent program for resolving low activity peaks is HYPERMET (Phillips and Marlow, 1976)

5. Protocols for short-lived radium isotopes: ^{223}Ra, ^{224}Ra

The method of choice for the analysis of ^{223}Ra (half life = 11.4 days) and ^{224}Ra (half life = 3.66 days) is the delayed coincidence technique of Moore and Arnold (1996). Samples are collected in 100-1000 liter tanks. In turbid waters samples are filtered (e.g., 1 µm Hytrex II cartridge). The filtrate is then passed through a column of MnO_2-coated acrylic fiber ("Mn-fiber") at <1 l/min to quantitatively remove radium (Moore et al., 1985). The amount of fiber needed should be adapted to the volume of water sampled, about 15-25 g dry MnO_2-coated fiber (Moore, 1976; Sun and Torgersen, 1998). It is advised to occasionally employ two fiber packages (A and B) in series to check the adsorption efficiency of each fiber package. Preparation of the Mn-fiber is described in Rutgers van der Loeff and Moore (1999).

Each Mn-fiber sample containing adsorbed Ra is washed with fresh water and partially dried by passing compressed air through a vertical tube containing the fiber for 1-3 min, which should then have a water-to-fiber weight ratio of 0.7 to 1.5 (Sun and Torgersen, 1998). The damp fiber is fluffed and placed in a tube connected to the closed loop circulation system described by Moore and Arnold (1996). Helium is circulated over the Mn fiber to sweep the ^{219}Rn and ^{220}Rn generated by ^{223}Ra and ^{224}Ra decay through a 1 L Lucas cell where alpha particles from the decay of Rn and daughters are recorded by a photomultiplier tube (PMT) attached to the scintillation cell. Signals from the PMT are routed to a delayed coincidence system pioneered by Giffin et al. (1963) and adapted for Ra measurements by Moore and Arnold (1996). The delayed coincidence system utilizes the difference in decay constants of the short-lived Po daughters of ^{219}Rn and ^{220}Rn to identify alpha particles derived from ^{219}Rn or ^{220}Rn decay and hence to determine activities of ^{223}Ra and ^{224}Ra on the Mn fiber. The system is calibrated using ^{232}Th and ^{227}Ac standards that are known to have their daughters in radioactive equilibrium and are adsorbed onto a MnO_2-coated fiber. The expected error of the short-lived Ra measurements is 8-14% (Garcia-Solsona et al., 2008).

After the ^{223}Ra and ^{224}Ra measurements are complete, the Mn fiber samples are aged for 2-6 weeks to allow initial excess ^{224}Ra to equilibrate with ^{228}Th adsorbed to the Mn fiber. The samples are measured again to determine ^{228}Th and thus to correct for supported ^{224}Ra. Another measurement after 3 months may be used to determine the ^{227}Ac, which will have equilibrated with ^{223}Ra (Shaw and Moore, 2002).

An alternate technique for measuring ^{224}Ra on the fiber utilizes a commercially available radon-in-air monitor (RAD-7, Durridge) to count ^{220}Rn released from the fiber. This has been described by Kim et al. (2001).

After the short-lived measurements are complete, the Mn fibers may be leached and used for long-lived Ra isotope measurements.

6. Notes on ^{223}Ra and ^{224}Ra measurements

1. Surface seawater supply. When collecting large sample volumes for short-lived radium isotopes the ships' seawater intake may not be appropriate if the pipes have scale containing Mn and Fe precipitates that sorb Th and ^{228}Ra, since all these may be a source of ^{224}Ra and ^{223}Ra. One should test the water from the pipes before relying on its use. A towed fish system such as described in Section 6.2.1 would eliminate this problem.

2. Standards. For the short-lived radium isotope counting via the delayed coincidence counter special care should be taken while preparing the standards from ^{232}Th and ^{227}Ac. Some issues have been described in Dimova et al. (2008) and Scholten et al. (2010). These studies found nearly quantitative adsorption of Th and Ac on Mn-fibers if standards were prepared from seawater.

3. Rinsing. Rinsing the Mn-fiber is very important both before and after sample collection. Since we do not have a very efficient way of rinsing the Mn-fiber after cooking, it has some residual Mn on it that can be washed out before passing the sample through. Ensure that the Mn-fiber is washed especially well before standard preparation.

4. For large volume samples use at least 25 g dry weight (~ 250 ml fluffed Mn-fiber). The Mn-fiber should be prewashed to remove unbound MnO_2 particles.

5. Column clogging. The outlet of the Mn-fiber column may become clogged with strings of Mn-fiber. Avoid this by putting a small plug of raw acrylic fiber at the base of the Mn-fiber.

7. References

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D. Protocols for ^{210}Po and ^{210}Pb

The determination of ^{210}Po and ^{210}Pb in particulate and dissolved water samples is routinely conducted on the same sample, first by measuring ^{210}Po (called 'in-situ' ^{210}Po) and then keeping the sample for a period of 6 months to 2 years for the in-growth of ^{210}Po from ^{210}Pb. The second ^{210}Po (called 'parent-supported') measurement provides the data on the concentration of ^{210}Pb. There is a number of important decay and in-growth corrections that need to be applied in the calculation of the final activities of in-situ ^{210}Po and ^{210}Pb activities. Reference can be made to Baskaran et al. (2013) and Rigaud et al. (2013) for evaluation of these corrections and basis for their calculations. Those desiring of more information as to details of the spread sheet calculations are encouraged to contact the first author of either paper.

1. Analytical instrument

The most widely used instrument for analyzing both dissolved and particulate ^{210}Po and ^{210}Pb in seawater is isotope dilution using alpha spectroscopy (Fleer and Bacon, 1984; Sarin et al., 1992; Radakovitch et al., 1998; Hong et al., 1999; Kim et al., 1999; Rutgers van der Loeff and Moore, 1999; Friedrich and Rutgers van der Loeff, 2002; Masque et al., 2002; Stewart et al., 2007; Baskaran et al., 2009).

2. Volume required

The volume required for analysis of dissolved and particulate ^{210}Po and ^{210}Pb ranges from a few liters (Hong et al., 1999) to 20-30 L (Sarin et al., 1992; Kim et al., 1999; Friedrich and Rutgers van der Loeff, 2002; Masque et al., 2002; Stewart et al., 2007; Baskaran et al., 2009). Due to finite blank corrections (reagents and spikes), the recommended water volume is at least 10 L for the dissolved ^{210}Po and ^{210}Pb measurements. As a general rule, the required volume for particulate ^{210}Po and ^{210}Pb measurements is at least 5 times the volume used for dissolved ^{210}Po and ^{210}Pb. Such volumes are most readily obtained using in situ pumps as on current GEOTRACES cruises.

3. Sampling

3.1 Dissolved

It has been established during GEOTRACES inter-calibration cruises that Niskin bottles with Teflon coated springs are applicable in the collection of seawater for ^{210}Po and ^{210}Pb (Church, et al., 2012). For operationally defined dissolved Po and Pb, the water samples should be filtered through the membrane or cartridge filters with a pore size of 0.4 μm. Since both Po and Pb are particle-reactive, it is strongly recommended to filter the samples as soon as possible after collection. From the intercalibration results, it was found that there was no significant difference between the particulate ^{210}Po and ^{210}Pb concentrations using 0.2 or 0.4 μm filters (Baskaran, et al., 2013). It was also found that the composition of the filter material (e.g., QMA) affects the particulate ^{210}Po and ^{210}Pb activity. It is not clear, however, if such differences are due to amounts of dissolved or colloidal Po or Pb sorbed or the differences in the retention of particulate Po and Pb. **Based on the Intercalibration results, it is recommended to use Supor 0.4 to filter cartridge (e.g., Acropak 500) to obtain the dissolved fraction.** Filtered seawater samples should be stored in acid-cleaned polyethylene (LDPE or HDPE) cubitainers or polycarbonate containers, and acidified as soon as possible (details given below). The cubitainer cap should be sealed with plastic wrap (e.g., Parafilm) and stored double bagged in plastic bags. The samples should be properly labeled with the GEOTRACES specific number ID according to sample station, date and depth. The date is requisite in the radionuclide decay and in-growth equations.

3.1.1 Sample weight or volume

The water samples are collected from the Niskin bottles in an acid-cleaned cubitainer. The total weight can be measured on a balance (precision ± 1 g). At sea, it may be difficult to obtain ± 1 g, but even ± 10 g error will only result in an error of ± 0.10% on a 10-L sample. Some labs use an electronic balance to weigh samples at sea, using a simple computer algorithm to average weights on the moving ship until a stable reading is obtained. Other labs weigh samples after they are returned to the home institution.

3.2 Particles

For particulate ^{210}Po and ^{210}Pb, standard filtering the requisite volume (10's of liters) through 0.45 μm Supor membrane filters can be very time consuming. Also prolonged contact time of the water with the filter material could result in the removal of dissolved ^{210}Po and/or ^{210}Pb. Although capsule filters are more efficient, quantitative removal of particulate matter from such filter cartridges is likely to be quite difficult. Results from the GEOTRACES Intercalibration exercise indicate 10-20 L water samples have a relatively high error on the particulate activities of ^{210}Po and ^{210}Pb (>20%). Hence it is recommended to collect at least 50 L for particulate ^{210}Po and ^{210}Pb measurements. In-situ pumps with Supor filters appear to be superior for collecting particulate matter from larger volumes of water. If in-situ pumps are not readily available, it is recommended to

use a 50 L volume composited from multiple Niskin bottles and passed through 0.45 μm, 142 mm diameter Supor filters.

4. Sample acidification and spiking

The water samples should be acidified immediately after filtration with reagent grade 6M HCl to pH < 2. It is highly desirable to spike the water sample with pre-weighed ^{209}Po, with a suggested activity of ~ 1-2 dpm for 10-L water sample, preferably using ^{209}Po (E_α = 4.881 MeV) US-NIST Standard Reference Material. The use of ^{208}Po (E_α = 5.115 MeV) as the primary tracer is generally discouraged, as the resolution with ^{210} Po (E_α = 5.304 MeV) becomes problematic by alpha spectrometry if the source is thick. However with good plates where the resolution can be corrected using peak overlapping equations (Fleer and Bacon, 1984), there may be an advantage of using both spikes. In this case ^{209}Po is used for the in-situ ^{210}Po and ^{208}Po for that ingrown from ^{210}Pb, which eliminates spike carry over in the absence of a separation procedure after the initial plate (Sec. 5). Both ^{209}Po and ^{208}Po are licensed radioactive material and hence require that proper protocol is followed for use onboard the ship. If the samples were not spiked onboard, it is recommended that the spikes are added to the acidified samples soon after at the shore-based laboratory and equilibrated for at least 24 hours with regular mixing. It is assumed that there is no loss of ^{210}Po and ^{210}Pb to walls of the container during acidified storage period. Differences in the activities between the samples spiked onboard and the ones spiked in the shore-based laboratory have not yet been evaluated. However, the differences are thought to be negligible in samples acidified (but not spiked) immediately after collection.

Stable Pb carrier (1 mg Pb/L of water) is added as $PbCl_2$, preferably from an ancient historical or mineral source. Note that some of the Pb carriers obtained commercially have a finite amount of ^{210}Pb in equilibrium with ^{210}Po, and hence in any case the blank level in Pb carrier should be quantified (Baskaran et al., 2013).

Iron carrier (5 mg Fe/L of water), in the form of $FeCl_3$ is also added and should be tested for blank levels of ^{210}Po and ^{210}Pb before its use. In any case, a number of total blanks of all reagents in the same amounts should be run separately along with regular samples.

5. Pre-concentration and onboard preliminary analysis

The acidified and spiked sample with stable Pb, ^{209}Po and Fe carriers should be allowed to equilibrate for about 24 hours. After equilibration, Pb and Po are simultaneously co-precipitated with $Fe(OH)_3$ by adding ammonium hydroxide to a pH of 8.0-9.0 maximum. Note some labs adjust the pH first to 4 and add 1 ml of 10% sodium chromate to enhance the Pb yields by co-precipitation of lead chromate. The precipitate and the solution can be separated either by successive decanting, followed by centrifugation or filtration. The precipitate is dissolved by adding a few milliliters of 6M HCl followed by washing of the centrifuged tube or filter paper with deionized water to bring the volume for plating to 0.2-0.5 N HCl. To this solution, 200 mg of ascorbic acid are added to yield a colorless solution and adjusted to pH ~2. Note while plating at lower pH (1M HCl) has been

successful, further experiments show that plating solutions with pH of 1.5 has the highest plating efficiencies (Lee et al., 2014). The Po isotopes are separated by spontaneous electroplating onto a polished silver disc, where the reverse side is covered by a neutral cement or plastic film/spray (Flynn, 1968). This residual solution is dried completely and the residue is taken in 5 ml of 9M HCl for the separation of residual Po from the Pb using an anion-exchange column such as AG1-X8 (Sarin et al., 1992). The purified Pb fraction should be spiked again with ^{209}Po and stored in a clean plastic bottle for at least 6 – 12 months after which the ^{210}Pb activity is measured by the ingrown activity of its granddaughter ^{210}Po. One can avoid the column separation of Pb and Po provided another ^{208}Po spike is added at the end of first plating. The correction for residual ^{210}Po is applied from the ^{210}Po/^{209}Po ratios in the first versus second plated counts. The ^{210}Po/^{208}Po ratio is then used to determine the activity of ^{210}Pb from the ingrowth of ^{210}Po in the background corrected second counts (as in Sec. 8.2). Note there is generally some amount of ^{209}Po in the ^{208}Po spike and hence a correction also may have to be applied, as well as possible peak overlap as described above. However this correction and the ^{209}Po contribution will only increase with time after calibration as the two isotope spikes have very different half-lives (^{208}Po only 2.8 years versus ^{209}Po of 125 years; Colle et al., 2014).

Note that some or all of the above procedures can be conducted onboard, depending on permission to use some of the reagents (e.g. ammonia) and radio tracer spikes (e.g. ^{209}Po). If taken through the iron co-precipitation step, it eliminates the need to transport large volume samples. If taken through the first plating stage, it insures separation of ^{210}Po in-growth from the ^{210}Pb grandparent over prolonged periods of time at sea (weeks to months).

It is also noted that if a suitable sample cannot be plated with adequate resolution of the alpha nuclides due to the thickness of the source usually from iron compounds, the Ag planchet can be leached for one hour with concentrated (~12 N) HCl. Then a major portion of the impurities plated on the Ag disk is removed, and the same cleaned plate can be recounted without further loss of Po and improved resolution. The procedure is detailed in Benoit and Hemond (1988).

6. ^{210}Pb yield determination

A precise aliquot of the stored solution (5%) is taken after column separation in an acid cleaned polyethylene bottle for stable Pb determination (either AAS, ICP-MS, or any other suitable instrument). It is important to account quantitative for the removal of this sub-sample from the ^{209}Po (or ^{208}Po) spiked solution kept for about a year in the determination of ^{210}Pb. It is this remaining solution that is utilized for the electroplating of ingrown ^{210}Po as described above. The final activity of ^{210}Pb calculation will involve the in-growth factor for ^{210}Po, decay of ^{210}Pb from collection to the second ^{210}Po plating, and chemical recovery of Pb, as described in detail in Section 8.

7. Digestion of filters containing particulate matter

A number of procedures have been followed in the digestion of the filter material. Since the particulate matter is adsorbed on the filter paper, digestion with a combination of HF (to break the Si matrix), HNO_3 (to break the organic matrix) and HCl (to convert to chloride medium) should be sufficient. However, most of the intercalibration groups could not dissolve the Supor filter completely. It is not assessed if there is any difference in the particulate activity between complete dissolution of the Supor filter (three times digestion with ~5 ml $HClO_4$) and partial dissolution (with 5 ml each of conc. HF-HNO_3-HCl, repeated three times). Since most of the particulate matter is biogenic, we do not recommend the total dissolution with $HClO_4$ since a special fume hood is needed and may not be readily available.

8. Model calculations for final activities of ^{210}Po and ^{210}Pb in seawater samples

8.1 In-situ ^{210}Po

Generally, it is important to correct the in situ ^{210}Po for both its decay and in-growth from in situ ^{210}Pb via ^{210}Bi. This occurs during the time elapsed between sampling and that of first initial separation by plating.

Calculation of the in-situ ^{210}Po activity involves the following specific corrections:
A) Background subtraction of the alpha spectrum for each detector for each ^{208}Po, ^{209}Po and ^{210}Po regions being used;
B) Decay of ^{210}Po from the time of plating on Ag planchets to mid-counting time of the sample;
C) Decay of ^{209}Po (or ^{208}Po) spike from the time of last calibration (or from the time certification for SRMs) to first plating. Note that the half-life has now been revised from 102 to 125 years (Colle et al., 2014).
D) In-growth correction from the decay of assayed in-situ ^{210}Pb via ^{210}Bi; and
E) Decay of ^{210}Po from the time of collection to first plating on Ag planchets.
In principle, a correction factor to the measured ^{210}Po activity from the decay of in-situ ^{210}Bi also needs to be applied. However, only a few labs have reported measurement of in-situ ^{210}Bi on the same sample (Tokieda, et al., 1994; Biggin, et al., 2002).
A detailed outline of these steps is presented. A set of model equations are offered that shows the step-by-step calculation. A spread sheet can be constructed with these equations to explicit decay/in-growth corrections, blank/background subtractions and error propagation. These can be confirmed in consultation as presented here (Baskaran et al., 2013) and elsewhere (Rigaud et al., 2013). Either should provide an accurate assay of in situ ^{210}Po and the ^{210}Pb grandparent.

The alpha spectrometer background should be obtained for every detector and its chamber geometry being used for a particular sample. The Ag planchets should be made from a pure reliable source, and checked for blank/background in each batch. The background is conducted by analyzing an unused cleaned Ag planchet, and subtracting the counting rate from the Po isotope regions of interest. It is also worth checking the detector chamber backgrounds without the Ag planchet to inspect for any spurious Po

contamination, such that the two backgrounds are the same within the counting uncertainty.

The ^{210}Po activity at the time of plating ($^{210}A'_{Po\text{-}210}$) is given by:

$$^{210}A'_{Po\text{-}210}\ (dpm) = (^{210}N_n/^{209}N_n)\ e^{\lambda Po210\ t1}\ e^{-\lambda Pos\ t2}\ A_{spike} \qquad (1)$$

where $^{210}N_n$ and $^{209}N_n$ are the background-subtracted net counts of ^{210}Po and ^{209}Po, respectively; t_1 is the time elapsed between the first plating and mid-counting; t_2 is time elapsed between spike polonium (either ^{209}Po or ^{208}Po) assayed and mid-counting; A_{spike} is the amount of Po spike added (dpm); and λ_{Po210} and λ_{Pos} are decay constants of ^{210}Po and the spike (either ^{209}Po or ^{208}Po), respectively.

Note that two sources of ^{210}Po contribute to the $^{210}A'_{Po\text{-}210}$ activity: i) *in-situ* ^{210}Po present in the sample that had decayed from sample collection until plating; and ii) in-growth from ^{210}Pb, between the time of sampling to the time of first plating. While *in-situ* ^{210}Po activity decreases with time from the time of collection, the amount of ^{210}Po derived from the in-growth of ^{210}Po via ^{210}Bi from the decay of *in-situ* ^{210}Pb increases with time. Thus the in-growth of ^{210}Po from the *in-situ* ^{210}Pb activity ($^{210}A_{in\text{-}growth}$) should be calculated using the Bateman's equation as:

$$^{210}A_{in\text{-}growth} = {}^{210}A_{Pb\text{-}in\text{-}situ}\ [\lambda_{Bi}\,\lambda_{Po}\,e^{-\lambda PbT} / (\lambda_{Bi} - \lambda_{Pb})\,(\lambda_{Po} - \lambda_{Pb}) + \lambda_{Bi}\,\lambda_{Po}\,e^{-\lambda BiT} /$$

$$(\lambda_{Pb} - \lambda_{Bi})\,(\lambda_{Po} - \lambda_{Bi}) + \lambda_{Bi}\,\lambda_{Po}\,e^{-\lambda PoT} / (\lambda_{Pb} - \lambda_{Po})\,(\lambda_{Bi} - \lambda_{Po})] \qquad (2)$$

where:

λ_{Pb}, λ_{Bi} and λ_{Po} are decay constants of ^{210}Pb, ^{210}Bi and ^{210}Po, respectively

T is the time elapsed between collection and first plating;

$^{210}A_{Pb\text{-}in\text{-}situ}$ ($= N_1{}^0\,\lambda_{Pb}$) denotes *in-situ* ^{210}Pb activity.

The amount of in-growth correction for ^{210}Po depends on the concentration of *in-situ* ^{210}Pb and the time elapsed between collection and *in-situ* ^{210}Po plating, as described in Section 8.2.

Thus the final correction will just be for the decay of in-situ ^{210}Po from the time of collection to first plating.

Thus the equation to calculate the *in-situ* ^{210}Po activity is given by:

$$A^{Po\text{-}210}{}_{in\text{-}situ}\ (dpm) = [^{210}A'_{Po\text{-}210}\ (dpm) - {}^{210}A_{in\text{-}growth}]\ e^{-\lambda PoT} \qquad (3)$$

8.2 Calculation of in-situ ^{210}Pb activity

The *in-situ* ^{210}Pb activity calculation involves the following corrections:

F) Background subtraction of the alpha spectrum for each detector and chamber geometry for each ^{209}Po ($^{209}N_{n2}$) (or ^{208}Po) and ^{210}Po ($^{210}N_{n2}$) regions being used;

G) Decay of ^{210}Po from the time of second plating to mid-counting (t_3);

H) Decay of ^{209}Po (or ^{208}Po) spike from the time of last calibration (or from the time of certification for SRM) to second plating (t_4);

I) In-growth factor for ^{210}Po from the decay of ^{210}Pb for the time elapsed between Po-Pb separation (after first plating) and second Po plating (t_5);

J) Chemical yield for ^{210}Pb; and

K) Correction factor for the decay of ^{210}Pb from the time of collection to the second plating (t_6)

The activity of ^{210}Po (in-grown, from the decay of ^{210}Pb) at the time of second plating, corrected for the decay of ^{210}Po from plating to mid-counting (term G above) and for the decay of spike due to time elapsed between the last assay of spike Po (^{209}Po or ^{208}Po) and the time of second plating (term H above) is given by:

$$^{210}A^{m}_{Po\text{-}210}\ (dpm) = (^{210}N_n/^{209}N_n)\ e^{\lambda Po210\ t3}\ e^{-\lambda Pos\ t4}\ A_{spike} \qquad (4)$$

The in-growth of ^{210}Po from the decay of ^{210}Pb during the time elapsed between Po and Pb separation after the first plating to second plating (term I above) is given by:

$$^{210}A_{Pb\text{-}210} = {}^{210}A^{m}_{Po\text{-}210}\ /[1- e^{-\lambda Po210\ t5}] \qquad (5)$$

The chemical yield of $^{210}A_{Pb\text{-}210}$ is corrected by (term J above):

$$^{210}A_{Pb}' = {}^{210}A_{Pb\text{-}210}\ /\ \text{chemical yield} \qquad (6)$$

where: the chemical yield (η_c) = amount of stable Pb carrier assayed/amount of stable Pb carrier added as described in Sec. 6.

The *in-situ* ^{210}Pb activity is corrected for the decay of ^{210}Pb from collection to plating is given by:

$$^{210}A_{Pbin\text{-}situ} = {}^{210}A_{Pb\text{-}210}'\ e^{\lambda Pb\ t6} \qquad (7)$$

where t_6 is the time elapsed between collection and 2nd plating and λ_{Pb} is the decay constant of ^{210}Pb.

Thus the equation to calculate the *in situ* ^{210}Pb activity is given by:

$$^{210}A_{Pb}\ in\text{-}situ = (^{210}A^{m}_{Po\text{-}210})\ e^{\lambda Pb\ t6}\ /\eta_c\ [1- e^{-\lambda Po210\ t5}] \qquad (8)$$

where $^{210}A^{m}_{Po\text{-}210}$ is calculated using equation (4).

9. Some issues that need to be considered

1) It has not been verified that dissolved sea water samples acidified and not spiked for prolonged periods after collection will retain their integrity to surface absorption before or after acidification. Indeed, prolonged periods of months without onboard separation only further compromise correction for the in-growth of unsupported ^{210}Po.

2) Note that some groups do not separate Pb and Po after the first electroplating of ^{210}Po, although some amount of residual Po is left behind. For example leaving the solution for about a year will result in 84% of residual ^{210}Po to decay away, but only <1% of ^{209}Po will decay and hence the residual ^{209}Po will affect the calculation of ^{210}Pb. Neither does additional plating with strips of Ag quantitatively remove residual Po from the solution. **Hence it is strongly recommended that the ion-exchange separation of Po and Pb be performed. If not, use of a double spike approach can be followed, first plating with ^{209}Po spike and second plating with ^{208}Po spike.**

3) The corrections for the in-growth of the ^{210}Po and decay of ^{210}Po and ^{210}Pb during the time elapsed between sample collection to first plating, separation of residual ^{209}Po (9M HCl ion-exchange column separation) to second plating (mid-counting of both Ag plates) needs to be applied. The recent papers of Baskaran et al. (2013) and Rigaud et al. (2013) outline how a spreadsheet can be constructed for these calculations.

4) There are alternative methods that have been reported for the separation of ^{210}Po and ^{210}Pb from sea water, such as co-precipitation with Co-APDC also used successfully during GEOSECS (Boyle and Edmond, 1975). This method while chemically more complex, does allow for co-precipitation of the nuclides under more acidic conditions. Two other methods are reported for the assay of ^{210}Po in fresh water samples published in an IAEA report (2009). It uses an initial separation by manganese co-precipitation followed either by DDTC complexation and solvent extraction into chloroform, or separation by Sr-resin before plating. These methods should be explored further for their efficacy in sea water.

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E. Protocols for anthropogenic radionuclides (^{239}Pu and ^{240}Pu, and ^{137}Cs) limited information on other isotopes (^{90}Sr, ^{237}Np, ^{241}Am)

Similar to some of the other TEIs, we do not recommend a specific sampling, processing, or analytical technique for the artificial radionuclides. Although the collection and analysis of separate dissolved and particulate phases would be ideal for some of the radionuclides (e.g. Pu isotopes, ^{241}Am), the large volumes required (100s-1000s of liters) to analyze these isotopes in the particulate phase and specialized equipment (i.e., large volume in-situ pumps) may or may not be available. Therefore, total analysis (i.e., unfiltered samples) may also be considered.

1. Analytical instruments

The different radionuclides require different analytical techniques. In some cases, different techniques can be used for the same radionuclide.

Accelerator mass spectrometry (AMS) and sector-field ICP-MS (multi or single collector) is suitable for Pu isotopes (except ^{238}Pu) including the separate quantification of ^{239}Pu and ^{240}Pu, and ^{237}Np; some methods for ^{241}Am as well (e.g., KENNA, 2002b; LEE et al., 2001; LINDAHL et al., 2005; YAMADA et al., 2006).

TIMS – Pu and Np a few TIMS methods exist – these require specialized/dedicated instruments (BEASLEY et al., 1998; e.g., BUESSELER and HALVERSON, 1987; KELLEY et al., 1999).

Alpha spectroscopy – suitable for ^{238}Pu , combined ^{239,240}Pu , and ^{241}Am (LIVINGSTON et al., 1975a; LIVINGSTON et al., 1975b; VAJDA and KIM, 2010).

Gamma spectroscopy (^{137}Cs) (e.g., AOYAMA et al., 2000; WONG et al., 1994)

Gas proportional or liquid scintillation counting – ^{90}Sr (e.g., BOWEN, 1970; LIVINGSTON et al., 1974; MOLERO et al., 1993)

2. Volume required

The volume required for analysis of the dissolved anthropogenic radionuclides range from 10-100 liters and is ultimately dependent on the method used as well as the geographic region of the sample. Analysis of ^{241}Am and or ^{90}Sr requires volumes towards the larger end of the range. For analysis of particulate matter, in situ pumping is likely the only viable option, with pumped volumes in the range of several 100s to 1000s of liters.

3. Sampling

As mentioned above, both dissolved (filtered) and total (unfiltered) are acceptable: Due to the significant volume requirements, dedicated hydrocasts will likely be necessary. Collection with a standard rosette system is adequate. Although not prone to contamination, we recommend that seawater samples be stored in acid-cleaned high or low density polyethylene (HDPE or LDPE) containers. Note that vertical concentration gradients may be large, so cross contamination is possible.

3.1 Dissolved and total

If seawater samples are to be analyzed for total concentrations, they may be simply drawn, unfiltered from the Niskin bottles. If separate collection of the dissolved phase is planned, general guidelines for Niskin filtering (i.e., gravity flow; Acropak 500) are recommended.

3.2 Sample volume or weight

A variety of approaches have been used to record sample weight and/or volume, and the literature should be consulted for the best one to use in a particular cruise. Since the majority of separations involve a co-precipitation step, this may be mitigated by the decision to spike and co-precipitate at sea or ship samples back to the laboratory for analysis.

3.3 In-situ filtration (Pu and Cs)

Although we did not intercalibrate on samples collected by in-situ filtration, in some cases, dissolved Pu can be collected on a series of MnO_2 coated fiber material. There is some evidence that this technique can be problematic for Pu because of the presence of multiple oxidation states with different adsorption efficiencies. This issue can be mitigated by the addition of additional in-line filters. Cesium-137 has been successfully collected using a series of potassium ferricyanide impregnated cartridges. Since we did not employ in-situ sample collection, we do not include methods in this document and suggest that the literature be consulted for additional details (BASKARAN et al., 2009 and references therein; BUESSELER et al., 1990).

3.4 Particles

The required volumes for particles are severe and almost certainly require an in situ filtration approach. These include MULVFS, McLane, and Challenger pumps.
QMA filters (quartz fiber ~1 um) are recommended for in-situ pumping specifically for there ease in digesting. QMA material does not appear to present a blank issue for the anthropogenic radionuclides.

4. Acidification, spiking and pre-concentration

As mentioned above, samples may be spiked and pre-concentrated at sea or acidified, and shipped to the home laboratory for spiking and pre-concentration. Given the large volumes, “at sea” processing is often the method of choice if sufficient personnel and shipboard space are available. Processing at sea avoids the necessity of shipping large quantities of seawater to the home laboratory. It does however require handling of radioisotopes at sea as well as more shipboard space and personnel.

4.1 Acidification

Although both HCl and HNO_3 are suitable, samples acidified to pH=2 with HCl have less shipping restrictions. Trace metal grade acid is sufficient. For safety, we recommend working with 6N HCl at sea rather than full strength. Samples appear to be stable after acidification.

4.2 Yield monitors

Measurements are done by isotope dilution using ^{242}Pu, ^{244}Pu, ^{239}Np, ^{236}Np, ^{134}Cs, ^{243}Am. In some cases ^{137}Cs is quantified without spiking by using stable Cs as the yield monitor.

4.3 Pre-concentration

With the exception of Cs-isotopes and ^{90}Sr, pre-concentration of the anthropogenic radionuclides is typically done by adsorption on a precipitate formed in seawater (scavenging), which is then recovered by decantation and centrifugation. The most commonly used scavenging method is Fe hydroxide, adding ~10mg Fe/liter of sample and raising the pH to 8-9. Another way to pre-concentrate Pu is by using MnO_2 coprecipitation. KMnO4 is added in excess to oxidize organic matter and to oxidize soluble Pu species to Pu(VI). After ~1 hour, the solution is made basic by adding NaOH, $MnCl_2$ solution and brown hydrated MnO_2 precipitates (LA ROSA et al., 2001). ^{137}Cs is pre-concentrated using the AMP (ammonium phosphomolybdate) method and ^{90}Sr is typically pre-concentrated using an oxalate precipitation (e.g., AOYAMA et al., 2000; LIVINGSTON et al., 1974; WONG et al., 1994). . Sequential techniques may be applied which allow to concentrate from a single water sample successively transuranics, Cs and Sr.

5. Spike calibrations

We recommend that a spike intercalibration be performed among participating laboratories with agreement on a primary Pu standard. If spike intercalibration can not be completed prior to the work, aliquots of the spikes used in GEOTRACES cruises should be archived for future inter-calibrations.

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V. Radiogenic Isotopes

Protocols for Nd isotopes $^{143}Nd/^{144}Nd$ ($\varepsilon_{Nd(0)}$)

Samples for Nd isotopes (as well as for REE analysis) should be collected using GO-FLO bottles (General Oceanics) or Niskin bottles with epoxy-coated stainless steel springs for trace elements, ideally Niskin-X bottles that have external springs. The samples should be filtered (0.4 to 0.8 μm pore size) to measure dissolved Nd.

1. Analytical instrument

The most widely used instrument for analysis of dissolved $^{143}Nd/^{144}Nd$ in seawater analysis is Thermal Ionization Mass Spectrometry, TIMS (Dahlqvist et al. 2005; Lacan and Jeandel, 2005; Shimizu et al., 1994; Piepgras and Wasserburg, 1987), but Multiple Collector Inductively Coupled Mass Spectrometry, MC-ICPMS, has also become an important method (e.g., Vance et al., 2004) and its importance will likely increase in the future.

2. Volume required

The volume of water required for analysis of dissolved $^{143}Nd/^{144}Nd$ depends on the sensitivity of the TIMS or MC-ICPMS instruments and methods. The amount of required Nd ranges from 1 to 30 ng with the lower range requiring either analysis of Nd on TIMS using NdO^+ beam or analysis with very sensitive MC-ICP-MS instruments, while the higher range allows analysis of Nd as metal by TIMS or analysis of Nd by less sensitive MC-ICP-MS instruments. The concentration of Nd in most open ocean water generally ranges from 0.5 to 6 ng/kg (Nozaki, 2001) and thus a 10L sample will yield between 5 to 60 ng of total Nd.

Analysis of particulate Nd isotopes requires filtration of larger volumes of water in most parts of the oceans (e.g., filtration with *in-situ* pumps). For example, Nd concentrations of particles in the Sargasso Sea vary between 2.9 to 12 μg/g, dependent on particle size (Jeandel et al., 1995). Assuming a minimum particle concentration in the sub-thermocline water column of about 10 μg/L, filtration of 400 liters would provide between 12 and 48 ng of Nd, comparable to 10L seawater samples.

3. Sampling

Five to 10 L (up to 20 L in the surface waters of the oligotrophic gyres) volumes are recommended. All seawater samples for operationally defined dissolved Nd should be filtered as soon as possible through membrane or depth filters with a pore diameter between 0.4 and 0.8 µm. At the time this document was written, there was no evidence that one type of filter is preferable to another (i.e., membrane filters, depth filters, and QMA filters gave the same result in open ocean conditions). Filtered seawater samples must be stored in acid-cleaned high or low density polyethylene (HDPE or LDPE) containers and must be acidified with HCl to a pH of 1.7 to 2.0 as soon as possible.

4. Sample Processing

Spiking is required if the goal is to measure Nd concentrations (using Isotope Dilution method) on the same aliquot as the one used for Nd isotope analysis. Some users prefer to determine the whole REE patterns (among them Nd) on a separate aliquot; in such cases, spiking the 10 L necessary for Nd isotopes is not required. Samples can be: i) spiked and pre-concentrated on the ship after sampling and filtration (reduces the volumes of water that needs to be shipped to land-based laboratories), or ii) acidified onboard and shipped to the laboratory where spiking, precipitation, separation chemistry and analysis take place.

Given the amount of water necessary to perform all suggested analyses within the GEOTRACES program, ideally, several isotope systems should be analyzed on the same samples (e.g., Be, Nd, Pa, Th and even ^{226}Ra, depending on the reagent used to preconcentrate). This last approach has the advantage of saving cable time, and therefore improving the sampling resolution.

4.1 Acidification

Add 1 mL concentrated HCl (ultraclean) per L of filtered seawater (pH 1.7-2). Following acidification, sample integrity should be protected by covering the cap and thread with Parafilm® or similar plastic wrap. Double plastic bags around each bottle/container are recommended. Labeling of samples should be made with a specific GEOTRACES # for each sample and depth.

4.2 Spiking

If the Nd concentration is measured on the same sample as Nd isotope ratios, an enriched isotope such as a ^{150}Nd spike can be used for determination of the Nd concentration in the filtered water. The spike addition is optimized to achieve a ^{150}Nd/^{144}Nd ratio in the spike sample mixture that introduces the smallest error on the Nd isotopic ratio measurement. The spiked seawater is left to equilibrate for at least 48 hours. If a small aliquot of ca. 500 ml or 1 L has been collected in order to measure all the REE including Nd on the same sample, only the aliquot will be spiked for ICP-MS concentration determination (Lacan and Jeandel, 2001).

4.3 Pre-concentration

Pre-concentration of Nd and REE could be done by adsorption on a Fe hydroxide precipitate (and/or Mn oxides) formed in seawater (scavenging), which is then recovered by decantation and centrifugation, or by pre-concentration onto C18 cartridges preconditioned with HDEHP/H2MEHP (see below).

4.3.1 Fe hydroxide

2-5mg of ultra-pure Fe (as $FeCl_3$) is added per liter of acidified and spiked seawater, stirred (e.g., by a magnetic stirrer for 2h or manual shaking) for complete mixing and left to equilibrate overnight. Thereafter, ~2-5 mL ammonium hydroxide (ultraclean) is added per L of sample to bring the pH to 7.5-8.5 and precipitate $Fe(OH)_3$. The sample is stirred (e.g., by a magnetic stirrer or manual shaking of the sample container) during ammonium addition. After 12-48 hours of settling, most of the supernatant is removed and the precipitate is centrifuged (or filtered).

4.3.2 C18 cartridges

Nd is sometimes pre-concentrated by adsorption onto C18 SepPak cartridges, which are loaded with a mixture of the strong REE complexants di(2-ethyl)hydrogen-phosphate and 2-ethylhexyldihydrogen-phosphate (HDEHP/H2MEHP) based on a method described by Shabani et al. (1992). This method has been applied extensively by Jeandel and co-workers (e.g., Jeandel et al., 1998; Lacan and Jeandel, 2005) and can be carried out at sea or in the home laboratory. Both of the above methods have been compared during the intercalibration of Nd isotopes and were found to yield the same isotopic results.

4.3.3 Mn oxides

Other works suggest to co-precipitate using 375 µl of 60 g/L $KMnO_4$ and 150 µl of 400 g/L $MnCl_2$, are successively added to the acidified/spiked sample and then pH is raised to 8 by addition of NH_4OH (Rutgers van der Loeff and Moore, 1999). Then, samples are shaken and left at least 24h for equilibration. The co-precipitated samples are then centrifuged or filtered. Mn oxides have been selected as the best scavenger for the simultaneous extraction of Ra, Nd, Th, Pa and U from the same sample (Jeandel et al., 2011).

While spiking and pre-concentration can be done aboard, dissolution of the recovered precipitate and subsequent separation of Nd by ion exchange column chemistry is always carried out in the home laboratory. Purification of Nd has to be as rigorous as possible during this stage; for TIMS analysis, traces of Ba will inhibit the Nd emission whereas traces of Sm will result in mass interferences. For MC/ICPMS (or NdO^+) analysis, critical interferences are expected from Ce and Pr.

4.3.4 Chelating resin

Using chelating resins is also a suitable pre-concentration technique for the determination of the concentration and isotopic composition of Nd in aqueous samples. The method uses a resin Nobias® PA1 (Hitachi High-Technologies®), which has a hydrophilic

methacrylate polymer backbone where the functional groups ethylenediaminetriacetic and iminodiacetic acids are immobilized. This pre-concentration method has been described and tested in Persson et al. (2011), can be used in the field, is easy, fast (about 8 h for a 3.6 kg sample), and reliable for pre-concentration of Nd from a seawater matrix.

5. Spike calibrations and blanks

Any spike used should be calibrated using a gravimetric Nd standard. Measuring different amounts of a calibrated standard solution mixed with the spike solution, and verifying the accuracy and reproducibility of the determined isotopic composition is also a good way to assess the quality and value of the spike. Laboratories participating in $^{143}Nd/^{144}Nd$ measurements in seawater should strive towards intercalibrations of their used spikes.

Blanks should be determined by isotope dilution and recorded for all batches of reagents and resins used in Nd chemistry. The total chemical procedure should be monitored for blank levels on a frequent basis.

6. Evaluation of analytical uncertainties

The reproducibility and precision of the mass spectrometric methods, TIMS or MC-ICPMS, should regularly be determined by analyzing international Nd standards (e.g., La Jolla Nd, Caltech nNdβ, or JNdi-1). The amount of standard used for the reproducibility runs should be comparable to the Nd amount extracted from seawater samples.

Precision of measurements and inter-laboratory accuracy for Nd concentrations and $^{143}Nd/^{144}Nd$ ratios have been determined during the GEOTRACES Intercalibration, and should be repeated at least at one cross-over or GEOTRACES Baseline Stations per GEOTRACES cruise.

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VI. Trace Elements

Foreword

The collection of dissolved and particulate trace elements is complicated by the issues of contamination, the existence of multiple chemical forms (speciation), differing protocols for the collection and handling of dissolved and particulate phases, and specialized procedures for different elements due to contamination and speciation effects. To simplify this section, the focus will first be on the collection and handling of dissolved trace elements, followed by protocols for mercury, and then two protocols for particulate

trace elements. Linkages between these protocols is done as much as possible for continuity, but to also allow the users to navigate through the protocols.

Acknowledgments

This set of protocols has benefited greatly from the generosity of the trace metal community to willingly share their experiences and information on oceanographic trace metal sampling. There is a caveat here: some of the vital information that was shared in the preparation of this cookbook section was about what not to do, and this knowledge had been gained through a combination of long term experience and common sense. However you will not find this information repeated here, as this cookbook is concerned only with working protocols.

1. Pre-cruise Preparations

1.1 Sampling bottles for collecting clean seawater

GO-FLO bottles (General Oceanics) are the generally-accepted device for collecting trace element depth profiles. Their interior surfaces should be Teflon-coated, the top air-bleed valve replaced with a Swagelok fitting to allow pressurization with clean nitrogen or filtered air, and the sample valve replaced with a Teflon plug valve (Cutter and Bruland, 2012). In addition, all the O-rings should be replaced with silicone (red) or Viton ones. In addition to GO-FLO bottles, Niskin-X (External spring water sampler) bottles have also been used successfully for water sampling, and should be modified in the same manner as the GO-FLOs (e.g., Teflon-coated). Most recently, the PRISTINE sampling bottles that are made of PVDF and titanium with butterfly closures (as of 2014 there are no publications with specifications for these bottles) have been used on the NIOZ "Titan" titanium sampling system (de Baar et al., 2008).

1.1.1 Requirements for deploying the Sampling Bottles

The GO-FLO or Niskin-X bottles should be deployed via one of the following methods (see also section 2.2):

(a) Individual Teflon-coated GO-FLO bottles hung manually on a Vectran (formerly referred to as Kevlar, or similar non-metallic) cable, this is the standard method used successfully for over three decades (Bruland et al., 1979). In addition to measuring wire out and angle, it is recommended that individual GO-FLO bottles be fitted with an internally recording depth sensor (e.g., RBR Depth Recorder, http://www.rbr-global.com/products/sm-single-channel-loggers/depth-recorder-rbrvirtuoso-d). The methods and data used in verifying depth should be documented in the metadata for the cruise.

(b) Teflon-coated GO-FLO bottles mounted on a trace metal-clean rosette system which uses a suitable trace-metal clean cable (Vectran conducting cable or similar). Examples of these systems include the CLIVAR 12 bottle rosette (Measures et al., 2008), the US

GEOTRACES apparatus (Cutter and Bruland, 2012), and programmed firing rosettes lowered on Vectran (e.g., Saito et al., 2013).

Weights to provide negative buoyancy for the Vectran line or rosette should be made of lead encased in epoxy. Information on the construction of these weights can be found in Measures et al. (2008).

It is recommended for the rosette systems that they use pressure housings made of titanium and examples of this include the US GEOTRACES system (Cutter and Bruland, 2012) and the TITAN system (de Baar et al., 2008). Zn anodes should be removed to prevent contamination.

1.1.2 Cleaning procedure for sampling bottles

(Note: There is some disagreement about whether cleaning these bottles is needed or desirable, but if GO-FLO bottles are cleaned; no acid should contact the outside of the bottle, the nylon components in particular.)

1. Fill bottles with detergent for one day.
2. Rinse 7x with deionized water (DIW) thoroughly until there is no trace of detergent
3. Rinse 3x with ultra high purity water (UHPW such as Milli-Q)
4. Fill bottles with 0.1M HCl (analytical grade) for one day, and empty out through the spigot to rinse these.
5. Rinse 5x with UHPW
6. Fill bottles with UHPW for more than one day before use
7. After discarding UHPW from bottles, deploy and trigger the bottles in open ocean water.
8. After discarding seawater from Teflon spigot, use bottles for sampling

Note: It is imperative that the Teflon spigots are cleaned during this process also, not just the inside of the bottles.

1.2 Sample Bottle Types for sample storage

For both total dissolvable and total dissolved trace metal analysis it is recommended that Low Density Polyethylene (LDPE) or High Density Polyethylene (HPDE) bottles be used. It is important to know whether the sample bottle manufacturers are using high quality resins and that there is little variation between batches. Good results have been found in the past (SAFE, GEOTRACES intercalibration) with bottles manufactured by both Nalgene; BelArt and HUB, though other bottles manufactured by other companies may also be suitable. Bottle caps with inserts are not reliable; caps made with PP are in general suitable for most metals. Aluminum and titanium must be sampled in bottles and caps made of 100% LDPE, although there are reports of FEP being acceptable.

Bottles for speciation samples and their cleaning are discussed below in Section 3.3. Polyethylene bottles are not recommended for Hg or metalloids (see Hg Section 5 for bottle types and cleaning).

1.3 Sample Bottle Cleaning

Please note this is a rigorous protocol, one of many that are currently employed by research groups with a long history of successful trace metal clean sampling. For more details on the cleaning procedure used by individual laboratories, please contact the authors of this report or directly with the labs themselves.

1.3.1 For LDPE and HDPE bottles (dissolved and dissolvable trace elements):

1. The bottles may need to be rinsed with methanol or acetone to release oils from manufacturing.
2. Soak bottles for one week in an alkaline detergent (e.g. Micro, Decon). This process can be sped up by soaking at 60°C for one day
3. Rinse 4x with ROW/DIW
4. Rinse 3x with UHPW under clean air.
5. Fill bottles with 6M HCl (reagent grade) and submerge in a 2M HCl (reagent grade) bath for one month. Again this can be sped up by heating for one week. Make sure threads and caps are leached! These acids don't need to be fresh each time; they can be reused several times (e.g. typically most groups replace the acid in the acid baths after every 4-6 cycles of bottles through the baths).
6. Rinse 4x with UHPW under clean air.
7. Fill bottles with 0.7 M HNO_3 (trace metal grade) or 1 M HCl (trace metal grade) for at least one month (i.e., transport on cruise filled with this). Should be stored doubled bagged. Note that you shouldn't use HNO_3 if you are intending to perform redox sensitive analysis. HNO_3 can also result in adsorption at neutral pH.
8. Rinse with UHPW, and ship the bottles empty and double bagged.

1.3.2 For PFA Teflon bottles:

Groups using Nalgene PFA bottles typically use the same cleaning protocol as for FEP Teflon found above (section 1.3.2. The following protocol was developed by Japanese colleagues for bottles manufactured by other companies, due to the variability in the quality of the PFA Teflon.

1. Soak bottles for one day in an alkaline detergent
2. Rinse 7x with DIW thoroughly until there is no trace of detergent
3. Rinse 3x with UHPW
4. Soak in 6 M reagent grade HCl bath for 1 day
5. Rinse 5x with UHPW
6. Fill bottles with a mixture of 1M (each) nitric acid, sulfuric acid and perchloric acid (analytical grade) and keep them at 100°C for 5 hours in a fume hood
7. Rinse 5x with UHPW water inside an ISO Class-5 laminar flow hood
8. Fill bottles with UHPW water and keep them at 80^{o}C for 5 hours
9. Rinse 5x with UHPW water inside an ISO Class-5 laminar flow hood. Should be stored doubled bagged

2. Sample Collection

2.1 Surface Sampling

It is recommended that a clean surface pump sipper/tow fish system which consists of (see also photo below):

a. A PTFE Teflon diaphragm pump (e.g. Almatec A-15TTT; or large peristaltic pump with silicone pump tubing (e.g., Vink et al. Deep-Sea Res. I, 47: 1141-1156, 2000)). Note: That there are still some issues with the use of these systems as not all metals have been tested at present. Diaphram pumps are in general preferred over peristaltic pumps, as the latter may disrupt or break zooplankton or phytoplankton cells.
b. PFA Teflon sample tubing; Bev-a-Line IV or Tygon 2275 may also be used, although Hg contamination may be an issue. Recommend a minimum 0.5'' OD, 3/8'' ID.
c. PVC depressor vane 1 m above a 20 kg weight enclosed in a PVC fish, alternatively a several groups have deployed a 50 kg stainless steel fish which does not require a separate depressor.
d. Polyester braided line connecting the fish to the depressor (if required) and then to the ship; the Teflon sampling tubing is run along this line.
e. PFA Teflon tubing is used on the other side of the pump to deliver seawater directly into a clean area for sampling.

For underway surface sampling at speeds from 1 to 12 knots, the sipper system is deployed off the side of the ship using the ship's crane to suspend the fish outside of the bow wake with the intake at approximately 2-m deep. Faster speeds are possible with this sipper design if there is little or no swell and the sipper remains outside of any breaking bow waves (Note: slight design changes to the fish and towing at 4-5 m allow sampling up to 15 knots). The sipper design also allows near-stationary sampling (moving forward into clean water at 0.5 to 1 knots) in order to collect large volumes of trace metal–clean seawater at depths up to 25 m.

A YSI Sonde (or equivalent) can also be attached to the bottom of the vane that allows accurate depth samples to be collected as well as providing T and S data. This system pumps water at ca. 5 L min^{-1} and is excellent for large volume collection.

It should be noted that there are currently several groups worldwide that operate systems capable of clean surface sampling for Fe similar to the one described in detail above. It is highly recommended that researchers wishing to develop their own system contact the existing groups directly for more information.

2.2 Depth Profiles

See Section VI.1.1.1 above on the pre-cruise preparations required for making trace element depth profiles. The following description is based on the US GEOTRACES program as information on this system is readily available (contact: Greg Cutter, ODU; also Cutter and Bruland, 2012; see de Baar et al., 2008 for a description of the TITAN system procedures).

The US GEOTRACES system consists of an epoxy powder-coated, aluminum rosette (Seabird) that holds 12-24 x 12 L GO-FLO bottles (or Niskin-X) and deployed on a Kevlar conducting cable allow rapid and contamination-free sampling. The bottles are sent down open, but when on-deck the open bottles are covered with plastic shower caps and the spigots have a sealed 3cm long piece of 3/8" Bev-a-line 4 tubing inserted into them. The shower caps are removed at the last minute before deployment and minimize contamination while on the deck. Sample bottles are triggered using Seabird software on the ascending cast (at 1-3 m min^{-1}).

Previously, the deployment of individual GO-FLO bottles (12-30 L) attached to a Kevlar cable and triggered with plastic messengers has served the community well in this respect. There are other rosette options (CLIVAR & TITAN) that have been successfully deployed in the past, the main criteria for any new rosette system is the demonstration of results identical to, or comparable to, data obtained by existing verified protocols from GEOTRACES Baseline stations.

Once onboard the GO-FLO bottle ends are covered with the plastic shower caps and transported to a clean area (Either a specialized lab container or a 'bubble' constructed from plastic sheeting) where sample handling is performed in clean HEPA filtered air. It should be noted that the GO-FLO bottles themselves can be placed outside the container and connected by tubing to the clean air zone inside the container. If the GO-FLO is pressurized then the entire bottle must be under clean air at all times. The critical point is that the sample water itself is only exposed to clean air.

3. Sample Handling

All sample handling should take place in a clean area, preferably an ISO Class-5 area (See Table 1). To minimize contamination, it is best to use two people for sampling handling. One person will open up the outside sample bottle bag and the other person can then open the inside bag and remove the previously labeled bottle and rinse/fill the bottle in the clean area.

The GO-FLO is pressurized using a low overpressure (<50 kPA, or <7 psi, maximum) of filtered (0.2 μm PTFE) high-quality nitrogen gas or compressed air to obtain a sufficient flow across the filters, while minimizing cell rupture or lysis. The GO-FLO is pressurized after connecting the polyethylene gas line to the Swagelok fitting on the GO-FLO. For filtered waters a capsule filter or membrane filter holder (see below) is connected to the GO-FLO's Teflon plug valve with Teflon PFA tubing (or clean equivalent) and the sample bottles are filled as above with the effluent from this filter (capsule filters should be rinsed with ca. 0.5 L of sample water prior to collection of the filtrate).

PE gloves are the cleanest for all metals and are recommended here if available. Gloves made from other materials (e.g., latex, nitrile) can be used but should be powder free and the users should ensure before use at sea that the gloves do not contaminate for any of the elements under investigation. If using nitrile gloves, rinse with clean water prior to use.

Table 1. New Clean Room Standards

OLD

Federal Standard 209E Airborne Particulate Cleanliness Classes											
		Class Limits									
		0.1μm Volume units		0.2μm Volume units		0.3μm Volume units		0.5μm Volume units		5μm Volume units	
Class Name											
SI	English	m^3	ft^3	m^3	ft^3	m^3	ft^3	m^3	ft^3	m^3	ft^3
M1		350	9.91	75.7	2.14	30.9	0.875	10.0	0.283	—	—
M1.5	1	1,240	35.0	265	7.50	106	3.00	35.3	1.00	—	—
M2		3,500	99.1	757	21.4	309	8.75	100	2.83	—	—
M2.5	10	12,400	350	2,650	75.0	1,060	30.0	353	10.0	—	—
M3		35,000	991	7,570	214	3,090	87.5	1,000	28.3	—	—
M3.5	100	—	—	26,500	750	10,600	300	3,530	100	—	—
M4		—	—	75,500	2,140	30,900	875	10,000	283	—	—
M4.5	1,000	—	—	—	—	—	—	35,300	1,000	247	7.00
M5		—	—	—	—	—	—	100,000	2,830	618	17.5
M5.5	10,000	—	—	—	—	—	—	353,000	10,000	2,470	70.0
M6		—	—	—	—	—	—	1,000,000	28,300	6,180	175
M6.5	100,000	—	—	—	—	—	—	3,530,000	100,000	24,700	700
M7		—	—	—	—	—	—	10,000,000	283,000	61,800	1,750

NEW

ISO/TC209 14644-1 Airborne Particulate Cleanliness Classes						
	Concentration Limits (particles/m^3)					
	0.1µm	**0.2µm**	**0.3µm**	**0.5µm**	**1µm**	**5µm**
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1,000	237	102	35	8	
ISO Class 4	10,000	2,370	1,020	352	83	
ISO Class 5	100,000	23,700	10,200	3,520	832	29
ISO Class 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO Class 7				352,000	83,200	2,930
ISO Class 8				3,520,000	832,000	29,300
ISO Class 9				35,200,000	8,320,000	293,000

Important Note: If using a waste bucket to collect water used in rinsing the sample bottles or otherwise, it is recommended to place a plastic mesh over the bucket to minimize aerosol generation and splash back.

3.1 Total Dissolvable (unfiltered) Samples

Prior to sampling, the sample bottles should be already empty of any solutions used in transport. The bottles should be rinsed at least three times with unfiltered samples from the GO-FLO bottles. Ensure that the caps are also rinsed by placing sample water in the bottle, screwing the lid back on, shaking, and then pouring the sample into the lid and then over the bottle threads. The sample should be filled to the bottle's shoulder. It is important that all bottles are filled to the same amount so that acidification of samples is equal (i.e., same pH in all bottles). Samples should then be acidified to pH 1.8 using Sea Star hydrochloric acid or 6M sub-boiled distilled trace metal grade HCl (4 mL per L sample), capped tightly, and resealed in the bags.

3.2 Total Dissolved (filtered) Samples

3.2.1 No particle collection

The first consideration is whether only the dissolved sample is being taken (no particle collection), or particle samples are being collected along with the dissolved sample (i.e., the filter and the filtrate will be analyzed). If only the filtered water sample is needed, then the use of a capsule/cartridge filter is recommended (see below) in combination with a slightly pressurized GO-FLO (see above for details on this). Gravity filtration is not recommended for 0.2 µm filters due to the slow flow rates.

For capsule filters where only the filtered water is sought, **it is recommended from the results of the SAFe and CLIVAR programs, the GEOTRACES intercalibration cruises (e.g., Cutter and Bruland, 2012), and subsequent GEOTRACES section cruises, to use the Pall Acropak Supor capsule filter (0.8/0.2 µm)**. Equivalent filters such as the **Sartorius Sartobran** have been found to perform similarly. These filters

were shown to be excellent for the following trace metals: Fe, Zn, Co, Cd, Mn, Pb, Cu and Ni. The following description of use is based on experiences with the Acropak or Sartobran capsule filters:

Clean tubing (Teflon or clean alternative) should be used to connect the filter cartridge to the pump outlet. The cartridge is acid cleaned as below, but then they are rinsed with 10 L of filtered open ocean seawater (either surface sipper/tow fish water or seawater from a near surface GO-FLO) before first use, and stored in a refrigerator until use (Note: Make sure they do not freeze). One filter capsule can be used for multiple depth profiles, working from surface to deep. Some groups use one for deep, and one for shallow, over several casts. When the filtration rate begins to noticeably slow down, the capsule is changed for a new clean one. As noted above the filters are rinsed between sample depths with ca. 0.5 L of sample water before final collection into the sampling bottle.

Cleaning method for capsule-type polysulfone filter (see also particle section):

1. Fill capsules with 0.1M HCl (trace metal grade) and keep them heated one day (< 80^{o} C to avoid damaging the filters).
2. Rinse capsules with UHPW thoroughly (more than 5x) until there is no residual acid
3. Fill capsules with UHPW and heat at about 70° C for one day
4. Rinse capsules 5x with UHPW
5. Fill and store capsules with UHPW

Some researchers have reported getting good data for some elements without any pre-cleaning. It is not recommended using nitric acid for this type of filter due to the risk of nitrate contamination.

3.2.2 Particle collection

Particle collection from GO-FLO samples is thoroughly discussed in Section IV.9 below. For the collection of water from samples from which particles are also being collected, the same method as above is used, but a 25, 47 or 142 mm polycarbonate or TFE Teflon filter holder and filter are used in place of the filter cartridge (filters discussed below in Section 8). The dissolved sample is collected as above, but the total volume of water passing through the filter must be recorded (e.g., (5) 2 L bottles filled + rinses = 12 L, etc. It is important to note that leaking membrane filter holders have been identified as a major source of contamination. Please see the Section IV.9 on GO-FLO particle collection for more details.

3.3 Speciation samples

Many of the trace elements in GEOTRACES that are core parameters exist as multiple species in the water column, in some instances in multiple redox states. Characterization of the speciation of these elements is often fundamental to understanding their properties, and many speciation studies have been conducted on GEOTRACES cruises to date.

The incorporation of speciation measurements into a large, multi-national section-based program like GEOTRACES poses important challenges:

(1) For many measurements, sampling must be carried out on board, particularly for species which are highly reactive, such as Fe(II).
(2) For some parameters, many measurements must be made on a single sample, such as complexometric titrations. Such measurements are labor intensive and require specialized instrumentation on board.
(3) Some measurements can be carried out ashore with frozen samples, but this requires large freezer capacity and careful attention to the conditions of freezing.
(4) Some methodologies are operationally defined, which can confound intercomparisons between different methods which are ostensibly determining the same parameter.

The protocols here apply to the determination of transition metal complexation by organic matter, and the determination of Fe(II) in seawater, since these parameters were examined as a part of the GEOTRACES Intercalibration program (e.g., Buck et al., 2012), but the protocols probably apply to other dissolved phase speciation measurements. This document does not cover particulate speciation protocols (for example selective leaching) that are covered elsewhere. Sampling in low oxygen environments requires special considerations and is discussed separately.

3.3.1 Sampling

Trace metal speciation should be carried out under the same rigorously clean conditions used for the determination of total dissolved metals. Contamination can completely alter the results, for example when metal-complexing ligands become saturated by a contaminant. Speciation samples should be collected from the same Go-FLO cast/depth and, preferably, bottle as the total dissolved metal samples, so that separate total analyses do not have to be performed on every speciation sample.

Results from the Intercalibration cruises revealed that all of the filter capsules used were acceptable for metal complexation measurements and the determination of Fe(II). The results also indicated that these samples can be collected directly from the pressurized Go-FLOs through capsule filters as for other samples, without a need for specialized plumbing. Therefore, complete integration of speciation sampling with other TM sampling is acceptable.

3.3.2 Sample handling

Two types of container are recommended for handling speciation samples: Teflon (FEP) and fluorinated linear polyethylene (FLPE). LDPE is not recommended because organic material leaches into the sample and interferes with many assays. These bottles should be cleaned using the same protocols for total dissolved metals, but special care must be taken to ensure there is no residual acid in the bottles. Even traces of acid might lead to pH-generated artifacts in species distribution. Samples for metal complexation can be refrigerated for several days, but must be frozen after that.

Samples for metal complexation measurements can be frozen in FLPE or FEP, but FLPE is recommended because of cost and because Teflon requires significant conditioning in seawater before routine use. The bottle should be filled to about 80% of capacity and

stored upright in a -20° C freezer. Rapid freezing in a -80° C freezer is not recommended for FLPE bottles; samples in FLPE were contaminated for Fe and Cu when frozen at -80° C. It is possible that such rapid freezing leads the bottle to become very brittle while the sample is still undergoing expansion during the freezing process.

3.3.3 Sampling Protocols for Fe(II)

Intercalibration results suggest that samples for Fe(II) can be collected from Go-FLOs in the same way as other samples, and transferred to another location on the ship for immediate analysis. If many samples are taken at once (i.e., if a complete profile is compiled) then it has been recommended by Kondo and Moffett (2013) to acidify the sample with MOPS buffer (3-(N-morpholino) propane sulfonate) at pH 7.2 so decay is slowed while all 24 samples are run. However, other groups do not acidify, but maintain the samples at either the in situ temperature or at 2-4° C using a water bath. Acidification to lower pH values is not recommend as it may lead to artificially high values over time. Freezing samples is not an acceptable preservation method for Fe(II).

3.3.4 Special consideration for samples collected from anoxic or suboxic zones

The top priority is to ensure that chemistry does not change significantly between bottle tripping and sample drawing. Concentrations of many TM, especially Fe and Mn are much higher in suboxic zones. It is important to exclude oxygen from these bottles and/or sample them quickly. Oxidation will compromise speciation data and also total data, since Fe(III) is more particle reactive and may adsorb onto the walls of the bottle, compromising total data and leading to memory effects on the next cast. One recommendation is to pressurize GO-FLO bottles from these depths with nitrogen, rather than compressed air. A secondary consideration is that waters from these depths are supersaturated in CO_2. Outgassing will lead to an elevation of pH which can influence speciation and exacerbate wall-loss artifacts, as observed for Fe on the SAFe cruise in 2004. Rapid sampling and capping bottles with no headspace, much like the methods used for collecting dissolved oxygen samples, are recommended.

3.3.5 Speciation Methodologies

Description of specific methodologies is beyond the scope of the proposed work. However, given that many techniques yield results that are operationally defined, thorough, detailed metadata is critical, including parameters such as reagents and their concentrations, pH, buffers used, and so forth.

3.4 Sample Acidification

Samples for total metal analysis should be acidified using HCl to below pH 1.8 (0.024M). HCl is preferred for a number of reasons over HNO_3, with a key reason being transport issues for samples containing a strong oxidizing agent.

Important Note: Some researchers prefer not to have their samples acidified at sea, but to receive unacidified samples that they then acidify later in their home laboratories. Thus, it is important that when samples are being exchanged between groups that this preference

is indicated at the earliest possible opportunity to avoid confusion and/or duplicate acid additions. The acidification procedure must be documented in the metadata.

4. Shipboard Determinations of Selected Dissolved Trace Metals

We recommend that shipboard determinations of Fe, Zn and Al are made onboard to check for contamination. This should be carried out on all sampling bottles (GO-FLO, Niskin-X, PRISTINE) at the start of the cruise and periodically throughout the cruise. The shipboard methods should be checked for accuracy using GEOTRACES and SAFe consensus samples.

It is strongly recommended that for onboard analysis samples are acidified to 0.024 M HCl (pH 1.7 – 1.8), as it was discovered during the SAFe cruise (Johnson et al., 2007) that total dissolved Fe was not rendered "reactive" to methods that only acidify to pH 3 for short exposure times prior to analysis. Alternatively microwaving to 60° C rendered the total dissolved Fe "reactive" within a few minutes; however acidification to 0.024M HCl (pH 1.7-1.8) was more effective overall. Presently it is suggested a combination of acidification and microwaving may be the best approach if the samples are to be measured immediately onboard, though there is currently no published study comparing these approaches.

Samples analyzed for dissolved cobalt should be UV irradiated prior to analysis (e.g., Milne et al., 2010). The exact irradiation time required will depend on the lamp type and strength and the optical characteristics of the sample bottle. For some analysis systems, samples for dissolved copper may also need to be UV irradiated.

Flow Injection techniques have been successfully used onboard ship for Fe and Al (e.g., Measures et al., 1995; Obata et al., 1993; Lohan et al., 2006; Brown & Bruland, 2008; and many others. For Zn, analysis at sea has typically been carried out using voltammetric analysis via either anodic or cathodic stripping voltammetry (e.g., Jakuba et al., 2008; Lohan et al., 2003) or using flow injection analysis (Gosnell et al., 2012).

5. Chemicals and Reagents

All chemicals and reagents used in sample analyses should obviously be of the highest quality possible. Researchers are encouraged to exchange information on their findings on the quality of the same chemical from different suppliers or different batches from the same supplier. Information on the shelf life and storage of analytical chemicals is also of use.

When primary standards are prepared from solids, the preparation method should be well described. Where possible, primary standards for TEIs should be exchanged between researchers to ensure analytical intercalibration.

6. Analytical Considerations: Precision and Accuracy

The precision and accuracy of each analytical procedure should always be reported. Accuracy is a measure of how close an analysed value is to the true value. In general, the accuracy of an analytical method is determined by the use of calibrated, traceable reference standards. However, it is important to bear in mind that the assessment of accuracy based upon primary standards can be misleading if the standards are not prepared in seawater because of matrix (i.e., salt) effects. In addition, it must be recognized that for many of the TEIs there are no readily available reference materials.

Precision is a measure of the variability of individual measurements (i.e., the analytical reproducibility) and for GEOTRACES two categories of replicates should be measured; field and analytical replicates. Analytical replication is the repeated analysis of a single sample and is a measure of the greatest precision possible for a particular analysis. Field replication is the analysis of two or more samples taken from a single sampling bottle and has an added component of variance due to sub-sampling, storage, and natural within sample variability. The variance of field and analytical replicates should be equal when sampling and storage have no effect on the analysis (assuming the analyte is homogenously distributed within the sampling bottle). Therefore, the difference between field and analytical replicates provides a first order evaluation of the field sampling procedure.

It should easily be apparent from these definitions that precision and accuracy are not necessarily coupled. An analysis may be precise yet inaccurate, whereas the mean of a variable result may be quite accurate. Therefore, precision and accuracy must be evaluated independently. The use of Certified Reference Materials is best for evaluating analytical accuracy, but for most trace elements there none available for seawater at appropriate concentrations as of this writing (2014). For the GEOTRACES Program, consensus intercalibration samples have been created.

It is recommended that the SAFe or GEOTRACES Consensus Samples should be used as a Reference Material (RM) to test of the accuracy of the methods used. As of 2014, consensus values for Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn (http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html) are available for SAFe and GEOTRACES Intercalibration samples. SAFe and GEOTRACES Intercalibration samples can be obtained by e-mailing: requestsafestandard@ucsc.edu and providing a shipping FED-Ex number. These samples are in LDPE bottles and have an individual sample number. Two general types of samples are available, surface and deep water samples.

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8. Protocols for Sampling and Determinations of Mercury and its Speciation

The intent of this document is to summarize the results of a recent NSF-sponsored international intercalibration/comparison exercise into the accurate and efficacious collection and analysis of open ocean seawater samples for total mercury (Hg) determinations as well as Hg speciation within the context of a GEOTRACES cruise. This report is not meant to be a standalone description of all aspects of on board collection activity during a GEOTRACES cruise, but rather those aspects that we have come to view as the "recommended practice" with regard to Hg determinations. These activities include bottle selection and cleaning, sample collection and handling on board, sample filtration, the recommended analytical procedures for both on board or on shore analyses and the latest view of optimal storage/preservation approaches if immediate analysis is not possible.

8.1 Sample Bottle Selection and Cleaning

As part of the Intercalibration Program, we revisited some of the most fundamental analytical considerations regarding bottle selection and cleaning. Particular care was taken to examine the susceptibility of sample bottles to the diffusion of elemental Hg (Hg^o) through the walls. Consideration of this potential contamination pathway is unique to mercury and is particularly important because many GEOTRACES cruises are likely to have large amounts of Hg^0 on board for electrochemical-based speciation analyses of Zn, Co, Pb and Fe. In addition, mercury is often used to preserve biological samples and there may be legacy Hg^0 in the ships laboratories from broken Hg thermometers. The potential for significantly elevated Hg^0 levels in shipboard laboratory spaces may result in airborne Hg concentrations that are highly elevated with respect to ambient air (ca. 1.5 ng m^{-3}). For example, on the two US GEOTRACES Intercalibration cruises, we found Hg^0 concentrations in the Hg Group work spaces that ranged from 20 to 50 ng m^{-3}. Given this range in ship-board air mercury concentrations, capturing Hg^0 from the shipboard laboratory air in a half-filled 500 mL sample bottle would result in a contamination increase ranging from 0.1-0.25 pM. Since the range of total Hg anticipated in open ocean seawater is around 0.25 to 2.5 pM, the potential impact from airborne contamination is

quite significant. While there are methods to fix this contamination (see below), every effort should be made to minimize work space Hg^o concentrations, including the use of activated charcoal scrubbers in laminar flow benches and the requisition of a separate laboratory van so that analyses may be performed outside of ship's lab spaces.

With Hg^o concentrations present in work spaces a potential problem, gas impermeability is an important consideration when selecting bottles to receive samples, especially for long term storage aboard ship. We found that glass and impermeable plastics (like polycarbonate) are the best for long-term (months) storage of seawater for Hg analysis.

Our recommended bottle cleaning procedure is shown below, and was found to be effective for the very low-level seawater concentrations, and resulted in low blanks for bottles made of almost any material. The key ingredient seemed to be BrCl, which is the commonly used wet chemical oxidant for digesting aqueous samples prior to total Hg analyses. The BrCl concentration used during cleaning should be greater than that used in subsequent sample digestion to ensure best results. Bottles used for minority species analyses (Hg^o, $(CH_3)_2Hg$ and $CH_3Hg(I)$) should be thoroughly cleaned of BrCl prior to use, to avoid destruction of these forms. For example, a rinse with low Hg NH_2OH (see below) following the BrCl cleaning could be useful; however, we have found that copious rinses with high-purity water are equally effective. In our recommended workflow described below, we also segregate the analysis of total Hg (which uses BrCl) and the minority species into different bottles, to avoid accidental oxidation.

6 day Citranox soak
>6 day 10% HCl
1 day 0.5% BrCl
pH 2 water rinse

Table 1. Recommended cleaning procedure for new bottles for Hg species in seawater.

We recommend that GEOTRACES samples for Hg be collected into those bottles that best fit the individual workflow of the cruise. For example, Teflon is recommended for short-term storage when samples will be analyzed within a few days as they are unquestionably clean, highly durable and less gas permeable than polyethylene. If longer term storage is intended, then collection in either polycarbonate or glass is recommended to provide the best protection against Hg^o diffusion. It should be noted that polycarbonate does not fare well when exposed to strong oxidizing acid (>4N HNO_3) or strong base for extended periods. Thus, if the cleaning regimen includes either of these solutions, polycarbonate is not recommended.

8.2 Sample Collection and Handling

We found that the collection of Hg is relatively insensitive to the sampling platform used (e.g., CLIVAR clean rosette, GEOTRACES carousel or GO-FLO bottle hung sequentially on a non-metallic hydrographic line, such as Kevlar). Thus, as long as the collection bottle (GO-FLO, X-Niskin or the equivalent) has been shown to be appropriately cleaned for other metals (e.g. Zn and Pb), it should be suitable for the collection of total Hg and Hg species. Furthermore, a number of different filtering strategies were tested, including the use of pressurized GO-FLOs and in-line capsule

filters (Osmonics 0.2 µm Teflon and the Acropak 0.2 µm Polyethersulfone) and as well as vacuum-assisted membrane filtration. The most commonly used membrane (0.45 µm pore size Nuclepore) and the capsule filters all seemed to compare well, suggesting that the particular filtering medium used is not critical, as long it has been previously tested to ensure a low blank.

Results from the highly oligotrophic Sargasso Sea (Bergquist and Lamborg, unpublished) suggest that there is essentially no "colloidal" Hg or $CH_3Hg(I)$ present in open ocean seawater, where colloidal was defined as particles between 0.02 – 0.45 µm effective size. Thus, we should not be surprised that different filtering media, assuming that they do not contribute a Hg blank or absorb Hg, should provide similar "dissolved" Hg results. Colloidal Hg is significant in coastal ocean environments, however, so that near-shore sampling should include a pore size-dependent definition of "dissolved" (e.g., Stordal *et al.*, 1996; Choe et al., 2003).

8.3 Sample Analysis

A major advancement in the determination of $CH_3Hg(I)$ in seawater was made during this project, which has lowered the detection limit, increased accuracy and facilitated a further streamlining of Hg species determinations (Bowman and Hammerschmidt, 2011). We now recommend this method and describe it below, as well as its integration into the general workflow.

During the Intercalibration Program, all but two of the participating laboratories used cold vapor atomic fluorescence spectroscopic (CVAFS) determination of Hg (as Hg^o). The other two laboratories employed the other commonly used analytical approaches, inductively coupled plasma-mass spectrometry (ICP-MS) (with isotope dilution) and cold vapor atomic absorption spectrometry (CVAAS). Both CVAFS and ICP-MS compared well, while the CVAAS did not exhibit adequate sensitivity to detect total Hg on the Intercalibration samples (250 mL). Thus, we recommend CVAFS or ICP-MS for Hg determinations. The CVAFS approach has the distinct advantage of being field employable allowing rapid determination of Hg^o and $(CH_3)_2Hg$ at sea. ICP-MS, especially when employed with isotope dilution, has the potential for a lower absolute detection limit. Thus, we recommend CVAFS for at sea determinations, but feel that either approach is appropriate for on shore analyses.

Our recommended workflow is illustrated in Figure 1. Details of instrument use are documented elsewhere (e.g., Fitzgerald and Gill, 1979; Gill and Fitzgerald, 1985; Gill and Fitzgerald, 1987; Horvat, 1991; Hintelmann and Wilken, 1993; Horvat et al., 1993; Hintelmann et al., 1997; Hintelmann, 1998; Hintelmann and Simmons, 2003; Bowman and Hammerschmidt, 2011). The workflow presented is oriented toward at-sea, multi-species determinations by CVAFS, but could be easily adapted for use with ICP-MS back on shore. A ready supply of high quality water (18 MΩ-cm resistivity) will be necessary for at sea or on shore cleaning, standard and reagent making. Most commercially available "ultrapure" water systems are adequate for Hg analyses, but a check of the ship's system should be done immediately, and it may be prudent to bring a back-up

system. Though not shown in the workflow, laboratories need to also do a very careful determination of analytical, bottle, and reagent blanks to assure that they are working at levels appropriate to the determination of open ocean seawater. If possible, this should be done on shore prior to a cruise as well as during the cruise. Replicate analyses on several samples to demonstrate precision is also a highly desirable when adequate sample is

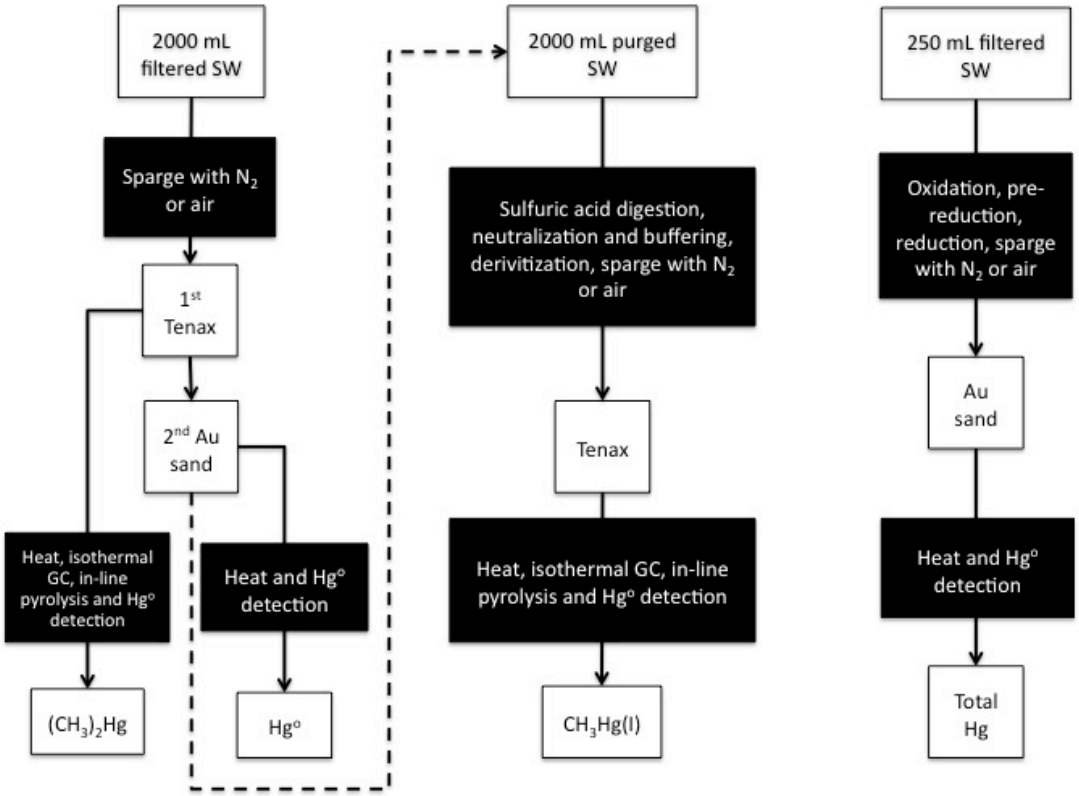

Figure 1. Our recommended workflow. All four analyses could be performed on one 2-L sample, but the reagents associated with analysis of $CH_3Hg(I)$ have a larger blank than those associated with total Hg determination. Therefore, for at-sea measurements, we recommend two separate aliquots be collected: one 250-mL sample for total Hg and one 2-L sample for Hg^o, $(CH_3)_2Hg$ and $CH_3Hg(I)$.

available. Standard spikes recoveries, especially for the $CH_3Hg(I)$ determination, should also be performed. These QA results should be reported along with the Hg results to demonstrate capability, reproducibility and accuracy.

8.*3.1 Total Hg*

During recent cruises, we have documented concentrations of total Hg in surface waters that are often highly depleted due to biological uptake and particle scavenging. Thus, GEOTRACES analysts should be prepared to deal with samples containing as little as 0.1 pM total Hg. As typical CVAFS arrangements have absolute detection limits on the order of 10 fmole, analyses performed on sample volumes of ca. 250 mL is recommended to ensure a resolvable signal.

Filtered aliquots of seawater should be pre-treated prior to analysis as follows: oxidize the sample with 0.05% (w/v) bromine monochloride (BrCl) solution or equivalent for at least 1 hour, removal of excess halogens with 0.05% v/v hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) solution for at least 5 minutes, and final reduction with 0.05% v/v stannous chloride ($SnCl_2$) solution followed by purging of Hg^o and trapping on gold or gold-coated sand (or the equivalent). Purging should progress until a volume of gas of at least 15 times the volume of liquid has been sparged, and at a volumetric flow rate of no more than 1 L min^{-1} (we recommend 0.5 L min^{-1}).

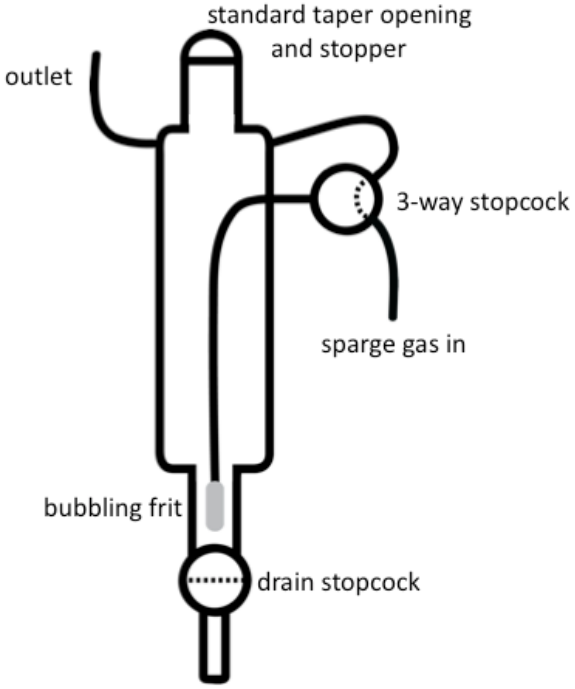

Figure 2. The sparging design developed at the University of Connecticut. It allows samples to be poured in at the top through the standard taper joint, while simultaneously allowing clean gas to vent the headspace. Emptying of the bubbler in preparation for another sample is achieve through the stopcock at the bottom, which will allow the bubbler to again fill with clean gas instead of room air. The three-way stopcock allows for the direction of sparging gas either through the headspace or the sparging frit at the bottom.

The sparging step should be conducted in a manner that minimizes introduction of shipboard laboratory air to the bubbler system. A closed sample introduction system is ideal, or a procedure that allows complete flushing of the headspace above the sample with Hg^0-free air (achieved using a Au trap column on the air inlet) prior to initiation of sample sparging. For samples less than about 300 mL in size, we recommend either a custom Fitzgerald Bubbler (diagram in Figure 2), or a 3 port bottle top sparging adaptor (e.g., Bio-Chem Omnifit #00945Q-3; fits any glass bottle with a GL45 thread) that can be fitted with a simple three-way manual valve (e.g. Cole-Parmer EW-30600-23) and attached to the sample bottle. Expelling the room air from the headspace of the Fitzgerald Bubbler is accomplished by having the purge gas flowing through the headspace and off-line with the collection gold trap for enough time to affect at least 5 volume exchanges. Entrainment of room air bubbles in the sample should also be avoided by decanting samples slowly and avoiding turbulent mixing after reagents have been added.

8.3.2 Hg^0 and $(CH_3)_2Hg$

Although these two dissolved gaseous mercury species are minor components (typically sub-pM concentrations) of the total mercury present in seawater, they are nonetheless highly important to measure as they are involved in air-sea exchange of Hg and probably in the formation of $CH_3Hg(I)$. Given the extremely low concentrations of these species, we recommend using 2 L sample sizes for analysis, with determination of Hg^o, $(CH_3)_2Hg$

and $CH_3Hg(I)$ all performed on the same aliquot. Procedurally, Hg^o and $(CH_3)_2Hg$ are the easiest of the species to measure, requiring only that a volume of stripping gas of at least 15x the volume of liquid be sparged through the fluid without further amendment. We have successfully used two sorption media in series to discriminate between these two gaseous mercury species. The gas exiting the sparger should pass first through a moisture trap (e.g., soda lime), then either Tenax or Carbotrap (or the equivalent) for $(CH_3)_2Hg$ collection, followed by Au or Au-coated sand for Hg^o collection (e.g., Bloom and Fitzgerald, 1988; Tseng *et al.*, 2004; Conaway *et al.*, 2009). Following sparging, the traps are analyzed separately using a CVAFS system that is equipped with a gas flow train. The Hg^0 collected on the gold trap is liberated for detection by simply heating (600-800 °C) in an argon gas-flow train connected to the CVAFS detector. The $(CH_3)_2Hg$ retained on the chromatography material trap is liberated under low heat (90-250 °C) and is passed first through a low temperature, isothermal chromatographic column (see in $CH_3Hg(I)$ section below) and then through a high temperature (600-800 °C) column packed with quartz wool to pyrolyze the $(CH_3)_2Hg$ to Hg^o and make it available for detection by CVAFS (Bloom and Fitzgerald, 1988). Tenax and Carbotrap columns should be rigorously preconditioned prior to use by sparging and heating them several times. Furthermore, they should be tested to ensure that they do not retain Hg^0 to a large degree. We recommend the use of Tenax rather than Carbotrap as it retains much less moisture and Hg^0. Fresh soda lime drying agent should be used on each sample, and can be recycled through baking.

8.3.3 $CH_3Hg(I)$

Following the sparging of Hg^o and $(CH_3)_2Hg$, the 2 L sample can be processed for $CH_3Hg(I)$ determination. The sample must first be "digested" for > 12 h, through addition of 40 mL of conc. H_2SO_4. Following digestion, the sample is first neutralized with ca. 60 mL of 50% KOH, and then buffered to ca. pH=5 with 30 mL of 2 M Na-Acetate/Acetic Acid buffer. The pH should be checked and adjusted as necessary with small additions of strong acid (H_2SO_4) or strong base (KOH).

To sparge the $CH_3Hg(I)$ from solution, it must first be derivatized or converted into a more volatile compound. Both alkylation (ethylation or propylation) and hydride generation have been used for this purpose. The new method described here, and in more detail in Bowman and Hammerschmidt (2011), makes use of a direct ethylation reaction applied to the seawater matrix. They have found that with the digestion step, close attention to pH and the use of fresh and cold ethylating agent (Na-tetraethylborate; NaTEB), quantitative ethylation in seawater can be achieved. This new proposed method eliminates the common practice currently employed of including a sample distillation step in the analysis to isolate the $CH_3Hg(I)$ from the matrix prior to the ethylation step.

As noted below, the ethylating agent is made up in small batches, but which often are not completely consumed within one week. After a week, even when kept frozen, the ethylating agent loses its potency and should be discarded. The thawed, working aliquot of 1% (wt:vol) NaTEB will also unavoidably lose potency throughout the course of the day, which can be slowed by keeping the solution cold. We recommend working samples in batches of four, by adding 1.5 mL of NaTEB directly to the buffered 2 L sample,

allowing each sample to react for at least 15 minutes, and then sparging the methylethyl mercury ($CH_3CH_2HgCH_3$) from the sample using a bottle top sparging adaptor as mentioned above.

The purge gas should first pass through a soda lime trap to remove moisture and then the $CH_3CH_2HgCH_3$ is collected on a Tenax trap column. Determination of $CH_3CH_2HgCH_3$ is conducted in an analogous way to $(CH_3)_2Hg$. The chromatographic separation is accomplished with a packed column (~0.5 cm diameter; ~60 cm length) of OV-3 on Chromosorb, held at about 60 °C.

8.4 Calibration and Comparability

One of the findings of the Intercalibration was that interlaboratory comparability was on the order of 50%. This lack of interlaboratory accuracy is unacceptable, as basin-to-basin variation in Hg concentrations (when comparing regions of similar productivity) can be expected to be considerably less. If datasets from cruises where different groups were involved are to be comparable, then overall accuracy must be improved. We therefore recommend that traceable Standard Reference Materials be included at numerous times during analyses. A list of Certified and Standard Reference Materials relevant to marine research is included below in Table 2. However, reasonably-sized seawater reference materials are not readily available for Hg determinations in the range that analysts will face in the open ocean. Therefore, we have set aside a large number of coastal seawater samples (125 mL), stored in BrCl cleaned glass vials for both total Hg and $CH_3Hg(I)$, where analysis of ca. 50 mL should provide similar absolute Hg species amounts as those in larger open ocean samples. These are available free of charge for use on any GEOTRACES cruise as a Consensus Value Reference Material. Participating laboratories should trace their analyses of this CVRM to a CRM in their laboratories prior to analysis. Analysis of the CVRM will ensure consistency across cruises, should the labs working Hg and $CH_3Hg(I)$ standards suffer from inaccuracy associated with dilution or handling. Contact Carl Lamborg to receive CVRM aliquots.

In order to achieve the most accurate results, we recommend analysts use the combination of both saturated vapor standard and aqueous standard calibrations. The combination of two working standards will aid in identification of gas leaks, column inefficiencies, standard degradation and low process yields. These processes can result in both random and systematic errors for individual samples as well as high- and low-biased calibrations.

8.5 Reagents

Hydroxylamine hydrochloride – dissolve 300 g of $NH_2OH \cdot HCl$ in 18 MΩ-cm water and bring to 1.0 L.

Stannous chloride – Bring 200 g of $SnCl_2 \cdot 2H_2O$ and 100 mL conc. HCl to 1.0 L with 18 MΩ-cm water. Purge with N_2 to lower blank. Store cold and tightly capped.

Bromine monochloride – In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Stir on stir plate if available. Slowly add 38 g $KBrO_3$ to the acid while stirring.

Acetate Buffer – Add 11.8 mL of glacial acetic acid and 2.2 g reagent grade sodium acetate trihydrate to ca. 50 mL 18 MΩ-cm water and shake until dissolved. Test pH, and adjust with acetic acid or sodium acetate to equal 5.5. Add more water to make up to 100 mL.

Sodium tetraethylborate – add 1 g of NaTEB (Strem 11-0575 or equivalent) to 100 mL of reagent-grade water. Divide the solution equally among plastic vials that then are capped and frozen. This solution should be kept frozen until used and made fresh every week or earlier.

Working Standards –We recommend making working standards from a stock solution of CH_3HgCl (Strem 80-2250 or equivalent) and $HgNO_3$ (reference solution; Fisher Scientific SM114-100 or equivalent). For $CH_3Hg(I)$, we have found that preservation with either 1) 2% glacial acetic acid and 0.2% concentrated HCl or 0.5% HCl to be useful. For Hg(II), preservation with 0.1% BrCl (see above) is sufficient.

Nitric Acid (for sample acidification) – J.T Baker Instra-analyzed trace metal grade. The acid blank should be determined prior to use (<0.01 ng/mL).

Argon – ultra-high purity grade with in-line gold and organic vapor removal traps

Soda Lime – ACS grade, 4-8 mesh, non-indicating, Alfa Aesar (stock number 36596). Approximately 5 cm length of soda lime is packed into ~0.5 cm (ID) by ~10 cm Teflon tubing and held in place with quartz or borosilicate glass wool. The columns are purged in a bubbler system for 10-15 minutes prior to use. Prepurging of soda lime columns is not necessary for trapping of methyl mercury.

Ultra-Pure Water – Obtained from a multi-column mixed-bed deionzing water system (e.g. Millipore Milli-Q Element system) that can produce 18 MΩ-cm water with a Hg content <0.1 ng/L.

8.6 References

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Agency	Item	Description	Certified for:	Amount
IAEA	IAEA-SL-1	Lake sediment	T	0.13
IRMM	BCR-060	Aquatic plant	T	0.34
IRMM	BCR-142R	Light sandy soil	T	0.067
IRMM	BCR-143R	Sludge amended soil	T	1.1
IRMM	BCR-145R	Sewage sludge	T	2.01
IRMM	BCR-145R	Sewage sludge	T	8.6
IRMM	BCR-277R	Estuarine sediment	T	0.128
IRMM	BCR-280R	Lake sediment	T	1.46
IRMM	BCR-320R	Channel sediment	T	0.85
IRMM	BCR-414	Plankton	T	0.276
IRMM	BCR-463	Tuna fish	T/M	2.85/3.04
IRMM	**BCR-579**	**Coastal sea water**	**T**	**1.9 ng/kg**
IRMM	ERM-CC580	Estuarine sediment	T/M	132/0.0755
IRMM	ERM-CE278	Mussel Tissue	T	0.196
IRMM	ERM-CE464	Tuna fish	T/M	5.24/5.50
NIST	SRM-1944	Harbor Sediment	T	3.4
NIST	SRM-1946	Lake Superior Fish Tissue	T/M	0.433/0.394 mg/kg wet
NIST	SRM-1947	Lake Michigan Fish Tissue	T/M	0.254/0.233
NIST	SRM-1974b	Mussel Tissue	T/M	167/69.6 µg/kg dry
NIST	SRM-2702	Marine sediment	T	0.4474
NIST	SRM-2703	Sediment	T	0.474
NIST	SRM-2781	Domestic sludge	T	3.64
NIST	SRM-2782	Industrial sludge	T	1.10
NIST	SRM-2976	Mussel Tissue	T/M	61.0/28.09 µg/kg
NRC-CNRC	DOLT-4	Dogfish liver	T/M	2.58/1.33
NRC-CNRC	DORM-3	Fish protein homogenate	T/M	0.382/0.355
NRC-CNRC	MESS-3	Marine sediment	T	0.091
NRC-CNRC	ORMS-4	River water	T	22.0 pg/g
NRC-CNRC	PACS-2	Marine sediment	T	3.04
NRC-CNRC	TORT-2	Lobster hepatopancreas	T/M	0.27/0.152
WHOI	WBW-1-2010	Coastal seawater	T/M	TBA /TBA

Table 2. Compilation of various marine relevant reference materials for total Hg and $CH_3Hg(I)$. All concentrations are mg/kg unless otherwise noted. $CH_3Hg(I)$ concentrations are as mass of Hg. T=total Hg, T/M=total and $CH_3Hg(I)$.
IAEA: International Atomic Energy Agency.

9. Collection of particulate samples from GO-FLO sampling bottles

The goal of sampling suspended particles from water sampling bottles mounted on a trace metal-clean rosette (e.g. GO-FLO bottles) is to allow analysis of particulate TMs if large volume *in situ* pumps are not available, and to complement pumping approaches to increase spatial resolution with minimal additional ship time expenditure. Hence these methods are the recommended for filtration of suspended particles from 5-12 L volumes, for purposes of analyzing for the key GEOTRACES trace elements, as well as additional elements as desired. Filtration may be done directly on-line from pressurized GO-FLO bottles, or off-line using a separate apparatus; recommendations for on-line filtration are given first, followed by procedural modifications for off-line filtration, and finally by analytical considerations.

9.1 Filter Type

We recommend Pall Gelman Supor 0.45 µm polyethersulfone filters. This recommendation is made after testing the properties of several candidate filter types. The factors that favored Supor filters were low metal blank in cleaned unused filters, mechanical strength and ease of handling, relatively high particle load capacity, low tendency to clog completely, and good filtration flow rate. A filter diameter of 25 mm works well for ~10 L volumes from most depths at open ocean stations, while 47 mm is preferred for shelf-slope stations where particle concentrations are higher, and may be used as well for upper euphotic zone samples at open ocean stations, as 25 mm filters may effectively clog before entire volume is filtered. Filter diameter should be minimized in general so that loading per area of filter is maximized in order that sample element concentrations exceed filter blank to the greatest degree possible.

An alternative filter type is mixed cellulose ester (we found **MF-Millipore type HAW** to work well), which is close in filtration performance to the Supor filters, but has higher blanks for some elements (e.g., Al, Th, Mn). Cellulose filters have the advantage that they will digest completely in nitric acid, which is not the case for Supor filters, though comparison of these filter types during GEOTRACES Intercalibration cruises suggests that this difference has no effect on completeness of dissolution of natural particles, using the digestion methods outlined below. However, we saw clear evidence that the type of filter used can affect the measured particulate TE concentrations, presumably due to differences in the effective size fractions and particle subpopulations sampled by each filter type. Clearly, particulate metal concentrations are operationally defined, and consistent filtration methods should be used for this reason. Filter choice should be consistent as the GEOTRACES program progresses and results are compared among various sampling programs.

Prefilter screens may be used upstream of main filters if size-fractionated sampling is desired, for example to provide samples comparable to size-fractionated sample collection by in situ pumping on the same cruise. In this case, prefilters can be mounted in separate filter holders connected to main filter holders. One convenient property of prefilters is that they pass air bubbles readily, and do not normally need inversion or other treatments to clear trapped head-space air. We recommend the use of 51 µm square weave polyester screens (#07-51/33 from Sefar Filtration) since they are also recommended for in situ pumping. Filter material can be punched to make circular filters before acid leaching as for other filter types. The use of prefilter diameters smaller than the main filter (e.g., 13 mm prefilters for 25 mm main filters) will increase particle loading per filter area on the larger size fraction and thus increase sample to filter blank ratio, a significant concern given relatively high prefilter blanks for some elements (Cullen and Sherrell, 1999). Resultant higher flow rates, however, can also disaggregate larger particles deposited on the prefilter, altering the apparent size fractionation in favor of small particles. Because filter blanks can be very large on these recommended filters for some elements (e.g., Cd, Cu; Cullen and Sherrell, 1999), we recommend collecting only one size fraction (>0.45 µm) as a default for the GEOTRACES program for cruises

during which particle sampling will be done exclusively from GO-FLO bottles, with no in situ pump sampling.

9.2 Filter holders

Filter holders should be compatible with trace metal clean procedures so that filtrate may be used for analysis of dissolved TMs if desired. Many types are available but none is ideal in design. **We used Advantec-MFS 47 mm polypropylene inline filter holders (type PP47; www.advantecmfs.com) and Millipore Swinnex polypropylene 25 mm filter holders (http://www.millipore.com/catalogue/module/C160).** These filter

Figure 1. Advantec-MFS polypropylene 47 mm filter holders.

holders are shown in Figures 1 and 2. **Any filter support screen on the upstream side of filter should be removed as it could act as an inadvertent prefilter.** The MFS filter holders have the advantage of closing by locking collar, so that filter is not subjected to twisting motion upon tightening, has convenient connectors for plumbing fittings and pressure applications, and is made of clean materials (e.g., red silicone o-rings). However, some effort is necessary to ensure proper sealing upon tightening, the blue polypropylene body is not transparent so headspace bubbles cannot be seen, and there is no air vent, requiring removal of headspace air by loosening the filter holder during initial flow (see "Attaching filter holders to GO-FLO bottles", below). Some other filter holder designs had some of these features, but had other disadvantages. The 25 mm Swinnex filter holders have no screen on the inlet side (not true of some other 25 mm in-line filter holders), but have imperfect sealing capabilities under pressure with the supplied white silicone gaskets, causing occasional slow drips to escape through the closure. Purchase extra silicone gaskets as these become easily distorted to imperfect circle shapes. Again, these choices were the best compromise we found, but other filter holders should be considered by future users. It is recommended that each filter holder

be marked with a unique number, so that samples can be kept organized while held in filter holders, and that persistent problems (e.g., blank, poor sealing) can be recorded and traced as necessary to particular filter holders. Advice in selection and operation is available from Rob Sherrell (sherrell@marine.rutgers.edu).

9.3 Cleaning Filters and filter holders

Filters are cleaned by the following protocol:

1. Pre-clean a 1000 mL LDPE pre-cleaned bottle by filling with 10% (v/v, or 0.12M) of TM Grade HCl, double bagging in 4mil Ziploc polyethylene bags, and placing in oven at 60°C for 4 hrs to overnight. Remove to fume hood and place inverted so that lid is acid-leached while acid cools. Pour out acid and rinse thoroughly at least 3 times with TM-clean deionized water (e.g., Milli-Q).
2. Fill the clean bottle 90% full with TM-clean deionized water.
3. Remove filters from original box using metal-free forceps (e.g., Bel-Art #379220000 Tefzel forceps, Product number 22-261826 from Fisher Scientific), grasping filters only on the edge so that sample region is not damaged, and carefully drop them in bottle. Make sure any separator papers from original packaging are not included. When 100 filters have been immersed in the water, fill last 10% of bottle volume with concentrated TM Grade HCl, cap tightly, mix gently so that filters do not crease, and place double bagged bottle in 60°,C oven overnight, as for bottle cleaning.
4. When bottle of filters is cool, slowly pour off acid to waste, retaining filters with cap held against bottle mouth. Keep filters in suspension by gentle hand-agitation while pouring off acid, to minimize folding and creasing when all solution is removed. Fill bottle slowly with DI water running gently down the inside wall, swirl gently, and pour out water, retaining filters with cap. Repeat 5 times. Leave last rinse in bottle and allow to sit at room temperature overnight so that any residual acid diffuses from pore spaces of filter. Repeat 3 more rinses the next day. Filters can be left in DI water suspension for use on ship from this supply, or can be loaded into individual Petri-slides for easy use, sampling, and replacing in Petri-slide. Use caution to avoid getting doubled filters, as they tend to stick to each other.

9.4 Attachment and use of filter holders on GO-FLO bottles

Filter holders require tight, metal-clean connections to GO-FLO bottles that can also be rotated so that filter holder can be inverted for clearing air from head space. Since the stopcocks on the US GEOTRACES GO-FLO bottles have 3/8" compression fittings, we used a ~4" length of 3/8" OD polyethylene or Bev-A-Line (Cole-Parmer) tubing, which was inserted into the stopcock fitting at one end and into a 90° elbow (white polypropylene) with 3/8" compression at one end and ¼" female NPT fitting at the other. This fitting can screw directly onto the inlet fitting of the MFS 47 mm filter holder, or can mate to a Luer-lock adapter that attaches to the inlet of the Swinnex 25 mm

filter holder (Fig. 2). It is recommended to minimize the length of small diameter tubing or Luer fittings, as they may cause flow restriction in early stages of filtration. The 90° fitting allows the filter holder to sit approximately horizontal during filtration, and also allows the 3/8" poly tube to be twisted in the stopcock fitting in order to allow clearance of air bubbles. **Clearance of trapped air is accomplished by opening stopcock with filter holder inverted, then unscrewing filter holder about ½ turn to allow a small volume of water to flow around filter, sweeping out trapped air.** Filter holder is then tightened securely, the 3/8" tube twisted again so that filter holder is right-side up, and filtrate flows normally with no seeping detected at threads of filter holder. Other solutions to the air-lock problem may be found, for example modifying the filter holder by making a larger ID inlet, but this was not thoroughly investigated. A clean outlet tubing (e.g., Bev-A-Line, C-Flex) can now be attached to the outlet of the filter holder if filtrate water is being retained in a sample bottle. Otherwise filtrate can flow to waste into a rectangular plastic waste bucket (ours were 11 L capacity). This allows filtered volume to be retained and measured later by repeated pouring into 2 L graduated cylinder. Alternatively, if volume in GO-FLO bottle is known, and volume is completely filtered, then volume measurement is not necessary. If the filter clogs, filtration should be stopped and either filtrate or residual water in GO-FLO bottle can be measured.

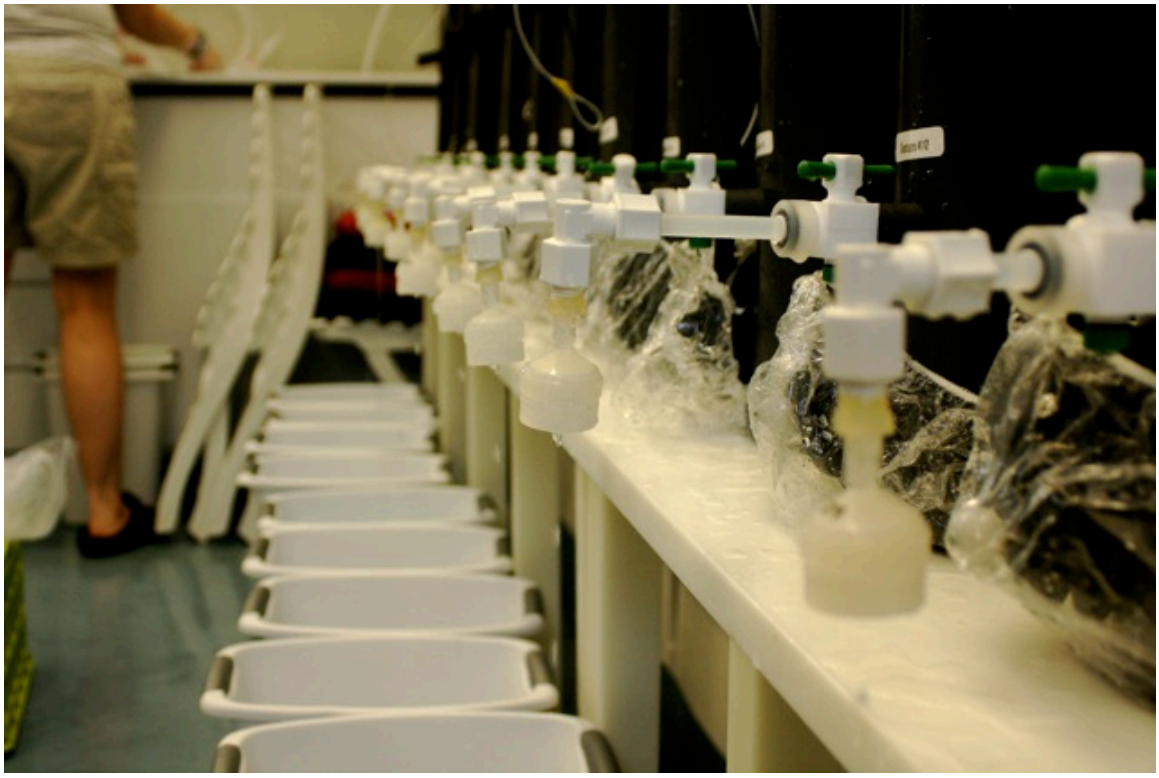

Figure 2. Swinnex 25 mm filter holders showing 3/8" OD tubing, 90° compression-NPT adapter, and NPT-Luer lock adapter. Note 11 L waste baskets for filtrate volume measurements.

9.5 Filtration time and particle settling artifacts

In order to optimize the ratio of particulate elemental concentrations to filter blank contributions, filters should be loaded as well as possible with sample. In practice, this means **filtering to the flow rate of about one drop per second through 0.45 µm Supor filters**, if possible. In our experience, this could be achieved within a 1-2 hour filtration period. Generally, at open ocean stations below 200 m, the full bottle volume of 10-11 L could be filtered through a 25 mm filter before this clogging point was reached, with the result of sufficient loading of the filter. In very clean deep water, two GO-FLO bottles (20-22 L) could be filtered through a single 25mm filter before clogging. However, volumes greater than 10 L were not deemed necessary for sufficient sample/blank ratio when filtering deep particulate matter.

Sample bias due to particulate sedimentation in water bottles prior to filtration has been a long recognized problem (Bishop and Edmond, 1976; Gardner 1977) and biases can be a factor of two or more. Allowing filtration times longer than 1-2 hours can lead to significant artifacts due to particle settling within the GO-FLO bottle. Settled particles tend to be larger aggregates, of course, and their loss by accumulation below the stopcock will affect measured particulate concentrations of elements differentially. Since particle settling can occur continuously during the period between GO-FLO closing at depth and initiation of filtration, **we recommend gentle mixing of GO-FLO bottles just before filtration, but after a small (0.5-1.0 L) volume is removed for oxygen, salinity, etc**. This small headspace allows effective mixing and homogenization of suspended particles. We recommend mixing by supporting the GO-FLO bottle horizontally and tilting slowly about 20° both directions, repeated three times, to achieve complete homogenization without unnecessary turbulence. Commence filtration immediately afterward. Alternative bottle designs with the stopcock at lowest point in bottle may alleviate this artifact, but users should be aware that at the low flow rates through these small filters, water movement near the bottom of the bottle is likely insufficient to resuspend and transport settled particles to the stopcock inlet. It is not clear that curved tubes attached to the inside of the stopcock and leading to the lowest point in the bottle are effective at re-entraining settled particles and aggregates. Demonstration that particle settling artifacts do not lead to inaccurate particulate elemental concentrations requires comparison to a collection method that is not vulnerable to this artifact, most notably *in situ* filtration.

9.6 Pressurizing water sampling bottles for filtration

Gas pressure applied to GO-FLO bottle is necessary to achieve acceptable filtration flow rates. **Recommended gas is clean air**, provided to a plastic tubing manifold by an oil-free compressor and **filtered (0.22 µm) at entrance to each sampling bottle**. We recommend **< 7 psi** (50 kPa) for filtration, a good compromise between high rate of filtration and minimization of cell lysis or other pressure-related artifacts. Nitrogen should be considered as a substitute when sampling suboxic waters.

9.7 Process blanks

It is highly recommended to collect filtration process (e.g., adsorption) blanks for comparison to unused filter blanks, in order to subtract an appropriate blank from concentrations measured on particulate samples. In our experiments, process filter blanks increase for some elements and decrease for others, to a significant degree, relative to blanks on unused, pre-cleaned, filters. We recommend using a 0.2μm pore size capsule filter (same Acropak as described in VI.3.2.1) on the outlet of the GO-FLO bottle, attaching the loaded filter holder to the capsule filter outlet, and filtering normally to a default volume of 2 L, so that TM-clean 0.2 μm filtered seawater passes through the particle sampling filter. Treat this filter thereafter as for normal samples. Such process blanks should be taken frequently enough during a sampling cruise that process blanks are representative of major water types (euphotic zone, thermocline, deep water column) and oceanic regimes being sampled (open ocean, slope water, shelf water), with some replication. This is necessary so that appropriate blanks can be compared to sample filters.

9.8 Storing Sampled Filters

When filtration is complete, residual headspace seawater may not flow through the nearly clogged filter. **We recommend attaching an all-polypropylene syringe, filled with air within a laminar flow bench, to the top of the filter holder and forcing residual seawater through the filter under pressure.** This will avoid spillage and loss of particulate material from face of filter when filter holder is opened, and will remove as much seawater as possible in order to reduce the residual sea salt matrix for analytical simplicity after the sample is digested. This method works well for key GEOTRACES trace metals, but may not be sufficient to reduce sea salt to a level where salt corrections are small enough for accurate determination of particulate Ca. In this case, a method for misting filters with DI water will need to be devised, as for in situ pumped samples (Section VI.9). In a laminar flow clean bench, filter holders can be disassembled and filters carefully removed using Tefzel forceps. If filters are still quite wet with seawater, they may be blotted by placing sample-face-up for a few seconds on an acid-cleaned quartz fiber filter, which will act as a wicking agent, further reducing the sea salt matrix. **Filters should be stored in a Petri-slide or similar suitable container and frozen at -20° C.** Freezing is recommended mainly as a way to physically stabilize the sample. Samples left at room temperature may allow residual seawater on the filter to slough off, leading to sample loss. **Drying in a TM-clean oven at 60° C is also acceptable to prepare samples for storage and shipping**. One group has noted that placing a wet filter in contact with a plastic surface and air-drying, oven-drying or freezing can lead to differential fractionation of major sea salt ions to the plastic surface when the filter is removed for later processing, such that Na, Ca, or Mg concentrations, used to correct particulate composition for sea salt contributions, are biased. This may be an issue for any particulate element with a substantial sea salt correction due to residual dried seawater on the filter.

9.9 Clean Up and Preparations After Sampling

All manipulations involving opening the filter holders should be done in a laminar flow clean bench. Once filters are removed to storage containers, filter holders should be rinsed on internal surfaces with a squirt bottle containing TM-clean DI water. In highly productive waters in particular, particles may adhere to the filter holder, and to the top headspace surfaces in particular. After shaking filter holder dry, new filters can be loaded into the filter holders in preparation for the next cast. Pre-sampling storage of the loaded filters in this manner is not problematic, as long as filter holders are stored in a metal-clean location (e.g., multiple layers of plastic bag or box).

9.10 Off-line Filtration

Filtration of seawater off-line, after collection from the GO-FLO sampling bottles into a secondary transfer container, has been shown to work as well, without large obvious artifacts (Experiments by R. Sherrell and J. Bishop). Off-line filtration allows rapid removal of seawater from the sampling bottle, decreasing between-cast turnaround time, and has the potential to minimize the particle settling loss artifact, which is a concern with on-line filtration. Off-line filtration may be the only practical alternative for some kinds of sampling systems

a. Removing volume for filtration: It is recommended to **mix the GO-FLO bottle, as described above, immediately before aliquoting volume for filtration. Volume to filter is suggested to be 5-10 L**, as practical. These volumes will load filters sufficiently to exceed filter blanks for nearly all samples and all analytes. Seawater should be drained cleanly and quickly into the transfer bottle or jug, which is then removed to a separate clean area for filtration.

b. Filtration Method: **A sample receiving bottle may be modified for direct filtration by inversion, with an air vent on bottom and a custom fabricated filter holder adapter that replaces the normal cap.** If the face of the filter is open to the bottle volume, without the normal constriction of typical in-line filter holders, then there will be no concerns with air lock or bubbles during filtration. If receiving bottle has tapered shoulders, this will be advantageous as particles will have reduced tendency to settle on shoulders during filtration.

   For this inversion method, a custom rack is recommended that supports the inverted bottles while still allowing them to be swirled periodically as filtration proceeds so that particles do not settle on bottom walls or shoulders. If bottle is not strong enough to be pressurized at 7 psi as for GO-FLO bottles (many plastic bottles are not sufficiently strong, or pose an explosion hazard), then vacuum can be applied to the filtrate outlet plumping (though difficult to integrate a vacuum method with clean collection of 5-10 L of filtrate), or the outlet flow can be passed through a clean peristaltic pump to provide suction.

Alternatively, the inversion method can be abandoned, and the unfiltered seawater in the receiving bottle could be poured in sequential aliquots into a conventional TM-clean filter funnel apparatus placed within a clean bench; this requires much more attention, whereas the bottle inversion methods should be largely self-tending. In either case, it is expected that the entire 5-10 L volume will be filtered through the filter types and sizes recommended above, so that the off-line method results in filters that are loaded to within a factor of 2 of those resulting from the on-line method, allowing reasonably large sample to filter blank ratios for all GEOTRACES key trace elements. If filtrate is needed for other analyses, secondary filtrate receiving bottles will be necessary. In this case, the entire procedure should be checked for freedom from procedural contamination.

c. Small volume off-line filtration method: **A smaller volume version of the offline inverted bottle filtration method may be employed if available volumes are limited.** A 1 liter sample receiving bottle may be modified for direct filtration by inversion, with an air vent on bottom and a custom fabricated filter holder adapter that replaces the normal cap (Fig. 3). This method has been used routinely on CLIVAR A16N, A16S, VERTIGO, and GEOTRACES IC expeditions, although not all key GEOTRACES TEs have been analyzed. In theory, if filter diameter is scaled down (e.g. 13mm) so that particle loading overcomes filter blank, this method could be used for all GEOTRACES key TEs. **This method does not permit filtrate collection.**

Figure 3. An example of a 1 L offline filtration method as used routinely on CLIVAR A16N, A16S, VERTIGO, and GEOTRACES IC expeditions. Pre-cleaned 1L LDPE bottles are modified with closing air vents at bottom. Sample is quickly transferred from the GO-FLO into the 1 L LDPE bottle which is then capped conventionally. Once returned to a Laminar Flow bench environment, the top is substituted for a tapered adaptor which has a mated 47 mm MFS filter holder with preloaded 0.45 µm Supor filter. The upstream orifice of the filter holder has been drilled out to twice standard diameter to minimize air-lock effects. Once samples are filtered under 25 to 40 mm Hg vacuum, they are transferred directly to sample bottles for further processing. Primary sample bottles and filter holders are reused after TM-clean DI water rinsing. More information available from J. Bishop (jkbishop@berkeley.edu) or Todd Wood (tjwood@lbl.gov).

9.11 Processing and analysis of particulate samples on filters

If the object is to achieve complete digestion of all particle types and therefore a total suspended matter analysis, we recommend below a procedure for the acid digestion of particulate samples, making the distinction between methods appropriate for Supor® (polysulfone) and MF-Millipore® (mixed cellulose ester) filters. Other methods may achieve comparable results for some or all key trace elements, but will need to be checked using appropriate certified reference materials and/or intercomparison with this method. The methodology for analysis of the resulting solution is the choice of the analyst, but guidelines are given, based on the ICP-MS methods developed during the GEOTRACES Intercalibration Program.

9.11.1 Digestion vial cleaning procedure
Savillex® 15 mL flat-bottom Teflon vials or equivalent are recommended.

- New Teflon vials and caps are cleaned in 1-3% solution of P-free lab detergent (e.g. Micro®).
- Teflon vials and caps are rinsed with Milli-Q water 3 times.
- Boiled in 50% TM grade HCl approximately 2 hours, in glass beakers on hot plate.
- Bulk rinsed with Milli-Q water and rinsed individually 3 times.
- Refluxed with cap tightened using 1-2 mL a solution of approximately 50% nitric acid, 10% hydrofluoric acid (this solution is recycled) for approximately 4 hours at 120˚C.
- Rinsed with Milli-Q water before reuse 3 times.
- Blank digest (no filter) should then be performed to determine metal blanks derived from Teflon vial walls. These should be compared to determined filter blanks and are expected to be at least several times lower. If they are not, vial cleaning procedure should be repeated until all vials meet digest blank criteria.

9.11.2 Cleaning of 15 mL archiving tubes
For storing digest solutions prior to analysis and for archiving, Corning® 15 mL clear polypropylene (PP) centrifuge tubes or equivalent are recommended.

- Filled with 1.2M TM grade HCl (this solution is recycled), capped tightly and placed in a plastic or polystyrene foam tube rack.
- Double-bagged in 4 mil plastic zip-lock bags, then heated in a 60˚ C oven for 4 hours to overnight.
- Turned upside down to cool in fume hood and leach caps.
- Rinsed with Milli-Q water 3 times, including careful rinsing of cap and tube threads.
- Shaken dry, and allowed to dry briefly but thoroughly in laminar flow clean bench.

9.11.3 Filter Digestion procedure

Ultrapure grade acids (e.g., Fisher Optima or equivalent) are recommended in this protocol.

- Digestion procedure is based on that developed by Sherrell (1991) and Cullen and Sherrell (1999).
- Ideally, 1 filter is to be digested per digestion vial.
- 10% HF/50% HNO_3 (v/v) digest solution is recommended in order to achieve complete dissolution of all particle types, and in particular to bring all lithogenic material in solution. Higher concentrations of HNO_3 have no effect on particle digestion effectiveness, but can increase filter blank.
- Polyethersulfone filters (Supor®) are placed against the wall of the vial, close enough to the top edge to avoid submerging any part of the filter in the digestion medium. This is done to allow refluxing, whereby the acid droplets to collect on the top of the vial (inside of cap), slide down the side of the vial over the sampled face of the filter and continue refluxing. Filters that are damp with residual seawater, or are dampened during the addition of digest acid, stick closely to the wall, so that refluxing acid passes over the face of the filter, not under it. The filter material stays relatively intact against the side of the vial but is never immersed fully in hot acid. Supor® filters do not fully dissolve in any case in this acid mixture, and hot immersion can increase the organic matter matrix of the digest solution, or occlude undigested particles in the resulting shrunken and distorted filter matrix.
- MF-Millipore filters are placed in the bottom of the vial because a complete digestion of the cellulose filter is achieved in under these conditions.
- 47 mm filters are cleanly cut in half using a ceramic blade scalpel, and the halves placed on opposite sides of the vial for refluxing.
- Typically, for a 25 mm diameter filter, add 1 mL of 50% HNO_3/10% HF solution to each vial. Roll acid around inside vial to ensure full contact with filter.
- Close the caps tightly and place vials on a Teflon or silicone surface hot plate at 130° C for 4 hours.
- After a cool down period, collect all the droplets from the cap and inside of the vials down to the bottom of the vial by either tapping the sealed vials or rolling the solution around.
- Dry down the solution on the hot plate at 130° C. Watch it until near dryness, reducing heat as necessary. Remove when droplet is reduced to <5 µL volume. This step reduces the HF in the sample, and allows the matrix to be switched to dilute nitric acid for analysis. Heat lamps cleanly mounted above the hot plate may help prevent condensation on vial walls.
- If desired, add 100 µL concentrated HNO_3, directly onto residual droplet, and dry down again to same size droplet. This ensures sufficient HF removal so that glass and quartz components of the introduction system of the analytical instrument are not etched or degraded.

9.11.4 Blanks

Vial blanks should be assessed, following the same protocol as described above, but deleting the filter. These are to be compared to digestions of unused filters and sampling process blank filters, in order to determine overall blank contributions and their sources.

9.11.5 Archiving procedure

The nearly dried residues are brought back into solution with 5% HNO_3 (for ICP-MS) or another acid mixture as required by the analytical method to be followed. The completeness of this redissolution can be checked with tracer elements and analysis of CRMs. This solution is referred to as the archiving solution hereafter.

- After the dry down step, add 3 mL of archiving solution to the Teflon vial, seal cap, and heat gently for 1 hour at 60° C to ensure a complete redissolution. This volume results in a solution for analysis (without further dilution) that contains relatively high concentrations of trace metals, minimizing effort expended to achieve extremely low instrument blanks during analysis. Roll the hot solution up on the walls of the vial to ensure that any digest solution dried to the surface of the filter is completely redissolved and quantitatively taken up.

- Pour or cleanly pipet this solution into precleaned 15 mL tubes and store them at 4° C to minimize evaporative loss.

9.11.6 Analysis procedures

The following in provided as an analytical guideline, not a rigid protocol; analysts may follow a variety of equally valid approaches. The procedure will also vary according to the type of mass spectrometric or other method to be used for analysis. However, the ideal procedure should consider the following aspects: reproducibility, precision, accuracy, and drift. We describe procedures used in the lab of R. Sherrell (Rutgers University) below, in order to show an example of the aspects of a successful analytical approach:

- Each sample should be spiked with a drift monitor (In, Sc) in order to make an accurate correction for drift and matrix-dependent sensitivity variations of the instrument. These element spikes can be added directly to the bottle of 5% HNO_3 archiving solution before adding 3 mL volumes to vials.
- External standard curves should be made in the archiving solution matrix, containing all elements of interest in appropriate ratios for typical expected sample composition. Since element concentrations may differ by many orders of magnitude (e.g., Ca vs. Co), single-element standards should be checked for cross-contamination before mixing. To be safe, two standard mixtures (high and low) are recommended. Standard curves of ~8 points should be constructed because element concentrations can vary greatly in natural samples (e.g., surface water vs. deep water), and curves used should contain points bracketing all sample concentrations encountered.
- Every 10 samples, a replicate analysis of a selected sample digest solution should be made.

- Spike recovery should be also assessed every 10 samples by spiking one additional sample aliquot with a known volume of a known composition solution.
- An aliquot of a representative large sample digestion solution should be run each analytical day as an internal laboratory consistency standard to check the inter-run long-term precision of the measurements.
- Since there is no certified reference material (CRM) for suspended oceanic particulate matter, a combination of CRMs like the ones specified here may be used instead:

  BCR-414a (http://irmm.jrc.ec.europa.eu/html/reference_materials_catalogue/)
  One or two of several available marine sediment CRMs (e.g., MESS, HISS).

The mass of certified standard used should be sufficient to be a representative subsample and its digestion volume should be scaled to mass as per oceanic particulate samples.

9.12 References

Bishop J. K. B. and J. M. Edmond (1976) A new large volume filtration system for the sampling of oceanic particulate matter. *J. Marine Res.* 34, 181-198.

Cullen, J.T. and R.M. Sherrell (1999) Techniques for determination of trace metals in small samples of size-fractionated particulate matter: Phytoplankton metals off central California, *Mar. Chem.* 67, 233-247.

Gardner W. D. (1977) Incomplete extraction of rapidly settling particles from water samples, *Limnol. Oceanogr.* 22, 764-768.

Sherrell, R.M. (1991) Collection of suspended oceanic particulate matter for trace metal analysis using a new in-situ pump, in Marine Particles: Analysis and Characterization, Eds. D.C. Hurd and D.W. Spencer, Amer. Geophys. Union, pp. 285-294.

10. In-situ Pumping Sampling Protocols For Particulate Trace Metals

In-situ filtration allows the collection of large volume size-fractionated samples of marine particulate matter from the water column. The ship-electricity powered Multiple Unit Large Volume in-situ Filtration System (MULVFS; Bishop et al., 1985) was designed to sample particle populations from 1000's to 10,000 L plus volumes of seawater accurately and without sampling bias or contamination in calm to harsh sea conditions including strong current regimes such as in the Gulf Stream. Its current depth capability is 1000 m. Commercially available battery-operated in-situ pumping systems (e.g., McLane, Challenger) can operate at any depth, and although scaled down in terms of volume filtered, can be used to achieve the same performance goals with modifications as detailed below. In addition to discussions below, please refer to the GO-Flo filtration section (VI.8) for further details on filter blanks and analytical details and the Particle Optics Protocols (Section VIII) for trouble-free transmissometer deployment. In the

discussion that follows, we identify protocols applicable to all in-situ filtration systems (MULVFS, McLane, and Challenger) with specific call outs where appropriate.

10.1 Cast documentation

Casts are identified by standard operation number, date, time of start of cast, filtration starting (time, lat., long.), filtration ending (time. lat., long.), and time of end of cast. Samples in each cast are identified by wire out depth, pump depth, electrical breakout number (for MULVFS), pump number/name, filter holder ID (especially for multiple filter holders per pump), filter type, and volume(s) of water filtered.

Volume(s) of water filtered is determined by flow meter readings before and after deployment. Electronic calculations of volumes filtered (as on McLane pumps) should not be trusted. Flow meters must be read twice prior to first deployment and must be verified against final readings from the previous deployment prior to each new deployment.

10.2 Protocols for deployment and recovery

As for any contamination-prone sampling, the bridge should be asked to stop grey water discharge for the duration of pump deployments. **Needle gunning, sweeping, or hosing on deck should also be suspended for the entire duration of sampling on station.**

10.2.1 Cable for deploying pumps

A metal-free line should be used to deploy McLane battery powered pumps. McLane pumps attach to a wire via 2 book-style stainless steel clamps. This requires a wire that does not compress very much when squeezed. Many braided metal-free lines (e.g., Amsteel, Kevlar) are unsuitable because they compress and prevent secure attachment of the pump onto the line. The US GEOTRACES North Atlantic Zonal Transect (GA03) used 3/8" OD Hytrel-coated Vectran, a liquid crystal polymer, for deploying up to 8 dual-flow upright McLane pumps at once (Figure 1). The Hytrel jacket is a thermoplastic polyester elastomer that is extruded over the Vectran strength member, and provides adequate grip and rigidity for clamping the pumps. On US ships, many winches, blocks, and level winds are optimized for 0.322" hydrowire, so the US GEOTRACES Eastern Pacific Zonal Transect (GP16) used a new Vectran wire with thinner Hytrel-coating (0.322" OD) to improve level-winding. We have used other types of metal-free wire on other cruises (1/4" OD Aracom Miniline, which has a Technora Aramid polyester strength core with a tightly woven over-braid of extremely thin polyester) (Figure 1). The polyester sheath of the Aracom Miniline provided much less grip than the Hytrel coating, so slippage in the pump clamp of several inches was occasionally observed and must be carefully monitored.

The MULVFS uses a dedicated 1000 m long Hytrel-jacketed electromechanical cable with 18 tapered electrical breakouts spaced along its length. MULVFS pumps are lifted onto and off of the wire with winch assist using a nylon-strap-tether that attaches to a nylon strap loop integrated into the cable above each connection point (Figure 2).

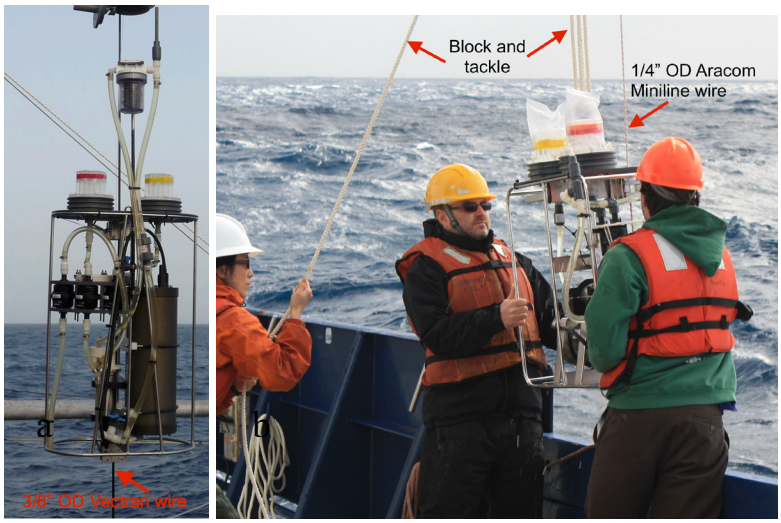

Figure 1. Dual-flow McLane WTS-LV pumps. a) upright WTS-LV used on US GEOTRACES GA03, deployed on 3/8" Vectran, and b) standard WTS-LV deployed on 1/4" OD Aracom Miniline cable on RR1202 showing block and tackle being used on recovery: two people steady the pump while a third person takes the weight of the pump using a block and tackle. Photos by Paul Morris and Rebecca Fowler.

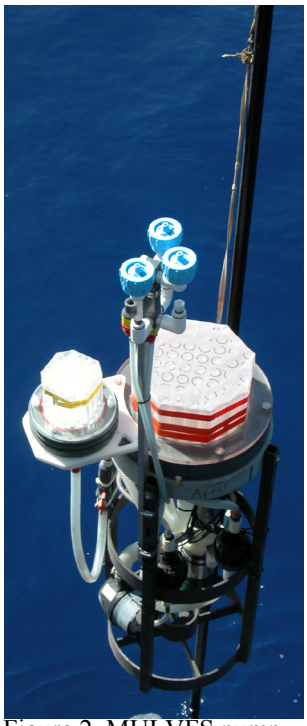

Figure 2. MULVFS pump showing nylon strap tether and main and 142 mm auxiliary "mini-MULVFS" filter holders. 47 mm filter holders also shown.

10.2.2 Deployment

Pumps are best deployed off the side of the ship to minimize vertical motion in high sea states and minimize particle contamination from ship propulsion systems. Wire angle must be maintained vertical to less than 5 degrees at all times during operations. It is often easier for the bridge to monitor wire angle if the pumps are deployed over the side.

If deployment must take place from the stern, the bridge must understand that propeller wash is to be avoided during deployment and recovery operations.

A self-recording CTD (e.g., SBE 19-plus) can be shackled to the end of the line to monitor depth and collect profile data during deployment and recovery to provide a hydrographic context (T, S, density) for the samples and ideally particle optics (transmissometer, scattering, fluorescence) data. At minimum, a self-recording depth sensor (e.g., Vemco Minilog, available to a maximum depth rating of 500 m or RBR depth loggers, available to full ocean depth) should be attached to a pump or directly to the line to monitor deviations from expected depths during pumping.

Pumps are attached at the appropriate wire-out readings (or breakout numbers in the case of MULVFS) that correspond to desired pumping depth. After attaching a pump to the line, the pump should sit just below the surface for ~30 s to allow for

bubbles to escape. In rough weather, a depth of 5 meters may be more practical. Alternatively, the pumps can be lowered at low (10 m/min) speed until 10 meters down. Winch speed should be ~30-45 m/min for deployment. Slower winch speeds must be used in high sea states.

10.2.3 During pumping

It is imperative to keep in good communication with the bridge to maintain a wire angle of less than 5 degrees during pumping, and especially to maintain a vertical wire angle during recovery of pumps to maintain an even distribution of particles on the filter to allow representative sub-sampling.

Pumping times will depend on the requirements for the types of analyses to be performed. McLane pumps are typically programmed to pump at 7-8 L/min for 2-4 hours (~1000-2000 L), depending on wire-time constraints, analytical requirements, and particle concentrations in the water column. McLane pumps slow down as filters are loaded, and shut off automatically once the pump rate reaches a minimum threshold (4 L/min for an 8 L/min pump head), regardless of whether the programmed pumping time has elapsed. This automatic shut off can occur using Supor filters after only 100-200 L are pumped through because of clogging. The automatic shut-off does not affect sample quality, but may limit the volume of water that can be pumped through, particularly in the euphotic zone. Thus far, the dual-flow version of the McLane pump (see section 10.3.4 below) loaded with paired QMA filters in one head and paired 0.8 μm Supor filters in the other head has not shut-off before the elapsed programmed pump times, as the effective filter area is doubled. For deep samples (>500 m), the particle concentrations are so low that clogging does not occur, and we expect a full 4 hours of pumping at 8 L/min (≤1900 L) to be required to maximize particle loading for many analyses.

MULVFS pumping times are typically 4 hours (2-3 hours in particle rich waters) and 10,000 L and 2000 L volumes of water are typically processed through main and auxiliary filter holders (Figure 2), respectively, below the euphotic layer.

10.2.4 Recovery

Winch speed should not exceed 30 m/min upon recovery. Filter holders should be covered with clean plastic bags or shower caps as soon as pumps are out of water and stable. Pumps must remain vertical as they are being taken off the wire. In the case of battery pumps, a good way to facilitate this is to have one person use a block and tackle to take the weight of the pump (Figure 1b, foreground) while two additional people take the pump off the wire. In the case of MULVFS, the pumps are lighter, and one of the recovery personnel can steady the pump as it is being detached from the electrical cable and lowered with winch assist to the deck.

Once the battery pump is on board, the quick release plumbing fittings from the bottom of filter holders should be disconnected from the pump and attached to vacuum lines to evacuate residual seawater in the filter holder headspace. After the headspace is evacuated, the filter holder should be disconnected from the pump and put into a clean container to bring into the lab. The pump can then be secured. Always keep the filter

holder upright to prevent particle redistribution on filter surface in the event that residual water remains in the filter holder.

For MULVFS pumps, a vacuum hose is attached to a side port on the main filter holder (and to the bottom of the auxiliary holder) while the pump is still on the wire and kept in place while the pump is unclamped and lowered to the deck (Figure 3). Pumps are secured as soon as possible to their mounting plate.

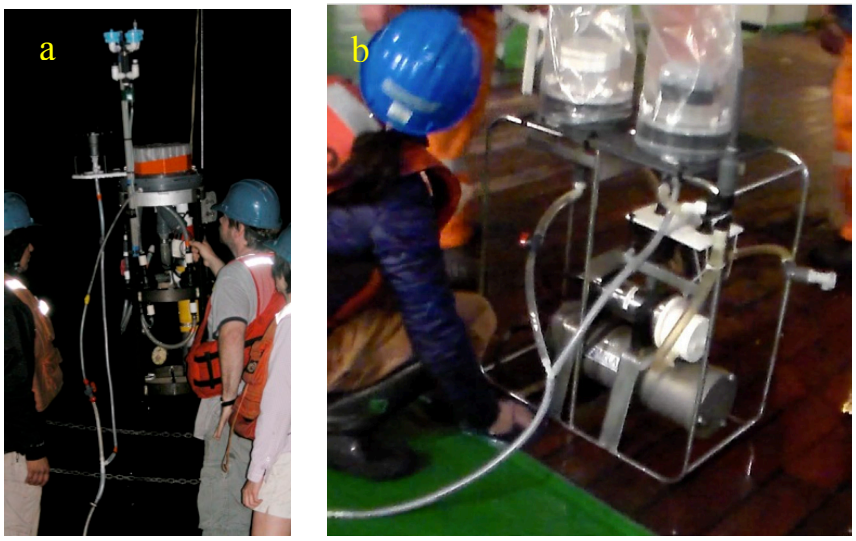

Figure 3. On-deck evacuation of seawater from filter holder headspace using vacuum lines. A) MULVFS pump during initial recovery operations on GEOTRACES IC2; vacuum hose connects to main and auxiliary filter holders. B) Dual-flow McLane pump during recovery on SANAE53; vacuum hose connects to each of the two filters.

10.3 Preparation and configuration of in-situ filtration systems

10.3.1 Filter Holder Design to prevent large particle loss

Commercially available (*e.g.* standard McLane WTS-LV holder) and "home made" single-baffle 142mm filter holders were found to lose major quantities of large particles during the two US GEOTRACES intercalibration cruises (Bishop et al., 2012). There is no doubt that particles are collected during operation of pumps; the loss of large particles clearly occurs from single baffle filter holders after the pumps shut down prior to and during the recovery process. It must be stressed that GEOTRACES IC work was undertaken in near waveless and windless conditions. **We thus strongly and urgently recommend use of filter holders that have multiple baffle systems similar to that used in the MULVFS system.** A "mini-MULVFS" design was tested and shown to be effective at retaining large particulates during the 2009 intercalibration cruise (Figure 2, 4b) (Bishop et al., 2012), and is now used exclusively for all US GEOTRACES cruises (e.g., Figure 1). McLane Research, Inc. now manufactures 142mm filter holders with multiple baffle systems based on the design tested during the GEOTRACES intercalibration cruises. Contact McLane for details (mclane@mclanelabs.com).

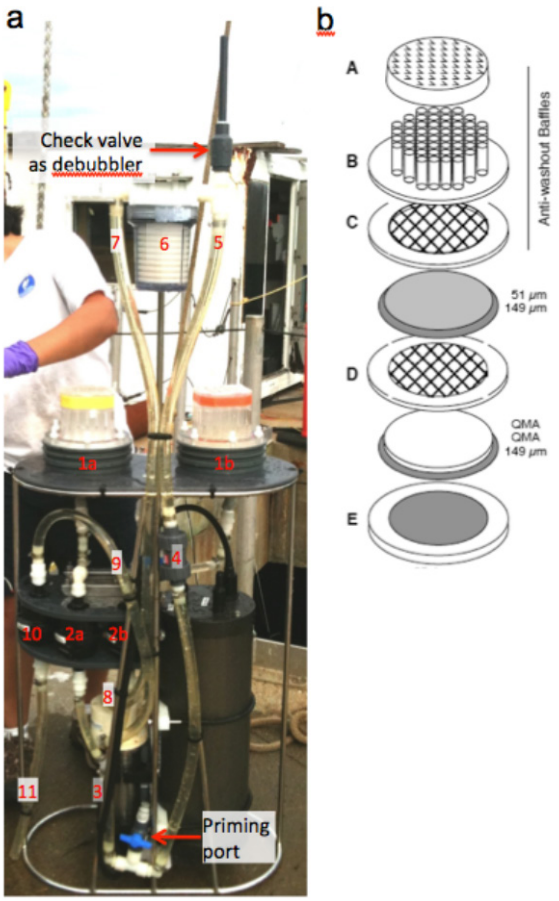

Figure 4a. Upright dual-flow battery operated in-situ pump (WHOI modification of McLane Research, Inc. Large Volume Water Transfer System sampler). Numbers mark the direction of flow during pumping, with flow entering the two "mini-MULVFS" style 142mm filter holders (1a, 1b) independently metered through two flowmeters (2a, 2b), then joining (3) to pass through the elevated Mn cartridge (6), pump head (8), and through a final flow meter (10). A restriction valve between 1b and 2b (not visible in picture) allows restriction of flow from second filter holder. A 1-way check valve (4) is placed between the filter holders and Mn cartridge to prevent backflow from the Mn cartridge, and another 1-way check valve is placed immediately upstream of the Mn cartridge as a debubbler. A priming port facilitates the introduction of distilled water to expel trapped air from the first two flowmeters.

Figure 4b. Schematic representation of multi-baffle "mini-MULVFS" holder design. A-C, anti washout baffles designed to eliminate effects of horizontal flows on collected large particle samples when pump is no longer running. Between C and D. 51 µm prefilter supported by 149 µm mesh. D prefilter support. Between D and E, paired filters QMA or Supor 0.8 µm (if QMA, supported by 149 µm mesh). E porous polyethylene frit as main filter support. From Bishop and Wood (2008).

10.3.2 Filter Holder Handling

As soon after a cruise as possible, the filter holder should be disassembled, rinsed in distilled water, and plastic components soaked in dilute detergent overnight. If holders were used in very productive environments, light scrubbing with a clean toothbrush of corners such as O-ring grooves can release dried-on plankton. The porous polyethylene frit should never be soaked in detergent, as it is too difficult to rinse out, and should simply be rinsed in distilled water before acid leaching. After rinsing copiously with distilled water to remove detergent, PVC and acrylic components should be leached in 1.2M HCl (trace metal grade) overnight at room temperature and well rinsed with Milli-Q (or similar ultrapure) water. Porous polyethylene frits retain acid that is not easily rinsed out. We find that an effective way to rinse out the acid is to replace the frit into its filter holder plate and apply a vacuum to the filter holder plate while pouring MQ-H_2O onto the frit. Rinse water pH should be monitored to ensure all acid is removed. All components should be dried in a laminar flow bench before being reassembled for transport. The white polyethylene frits should be packaged separately from the filter holders to prevent residual acid fumes from degrading filter holder components in transit.

Note: Most PVC and acrylic components of the filter holders can be leached in 1.2M HCl, but acetal (Delrin) components and silicone O-rings (if present) are not acid-resistant and should be soaked for at most a few hours in 0.1M HCl. Metal components such as

threaded stainless steel rods should not be acid-leached, but simply rinsed well in distilled water!

Filter holders should be rinsed with Milli-Q water after each deployment and stored in plastic containers between uses.

At the end of a cruise, the polyethylene frits should be removed from the filter holders and dried as much as possible before packaging for transit. If it is kept damp in the filter holder, it can get moldy and must then be discarded.

10.3.3 System configuration: debubblers and backflow check valves

Based on extensive experience with MULVFS, we highly recommend incorporating a one-way check valve (e.g., PVC ball check valve) as a debubbler to allow escape of air bubbles trapped in pump components when the pumps are first submerged in the water. All in-situ pumps induce water flow by inducing suction below the filter holder. Pumps operated in shallow water (depths less than 50 m) will separate significant quantities of dissolved gases from water as samples are filtered. Failure to allow this air to escape can result in filter tearing as expanding bubbles force their way through the filter during recovery. The debubbler should be located at the highest point in the plumbing (Figures 4a and b – McLane setup; 2 and 5 – MULVFS) and thus provide an escape route for air bubbles (e.g., Bishop and Wood, 2008). Winch speeds on recovery should be <30 m/min within 50 m of the surface to permit air sufficient time to escape.

Additional one-way check valves are recommended between the base of the filter holder and pump to prevent backflow and loss of particles and to isolate sources of contamination (*e.g.,* rusty pump components, MnO_2-coated cartridges, see below) from the underside of the filter (Figure 4a). PVC Y-check valves or ball check valves can be used for this purpose. If the latter, the valve may need to be retrofitted with a buoyant ball (*e.g.* ¾" polypropylene ball for a ½" NPT PVC ball check valve) to allow for a seal if the valve is oriented "upside down" (downflow).

10.3.4 Dual-flow modification for McLane pumps

Based on successful multipath filtration achieved by MULVFS, dual flow battery operated pumps were developed and tested by the US group at WHOI for deployment on the US GEOTRACES North Atlantic Transect to allow the simultaneous use of quartz fiber filters (Whatman QMA) and hydrophilic polyethersulfone (Pall Supor) filters and MnO_2-coated adsorption cartridges (Figure 4, above) (Lam and Morris, Patent pending). Main modifications include two additional flow meters to separately measure the flow through each filter holder, and a final flowmeter to measure total outflow for a total of three flowmeters (Figure 4a). A ball valve below one of the flow paths allows flow to be turned off if a single flow path is desired. Using paired QMA filters in one holder and paired 0.8μm Supor filters in the other holder (see section 10.4) typically results in a 2:1 volume ratio filtered between the QMA and Supor holders because of higher flow rates through the QMA compared to Supor filters.

The WHOI upright dual-flow version has a priming port (Figure 4) to expel trapped air from the initial 2 flowmeters. Milli-Q water (or similar) should be used to prime the pump before attaching the filter holders and should flood both initial flowmeters. After the first deployment, seawater is retained in the plumbing lines and subsequent deployments do not require priming.

McLane Research Laboratories, Inc., now offers a dual-flow option (WTS-LVDF--http://mclanelabs.com/master_page/product-type/samplers/wts-lv-large-volume-pump). Contact McLane for details (mclane@mclanelabs.com).

10.3.5 Mn cartridge

Samples for short-lived radionuclides are often collected using a Mn-coated cartridge plumbed in line or into a separate flow path of an in-situ pump (e.g., (Charette et al., 1999; Hancock et al., 2006; Luo et al., 1995). Simultaneous collection of particulates for trace metal analysis and with a MnO_2-coated cartridge downstream is possible (e.g., (Bishop and Wood, 2008), but plumbing modifications (debubblers, check valves) mentioned above become essential. Since the Mn cartridge is downstream of the filters, contamination is not an issue during pumping. The biggest opportunity for contamination is when the pump is first submerged and seawater floods the plumbing to displace air, potentially backflushing through the Mn cartridge and up into the filter holder. **Placement of the Mn cartridge must be higher than the filter holder to minimize contamination of filters due to backflow (Figures 4 and 5).**

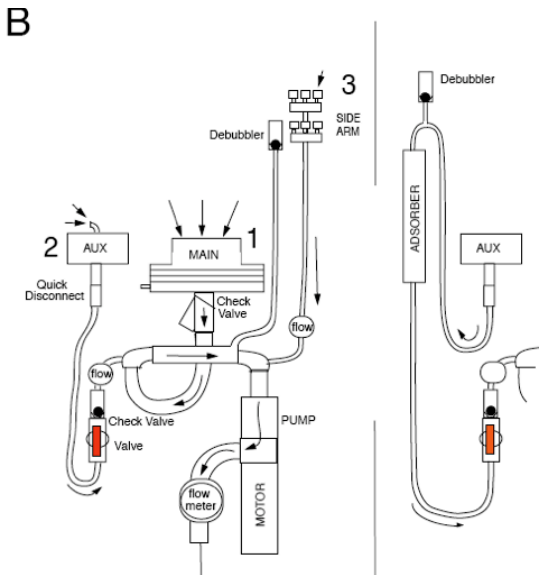

Figure 5: Schematic of Mn cartridge placement on MULVFS. From Bishop and Wood, 2008.

The placement of the Mn cartridge above the filter holder minimizes the backflushing through the Mn cartridge and into the filter holder as air is forced out of the system through the debubbler. The placement of a debubbler at the highest point in the plumbing and next to the Mn cartridge further allows excess Mn to escape as the plumbing floods with seawater. A one-way check valve is placed just upstream of the Mn cartridge as an additional safeguard from contamination from the Mn cartridge (Figure 4a). Finally, the outflow from the pump should point downward and be vertically separated from expected intake for the filter holders. We have found that an outflow separated by ~1m from the filter holder is sufficient for horizontal currents to carry the Mn-rich effluent away.

10.4 Filter type selection: quartz (QMA) and plastic (PES)

No single filter type can accommodate the needs of all desired measurements. Ideally, a combination of quartz and plastic filters are deployed on a multiple flow path pump.

10.4.1 Quartz fiber filters

QMA filters have a nominal pore size of 1μm for seawater filtration, have a long track record of use in in-situ filtration, have the best flow characteristics, and result in even particle distribution. QMA filters can be pre-combusted for particulate organic carbon (POC) concentration and isotopic analyses, and are suitable for analyses of most trace metals when using leaches (e.g., hot 0.6M HCl; HNO_3:HCl) which leave the filter matrix intact. Some elements (documented for Al and U (Bishop; Geotraces – unpublished data); suspected for Pa (M. Fleisher pers. communication, 2009) and possibly Th, do adsorb significantly to QMA filters, and appropriate flow-dependent blanks must be collected to determine these (see below). QMA filters are unsuitable for total digests using hydrofluoric acid (HF), as blanks for some elements are high (Cullen and Sherrell, 1999).

We recommend deploying paired QMA filters (e.g., Whatman) supported by a ~150 μm (or 149 μm) polyester mesh (*e.g.* 07-150/41 from Sefar Filtration) as a physical support for the fragile QMA filters during pumping and for ease of handling post sampling. QMA filters should be loaded in the filter holder one on top of the other with the small gridded mesh pattern (visible on some batches of QMA filters) down, and on top of the ~150 μm mesh support filter.

Paired filters (2 filters sandwiched together) increase particle collection efficiency to capture a portion of the sub-micron particle population (Bishop et al., 2012; Bishop et al., 1985; Bishop and Wood, 2008), important for some biologically associated elements (e.g., P and Cd, where the sub-micron contribution would be expected to scale with picoplankton abundance). For other elements, the bottom filter can act as a flow-through blank (e.g., Al, which exhibits significant flow-dependent adsorption to QMA). In a worst-case scenario in which all plumbing safeguards detailed in section 3 above fail, the bottom filter can act as a barrier to unexpected contamination (e.g., from Mn cartridge or Fe from rusty pump components downstream), allowing the top filter to still be analyzed. The top and bottom filters should thus by analyzed separately.

10.4.2 Hydrophilic polyethersulfone (PES) membrane filters

Hydrophilic polyethersulfone (PES) membrane filters (e.g., Pall Supor) have low unused filter blanks and have the best flow characteristics of the available plastic filters, and are thus currently the plastic filter of choice (see GO-Flo filtration cookbook). Mixed cellulose ester filters (e.g., MF-Millipore type HAW), which may be a suitable alternative for GO-Flo filtration, become very brittle upon drying and are thus more difficult to handle for the larger sizes used for in-situ filtration. Supors are suitable for digestions that use HF, although the filters are difficult to get completely into solution unless very strong oxidizers such as perchloric acid (Anderson et al., 2012) or Piranha reagent (3:1 H_2SO_4: H_2O_2) (Ohnemus et al., 2014) are used. In-situ adsorption blanks are still being investigated and are significant for some elements (including P).

Supors and plastic filters in general do have serious drawbacks, however, the greatest of which is the poor (heterogeneous) particle distribution beginning to be observed on deep

(>200 m) samples. The particle distribution on the filter worsens with depth and with decreasing pore size. This issue may not be resolvable when using Supors, as it may have to do with the manufacturing process and be inherent to the filter medium itself. To our knowledge, other U.S. manufacturers of PES membrane filters either do not make a 142 mm diameter filter size or do not have pore sizes greater than 0.2 μm.

For in-situ filtration, we currently recommend paired 0.8 μm PES filters (e.g., Supor 800) as the best compromise. As with the QMA, paired 0.8 μm Supor filters increase particle collection efficiency and collect in total a similar particle population to a single 0.45 μm Supor filter, while having better flow characteristics and better particle distribution compared to a single 0.45 μm Supor (Bishop et al., 2012). Flow rates achieved are approximately 40% of that through QMA filter pairs (Bishop et al., 2012). Also like the QMA, the bottom Supor can act as a cross check for adsorption blanks and acts as a barrier to particulate contamination if necessary. **Supors should not be supported with a 150 μm mesh filter, as this prevents an adequate seal in the filter holder stage.**

10.4.3 Prefilter Mesh

For large (>51μm) particle collection, 51μm polyester square weave mesh (e.g., 07-51/33 from Sefar Filtration) loaded upstream of QMA or Supor filters is the best known option, supported by a 150 or 149 μm polyester mesh as for the QMA for ease of handling (51μm filter should be loaded directly on top of the 150 μm support filter in the filter holder). Polyester has acceptable blanks for typical particle composition and filter loading for leach conditions that do not destroy the filters (e.g., 0.6M HCl), but it has known high concentrations of Mn, Ti, and P (Cullen and Sherrell, 1999; Lam et al., 2006), making this filter unsuitable for total digestion when these elements are low in the samples.

For total digestion of the >51 μm size fraction, we recommend rinsing freshly collected particles from a pie slice subsample of the prefilter of known area onto a 25mm Supor filter using trace-metal clean filtered (0.2-0.45 μm) seawater (such as from a towed fish).

10.4.4 Filter Blanks

Filter blanks are determined using cleaned unused and process blank filters. A process ("dipped") blank filter is one that is deployed at depth on a pump but has no water actively pumped through it. Ideally, this is accomplished by loading a regular filter set into a filter holder that is attached to a pump but not connected to the pumping system. A 0.2 μm Supor (or similar) filter placed at the top of the stack will ensure that the dipped blank filter set are exposed to seawater but do not have particles on them. If an extra filter holder is not available, a dipped blank filter set can be sandwiched between acid-leached 1 μm polyester mesh and deployed in acid-leached polyethylene containers that have had holes punched through them (Figure 6). This filter is processed in an identical way to samples. Process blanks should be obtained at least once every other station. One unused filter set should be retained for blank purposes at least once every 30 samples.

Figure 6: Dipped blank filter sets sandwiched between 1 μm polyester mesh, and deployed in perforated plastic containers that are attached to the pump with cable ties.

10.5 Filter cleaning procedure

All filter cleaning and handling should be done in a HEPA-filtered environment.

10.5.1 Preparation and cleaning of QMA filters

Cleaning procedures for QMA filters generally follow those described in (Bishop et al., 1985). The protocol that follows has been demonstrated effective during GEOTRACES IC expeditions.

Whatman QMA filters are typically sold as 8”x10” sheets in the U.S. 142 mm diameter circles are punched using a sharpened 142mm-diameter template (made of stainless steel, if possible). 293 mm QMA filters for MULVFS are available by special order from Whatman and have been cut from bulk roll material in the past.

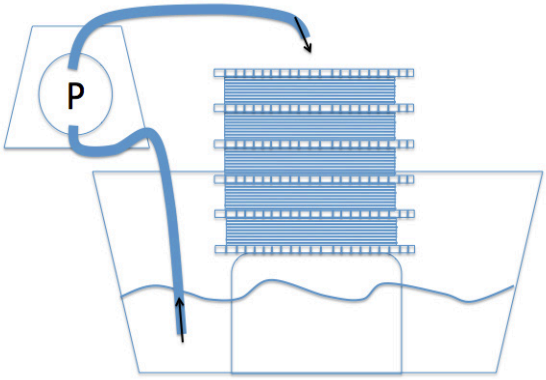

Figure 7: Schematic of filter rinsing procedure.

Briefly, ~5 stacks of 10 cut filters, each separated by a polystyrene grid (see Materials List), are leached at room temperature in a recirculating bath system in two overnight batches of 1.2M trace metal grade HCl in series and rinsed copiously (over 2-3 days) with Milli-Q water until the pH of the rinse water indicates that all the acid is rinsed out.

For the acid-leaching steps, the filters can be submerged in the acid with a peristaltic pump to aid in recirculation. The elevation of the stack of filters above the level of the acid solution – specifically required for larger 293 mm filters (Bishop et al., 1985), guarantees that acid flows through all filters (Figure 7). For rinsing, it is important to elevate the filter stack above the level of the rinse water, and to pump water from the bottom of the tub using a peristaltic or similar pump and to dispense it onto the top of the filter stack to allow milli-Q water to gravitationally drip through the stack to rinse out residual acid (Figure 7). The pump rate should exceed the ability of the filters to absorb the liquid (~600 mL/min for 142 mm filters). The rinse water should be changed periodically until the pH of the rinse water indicates that all acid has been rinsed out (pH~5). **Simply soaking filters in Milli-Q water will not get residual acid out, and pH of rinse water must be monitored to determine when rinsing is complete.**

Filter stacks are then dried in a laminar flow hood (~2 days). After drying, the 5 filter stacks are placed into a clean Pyrex baking dish, each stack of 10 separated by 2 Pyrex rods, and the entire stack covered with an inverted Pyrex dish to guard against contamination, and combusted at 450° C for 4 hrs in a clean muffle furnace that is dedicated to combusting unused filters.

When cool, the topmost and bottommost QMA filters in the entire stack are discarded after combustion, and the remaining QMA filters are packaged in polyethylene clean room bags. If a clean muffle furnace is not available, QMA filters can be combusted before acid leaching, although DOC contribution from the acid-leaching process has not been tested.

10.5.2 Supor filters

Supor filters are leached overnight in a non-recirculating 1.2M HCl (trace metal grade) bath at 60° C, then rinsed copiously with Milli-Q water until the pH of rinse water indicates that all acid has been rinsed out. An elevated recirculating system as for the QMA filters (Figure 7) accelerates the rinsing process, though is not as crucial as for the QMA filters. **Regardless, the pH of the rinse water must be monitored to determine when acid has been rinsed out.**

Use in pumps: The manufacturer (Pall) indicates that slightly better flow rates may be obtained by retaining the filter side facing up in the package as the upstream side. It is important to keep track of which side is up during the cleaning process, as there are no visual cues once the filters are out of the box.

10.5.3 Polyester filters

51 μm and 150 μm polyester mesh filters are leached overnight at room temperature in 1.2M HCl (trace metal grade) in a non-recirculating bath, soaked overnight in Milli-Q water, then rinsed with Milli-Q water. They are air dried in a laminar flow bench.

10.6 Particle Sample Handling

All filter handling should be done in a HEPA filtered environment (flow hood or bubble) wearing powder-free nitrile or vinyl gloves.

Filter samples should be transferred to a filter stand in the lab that is pulling a 0.25 – 0.5 atm vacuum to remove as much residual seawater as possible from filter pores to reduce sea salt on the sample. A slightly modified extra base plate with vacuum line can be used as a filter stand. If rinsing with Milli-Q water, this should also be done under vacuum, and we recommend using an aerosol mister to minimize the volume of water used (e.g., Figure 8). We find that ICP-MS results are more stable with reduced salt. **Isotonic rinses (e.g. ammonium formate) are to be avoided since weakly associated metals are easily lost.** Previous reports have suggested the extreme lability of some elements such as P

Figure 8. Nalgene Aerosol Spray Bottle has no metal parts, and can be acid cleaned

upon leaching with distilled water (Collier and Edmond, 1984). Tests on the 2009 IC2 cruise comparing MQ-water misted and unmisted sections of QMA filter found that misting as described above with a small volume of MQ-water resulted in a relatively modest loss of P (~9%) for euphotic zone samples, but no significant loss in samples below 120 m (Figure 9). There was no significant loss in other elements such as Cd, and Na from salt was reduced by more than 30% (Bourne and Bishop, unpublished).

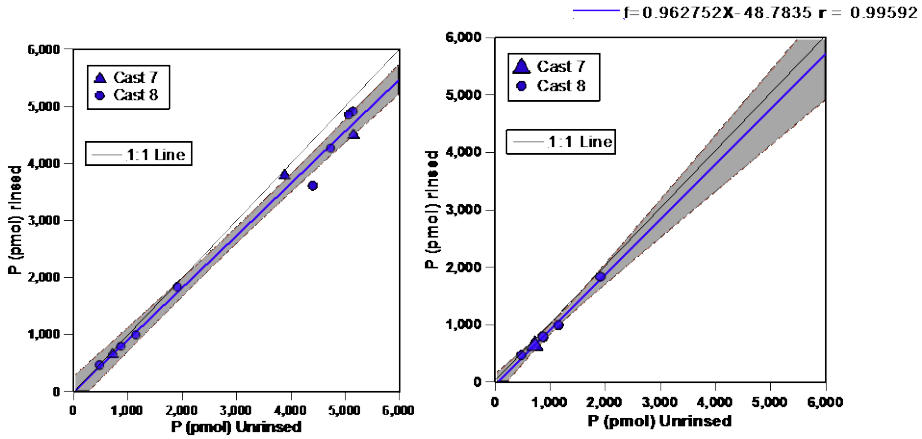

Figure 9: Effect of misting with MQ-H2O on P on samples from SAFe. a) misting leads to a ~9% loss of P (P_{rinsed}=0.912*$P_{unrinsed}$+0.49, r=0.994) P loss is restricted to euphotic zone samples, as B) isted vs. unisted samples deeper than 120 m were not significantly different (H. Bourne and J. Bishop, unpublished).

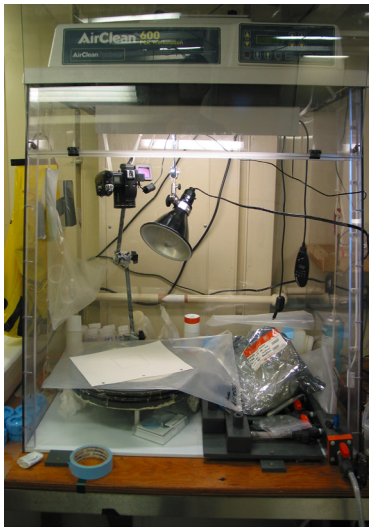

Figure 10. Laminar Flow bench setup used for MULVFS sample processing (rinsing/suction stand at left). Remotely controlled digital camera and lighting for photo-documentation.

10.6.1 Photo documentation of filters

Filter samples should be photographed under fixed lighting and camera geometry to document particle distribution (Figure 10). A white target photographed at varying camera shutter speeds is used for image calibration. Digital photographs or dried filters can be quantitatively processed to achieve accurate representation of particle profiles (Lam and Bishop, 2007).

10.6.2 Filter drying

Filter samples for particulate trace metal analysis are typically dried on square [15 cm (for 142 mm) or 30 cm (for 293 mm)] acid-leached polystyrene grids (see materials list) in a clean oven at 60° C. This grid material is the same as used for prefilter support in MULVFS and mini-MULVFS filter holders. The low surface area contact of the filter on the grids promotes drying and minimizes fractionation of elements. Drying is complete in 1-2 days for QMA filters, and ~1 day for prefilter or Supor filters, depending on filter loading.

We have shown that a regular gravity-flow stainless steel oven dedicated to filter drying, with stainless steel oven racks replaced with polystyrene grids, is suitable for sample drying. The oven ideally should be situated in a HEPA-filtered clean bubble, and vented outside of the bubble to prevent overheating. Dried samples are stored in polyethylene clean room bags or acid leached plastic containers.

Storage of wet samples in plastic containers is to be avoided because of (1) sample degradation (e.g., for POC analysis), and (2) fractionation of salt-associated elements to the dish.

For samples that are not sensitive to degradation (e.g., Supor filters for trace element analysis), filters can be dried at room temperature in a laminar flow bench on polystyrene grids.

10.6.3 Particle subsampling

QMA filters are easily subsampled using a sharpened and acid-leached acrylic or polycarbonate tube of any diameter. Round punches are not practical with Supor filters, and we recommend cutting straight-edge slices using a stainless steel scalpel or ceramic blade, either in pie-wedges (e.g., ¼, 1/8, 1/16) or by tracing around a straight-edged hard-plastic template. Subsampling can be done when filters are wet or dry, depending on the analytical needs.

Because of sometimes unavoidable heterogeneity in particle distribution on Supor and Polyester filters, we recommend photo documentation of the filter using fixed lighting and camera geometry (Figure 10) before and after subsampling to document heterogeneity. Details of the procedures are described in Lam and Bishop (2007).

Great care should be taken to sample as representatively as possible, including taking multiple smaller subsamples across heterogeneous areas, or subsampling larger pie-slices (quarters or eighths) to average out the heterogeneity. Final subsampling shape and size will depend on particle distribution and analysis needs.

10.7 List of materials (and example U.S. suppliers)

- 51 μm polyester prefilter: precision woven open mesh polyester fabric. Sefar PETEX 07-51/33 from Sefar filtration (filtration@sefar.us): available in the U.S. per meter on a large roll, or Sefar will laser-cut discs to specified diameters for a minimum order of 250 pieces (~US$1/142mm disc in 2009).
- ~150 μm support: Sefar PETEX 07-150/41 from Sefar Filtration; otherwise as above
- 1 μm mesh for dipped blanks: Sefar PETEX 07-1/1 from Sefar Filtration; buy by the meter and cut out a rectangle to fold over the dipped blank filter set
- Quartz fiber filter: Whatman QMA available in the U.S. as 8”x10” sheets from Fisher Scientific, and must be cut manually. Larger 293 mm Filters for MULVFS must be custom ordered.
- Hydrophilic polyethersulfone (PES) membrane filters: available in 142 and 293 mm diameter from Pall Corporation (“Supor800 PES Membrane Disc Filters”)

- Plastic (poly)styrene grids: called “egg crate louvers” or “(poly)styrene fluorescent light diffusing panels”. 2’x4’x~3/8” sheets available at U.S. hardware stores in the lighting/electrical section or online (*e.g.* www.edee.com/eggcrate.htm). Very versatile—used as anti-washout baffles in filter holders, stack separators during filter cleaning, oven racks, and filter support grids during oven drying.
- Vemco Minilog ((http://vemco.com/products/minilog-ii-t/) or RBR Virtuoso (http://www.rbr-global.com/products/sm-single-channel-loggers/depth-recorder-rbrvirtuoso-d). Recording pressure loggers.
- Debubbler: *e.g.* ¼” NPT trim check valve (PVC ball check valve) from Hayward™
- Check valves below filter holders: *e.g.* 1/2” NPT true union design ball check valve from Hayward™
- Flowmeters: *e.g.* Elster AMCO Water, Inc.
- Polyethylene clean room bags: *e.g.* KNF FLEXPAK Clear Polyethylene Clean room bags

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VII. Nitrate and Silicon Isotopes

A. Protocols for Nitrate Isotopes

1. Sampling

- Given that nitrate is not contamination-prone, sample collection via the ship's rosette is adequate.
- Water volumes of approximately ~250 mL per depth are needed for triplicate 50 mL samples, plus bottle rinses.
- Samples for nitrate isotope analysis should be filtered then frozen at -20 ˚C (see below for more details on filtration and sample storage).
- Sample containers (60 mL square wide-mouth HDPE bottles, Thermo Scientific No. 2114-0006) need not be precleaned, but should be triple-rinsed with seawater prior to sample collection.

2. Storage

- **It is recommended that samples be filtered and stored frozen at -20˚ C.**
- Filtration on Intercalibration Cruises 1 and 2 (IC1 and IC2) was achieved via pressure filtration through 0.22 µm Sterivex filter capsules. However, on section cruises, we plan to switch to gravity filtration through stacked 0.8/0.45 µm polyethersulfone membrane filters (e.g., Acropak 500) to coordinate sampling with other (e.g., radioisotope) groups. It is not known whether this will have an adverse effect on nitrate isotope storage. However, storage tests during IC1 showed no difference between filtered (0.2 µm) and unfiltered seawater stored at -20 ˚C for up to 18 months in waters collected at BATS from 150 m, 500 m, and 800 m with nitrate concentrations ranging from 2-22 µM. Filtration is still recommended, however, as it adds an extra layer of protection against biological activity altering nitrate isotope ratios during freezing and thawing in samples

collected from more highly productive waters or in samples with lower nitrate concentrations.

3. Analysis

- The nitrate isotope intercalibration included analyses via the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002) and the Cd/azide method (McIlvin and Altabet 2005). According to the published protocols, the precision should be similar between the methods, or approximately 0.2‰ for $\delta^{15}N_{NO3}$ and 0.5‰ for $\delta^{18}O_{NO3}$. Either method should provide the necessary sensitivity and throughput for nitrate isotope analyses in GEOTRACES.
- Regardless of analytical technique, it is recommended that each sample be analyzed in duplicate. Given that replicate analyses run on different days show more variability than replicates within a given day's run (especially for $\delta^{18}O_{NO3)}$, it is recommended that replicate analyses be performed on separate days to capture the day-to-day variability.
- During the intercalibration exercises, several procedural modifications were tested that can be used to minimize sample drift and therefore improve analytical precision. Grey butyl vial septa (MicroLiter part #20-0025) were found to be gas-tight (for up to six months), yet adequately pliable to use in an autosampler (McIlvin et al., in prep). In addition, we found that backflushing a portion of the GC column between samples kept backgrounds low for m/z 44, 45, and 46 and increased analytical precision (McIlvin et al., in prep).

4. Calibration

- International reference materials available for nitrate isotopes ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) should be used to calibrate measured $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ (Table 1; Sigman et al., 2001; Casciotti et al., 2002; Böhlke et al., 2003). It is recommended that at least two bracketing standards be chosen to calibrate $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$. Note that due to a ^{17}O anomaly (Böhlke et al. 2003), USGS-35 should not be used to calibrate $\delta^{15}N_{NO3}$ via N_2O-based methods.
- The number of standard analyses per run and their distribution over the run may vary; however, standards should each be analyzed at least in triplicate with a given batch of samples, and the standard deviation of these standard analyses should be less than 0.2‰ for $\delta^{15}N_{NO3}$ and less than 0.5‰ for $\delta^{18}O_{NO3}$.
- Internal laboratory standards can be used to ensure day-to-day consistency of sample calibration.
- Standards should be made up in high purity water (> 18 MΩ - cm) or in nitrate-free seawater. To ensure proper blank correction (Casciotti et al., 2002), standard injections should closely match the nmole amounts and volumes (where possible) of the samples in the run.
- If more than one laboratory is involved in analyzing nitrate isotopes from a given oceanographic section, it is recommended that some profiles be measured by both laboratories to ensure that proper intercalibration is maintained.

Table 1: Nitrate isotope reference materials (Böhlke et al., 2003)

Standard	$\delta^{15}N$ (‰ vs. AIR)	$\delta^{18}O$ (‰ vs. VSMOW)
USGS-32	+180.0	+25.7
USGS-34	-1.8	-27.9
USGS-35	+2.7	+57.5
IAEA NO3	+4.7	+25.6

5. References

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Casciotti, K. L., D. M. Sigman, M. Galanter Hastings, J. K. Böhlke, and A. Hilkert. 2002. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Analytical Chemistry 74: 4905-4912.

Granger, J., and D. M. Sigman. 2009. Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method. Rapid Communications in Mass Spectrometry 23: 3753-3762.

McIlvin, M. R., and M. A. Altabet. 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. Analytical Chemistry 77: 5589-5595.

B. Protocols for Silicon Isotopes

1. Sampling

- Water samples for silicic acid and biogenic silica isotope analysis should be gravity filtered through 0.45 µm, polycarbonate or polyethersulfone membrane filter cartridges using silicone tubing and then stored at room temperature in the dark. For larger sample volumes a peristaltic pump can be inserted on the silicone tubing between the Rosette sampling bottle and the filter cartridge.
- Water volumes of between 1.0 and 4.5 L per depth are required for triplicate analysis, plus bottle rinses. Sample volume will depend upon the needs of the sample preparation and analytical method employed. Triethylamine silico molybdate purification coupled to MC-ICP-MS (Abraham et al., 2008) and IRMS methods (Brzezinski et al. 2006) have higher mass requirements (~2-3 µmol Si) and 4 L samples are recommended in oligotrophic surface waters. The sample mass requirements for cationic chromatography followed by MC-ICPMS (Georg et al. 2006) are lower and a 1 L sample is recommended. For deeper waters with higher $[Si(OH)_4]$ (> 10 µM) a sample volumes of 1.0 L is sufficient for both methods.

- Suggested seawater sample containers are HDPE or PP bottles.
- Sample containers should be pre-cleaned by soaking overnight in 10% HCl, followed by triple rinsing with high purity water (> 18 MΩ - cm). Bottles should be triple-rinsed with seawater prior to sample collection.
- For particulate biogenic silica, samples are collected onto polycarbonate or polyethersulfone filters using in-situ pumping devices. In oligotrophic or deep waters 100-400 L of water should be filtered to obtain sufficient mass for analysis. Membranes should be dried in a clean environment overnight at 60° C.

2. Storage

- It is recommended that filtered water samples be stored in the dark at room temperature. There is no need to acidify samples.
- Dried filters containing particulate Si can be stored in polypropylene tubes.

3. Analysis

- The silicon isotope intercalibration included analyses via MC-ICPMS (Abraham et al. 2008; Georg et al. 2006) and IRMS (Brzezinski et al. 2006).
- For silicic acid in low Si seawater, magnesium co-precipitation (Reynolds et al. 2006a) proved to be an effective means of concentrating Si however recovery should be checked and the addition of base adjusted to ensure quantitative recovery of Si. Purification can then be processed using either cationic chromatography (Georg et al., 2006) or reaction of silicic acid to silicomolybdic acid and precipitation with triethylamine (De La Rocha et al. 1996), providing residual Mo and major elements are checked to be negligible to avoid matrix effect when using MC-ICPMS.
- For biogenic silica, a 1-step leaching (0.2M NaOH, 40 mins., 100° C) adapted from Ragueneau et al. (2005) or Varela et al. (2004) should be applied first. Potential lithogenic contamination can be monitored by measuring Al content in the leachate.
- Regardless of analytical technique, it is recommended that each sample be analyzed at least in duplicate. Given that replicate analyses run on different days show more variability than replicates within a given day's run it is recommended that replicate analyses be performed on separate days to capture the day-to-day variability.

4. Calibration

- NBS 28 silica sand (NIST RM 8546) is the preferred primary reference material for silicon isotopes, i.e. $\delta^{30}Si = 0$ ‰ (Reynolds et al. 2006b). Unfortunately, despite a huge stock, this reference material is currently no longer being distributed by NIST. It is required to calibrate any in-house standard or secondary reference material.

- Two well characterized in house standards are “diatomite” and “Big Batch” (Reynolds et al. 2007). Laboratory in-house standards can be used to ensure day-to-day consistency of sample calibration.
- The number of in-house standard analyses per run and their distribution over the run may vary; however, standards should each be analyzed at least in triplicate with a given batch of samples, and the standard deviation of these standard analyses should be less than 0.1‰ for $\delta^{30}Si$.
- If more than one laboratory is involved in analyzing Si isotopes from a given section, it is recommended that some profiles be measured by both laboratories to ensure that proper intercalibration is maintained.

5. References

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VIII. Protocols for Optics: Transmissometer and Scattering Sensors

In this document we present the methodology for optical characterization of particles using transmissometer and scattering sensors during CTD casts. The examples cited apply to WETLabs, Inc. C-STAR red (660 nm) transmissometers and Seapoint Inc. turbidity (810 nm) sensors but apply to all similar instruments. The treatment of data from similar optical sensors should follow recommendations outlined below. Methodology closely follows Bishop and Wood (2008).

1. Transmissometers and Scattering sensors

Transmissometers are the most sensitive sensors for particle distributions in seawater and track closely the variations of POC in the water column (e.g. Bishop 1999; Bishop and Wood, 2008). They have had 3 decades of development and have found worldwide deployment. With the protocols below, it is possible to achieve an absolutely calibrated data set on particle abundance, not only in surface waters, but also throughout the entire water column. Scattering sensors are often deployed together with transmissometers and are more sensitive to variations of particle size and refractive index.

The physically meaningful parameter derived from a transmissometer is beam attenuation coefficient, $\boldsymbol{c}$, which is the light loss from a collimated* beam due to combined effects of absorption and scattering by particles and absorption by water. Effects of light absorption by water are assumed constant at 660 nm and are eliminated by defining 100% transmission as the transmissometer reading in particle-free water.

** In practice, transmissometer beams are usually divergent, and the detector view of the beam is also divergent (e.g. 1.5° in C-Star transmissometers; 0.92° in C-Rover transmissometers; 0.5° in old Sea Tech instruments) and thus at wider view angles, the increased detection of forward scattered light by particles can lower sensitivity (Bishop and Wood, 2008). For additional discussion consult (Boss et al. 2009).*

Accurate determination of particle beam attenuation coefficient, $\boldsymbol{c_p}$, requires (1) care in mounting sensors, (2) elimination of optics contamination while the sensor is not in the water, (3) compensation for sensor drift, and compensation for the specific analogue to digital conversion electronics of the equipment being used to read the sensor.

1.1 Sensor mounting

Transmissometer sensors are best mounted horizontally with the water path unimpeded to water flow during down and up casts (Figure 1). The sensor must be supported, but not stressed by mounting clamps/hardware. Mounting is facilitated

by use of all-stainless steel hose clamps and backing the sensor with 2 – 3 mm thick silicone rubber. Use black electrical tape to cover any shiny band material in proximity to the light path of the instrument. The CTD and sensors should be covered to prevent baking in strong sunlight between stations.

Figure 1. Mounting of 2 transmissometers and PIC sensor on the GEOTRACES rosette system during the 2008 and 2009 Intercalibration Expeditions. Plastic caps prevent optics contamination see section 3.0. Methodology from Bishop and Wood (2008).

For Rosette/Carousel Systems: It is <u>not recommended</u> to mount transmissometers vertically clamped to the CTD (Figure 2, left). This arrangement makes it extremely difficult to service/clean optical windows and to place or remove plastic caps (to prevent optics contamination) when the rosette is populated with bottles. The use of bulky clamps close to the optical path further results in flow separation during up and down casts and can lead to biased profiles.

For logging CTD packages deployed during in-situ pump casts, transmissometer sensors must be mounted vertically due to smaller frame dimensions. Note: clamping is away from the optical path of the C-Rover instrument.

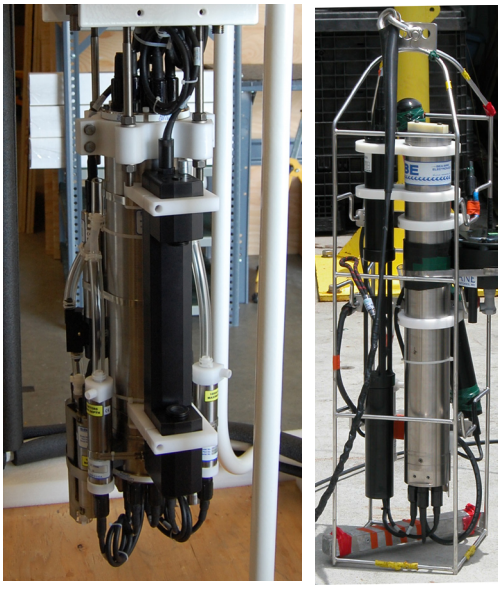

Figure 2. Vertical mounting of transmissometers close to the CTD unit (SBE 911 shown) at the center pylon of rosette/carousel frames (left) results in cleaning access difficulty with bottles emplaced and possible flow separation from optics during casts. Vertical mounting of transmissometers on autonomous logging CTD's (right) is sometimes unavoidable due to geometric constraints. Unit shown on right is the SBE 19plus, WETLabs Inc. C-ROVER transmissometer, Seapoint scattering sensor package deployed with MULVFS during GEOTRACES IC expeditions.

Scattering sensors. Scattering sensors must be mounted in a way where water flows past the sensor windows tangentially and in a way where the sensor is not influenced by structures on the frame to which it is mounted. In the case of Seapoint sensors, structures (Rosette frame, bottles, etc.) must be at a distance of 50 cm or more otherwise profiles are offset high. The signal from scattering sensors is 'bottom up' and thus the major concern when deploying scattering sensors on CTD's is the accurate determination of the signal when 'zero' particles are present. This can be assessed by pressing a strip of black rubber sheeting onto the source and detector windows and reading recording 10 sec averaged 24 Hz data. Seapoint sensors must be operated at 100x gain to be useful in the ocean.

2. Avoiding optical data dropouts

When optical sensors are mounted on CTD's at the beginning of an expedition, it is important to carefully inspect cables, clean all connector contacts, and to avoid any stress on the wiring harness from the CTD at the point where the connector mates with the transmissometer. In other words, there should be no bending stress of the connector at the point where it is connected. Data dropouts during a cast will lead to unexpectedly low transmissometer voltage readings even in parts of the profile where data are not interrupted. If dropouts develop during an expedition, cabling stress is almost always the primary cause.

3. Elimination of optics contamination and cast-to-cast offsets

Contamination of transmissometer optics while the CTD-rosette system is on deck has been a major and recurring problem preventing absolute measures of light transmission in the water column (Bishop, 1999). In many cases, an assumption of constant and low c_p is assumed for deep (2000 m) waters (e.g. Gardner et al., 2006) and cast data can be offset to superimpose in deep water. This offsetting protocol will not work close to continental margins.

3.1 Preinstallation Cleaning and Cap Protocol

Prior to installation of the transmissometer on the CTD, optical windows must be cleaned thoroughly with Milli-Q (or other clean deionized) water and dried with lint-free wipes. We found that monitoring transmission output with a 4.5 (4 or 5) digit voltmeter to be a useful guide to cleanliness. We aim for readings that are stable to better than 1 mV. Once clean, plastic bottle caps (from 125 mL Nalgene polyethylene bottles) are installed to isolate the transmissometer windows from further contamination. Caps remain in place to protect the transmissometer while it is being mounted on the CTD, and until CTD deployment.

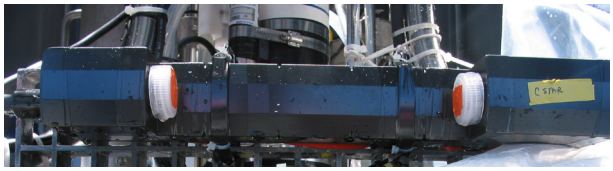

Figure 3. CSTAR transmissometer with plastic bottle caps installed on optical windows that are effective at preventing optics contamination while not deployed.

If the transmissometer is already mounted to a CTD / Rosette system, then the entire package must be clean and dry in a dry low humidity environment and digitizing software for the CTD can be used for pre-cruise calibration; one will need to digitally record 10 second averages of 24 Hz data to gain sufficient precision to follow cleaning progress and the CTD computer display should be conveniently located near to the rosette.

3.2 Deployment

Just prior to each CTD cast (at the same time when salinity sensors are serviced) caps are removed and transmissometer source and detector windows are rinsed with Milli-Q water. When the rosette cast returns (before water sampling from the rosette begins), windows are re-rinsed with Milli-Q water and plastic bottle caps are reinstalled to seal the transmissometer windows from the deck environment. Windows can remain wet with Milli-Q water. The Milli-Q water quenches any biofouling of the optics between casts.

4. Compensation for Transmissometer Drift and CTD Digitizing Electronics

Manufacturers (e.g., WETLabs, Inc.) provides calibration readings of transmissometer voltages in air, in particle-free water, and with beam-blocked, referred to specifically as $Vair_{CAL}$, $Vref_{CAL}$, and $Vzero_{CAL}$. Ideally, these numbers should be provided at millivolt (or better) accuracy/precision.

4.1. On CTD Calibration

Assuming that the transmissometer is already cleans and 'lab' calibrated on the ship (section 2.1), 'On-CTD' air and beam-blocked measurements, $Vair_{CTD}$ and $Vzero_{CTD}$ (after careful cleaning of optics) must be performed before the first and after the final CTD deployment of a specific GEOTRACES leg. We note that $Vair_{CTD}$ values can often be over 1 percent lower than $Vair_{CAL}$ (the manufacturer's air calibration data) even for fresh out-of-the-box instruments when they are attached to low input impedance CTDs such as the SeaBird 911. $Vzero_{CTD}$ will often be different from $Vzero_{CAL}$.

$Vzero_{CTD}$ is measured with plastic caps in place with CTD in acquire mode (collecting 24 Hz data). Provided that the transmissometer windows are dry and the environment on deck is sheltered from salt spray, rain etc., $Vair_{CTD}$, can be determined at the same time by removing the plastic caps from the transmissometer for 1 minute while recording CTD

data at 24 Hz. This procedure should be repeated at the end of the expedition after rinsing and drying the windows.

4.2 Compensation for drift

Loss of transmissometer beam intensity over a cruise is significant and must be corrected for. For example during the VERTIGO ALOHA expedition (2004), $Vair_{CTD}$ showed a -0.76% loss of transmission over 56 hours of CTD use and 103 casts; for the VERTIGO K2 expedition (2005), transmission loss was -0.29% over 95 hours and 86 casts in the colder waters. Drift may be temperature dependent.

The drift of $Vair_{CTD}$ for any expedition should be interpolated over the accumulated CTD operation time to provide $Vair_{CTD}$-n, where n is the cast number. Scaling by elapsed sensor "on" time is reasonable based on known aging properties of LED light sources; we have found $Vzero_{CTD}$ to be invariant during any one expedition.

$$Vair_{CTD}\text{-n} = Vair_{CTD\text{-cal1}} - R(Vair_{CTD\text{-cal1}} - Vair_{CTD\text{-cal2}}) \quad (1)$$

Here $Vair_{CTD\text{-cal1}}$ and $Vair_{CTD\text{-cal2}}$ are the pre and post expedition on-CTD air calibrations and R is the fraction of CTD "on" time elapsed at the time of the cast-n.

> *Transmissometers deployed with logging CTDs (such as those deployed with pumping systems) should be cleaned and air calibrated ($Vair_{CTD}$-n determined for each cast) in the dry environment of the ship's laboratory every time they are deployed. In this case $\boldsymbol{c_p}$ may be calculated accurately after each cast.*

$Vref_{CTD}$-n, the voltage the sensor would read in particle free water at the time of the specific CTD cast, is derived according to Equation 2.

$$Vref_{CTD}\text{-n} = (Vair_{CTD}\text{-n} — Vzero_{CTD})/(Vair_{CAL} — Vzero_{CAL})*(Vref_{CAL} — Vzero_{CAL}) + Vzero_{CTD} \quad (2)$$

Transmission (T) is calculated using Equation 3:

$$T = (Vread\text{-n} — Vzero_{CTD})/(Vref_{CTD}\text{-n} — Vzero_{CTD}) \quad (3),$$

where Vread-n is the instantaneous voltage reading of the transmissometer at different depths during the specific cast. Particle beam attenuation coefficient, $\boldsymbol{c_p}$, is calculated:

$$\boldsymbol{c_p} = -(1/0.25)*\ln(T) \ \ m^{-1} \quad (4),$$

where the 0.25 is the path length of the transmissometer in meters.

Given the requirement for pre and post expedition "on CTD" calibrations, The CTD data must be post-processed after completion of each leg in order to arrive at accurate values for $\boldsymbol{c_p}$.

Other NOTES: Raw data profiles should reproduce on up and down casts by better than 1 mV (the precision of CTD digitization) except when thermal structure of the water column is highly variable (Figure 4, below).

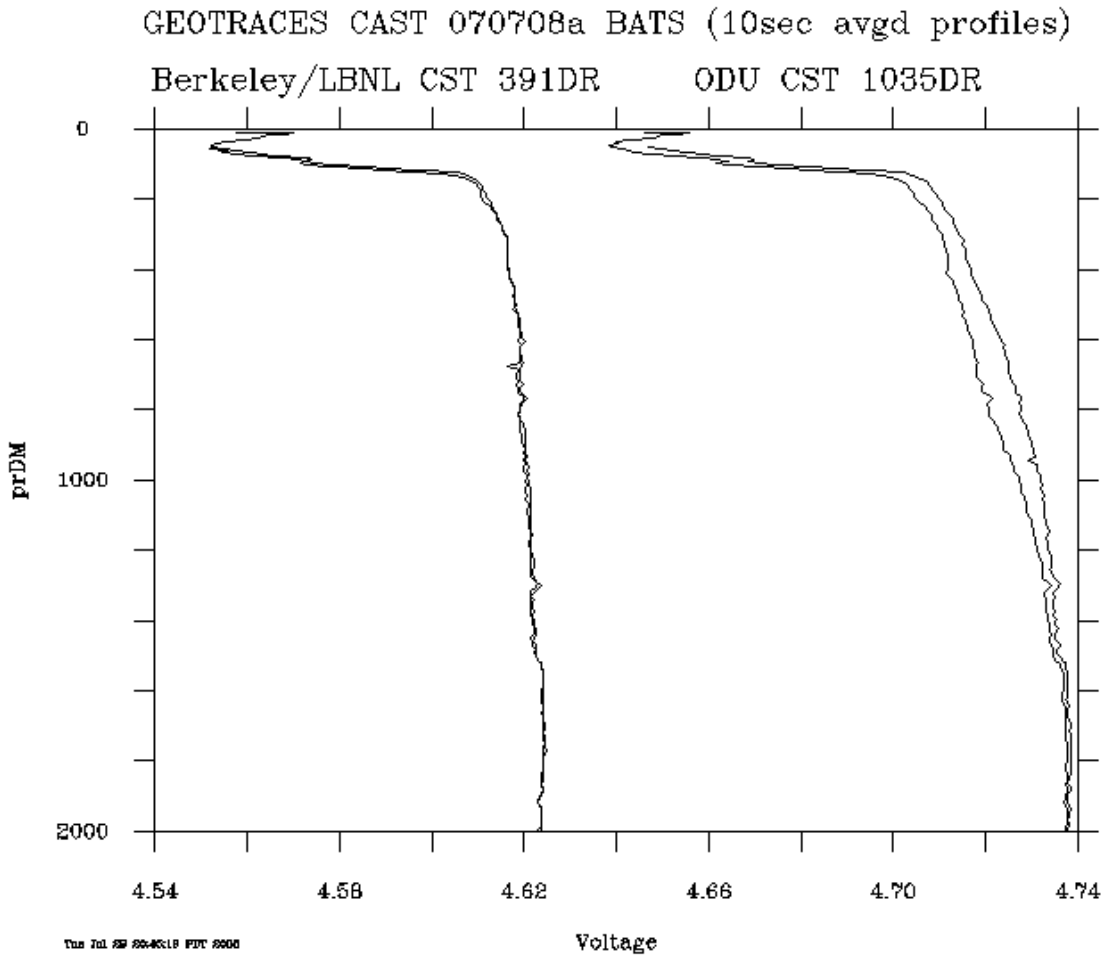

Figure 4. Examples of good (left) and poor (right) reproducibility of transmissometer data during GEOTRACES IC1 – Cast 070708a near the Bermuda Time Series Station. The profile on the right shows moderate thermal hysteresis of the C-STAR (1035DR) response during down and up (shifted to higher voltage) profiles. Profile on the left (CST 391DR) shows profile repeatability to better than 1 mV – the digitizing precision of the CTD. Profile data are raw 24Hz transmission voltages with 10 second averaging.

5. References

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.
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IX. Glossary of Terms

Terminology relevant to GEOTRACES Standards and Intercalibration Activities (not in alphabetical order, but by category)

Accuracy – The degree of agreement of a measured value with the true or expected value of the quantity of concern (Taylor, J.K. 1987. *Quality Assurance of Chemical Measurements*. Lewis Publishers, Michigan, 328 pp.). Accuracy therefore includes random and systematic errors.

Precision – The degree of mutual agreement characteristic of independent measurements as the result of repeated application of the process under specified conditions. It is concerned with the closeness of results (Taylor, 1987). Precision therefore is a measure of random errors in a method or procedure.

Standard (also, measurement standard or étalon) – Material measure, measuring instrument, reference material or measuring system intended to define, realize, conserve or reproduce a unit or one or more values of a quantity to serve as a reference (ISO. 1993. *International Vocabulary of Basic and General Terms in Metrology, Second Edition*. International Organization of Standardization, Switzerland, 59 pp.). See Primary Standard for a definition more relevant to GEOTRACES.

Primary Standard – Standard that is designated or widely acknowledged as having the highest metrological qualities and whose value is accepted without reference to others standards of the same quantity (ISO, 1993).

Reference Material – Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials (ISO, 1993).

Certified Reference Material – Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence (ISO, 1993).

Standard Reference Material – Reference material which by community agreement can be used as an intercomparison sample for stated TEIs. Validation of the SRM is carried out by repeated analysis during an intercalibration exercise.

Intercalibration – The process, procedures, and activities used to ensure that the several laboratories engaged in a monitoring program can produce compatible data. When compatible data outputs are achieved and this situation is maintained, the laboratories can be said to be intercalibrated (Taylor, 1987). Intercalibration therefore is an active process between laboratories that includes all steps from sampling to analyses, with the goal of achieving the same accurate results regardless of the method or lab.

Intercomparison – This is not well defined in the literature, but by implication is the comparison of results between laboratories, but is not the active process of ensuring that the same results are achieved as in an Intercalibration. It also may not include all steps, for example, sampling, sample handling, and analyses.

Appendix 1
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Appendix 2, Version 2.0

JGOFS Report 19, Determinations of Pigments (HPLC and Fluorometric) and POC/PON, for GEOTRACES Cruises

Preface

The Joint Global Ocean Flux Study relies on a variety of techniques and measurement strategies to characterize the biogeochemical state of the ocean, and to gain a better mechanistic understanding required for predictive capability. Early in the program, a list of Core Measurements was defined as the minimum set of properties and variables JGOFS needed to achieve these goals. Even at the time of the North Atlantic Bloom Experiment (NABE), in which just a few nations and a relatively small number of laboratories contributed most of the measurements, there was a general understanding that experience, capability and personal preferences about particular methods varied significantly within the program. An attempt to reach consensus about the best available techniques to use is documented in JGOFS Report 6, "Core Measurement Protocols: Reports of the Core Measurement Working Groups". As JGOFS has grown and diversified, the need for standardization has intensified. The present volume, edited by Dr. Anthony Knap and his colleagues at the Bermuda Biological Station for Research, is JGOFS' most recent attempt to catalog the core measurements and define the current state of the art. More importantly, the measurement protocols are presented in a standardized format which is intended to help new investigators to perform these measurements with some understanding of the procedures needed to obtain reliable, repeatable and precise results.

The job is not finished. For many of the present techniques, the analytical precision is poorly quantified, and calibration standards do not exist. Some of the protocols represent compromises among competing approaches, where none seems clearly superior. The key to further advances lies in wider application of these methods within and beyond the JGOFS community, and greater involvement in modification and perfection of the techniques, or development of new approaches. Readers and users of this manual are encouraged to send comments, suggestions and criticisms to the JGOFS Core Project Office. A second edition will be published in about two years.

JGOFS is most grateful to Dr. Knap and his colleagues at BBSR for the great labor involved in creating this manual. Many scientists besides the Bermuda group also contributed to these protocols, by providing protocols of their own, serving on experts' working groups, or reviewing the draft chapters of this manual. We thank all those who contributed time and expertise toward this important aspect of JGOFS. Finally, we note the pivotal role played by Dr. Neil Andersen, US National Science Foundation and Intergovernmental Oceanographic Commission, in motivating JGOFS to complete this effort. His insistence on the need for a rigorous, analytical approach employing the best available techniques and standards helped to build the foundation on which the scientific integrity of JGOFS must ultimately rest.

Hugh Ducklow
Andrew Dickson
January 1994

Chapter 1. Introduction

The Joint Global Ocean Flux Study (JGOFS) is an international and multi-disciplinary study with the goal of understanding the role of the oceans in global carbon and nutrient cycles. The Scientific Council on Ocean Research describes this goal for the international program: “To determine and understand the time-varying fluxes of carbon and associated biogenic elements in the ocean, and to evaluate the related exchanges with the atmosphere, sea floor and continental boundaries.” As part of this effort in the United States, the National Science Foundation has funded two time-series stations, one in Bermuda and the second in Hawaii and a series of large process-oriented field investigations.

This document is a methods manual describing many of the current measurements used by scientists involved in JGOFS. It was originally based on a methods manual produced by the staff of the US JGOFS Bermuda Atlantic Time-series Study (BATS) as part of their efforts to document the methods used at the time-series station. It has been modified through the comments of many JGOFS scientists and in its present form is designed as an aid in training new scientists and technicians in JGOFS style methods. An attempt was made to include many JGOFS scientists in the review of these methods. However, total agreement on the specifics of some procedures could not be reached. This manual is not intended to be the definitive statement on these methods, rather to serve as a high quality reference point for comparison with the diversity of acceptable measurements currently in use.

Presented in this manual are a set of accepted methods for most of the core JGOFS parameters. We also include comments on variations to the methods and in some cases, make note of alternative procedures for the same measurement. Careful use of these methods will allow scientists to meet JGOFS and WOCE standards for most measurements. The manual is designed for scientists with some previous experience in the techniques. In most sections, reference is made to both more complete detailed methods and to some of the authorities on the controversial aspects of the methods.

The organization and editing of this manual has been largely the effort of the scientists and technicians of the BATS program as administered by the Bermuda Biological Station For Research, Inc. (Dr. Anthony H. Knap as principal investigator). A large number of scientists from around the world submitted valuable comments on the earlier drafts. We acknowledge the considerable input from our colleagues at the Hawaii Ocean Time-series (HOT) and members of the methods groups of the international JGOFS community. The Group of Experts on Methods, Standards and Intercalibration (GEMSI), jointly sponsored by the Intergovernmental Oceanographic Commission and the United Nations Environment Programme, have also reviewed this document. The support for compilation of this work was provided in part by funds from the United States National Science Foundation OCE-8613904; OCE-880189.

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Chapter 13. Measurement of Algal Chlorophylls and Carotenoids by HPLC

1.0 Scope and field of application

Many individual algal pigments or pigment combinations and ratios are taxon-specific. Therefore, analysis of the chlorophylls and carotenoids present in a seawater sample can reveal the taxonomic composition of natural algal populations. This technique allows for the rapid separation of important phytoplankton pigments with detection limits for chlorophylls and carotenoids (using absorbance spectroscopy as analyzed by HPLC) on the order of 1 ng (Bidigare, 1991). The HPLC method described here is a modified version of Wright et al. (1991), provided by Bidigare (in press). Scientists who employ this or other methods to measure pigments should make themselves aware of the current and historical issues that surround these techniques and make appropriate decisions about specific methodologies for their application based on the scientific requirements and constraints of their individual programs.

2.0 Definition

The concentration of all pigments is given as ng kg^{-1} in seawater.

3.0 Principle of Analysis

The reverse phase high performance liquid chromatography method described here separates all the phytoplankton pigments listed below in order of polarity upon passage through a column. The most polar pigments are removed earlier than the less polar pigments.

Chlorophyllide *b*
Chlorophyllide *a*
Chlorophyll c_3
Chlorophyll c_1+ c_2 and Chlorophyll Mg 3,8DVP a_5
Peridinin
19' - Butanoyloxyfucoxanthin
Fucoxanthin
19' - Hexanoyloxyfucoxanthin
Prasinoxanthin
Pyrophaeophorbide *a*
Diadinoxanthin
Alloxanthin
Diatoxanthin

Lutein
Zeaxanthin
Chlorophyll *b*
Chlorophyll *a*
Phaeophytin *b*
Phaeophytin *a*
α Carotene
β Carotene

Picoplanktonic prochlorophytes are abundant in tropical and subtropical seas and oceans. They contain divinyl-chlorophyll *a* and divinyl-chlorophyll *b* (more appropriately called 8-desethyl, 8-vinyl Chlorophyll), both co-eluting with "normal" chlorophyll *a* and *b* with this reverse phase liquid chromatography technique.

4.0 Apparatus and Reagents

4.1 *Filtration System* and *Whatman 47 mm GF/F filters*

4.2 *Liquid nitrogen* and freezer for storage and extraction

4.3 *Glass centrifuge tubes* for extraction, 15 ml

4.4 High pressure liquid chromatograph capable of delivering three different solvents at a rate of 1 ml/minute.

4.5 *High-pressure injector valve* equipped with a 200 μL sample loop.

4.6 *Guard Column* (50 x 4.6 mm, ODS-2 C18 packing material, 5 μm particle size) for extending life of primary column.

4.7 *Reverse phase HPLC Column* (250 x 4.6 mm, 5 μm particle size, ODS-2 Spherisorb column).

4.8 *Absorbance detector* capable of monitoring ar 436 nm, or preferably, an *on-line diode array spectrophotometer.*

4.9 Data recording device: strip chart recorder or, preferably, an electronic integrator or computer equipped with hardware and software for chromatographic data analysis.

4.10 Glass syringe, 500 µl

5.0 Eluants

Eluant A (80:20, v:v, methanol: 0.5 M ammonium acetate, aq., pH=7.2), eluant B (90:10, v:v, acetonitrile:water), and eluant C (ethyl acetate). Use HPLC-grade solvents, measure volumes before mixing. Filter eluents through a solvent-resistant 0.4 µm filter before use and de-gas with helium.

The gradient program is listed in Table 13-1.

6.0 Sample Collection and Storage

Water samples are collected from niskins into clean polyethylene bottles with Tygon® tubing. Samples are immediately filtered through 47 mm GF/F filters using polycarbonate in-line filter holders (Gelman) and a vacuum of less than 100 mm Hg. Filters are folded in half twice and wrapped in aluminum foil, labeled, and stored in liquid nitrogen (to avoid formation of degradation products) until on-shore analysis.Alternatively, filters can be immediately placed in acetone for pigment extraction if analysis is to be carried out onboard ship. Samples collected for HPLC analysis can also be used in the measurement of chlorophyll *a* and phaeopigments by fluorometric analysis.

Filtration volume will vary with sampling location. For oligotrophic waters, 4 liters are filtered, whereas in coastal regions a smaller volume (0.5-1.0 liters) may be appropriate. In this case, a 25 mm GF/F filter is recommended.

7.0 Procedure

7.1 After removal from liquid nitrogen, the pigments are extracted by placing the filters in 5.0 ml 100% acetone. For 47 mm GF/F filters, 0.8 ml of water is retained on the filter, adjusting the final extraction solution to 86% acetone and the final extraction volume to 5.8 ml. In order to correct for any errors introduced by evaporation or experimental losses, 100 µl of an internal standard (canthaxanthin in acetone, Fluka) is added to each sample which elutes after zeaxanthin and before chlorophyll *b.* The samples are covered with Parafilm to reduce evaporation, sonicated (0°C, subdued light) and allowed to extract for 4 hours in the dark at -20°C. Following extraction samples are vortexed, filters are pressed to the bottom of the tube with a stainless steel spatula, and centrifuged for 5 minutes to remove cellular debris. External standards are also run before each sample set for daily HPLC calibration.

The addition of 5.0 ml acetone for pigment extraction is necessary to completely submerge 47 mm GF/F filters in 15 ml centrifuge tubes. However, this volume can be altered depending on the sizes of the filter and the extraction tube.

7.2 The HPLC system is setup and equilibrated with solvent system A at a flow rate of 1 ml/min.

7.3 Samples and standards are prepared for injection by mixing a 1 ml aliquot of the pigment extract with 300 µl of distilled water in a 2 ml amber vial. Shake and allow to equilibrate for 5 minutes prior to injection.

7.4 Approximately 500 µl of a sample is injected into the 200 µl sample loop and the three-step solvent program initiated is on closure of the injection valve. The chromatogram is then collected on a recording device.

7.5 The identities of the peaks from the sample extracts are determined by comparing their retention times with those of pure standards and algal extracts of known pigment composition. Peak identities can be confirmed spectrophotometrically by collecting eluting peaks from the column outlet.

7.6 *Calibration:* The HPLC system is calibrated with pigment standards obtained commercially (chlorophylls *a* and *b*, and ß-carotene can be purchased from Sigma Chemical Co., and zeaxanthin and lutein from Roth Chemical Co.) and/or by preparative scale HPLC (collecting and purifying HPLC fractions and placing in standard solvents) standards. Concentrations of pigment standards should be determined using a monochromator-based spectrophotometer in the appropriate solvents prior to the calibration of the HPLC system. The recommended extinction coefficients for most of the common algal pigments are provided in Table 13-2 (Bidigare 1991). Pigment standard concentrations are calculated as follows:

$$C_s = \frac{(A_{\max} - A_{750nm})}{E \times l} \times \frac{1000\text{mg}}{1\text{g}}$$

where:

Cs	=	pigment concentration (mg l^{-1})
$A_{\max}$	=	absorbance maximum (Table 2)
$A_{750\ nm}$	=	absorbance at 750 nm to correct for light scattering
E	=	extinction coefficient (L g^{-1} cm^{-1}, Table 2)
l	=	cuvette path length (cm)

Standards stored under nitrogen in the dark at -20°C are stable for approximately one month.

After determining the concentrations of the pigment standard they are injected onto an equilibrated HPLC system to calculate standard response factors (RF). Response factors are calculated as weight of standard injected (determined spectrophotometrically) divided by the area of the pigment standard plus areas of structurally related isomers, if present.

8.0 Calculation and expression of results

Concentration of the individual pigments in the sample are calculated using the following formula:

$$C_i = (\mathrm{A}) \times (\mathrm{RF}) \times \left(\frac{1}{\mathrm{IV}}\right) \times (\mathrm{EV}) \times \left(\frac{1}{\mathrm{SV}}\right)$$

where:

C_i	=	individual pigment concentration (ng per liter)
A	=	integrated peak area
RF	=	standard response factor
IV	=	injection volume
EV	=	extraction volume with internal standard correction
SV	=	sample volume

The units of ng kg^{-1} can be obtained by dividing this result by the density of the seawater.

9.0 References

Bidigare, R. (1991). in Spencer and Hurd (eds.). The analysis and characterization of marine particles. American Geophysical Union, Washington D.C.

Herbland, A., A. Le Bouteiller, and P. Raimbault. (1985). Size structure of phytoplankton biomass in the equatorial Atlantic Ocean. *Deep-Sea Res*. **32**:819-836.

Holm-Hansen, O., and B. Riemann. (1978). Chlorophyll *a* determination: improvements in methodology. *Oikos*, **30**: 438-447.

Wright, S.W., S.W. Jeffrey, F.C. Mantoura, C.A. Llewellyn, T. Bjørnland, D. Repeta, and N. Welschmeyer (1991). Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* **77**:183-196.

Table 13-1. HPLC solvent system program.

Time	Flow Rate	%A	%B	%C	Conditions
0.0	1.0	100	0	0	Linear gradient
2.0	1.0	0	100	0	Linear gradient
2.6	1.0	0	90	10	Linear gradient
13.6	1.0	0	65	35	Linear gradient
18.0	1.0	0	31	69	Hold
23.0	1.0	0	31	69	Linear gradient
25.0	1.0	0	100	0	Linear gradient
26.0	1.0	100	0	0	Hold
34.0	1.0	100	0	0	Inject

Table 13-2:

Pigment	**Wavelength (solvent)**	**E 1cm(L $g^{-1}cm^{-1}$)**
Chlorophyll *a*	664 nm (90% acetone)	87.67
Chlorophyll *b*	647 nm (90% acetone)	51.36
Chlorophyll c_1+c_2	631 nm (90% acetone)	42.6
Chlorophyllide *a*	664 nm (90% acetone)	128.0
Fucoxanthin	449 nm (EtOH)	160.0
19’ - Hexanoyloxyfucoxanthin	447 nm (EtOH)	160.0
19’ - Butanoyloxyfucoxanthin	446 nm (EtOH)	160.0
Lutein	445 nm (EtOH)	255.0
Zeaxanthin	450 nm (EtOH)	254.0
Prasinoxanthin	454 nm (EtOH)	160.0
Alloxanthin	453 nm (EtOH)	262.0
Peridinin	472 nm (EtOH)	132.5
Diadinoxanthin	446 nm (EtOH)	262.0
Diatoxanthin	449 nm (EtOH)	262.0
β Carotene	453 nm (EtOH)	262.0
Phaeophorbide *a*	665 nm (90% acetone)	69.8
Phaeophytin *a*	665 nm (90% acetone)	49.5

Chapter 14. Measurement of Chlorophyll *a* and Phaeopigments by Fluorometric Analysis

1.0 Scope and field of application

Chlorophyll *a* measurements have historically provided a useful estimate of algal biomass and its spatial and temporal variability. The fluorometric method is extensively used for the quantitative analysis of chlorophyll *a* and phaeopigments. However, errors can be introduced into the results when chlorophylls *b* and/or chlorophylls *c* are present. Chlorophyll *b* is the main source of error in this method. While generally not abundant in surface waters, chlorophyll *b* can be as high as 0.5 times the chlorophyll *a* concentration in the deep chlorophyll maximum, causing slight underestimations of the chlorophyll *a* concentration, and drastic overestimations of the phaeopigment concentrations. Divinyl-chlorophyll *a* also interferes and is taken as chlorophyll a by this method. The procedure described here is appropriate for all levels of chlorophyll *a* concentration in the marine environment. Filtration volumes should be modified for the different environments. Scientists who employ this or other methods to measure pigments should make themselves aware of the current and historical issues that surround these techniques and make appropriate decisions about specific methodologies for their application based on the scientific requirements and constraints of their individual programs.

2.0 Definition

The concentrations of chlorophyll *a* and phaeopigments in seawater are given as $\mu g\ kg^{-1}$.

3.0 Principle of Analysis

Algal pigments, particularly chlorophyll a, fluoresce in the red wavelengths after extraction in acetone when they are excited by blue wavelengths of light. The fluorometer excites the extracted sample with a broadband blue light and the resulting fluorescence in the red is detected by a photomultiplier. The significant fluorescence by phaeopigments is corrected for by acidifying the sample which converts all of the chlorophyll a to phaeopigments. By applying a measured conversion for the relative strength of chlorophyll and phaeopigment fluorescence, the two values can be used to calculate both the chlorophyll a and phaeopigment concentrations.

4.0 Apparatus

4.1 Filtration system and Whatman GF/F filters

4.2 Liquid nitrogen and freezer for storage and extraction

4.3 Glass centrifuge tubes for extraction, 15 ml

4.4 Turner fluorometer, fitted with a red sensitive photomultiplier, a blue lamp, 5-60 blue filter and 2-64 red filter.

5.0 Reagents

5.1 100% acetone

5.2 90% acetone

5.3 1.2M HCl (100 ml HCl in 900 ml de-ionized water)

6.0 Sample Collection and Storage

Water samples are collected from niskins into clean polyethylene bottles with Tygon® tubing. Samples are immediately filtered through 47 mm GF/F filters using polycarbonate in-line filters (Gelman) and a vacuum of less than 100 mm Hg. Filters are folded in half twice and wrapped in aluminum foil, labeled, and stored in liquid nitrogen (to avoid formation of degradation products) until shore analysis. Alternatively, filters can be placed immediately in acetone for pigment extraction if analysis is to be carried out onboard ship.

In oligotrophic waters, for this measurement coupled with HPLC determined pigments, 4 liters are filtered. For fluorometric analysis alone, a smaller volume (0.5 -1.0 l) may be sufficient. In coastal regions, a volume of 0.1-0.5 l may be adequate. In this case, use of 25 mm GF/F filters may be appropriate.

7.0 Procedure

7.1 After removal from liquid nitrogen or freezer), the pigments are extracted by placing the filters in 5.0 ml 100% acetone. For 47 mm GF/F filters, 0.8 ml of water is retained adjusting the final extraction solution to 86% acetone and the final extraction volume to 5.8 ml. The samples are covered with Parafilm to reduce evaporation, sonicated (0°C, subdued light) and allowed to extract for 4 hours in the dark at -20°C. Following extraction, samples are vortexed, filters are pressed to the bottom of the tube with a stainless steel spatula and spun down in a centrifuge for 5 minutes to remove cellular debris. For fluorometric analysis (not HPLC), decantation can replace centrifuging.

7.1.1 The addition of 5.0 ml acetone for pigment extraction is necessary to completely submerge 47 mm GF/F filters in 15 ml centrifuge tubes. This volume may be altered depending on the size of the filter and volume of the extraction tube.

7.2 The fluorometer is allowed to warm up and stabilize for 30 minutes prior to use.

7.3 The fluorometer is zeroed with 90% acetone.

7.4 1.0 ml of pigment extract is mixed with 4.0 ml 90% acetone in a cuvette and read on the appropriate door to give a reading between 30 and 100. The sample is then acidified with 2 drops of 1.2 M HCl. Further dilutions may be necessary for higher chlorophyll *a* concentrations.

7.5 *Standardization*

7.5.1 For laboratory use, the fluorometer is calibrated every 6 months with a commercially available chlorophyll *a* standard (*Anacystis nidulans*, Sigma Chemical Company). If the fluorometer is taken to sea, it is recommended that the fluorometer be calibrated before and after each cruise.

7.5.2 The standard is dissolved in 90% acetone for at least 2 hours and it's concentration (mg l^{-1}) is calculated spectrophotometrically as follows:

$$\text{chl}a = \frac{(A_{\max} - A_{750nm})}{E \times l} \times \frac{1000\text{mg}}{1\text{gram}}$$

where:

$A_{\max}$ = absorption maximum (664 nm)

$A_{750\ \text{nm}}$ = absorbance at 750 nm to correct for light scattering

E = extinction coefficient for chl a in 90% acetone at 664 nm (87.67 L g^{-1} cm^{-1})

l = cuvette path length (cm)

7.5.3 From the standard, a minimum of five dilutions are prepared for each door. Fluorometer readings are taken before and after acidification with 2 drops 1.2 M HCl.

7.5.4 Linear calibration factor (K_x) are calculated for each door (x) as the slope of the unacidified fluorometric reading vs. chlorophyll *a* concentration calculated spectrophotometrically.

7.5.5 The acidification coefficient (F_m) is calculated by averaging the ratio of the unacidified and acidified readings (F_o/F_a) of pure chlorophyll *a*.

7.5.6 Samples are read using a door setting that produces a dial reading between 30 and 100. The fluorometer is zeroed with 90% acetone each time the door setting is changed.

8.0 Calculation and expression of results

The concentrations of chlorophyll *a* and phaeopigments in the sample are calculated using the following equations:

$$\text{Chl } (\mu g/l) = \left(\frac{F_m}{F_m - 1}\right) \times (F_o - F_a) \times K_x \times \left(\frac{\text{vol}_{ex}}{\text{vol}_{filt}}\right)$$

$$\text{Phaeo (chl equiv. weights)} = \left(\frac{F_m}{F_m - 1}\right) \times [(F_m \bullet F_a) - F_o] K_x - \text{vol}_{ex}$$

where:

F_m	=	acidification coefficient (F_o/F_a) for pure Chl *a* (usually 2.2).
F_o	=	reading before acidification
F_a	=	reading after acidification
K_x	=	door factor from calibration calculations
vol_{ex}	=	extraction volume
vol_{filt}	=	sample volume

9.0 References

Herbland, A., A. Le Bouteiller, and P. Raimbault. (1985). Size structure of phytoplankton biomass in the equatorial Atlantic Ocean. *Deep-Sea Res.*, **32**: 819-836.

Holm-Hansen, O., and B. Riemann. (1978). Chlorophyll *a* determination: improvements in methodology. *Oikos*, **30**: 438-447.

Chapter 15. Determination of Particulate Organic Carbon and Particulate Nitrogen

1.0 Scope and field of application

This procedure describes a method for the determination of particulate organic carbon and particulate nitrogen in seawater. The assay is appropriate for measuring oceanic levels of particulate organic carbon (5.0 - 500.0 µg C/kg) and particulate nitrogen (0.5 - 100.0 µg N/kg).The principles for this method were first described by Gordon (1969) and Kerambrun and Szekielda (1969). Sharp (1974) describes a number of useful modifications to the existing method applied here. Detailed description of the analytical procedure is given by the manufacturer (Control Equipment Corporation 1988). Some of the details of the actual measurement of carbon and nitrogen in this method are specific to the Control Equipment Corporation (CEC) 240-XA Elemental Analyzer hardware used at the Bermuda Atlantic Time-series Study. Scientists who employ this or other methods to measure POC and PN should make themselves aware of the current and historical issues that surround these techniques and make appropriate decisions about specific methodologies for their application based on the scientific requirements and constraints of their individual programs.

2.0 Definition

2.1 The concentration of particulate organic carbon is given in µg C/kg seawater.

2.2 The concentration of particulate nitrogen is given in µg N/kg seawater.

3.0 Principle of Analysis

A dried, acidified sample of particulate matter is combusted at 960°C. The organic carbon is converted to CO_2 and the nitrogen oxides are subsequently reduced to N_2 gas. Both gases are measured by thermal conductivity.

4.0 Apparatus

4.1 *Control Equipment Corporation (CEC) 240-XA Elemental Analyze*r (Leeman Labs, Inc.)

4.2 CAHN Model 4400 Electrobalance

4.3 Hewlett Packard (HP-150) Analytical Software

5.0 Reagents

5.1 *Hydrochloric acid* (concentrated HCl: reagent grade)

5.2 *Acetanilide* (reagent grade): Acetanilide has 0.7109 g C and 0.1036 g N per g total mass.

6.0 Sampling

The POC/PN samples are taken after oxygen, CO_2, salinity and nutrient samples have been removed, approximately 30–60 minutes after the CTD/rosette reaches the surface. Settling of large particles in the Niskin bottles will create a non-uniform distribution of the particles within this period of time. For best results, the Niskin bottle should therefore be shaken before sampling or the entire volume filtered (including the volume below the spigot).

Samples are collected in 4 liter polypropylene bottles equipped with a 1/4" outlet at the base. The filtration is "in-line" with the filter mounted in a Delrin filter holder. The holder is connected to the outlet at the bottom of the 4 liter bottle on one end and a vacuum system (liquid container and pump) on the other. Two liters are normally filtered at all depths (although this volume may not be adequate for all systems) from surface to 1000 m onto precombusted (450°C, 5 hours) 25 mm Whatman GF/F filters (nominal pore size 0.7 μm). The filter is removed, wrapped in precombusted aluminum foil and stored frozen in a deep freezer (-20°C) until processed.

7.0 Procedures

7.1 *Sample Analysis*

7.1.1 Prior to analysis, the filters are thawed, allowed to dry overnight at 65°C in acid washed and precombusted (450°C, 2 hours) scintillation vials and then placed overnight in a desiccator saturated with HCl fumes. The air in the desiccator is kept saturated by leaving concentrated HCl in an open container in the lower compartment of the desiccator. Thereafter, the filters are dried again at 65°C and packed in precombusted (850°C, 1 hour) nickel sleeves.

7.1.2 The samples are analyzed on a Control Equipment Corporation (CEC) 240-XA Elemental Analyzer following the guidelines given by the manufacturer. Sixty-four samples are run at a time on the auto-sampler, of which one is a standard (see below) and approximately nine are Ni sleeve blanks. The machine operator checks on the machine regularly to ensure that problems

have not developed. Data are collected and stored by a microcomputer automatically.

7.2 *Standardization and blank determination:* Acetanilide standard and blanks (empty Ni sleeves) are measured prior to each batch run of samples (64 samples). A minimum of three empty filters are processed as an ordinary sample and analysed for each cruise as filter blanks. The acetanilide standard is weighed in acetone washed tin capsules on a CAHN Electrobalance. Standard weights are usually between 0 and 2.0 mg. The tin capsule with the standard is put into a nickel sleeve and run on the Elemental Analyzer. The empty filter blanks should be treated exactly like sample filters except that no sample water is passed through them.

8.0 Calculation and expression of results

The POC and PN weights of each of the samples are integrated and estimated automatically by the Hewlett Packard (HP-150) Analytical Software, supplied with the CEC instrument. The program automatically includes the latest Ni sleeve blank into its calculations. The *in-situ* concentration is estimated:

$$\mu g/kg = \frac{S - B}{V \rho}$$

Where:

S = the result for the filtered sample
B = the measured filter blank
V = volume filtered (liters)
ρ = density (a function of T, S and P, where T = model temperature at filtration, S = salinity of the sample, and P = atmospheric pressure)

9.0 References

Control Equipment Corporation. (1988). The automated and advanced Model 240-XA Elemental Analyzer. Lowell, MA.

Gordon, Jr. D.C. (1969). Examination of methods of particulate organic carbon analysis. *Deep-Sea Research* **16**:661-665.

Kerambrun, P. and K.H. Szekielda. (1969). Note technique. *Tethys* **1**:581-584.

Sharp, J.H. (1974). Improved analysis for “particulate” organic carbon and nitrogen from seawater. *Limnology and Oceanography* **19**:984-989.

Appendix 3

PICES Report 34, Determinations of DOC and DON, for GEOTRACES Cruises

Determination of dissolved organic carbon and total dissolved nitrogen in sea water

1. Scope and field of application

This procedure describes a method for the determination of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) in sea water, expressed as micromoles of carbon (nitrogen) per liter of sea water. The method is suitable for the assay of oceanic levels of dissolved organic carbon (<400 $\mu mol \cdot L^{-1}$) and total dissolved nitrogen (<50 $\mu mol \cdot L^{-1}$). The instrument discussed and procedures described are those specific to the instrument employed in the Hansell Laboratory at the University of Miami. Instruments produced by other manufacturers should be evaluated for suitability.

2. Definition

The dissolved organic carbon content of seawater is defined as:

> *The concentration of carbon remaining in a seawater sample after all particulate carbon has been removed by filtration and all inorganic carbon has been removed by acidification and sparging.*

The total dissolved nitrogen content of seawater is defined as:

> *The concentration of nitrogen remaining in a seawater sample after all particulate nitrogen has been removed by filtration.*

3. Principle

A filtered and acidified water sample is sparged with oxygen to remove inorganic carbon. The water is then injected onto a combustion column packed with platinum-coated alumina beads held at 680°C. Non-purgeable organic carbon compounds are combusted and converted to CO_2, which is detected by a non-dispersive infrared detector (NDIR). Non-purgeable dissolved nitrogen compounds are combusted and converted to NO, which when mixed with ozone chemiluminesces for detection by a photomultiplier.

4. Apparatus

Shimadzu TOC-V_{CSH} with ASI-V auto sampler and TNM-1 Total Nitrogen detector (or equivalent).

5. Reagents

5.1. Compressed gas

Ultra High Purity (UHP 99.995%) oxygen is used as the carrier gas for the Shimadzu TOC-V. High quality carrier gas is required to obtain low background levels in the detector. Oxygen is used to ensure complete combustion of all organic material.

5.2. Combustion Column Catalyst

The carrier gas passes through a column packed with 2 mm platinum-coated alumina beads (Shimadzu P/N 017-42801-01), held at 680°C.

5.3. Platinum Gauze

Pure platinum wire gauze (52 mesh woven from 0.1 mm diameter wire) is roughly formed into cubes (≈0.5 cm to a side) and several (3-5) are placed on top of the combustion column bed. The platinum gauze improves analytical reproducibility and retains injected salt.

5.4. Acidification of Sample

Trace-impurity analyzed concentrated hydrochloric acid is used to acidify samples prior to analysis. Approximately 0.1% by volume of the concentrated acid is added to each sample prior to analysis to lower the pH of the sample to <pH 2. At this pH and with sparging, all inorganic carbon species are converted to CO_2 and removed from the sample. Automated acidification by the TOC-V is not used as with time the blank using this acid solution increases. By manually acidifying the sample with acid freshly taken from a sealed bottle, the increase in blank has not been observed.

6. Sampling

Proper sampling techniques and handling are essential to good quality data. Care must be taken to minimize contamination of the sample. Sampling from the rosette should be done using clean silicone tubing. Gloves should be worn during sampling. It is recommended that anyone sampling from the rosette prior to collection of the samples (e.g., gases) also wear gloves. If that it not possible, every effort must be made not to touch the sample nipple (the path of the water stream, from Niskin to sample bottle, must be kept very clean). Grease (whether mechanical grease from ship operations or sealing grease as employed for some gas sampling) should never be allowed to come in contact with the sample nipple.

6.1 Sample preparation

Prior to sampling, 60 ml High Density Polyethylene (HDPE) bottles are cleaned, first by rinsing with distilled water, followed by a 4 hour soak in 10% hydrochloric acid, and then copiously rinsed with distilled water, inverted onto a clean surface and allowed to air dry.

All tubing and the polycarbonate inline filter holder should be acid washed and rinsed with copious quantities of distilled water prior to use. Tubing should be silicone; under no circumstances should Tygon® tubing be used as it is a source of contamination.

GF/F filters should be combusted at 450°C for at least 4 hours prior to use and stored in a glass airtight container.

6.2 Sample Collection

Whether or not a sample is filtered prior to analysis depends on the goal of the measurement. If DOC(N) is the variable of interest, then ideally all samples must be filtered. However, the handling of water required for filtration can introduce contaminants, so in some cases filtration may be bypassed. In oligotrophic waters, for example, where particulate organic carbon concentrations may be a very small fraction of the total organic carbon, filtering may not be necessary. Since the particles are generally small and homogeneously distributed in a sample, the analysis of unfiltered water results in a good measure of total organic carbon (TOC). Likewise, samples collected at depths >250 meters may be left unfiltered as water from these depths normally have low particulate organic carbon loads (<1 μmole/liter).

In high productivity areas (nutrient rich zones), a substantial portion of the total carbon may be present in particulate form, and many of those particles may be large and so not homogeneously and representatively assessed in the DOC analyzer. In those situations, samples collected between the surface and 250 m are filtered through a precombusted GF/F filter. For consistency, when sampling in both oligotrophic and eutrophic environments as part of a study, prefiltering is recommended for all upper layer waters.

The GF/F filters are housed in a polycarbonate inline filter holder connected to the Niskin bottle sample nipple with silicone tubing, with collection of filtrate into a precleaned 60ml HDPE bottle. HDPE sample bottles should be labeled with sample-specific information, such as the cruise designation, cast number, and Niskin bottle number. The filter holder, with filter in place, must be well flushed with sample prior to collection into the bottles. The sample bottles should be rinsed 3 times with sample prior to filling. Bottles should be filled to between 75 and 90%, or 45 to 55 ml into the 60 ml bottle. This volume provides room for expansion of the water on freezing. The sample bottles are then capped tightly and frozen upright.

7. Procedures

Water samples are collected from the rosette. Water taken from the surface to 250 m is filtered using precombusted (450°C) GF/F inline filters as they are being collected from the Niskin bottle. At depths >250 meters, the samples are collected without filtration. After collection, samples are frozen upright in 60 ml acid-cleaned HDPE bottles, and remain cold until analysis. Prior to analysis, samples are returned to room temperature and acidified to pH <2 with concentrated hydrochloric acid. Analysis is performed using a Shimadzu TOC-V_{CSH} Total Organic Carbon Analyzer with the TNM-1 Total Nitrogen detector.

Instrument conditions are as follows:

Combustion temperature	680°C
Carrier gas	UHP Oxygen
Carrier flow rate	150 ml/min
Ozone generation gas	Zero Air from Whatman TOC Gas Generator
Ozone flow rate	500 ml/min
Sample sparge time	2.0 minutes
Minimum number of injections	3
Maximum number of injections	5

Number of washes	2
Standard deviation maximum	0.1000
CV maximum	2.00%
Injection volume	100 µl

Each detector functions independently with respect to the acceptance values above. If DOC meets the required specifications, but TDN does not, the instrument will continue making injections until either the criteria are met or the maximum number of injections has been reached. The same is true for the situation where TDN has met the criteria and the DOC has not.

The DOC system is calibrated using potassium hydrogen phthalate and the TDN system using potassium nitrate, both in Milli-Q water. System performance is verified daily using Consensus Reference Water (http://www.rsmas.miami.edu/groups/biogeochem/CRM.html). This reference water is deep Sargasso Sea water (DSR) that has been acidified and sealed in 10 ml ampoules, the concentrations of which (of DOC and TDN) has been determined by the consensus of up to six expert and independent laboratories. Low Carbon Water (LCW) that has gone through the same acidification, sealing process, and consensus verification program as the DSR and has an agreed upon carbon concentration of 1 to 2 µmoles C/L is also analyzed and used to determine the instrument blank. After verifying proper operation of the TOC/TN instrument, samples are placed on an auto sampler for analysis. The run starts with a QW (Q Water) blank and a reference seawater analysis. Then six samples are analyzed, followed by another QW blank and reference seawater. This sequence is repeated until all samples for that run are analyzed. The run ends with a QW blank, reference water, and a QW blank that had not been acidified. This last blank verifies that the hydrochloric acid used to acidify the samples is not contaminated. QW blanks and reference water samples are used to evaluate system performance during the analytical run. If a problem is detected with the blanks or reference waters, the samples are reanalyzed.

8. Calculation and expression of results

The Shimadzu TOC-V is calibrated for carbon using a 4 to 5 point analysis of potassium hydrogen phthalate in Milli-Q water. Since the instrument performs using units of parts per million (ppm), the concentration of the sample in µM (micromolar or micromoles per liter), and correction for the instrument blank, is calculated as:

$$[(\text{Sample (ppm)} - \text{LCW (ppm)}) \times 83.33333] + \text{LCW value (µM)}$$

where Sample and LCW are the concentrations determined by the TOC-V, 83.33333 is a conversion factor converting ppm to µM and LCW is the carbon

concentration of the Low Carbon Water CRM. Subtracting the LCW (ppm) from the sample removes both instrument blank and carbon content of the LCW. The carbon content of the LCW is added again (final term in equation) to calculate the correct sample concentration.

For total dissolved nitrogen, the instrument is calibrated using a similar method to that used for calibrating total carbon. The standard is potassium nitrate in Milli-Q water. Again the instrument is calibrated in ppm and the following calculation is used to convert from ppm to μM:

Sample (ppm) X 71.43

where sample is the concentration determined by the TOC-V and 71.43 is a conversion factor from ppm to μM. An instrument blank has not been detected for the nitrogen system. Dissolved Organic Nitrogen (DON) is calculated by subtracting inorganic nitrogen (NO_2, NO_3, etc) from the total dissolved nitrogen determined by the TOC-V.

9. Quality assurance

On a daily basis, Consensus Reference Water (CRM) is analyzed to verify system performance. If the value of the CRM does not fall within the expected range, samples are not analyzed until the expected performance has been established.

The QW blanks and reference seawater samples analyzed with the samples are used for quality assurance and quality control (QA/QC). By evaluating the performance of these reference waters, instrument drift and performance can be evaluated. If a problem is detected with either drift or performance, the samples are reanalyzed.

Citation:

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO2 measurements. PICES Science Report No. 34.