Sampling and Sample-handling Protocols for GEOTRACES Cruises

Version 3 edited by the 2017 GEOTRACES Standards and Intercalibration Committee:

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Table of Contents

I. INTRODUCTION 4

II. GENERAL CONSIDERATIONS.. 4

III. HYDROGRAPHY AND ANCILLARY PARAMETERS... 5

IV. RADIOACTIVE ISOTOPES 8

A. Protocols for ^{230}Th and ^{231}Pa.. 8

B. Protocols for ^{234}Th... 12

C. Protocols for ^{226}Ra and ^{228}Ra Measurements in Sea Water 15

D. Protocols for ^{210}Po and ^{210}Pb... 27

E. Protocols for ^{7}Be.. 36

F. Protocols for anthropogenic radionuclides (^{239}Pu and ^{240}Pu, and ^{137}Cs) and limited information on other isotopes (^{90}Sr, ^{237}Np, ^{241}Am, ^{236}U and ^{129}I).................................... 37

V. RADIOGENIC ISOTOPES 42

A. Protocols for $^{143}Nd/^{144}Nd$ 42

B. Protocols for $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$ 48

VI. TRACE ELEMENTS 53

1. Pre-cruise Preparations 53

2. Sample Collection 56

3. Sample Handling.. 58

4. Shipboard Determinations of Selected Dissolved Trace Metals................................. 63

5. Chemicals and Reagents 63

6. Analytical Considerations: Precision and Accuracy.. 64

7. References.. 65

8. Protocols for Sampling and Determinations of Mercury and its Speciation 66

9. Collection of particulate samples from GO-FLO sampling bottles 76

10. In-situ pump sampling protocols for particulate trace metals................................... 91

11. Shipboard Aerosol Sampling 107

VII. NITRATE, SILICON, AND CARBON ISOTOPES 118

A. Protocols for Nitrate Isotopes 118

C. Protocols for Carbon Isotopes in Dissolved Inorganic Carbon (DIC) 123

VIII. PROTOCOLS FOR OPTICS: TRANSMISSOMETER AND SCATTERING SENSORS 123

1. Transmissometers and Scattering sensors.. 124

2. Avoiding optical data dropouts.. 126

3. Elimination of optics contamination and cast-to-cast offsets 126

4. Compensation for Transmissometer Drift and CTD Digitizing Electronics 127

5. References.. 129

IX. BIOGEOTRACES PARAMETERS 130

A. Active fluorescence (i.e., F_v/F_m and other biophysical metrics) 130

B. Metagenomics 134

X. GLOSSARY OF TERMS 138

Appendix 1. Contributors to the GEOTRACES Cruise Protocols, Version 3.0

Appendix 2. GEOTRACES-recommended modifications to JGOFS 19 protocols

Appendix 3. PICES Report 34, Determinations of DOC and DON, for GEOTRACES Cruises

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 from 2010-2017, 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, Nutrient Isotopes, Optics, and BioGEOTRACES parameters. 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 GEOTRACES Section 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. Cruises without a crossover station are required to sample at least 3 depths in replicate at 2 different stations, at least for all key parameters (defined in Table 2 of the GEOTRACES Science Plan), and samples from these intercalibration depths must be distributed to at least one other laboratory for TEI determinations. Where two labs share the analyses for a parameter on a cruise, this requirement can be satisfied by alternating samples between labs. Exceptions to this requirement can be made if an alternative method to demonstrate intercalibration can be provided and approved by the Standards and Intercalibration Committee. The results from this effort should be examined later for data integrity and coherence.

C. The number of detectable parameters is constantly growing, and the analytical state-of-the-art for certain transient and/or large volume parameters sometimes does not allow the use of crossover or baseline stations, or sample exchanges. Therefore, the intercalibration for such short-lived, transient, very large volume, or new parameters may include different approaches for establishing accuracy. This can include the measurement of reference materials, comparisons with nearby stations, analyses of sites with known concentrations, or other methods of external validation. The Standards and Intercalibration Committee and elemental coordinators will assist in determining an appropriate method for the intercalibration of a specific parameter.

D. Nutrient and salinity samples should be taken along with all trace element samples 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.

E. 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.

F. 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) - $\mu mol\ l^{-1}$ 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), nutrients, and carbon system parameters 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.

There are an additional suite of ancillary parameters that are not required for every GEOTRACES cruise, but provide invaluable information on water mass tracing and transport - the chlorofluorocarbons, sulfur hexafluoride, and ^{3}He. The GO-SHIP hydrography manual (Hood et al., 2010) fully describes methods for sampling and analyses for these parameters, and their associated quality assurance/intercalibration protocols.

The JGOFS Report 19 sections that include 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), Hooker et al. (2005) 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 (2017).

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
Carbon system Parameters	Coulometry, etc.	NA	Hood et al., 2010
Pigments*	Fluorometry and HPLC	NA	Hooker et al. (2005)
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

Hooker, S.B., Van Heukelem, L., Thomas, C.S., Claustre, H., Ras, J., Schluter, L., Perl, J., Trees, C., Stuart, V., Head, E., Barlow, R., Sessions, H., Clementson, L., Fishwick, J., Llewellyn, C., Aiken, J., 2005. The Second SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-2). NASA Tech. Memo. 2005-212785, NASA Goddard Space Flight Center, Greenbelt, Maryland, 112 pp.

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

* Pigments are also considered a “BioGEOTRACES” parameters whose intercalibration protocols are described in Section IX

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 instruments

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. For ^{230}Th and ^{231}Pa as key parameters, crossover stations or duplicate/alternate sampling schedules are required. As a working standard, a limited amount of a fortified sea-water sample has been prepared at LDEO and has been distributed on request.

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 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 should 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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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) 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. It should be noted that for very small samples, inhomogeneous distributions of larger Th-containing particles might introduce some additional scatter in total ^{234}Th.

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. 2003) 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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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 clean-up 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 clean-up 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.

^{226}Ra and ^{228}Ra have now been successfully measured by ICP-MS and TIMS (Foster et al., 2004; Olivier et al., 2008; (Bourquin et al. 2011; Hsieh and Henderson 2011)). 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 submitted; 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.

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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. Generally, 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 µm 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 up 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. 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:

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

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:

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

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:

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

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:

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

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

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

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:

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

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:

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

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.

10. References

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E. Protocols for ^{7}Be

^{7}Be is produced naturally in the atmosphere. Its short half-life of 53 days and its deposition with precipitation leads to transient signals that have successfully been used to determine a variety of ocean processes at the sea surface (e.g. vertical mixing, atmospheric deposition, sea-ice transport). The requirement of large sample volumes and its short half-life create difficulties with its intercalibration. No reference materials are available for ^{7}Be, and the only way of measuring it in environmental concentrations is by gamma spectrometry, preventing the use of alternative detection methods for validation

purposes. Nevertheless, it can be measured reliably when extracted from large volumes of sea-water (typically 10-100s of liters) with Fe-hydroxide coated adsorbers or by precipitation with $Fe(OH)_3$. Chemical recoveries after the adsorption step can be monitored by adding stable Be tracer. The absence of a radioactive spike and of alternative detection methods means that a careful evaluation of adsorption efficiencies and energy-specific detection efficiencies of the gamma-spectrometer are crucial. Due to very different activities, variable pre-concentration factors are required for sea-water, snow, rain, sea-ice or aerosols. Pre-concentration is of performed with $Fe(OH)_3$, either on adsorbers or as a precipitate. For small volumes with low salinity (e.g. rain), a complete evaporation of the sample can alternatively be considered. An example of ^{7}Be analyses of various phases and more references regarding ^{7}Be analyses in the marine environment can be found in Kadko et al. (2016).

Kadko, D., B. Galfond, W. M. Landing, and R. U. Shelley. 2016. Determining the pathways, fate, and flux of atmospherically derived trace elements in the arctic ocean/ice system. Marine Chemistry **182:** 38-50.

F. Protocols for anthropogenic radionuclides (^{239}Pu and ^{240}Pu, and ^{137}Cs) and limited information on other isotopes (^{90}Sr, ^{237}Np, ^{241}Am, ^{236}U and ^{129}I)

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.
Due to the currently small number of laboratories able to analyze these parameters and due to the large volumes required, obtaining reference materials for artificial radionuclides is challenging. For some of the more commonly studied isotopes (^{137}Cs, ^{239}Pu, ^{240}Pu), a limited choice of materials may be available, e.g. through the Marine Environment Laboratory of the IAEA. For some new and demanding parameters, groups have resorted to share dedicated samples to externally compare laboratories' results. An overview of sampling methods for many anthropogenic radionuclides can be found in Kenna et al. (2012).

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). For ^{236}U and ^{129}I (e.g. Casacuberta et al. 2016), AMS is the method of choice.

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 their 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 cannot 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

A. Protocols for $^{143}Nd/^{144}Nd$

Samples for Nd isotopes ($^{143}Nd/^{144}Nd$, typically expressed as ε_{Nd}) as well as for rare earth element (REE) concentration analysis should be collected using GO-FLO bottles (General Oceanics) or Niskin bottles with epoxy-coated stainless-steel springs for trace elements. The samples should be filtered (0.2 or 0.45 µm pore size) to measure dissolved Nd.

1. Analytical instrument

The two instruments used for analysis of dissolved $^{143}Nd/^{144}Nd$ in seawater are Thermal Ionization Mass Spectrometry, TIMS (Piepgras and Wasserburg, 1987; Shimizu et al., 1994; Dahlqvist et al. 2005; Lacan and Jeandel, 2005), and Multiple Collector Inductively Coupled Mass Spectrometry, MC-ICP-MS (e.g., Vance et al., 2004). Both instruments have been shown to produce precise and accurate Nd isotope ratios (Pahnke et al., 2012; van de Flierdt et al., 2012).

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-ICP-MS instrument and method. The amount of Nd required per analysis ranges from 1 to 30 ng, with the lowest part of the range being only feasible using NdO^+ beams on a TIMS and very sensitive MC-ICP-MS instruments. The higher part of the concentration range allows analysis of Nd as metal by TIMS or analysis of Nd by less sensitive MC-ICP-MS instruments. Note that new generation TIMS instruments may enable accurate isotope analysis of as little as 5 ng of Nd as metal if chemical separation from other REEs and Ba is sufficiently complete. The concentration of Nd in most open ocean water generally ranges from 0.5 to 6 ng/kg (e.g. Nozaki, 2001; see also more recent compilations by van de Flierdt et al. (2016) and Tachikawa et al. (2017)). 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.

For the analysis of dissolved REE concentrations, the required volume depends on the method that is applied for the pre-concentration of REEs from seawater. In the past, pre-concentration via Fe co-precipitation or liquid-liquid extraction has been applied, typically requiring 200-500 mL of seawater (e.g., van de Flierdt et al., 2012; Jeandel et al., 2013). Recently, automated pre-concentration using a seaFAST system (Elemental Scientific) has been successfully applied on 4 mL in online mode (Hathorne et al., 2012) and 10 mL in offline mode (Behrens et al., 2016).

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 0.2 and 0.45 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; Pahnke et al., 2012). It was however noted that blanks from QMA filters were elevated (Jeandel, personal communication; see also section 10.2.1). Filtered seawater samples for Nd isotopes and REE concentrations must be stored in acid-cleaned high or low-density polyethylene (HDPE or LDPE) containers and must be acidified with ultra-clean HCl or HNO_3 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), ii) acidified and pre-concentrated onboard (for Nd isotope analysis only), or iii) acidified and shipped to the home laboratory where spiking, pre-concentration, 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. See Struve et al. (2016) for an example of combined extraction for Pa, Th and Nd isotopes or Jeandel et al. (2011) for the sequential extraction of Ra, Nd, Th, Pa, and U for isotope analysis.

4.1 Acidification

Add 1 mL concentrated HCl (ultraclean) per liter 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.

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 to measure all the REEs, including Nd on the same sample, only the aliquot will be spiked for ICP-MS concentration determination (Lacan and Jeandel, 2001; Behrens et al., 2016).

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). For separate REE analyses, pre-concentration can also be achieved by using an automated online or offline seaFAST system (Elemental Scientific) containing a cartridge with a chelating resin with ethylenediaminetriacetic and iminodiacetic acid functional groups (see also 4.3.4 below; e.g., Hathorne et al., 2012; Behrens et al., 2016).

4.3.1 Fe hydroxide
2 to 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 to 5 mL of ultraclean ammonium hydroxide is added per liter 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
Neodymium 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) or just HDEHP 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 (Pahnke et al., 2012; van de Flierdt et al., 2012).

4.3.3 Mn oxides

Other works suggest to co-precipitate using 375 µl of 60 g/L $KMnO_4$ or 150 µl of 400 g/L $MnCl_2$, which are successively added to the acidified/spiked sample and then pH is raised to 8 by addition of NH_4OH (Rutgers van der Luff and Moore, 1999). 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).

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.

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, ideally in a metal- and particle-free environment (i.e. metal-free clean 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-ICP-MS (or NdO^+) analysis, critical interferences are expected from Ce, Pr and Sm.

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-ICP-MS, 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. It is furthermore recommended to constrain the true external reproducibility by repeat analyses of an in-house seawater standard (REEs), an artificial seawater standard (Nd

isotopes, REEs) or USGS reference materials even though they will have a different matrix.

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. If not possible, samples from duplicate sampling at multiple water depths >1000m water depth and preferably different stations should be exchanged with at least one other laboratory.

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B. Protocols for $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$

Samples for the stable Pb isotope composition of seawater ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) are particularly difficult to collect without contamination by shipboard equipment and sampling devices. Clean sampling methods are however also essential for other contamination prone elements, such as Zn and Fe, and successful intercalibration for all of these elements has been demonstrated, including Pb (http://www.geotraces.org/science/intercalibration/322-standards-and-reference-materials) and Pb isotopes (Boyle et al., 2012).

1. Analytical instruments

The instruments used for analysis of dissolved Pb and Pb isotopes in seawater include Thermal Ionization Mass Spectrometry (TIMS; e.g. Paul et al., 2015), single-collector Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Wu and Boyle, 1997; Sohrin et al., 2008; Lee et al., 2011; Zurbrick et al., 2013; Middag et al., 2015), and Multiple Collector Inductively Coupled Mass Spectrometry (MC-ICP-MS; Reuer et al., 2003). Differential Pulse Anodic Stripping Voltammetry (DPASV) has also been employed (Helmers and Rutgers van der Loeff, 1993) but is not commonly utilized at present. Although useful Pb isotope data has been obtained by single-collector ICP-MS with ion counting detection, higher isotope ratio precision is possible using TIMS or MC-ICP-MS with Faraday cup detection.

2. Sample Volume

Lead concentrations in the ocean in 2016 are in the range of a few tenths of a pmol/kg to ~100 pmol/kg. With modern ICP-MS instruments and rigorous blank control, Pb concentrations can be determined at those concentrations by samples ranging from a few mL to ~100mL. In order to obtain precise and accurate stable isotope data, samples of 0.5L to 10L are required to have a sufficiently high ion signal to overwhelm detector noise and blank levels. The exact volume depends on the analytical equipment used and ratios targeted (i.e., less seawater is required if only high abundance ratios ($^{208,207,206}Pb$) are measured; more is required if precise ^{204}Pb data are part of the goal.

3. Sampling

3.1. Sampling Systems

Several different types of samplers have been used over the years that have been capable of collecting uncontaminated Pb samples. There are streaming sample collectors for underway surface water sampling: see section VI.2. Depth profile samples have been collected using the CalTech "Moon Lander" (Schaule and Patterson, 1981), MIT Vane Bulb (Boyle et al., 1986), GO-FLO bottles mounted on Kevlar cable (Bruland, 1979), MITESS (Bell et al., 2002), and the systems described in Section VI.2. For recent high throughput GEOTRACES cruises, the trace element rosette-mounted GO-FLO and Niskin X and Titan samplers have been used most frequently. Sampler cleaning is discussed in the contamination-prone trace element section (section VI.1.1.2), but it is highly recommended that prior to each use at sea the samplers are filled with trace metal clean seawater (natural pH) and left standing for about a day. At least for GO-FLO samplers, it has been observed on at least one cruise that a few individual samplers continued to contaminate samples throughout the cruise. Because Pb concentrations are not analyzed at sea, this will not be known much later after analysis in the home laboratory. To prevent this problem from creating false systematic signals (e.g., the sample nearest the bottom always has higher Pb), a regular bottle rotation on the rosette should be employed so that the same bottle isn't always in the same depth position.

Ordinary Niskin bottles should NOT be used as several components of them (PVC walls, internal spring closures, O-rings) have been shown to contaminate for Pb.

3.2. Filtration

For many years, clean filtration was sufficiently difficult that "total dissolvable" Pb from unfiltered samples was preferred (which is generally acceptable as >90% of the Pb in most samples is in the dissolved state). But even then, some investigators obtained good results with clean Nuclepore® filters in specially-cleaned filter holders. More recently, high-volume GEOTRACES cruises have preferred pressure filtration direction from the sampler using inline capsule or cartridge filters that can filter large volumes for multiple samples (see Section VI.3.2; e.g., Acropak Supor capsule filter (0.8/0.2µm) and 0.2 µm Sartobran-300 (Sartorius) filter cartridges (e.g., Bridgestock et al., 2016)). Filter cleaning is discussed in the contamination-prone trace element sampling section (VI.3.2.1), but typically involves a prolonged acid leach followed by rinsing with seawater and several liters of clean seawater before being used for samples.

3.3. Sample Handling and Storage

Any steps involving exposure of the samples to the atmosphere should be done in a HEPA-class 6 filtered air environment, as should any other steps involving open sample bottles. Sample containers made out of LDPE and HDPE have been proven reliable, but bottles should ideally be blank tested prior to using a specific vendor. They should be cleaned in the similar fashion as described in the contamination-prone trace element

section (VI.1.3), and it is recommended to store them after cleaning in clean containers/laboratory environments inside double Ziploc polyethylene bags. Sample containers should be rinsed with three small aliquots of seawater sample before final collection. In order to prevent adsorption of Pb to the container walls, samples should be acidified to ~pH 2, usually with trace metal clean 6M HCl. When ultraclean lab facilities are available, samples can be acidified at sea, but it is not necessary to do this so long as the acidification is done within a short time after being returned to the shore lab. In such cases, a few weeks should be allowed for any adsorbed Pb to be released from the container walls. Upon completion of seaboard handling, samples should be stored in clean closed containers inside double Ziploc polyethylene bags.

4. Sample Processing

Co-precipitation / pre-concentration: Analysis for both Pb and Pb isotopes requires separation from the major ion salt and some degree of concentration to smaller volumes. There are many ways this can be done that have been used over the years, but two methods are commonly employed for concentration and purification. (1) In recent years $Mg(OH)_2$ co-precipitation has been popular as a low-blank pre-concentration method. A small amount of ammonia is added to raise the pH above the solubility of $Mg(OH)_2$, then the precipitate is concentrated by centrifugation, settling or filtration. The precipitate is dissolved in acid and then further purified by anion exchange chromatography for isotope analysis. (2) A chelating resin is used to selectively concentrate Pb relative to the major salt ions followed by anion exchange purification for isotope analysis. One known issue with the $Mg(OH)_2$ co-precipitation method is that when used for large volumes (>500 ml) of high-Si seawater (>30 µmol/kg), the silica is also concentrated and can precipitate as a gel when the precipitate is dissolved, requiring additional steps to be taken to avoid the precipitation of silica gel (Boyle et al., 2012; Paul et al., 2015).

5. Spiking and Blanks

In general, quantification of Pb concentrations can either be done by calibration of Pb recovery efficiencies using standard-addition spiked seawater samples or by isotope dilution methods with the isotope spike added to the seawater before Pb pre-concentration. Both methods have been shown to work when handled carefully, although isotope dilution has some advantages in compensating for unexpected Pb losses (e.g. because of strong organic complexation in some samples) and matrix-dependent sensitivity issues.

Control of blanks is essential and should be monitored for each analytical session by the analysis of acidified low-Pb seawater (which can be prepared by $Mg(OH)_2$ co-precipitation or by passing the seawater through a chelating column). Reagent blanks should be checked before use. Volatile reagents which cannot be run directly by most instrumental methods (such as concentrated acids or NH_3 solution) can be evaporated to dryness followed by redissolution in a small volume of dilute acid.

6. Evaluation of Analytical Uncertainties

Despite the care with which the Pb concentration analyses are taken, small things can go wrong that affect the accuracy of the final result. For example, a micropipette might be miscalibrated on one day with the mistake propagating into the final results for all samples from that session. The best way to detect issues like these is to have one or more large-volume in-house seawater samples that are run on every analytical session, and with overlaps as one large-volume sample is depleted and another takes its place. Ideally that large-volume sample would be some international reference material, but in actuality for high-volume GEOTRACES sections, existing reference materials are not available in sufficient quantities to allow for this. So, the best alternative is that the international reference materials are run along with the large-volume in-house reference seawater samples for several analytical sessions so that the in-house sample is traceable to the international reference material.

There is no evidence that Pb isotope fractionation occurs during sample pre-concentration, so it is not necessary to run seawater Pb isotope reference materials on each analytical session (although it would behoove the analyst to demonstrate this for their own methods by spiking a seawater sample with NBS-981 to show that the added Pb is recovered with no isotope fractionation). Instead, it has proven sufficient to compare the concentrated and purified seawater Pb with a stock NBS-981 solution for each analytical session, and correcting the result for offsets from established NBS-981 isotope ratios (Thirlwall, 2002; Baker et al., 2004).

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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, then two protocols for particulate trace elements, and finally methods for collecting atmospheric particulate (aerosol) 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 and OTE (Ocean Test Equipment; both 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 (Rijkenberg et al., 2015)) 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, Niskin-X, or OTE 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. A complete video on GO-FLO modifications, repairs and cleaning is available at: https://youtu.be/bshM0G3GQac)

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 analyses, 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 1 M HCl (trace metal grade) for at least one month. Should be stored doubled bagged. Bottles should be emptied of all acid before transporting to the ship.
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 1M nitric 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 80ºC for 5 hours
9. Rinse 5x with UHPW water inside an ISO Class-5 laminar flow hood. Should be stored doubled bagged

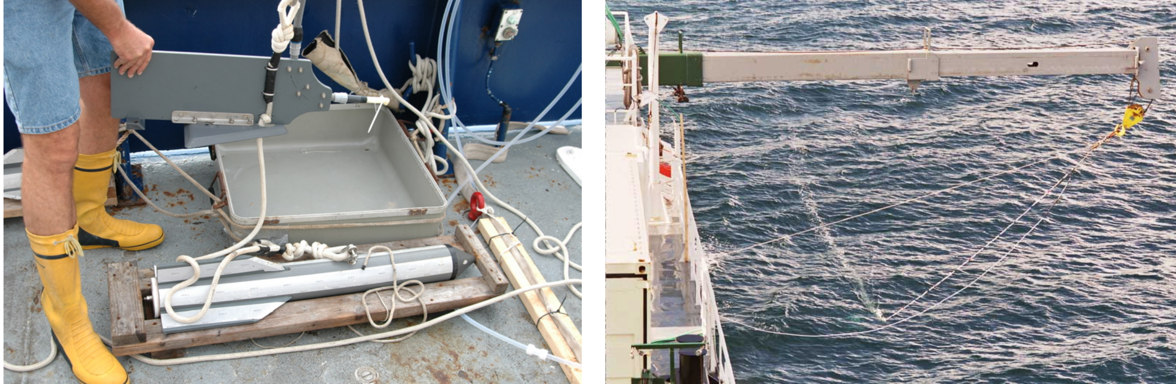
2. Sample Collection

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. Diaphragm 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									
Class Name		0.1μm Volume units		0.2μm Volume units		0.3μm Volume units		0.5μm Volume units		5μm Volume units	
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 0.024 M HCl using Sea Star hydrochloric acid or 6M sub-boiled distilled trace metal grade HCl), 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° 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 or 47 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 and transient, 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 (-20° C), but this requires large freezer capacity and careful attention to the conditions of freezing. Note: freezing or transport with dry ice can be problematic for analysis on thawing due to the high uptake of CO_2 by the samples. There is also anecdotal evidence of plasticizer release from LDPE bottles when stored at -80° C.
(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 another 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. Filtered 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 (see Section 3.3.4 below). However, sample handling after acquisition is still not well established as of 2017. Collection using 50 mL all polyethylene syringes equipped with polycarbonate 3-way Luer valves to eliminate all overlying air and facilitate hermetic transfers have been employed; analyses are performed within one hour (e.g., Cutter et al., 2017). 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. Samples for total Cu and/or Cu speciation collected in sulfidic environments may require an additional oxidant (e.g., H_2O_2) to recover stable Cu sulfides from the sample bottle as acidification with nitric acid has been shown to not recover these species which adsorb to the bottle walls (Teflon and LDPE).

3.3.5 Speciation Methodologies

Description of specific methodologies is beyond the scope of this publication. However, given that many techniques yield results that are operationally defined, thorough, detailed metadata are 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, OTE, 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 dissolved Fe was not rendered "reactive" to methods that only acidify to pH 3 for short exposure times prior to analysis.

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 been carried out using flow injection analysis (Gosnell et al., 2012; Wyatt et al., 2014), as well as anodic stripping voltammetry (e.g., Jakuba et al., 2008).

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 using calibrated volumetric and gravimetric equipment, and 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 (2017). 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 2013, consensus values for Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn are available for SAFe and GEOTRACES Intercalibration samples (http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html). James Moffett is maintaining an archive of deep Pacific samples from the SAFe cruise in 2004 and surface samples from the 2009 GEOTRACES Intercalibration Cruise

(GSP -open ocean- and GSC -coastal). He can be reached at jmoffett@usc.edu. They are available at no cost, but recipients must pay for shipping. These samples are in LDPE bottles and have an individual sample number. Two general types of samples are available, surface and deep water samples from both coastal and open ocean Pacific Ocean.
Updated consensus values for D1 will be available at http://earth.usc.edu/labs/moffett/index.html. There are no consensus values for GSP or GSC because few investigators have reported values.

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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 the 2008 and 2009 GEOTRACES Intercalibration cruises, as well as the 2012, 2013, 2014, and 2015

GEOTRACES international intercalibration exercises for Hg species in seawater. The collection and analysis of open ocean seawater samples for total mercury (Hg) determinations as well as Hg speciation within the context of GEOTRACES cruises are described. 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 regards 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

The susceptibility of sample bottles to the diffusion of elemental Hg (Hg^o) through the walls must be evaluated. 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, Cu, and Fe. In addition, $HgCl_2$ is often used to preserve biological and carbon parameter samples. 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 2008 and 2009 GEOTRACES Intercalibration cruises, Hg^0 concentrations in the Hg Group work spaces ranged from 20 to 50 ng m^{-3}. Given this range in ship-board air Hg 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. Glass, thick-walled PFA Teflon, and impermeable plastics (like polycarbonate) are the best for long-term (months) storage of seawater for Hg analysis.

For the recommended Teflon and plastic bottle cleaning procedure is shown below, and is found to be effective for the very low-level seawater concentrations, and results in low blanks for bottles made of almost any material. The key ingredient is 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 Hg species analyses (Hg^o, $(CH_3)_2Hg$ and $CH_3Hg(I)$) should be in contact with BrCl prior to use to avoid destruction of these forms. In the recommended workflow described below, the analysis

of total Hg (which uses BrCl) and the minor species are segregated into different bottles to avoid accidental oxidation. Alternatively, heating glass bottles over 500°C for 2h or more shows good results as well.

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 total Hg in seawater.

GEOTRACES samples for Hg should be collected into those bottles that best fit the individual workflow of the cruise. For example, FEP Teflon is suitable for short-term storage when samples will be analyzed within a few hours as they are unquestionably clean, highly durable and less gas permeable than polyethylene. If longer term storage is intended, then collection in thick-walled PFA Teflon, 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

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, NIOZ Pristine bottles 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 can be used, 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 compare well, suggesting that the particular filtering medium used is not critical as long it has been previously tested to ensure a low blank. In this respect, several batches of Sartobran 300 cartridge filters have shown contamination issues for total Hg.

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. 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 recently, which has lowered the detection limit, increased accuracy and facilitated a further streamlining of Hg species determinations (Bowman and Hammerschmidt, 2011). The

implementation of isotope dilution techniques shows equally low detection limits, high accuracy and traceable recoveries for complex matrices (Heimbürger et al., 2015).

During the Hg Intercalibration programs, most of the participating laboratories used cold vapor atomic fluorescence spectroscopic (CVAFS) determination of Hg (as Hg^o). Some 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 compare well, while the CVAAS did not exhibit adequate sensitivity to detect total Hg on the Intercalibration samples (250 mL). Thus, CVAFS or ICP-MS are recommended for Hg determinations. The CVAFS approach has the distinct advantage of being field use, allowing rapid determination of total Hg and DGM (Hg^o + $(CH_3)_2Hg$) at sea. ICP-MS, especially when employed with isotope dilution, has the potential for a lower absolute detection limit. Thus, CVAFS is recommended for at sea determinations, but either CVAFS or IC-MS methods are appropriate for on shore analyses.

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

8.3.1 Total Hg

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. Additionally, an alternate method on 40 mL samples allows higher throughput with shorter purging times with an optimized CVAFS setup (Heimbürger et al., 2015).

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 20 minutes, 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} (0.15 - 0.5 L min^{-1} is recommended).

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.

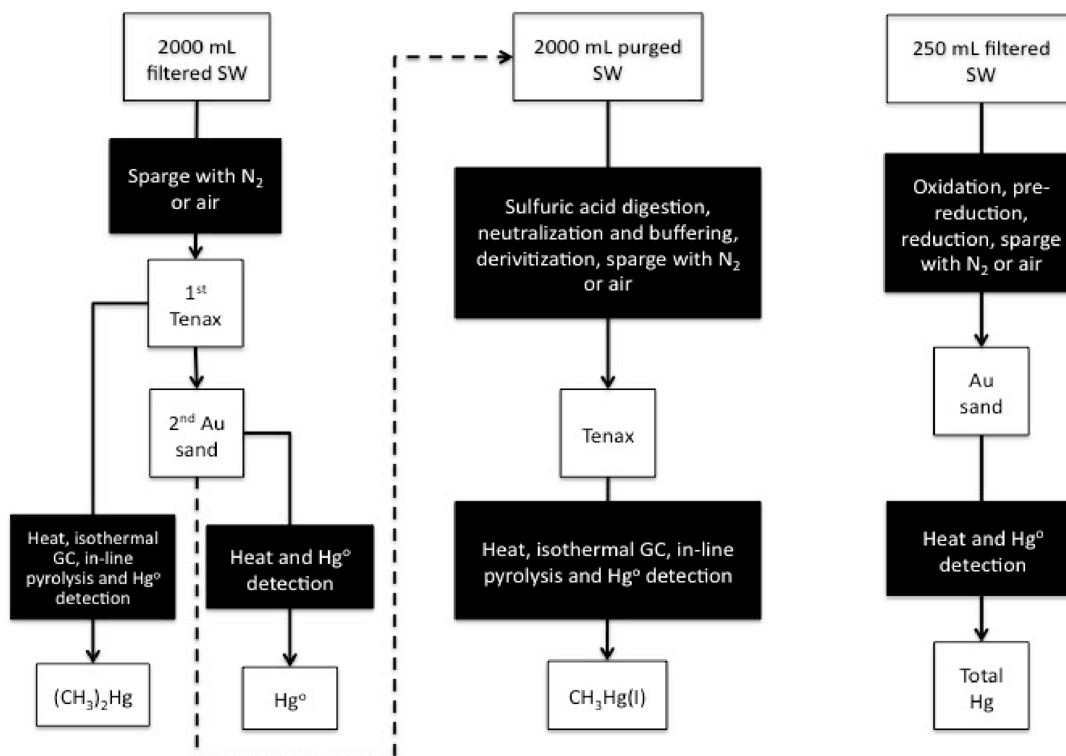

Figure 1. Recommended Hg workflow. All four analyses could be performed on one 2-L sample, but the reagents associated with analysis of $CH_3Hg(I)$ have a larger blank than those associated with total Hg determination. Therefore, for at-sea measurements two separate aliquots should be collected: one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

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, 2 L sample sizes are recommended 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. Two sorption media in series can be used 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) it 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. The use of Tenax rather than Carbotrap is recommended 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. Working samples in batches of four are recommended, 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 60 °C.

Alternatively, total methylated Hg and $CH_3Hg(I)$ may be analyzed via state of the art isotope dilution techniques. Such methods require sample treatment and preservation on the ship for later measurements in the home laboratory. $(CH_3)_2Hg$ converts to $(CH_3)Hg(I)$ upon acidification to preserve samples for total methylated Hg (MeHg = $(CH_3)_2Hg$ + $(CH_3)Hg(I)$). The recommendation is to measure DGM (($(CH_3)_2Hg$ + Hg°) from a larger sample (>250 mL) directly onboard, and then preserve an aliquot of the sparged seawater sample with bidistilled HCl for later $(CH_3)Hg(I)$) measurements. From both measurements, $(CH_3)_2Hg$ can then be calculated as MeHg minus $(CH_3)Hg(I)$.

MeHg and $(CH_3)Hg(I)$) is analyzed via isotope dilution (ID), using a high sensitivity coupled gas chromatography - sector field ICP-MS (GC-SF-ICP-MS) [*Heimbürger et al.*, 2015]. Briefly, enriched spikes of ^{199}iHg and ^{201}MeHg (ISC Science, Spain) are added to a 115 mL aliquot of the seawater samples. After 24h equilibration, pH is adjusted to 3.9 with NH_3 (ULTREX® II Ultrapure Reagent, J.T. Baker, USA) and a buffer solution made up with acetic acid (glacial, ULTREX® II Ultrapure Reagent, J.T. Baker, USA)/sodium acetate (J.T. Baker, USA). Sodium tetra propyl borate (1mL, 1%, v:v; Merseburger Spezialchemikalien, Germany) is then added together with 200 µL hexane (Sigma Aldrich, USA). The glass bottles are hermetically sealed with Teflon-lined caps and vigorously shaken for 15 minutes. The organic phase is recovered and 2 µL are injected into the GC (Thermo Trace Ultra) coupled to a SF-ICP-MS (Thermo Element XR). Detection limits of 0.025 and 0.001 pM for inorganic Hg and MeHg/$(CH_3)Hg(I)$), respectively, can be achieved this way.

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. Therefore, it is strongly recommended 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 certified seawater reference materials are not readily available for Hg determinations in the range that analysts will face in the open ocean. Therefore, a consensus reference material can be used: 1200 seawater samples (125 mL), stored in pre-baked (550°C, 4h) glass vials for both total Hg and $CH_3Hg(I)$. The GEOTRACES MED-400 samples 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 Lars-Eric Heimbürger at the Mediterranean Institute of Oceanography (lars-eric.heimburger@mio.osupytheas.fr) to receive CVRM aliquots.

In order to achieve the most accurate results, it is recommended that 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 30 g of $NH_2OH \cdot HCl$ in 18 MΩ-cm water and bring to 100 mL.

Stannous chloride – Bring 20 g of $SnCl_2$ (anhydrous) and 10 mL conc. HCl (trace metal grade or bi distilled) to 100 mL with 18 MΩ-cm water. Purge with Ar or N_2 to lower blank. Store cold and tightly capped.

Bromine monochloride – Heat KBr and $KBr0_3$ to 250 for at least 2h. In a fume hood, dissolve 2.7 g of KBr in 250 mL of trace metal grade or bidistilled HCl. Stir on stir plate if available. Slowly add 3.8 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 – It is recommended that 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) be made. For $CH_3Hg(I)$, preservation with either 1) 2% glacial acetic acid and 0.2% concentrated HCl or 0.5% HCl is used. For Hg(II), preservation with 0.1% BrCl (see above) is sufficient.

Hydrochloric acid (for sample acidification and reagent preparation) trace metal grade or bidistilled. Glass of thick-walled Teflon bottles are preferred, as acids may pick up Hg through the bottle walls. The acid blank should be determined prior to use (<0.01 ng/mL).

Working Standards – It is recommended that 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) be made. For $CH_3Hg(I)$, preservation with either 1) 2% glacial acetic acid and 0.2% concentrated HCl or 0.5% HCl is used. For Hg(II), preservation with 0.1% BrCl (see above) is sufficient.

Hydrochloric acid (for sample acidification and reagent preparation) trace metal grade or bidistilled. Glass of thick-walled Teflon bottles are preferred, as acids may pick up Hg through the bottle walls. The acid blank should be determined prior to use (<0.01 ng/mL).

Nitric Acid (for sample acidification) – J.T Baker Instra-analyzed trace metal grade. Glass of thick-walled Teflon bottles are preferred, as acids may pick up Hg through the bottle walls. 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. 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
	ERM-CA400	Total mercury in seawater	T	16.8 ng/L
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-5**	**River water**	**T**	**26.2 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 (not certified)	TBA /TBA
MIO	GEOTRACES MED 400	seawater	Not certified	

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.
IRMM: European Commission-Joint Research Centre-Institute for Reference Materials and Measurements.
NIST: National Institute of Standards and Technology (USA).
NRC-CNRC: National Research Council Canada.

Gill, G.A. and W.F. Fitzgerald (1987) Picomolar mercury measurements in sea water and other materials using stannous chloride reduction and two-stage gold amalgamation with gas phase detection, *Marine Chemistry*, 20, 227-243.

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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 TEIs in parallel with dissolved TEIs to match spatial resolution with minimal additional ship time while complementing large volume *in situ* pumping approaches which offer replicate particle subsampling for both concentration and isotopic composition studies. The following methods are recommended for the 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 blanks 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 the entire volume is filtered. Filter diameter should be minimized in general so that particle loading per area of filter is maximized in order that sample element concentrations exceed the filter blank to the greatest degree possible.

An alternative filter type is mixed cellulose ester (e.g. MF-Millipore type HAW), which is close in filtration performance to the Supor filters, but has higher blanks for most trace elements (Planquette and Sherrell, 2012). Cellulose filters do 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 (Planquette and Sherrell, 2012; Ohnemus et al., 2014). 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 (Planquette and Sherrell, 2012). Clearly, particulate metal concentrations are operationally defined, and consistent filtration methods should be used for this reason. Supor filters have been used for all U.S. GEOTRACES cruises to date, on GA01 and GA02N cruises and indeed for all international GEOTRACES cruises when particles were sampled from GO-FLO bottles.

Prefilter screens may be used upstream of main filters if size-fractionated sampling is desired, e.g. to provide samples comparable to the size-fractionated samples collected 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/cleaning. 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 complementary *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 holders are shown in Figures 1 and 2. **Any internal filter support grid on the upstream side of filter should be removed as it could act as an inadvertent prefilter.** The MFS and Swinnex filter holders have the advantage of closing by locking collar (so that the filter is not subjected to twisting motion upon tightening), have convenient NPTM/Luer connectors for plumbing fittings and pressure applications, and are 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 of the MFS filter holders is not transparent so headspace bubbles cannot be seen, and there is no air vent, which requires loosening the filter holder during initial flow to remove air in the headspace (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 grid 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. Users should purchase extra silicone gaskets as these become easily distorted to imperfect circle shapes. Again, these choices are the best compromise found to date, 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. Additional 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 1.2M) of TM Grade HCl, double bagging in heavy duty (e.g. 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.

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

3. Remove filters from the 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 the sample region is not damaged, and carefully drop them into the bottle. Make sure any separator papers from the original packaging are not included. When 100 filters have been immersed in the water, fill the last 10% of bottle volume with concentrated TM Grade HCl, cap tightly, mix gently so that the filters do not crease, and place the double bagged bottle in a 60°C oven overnight, as for bottle cleaning.
4. When bottle of filters is cool, slowly pour off acid to waste, retaining filters with the cap held against the bottle mouth. Keep filters in suspension by gentle hand-agitation while pouring off acid, to minimize folding and creasing while all the solution is removed. Fill the bottle slowly with DI water running gently down the inside wall, swirl gently, and pour out the water, retaining filters with the cap. Repeat 5 times. Leave the last rinse in the bottle and allow to sit at room temperature overnight so that any residual acid diffuses from the pore spaces of the filters. Repeat 3 more rinses the next day. Always check the pH to ensure no acid remains as Supor filters can take many rinses to remove all traces of acid. Filters can be left in the DI water suspension until used on ship, or can be loaded in advance into individual Petri-slides for easy access and storage in the same Petri-slide. Use caution to avoid getting doubled filters, as the Supor filters 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. 2a). Alternatively, a smaller configuration can be assembled from polycarbonate components from Nordson Medical (Value Plastics), which include a male-to-female Luer thread elbow (p/n LE87-9), a lock ring-to-barb fitting (p/n MTLL055-9) for connecting the 3/8" tubing to the elbow, and a lock ring (p/n FSLLR-9) for securing the Luer end of the elbow to the Swinnex-type filter holders (Fig. 2b). 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 (Fig. 2). **Clearance of trapped air is accomplished by opening stopcock with filter holder inverted i.e. outlet facing up), 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, e.g. by modifying the filter holder with a larger ID inlet, but this possibility has not been 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 a 2 L graduated cylinder. Alternatively, if the volume in a GO-FLO bottle after initial sampling (e.g. salinity, nutrients) is known, and the volume is completely filtered, then volume measurement is not necessary. If the filter clogs, filtration should be stopped and either the filtrate or the residual water in the GO-FLO bottle can be measured.

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 much 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, particle-deplete water, two GO-FLO bottles (20-22 L) could be filtered through a single 25mm filter before clogging.

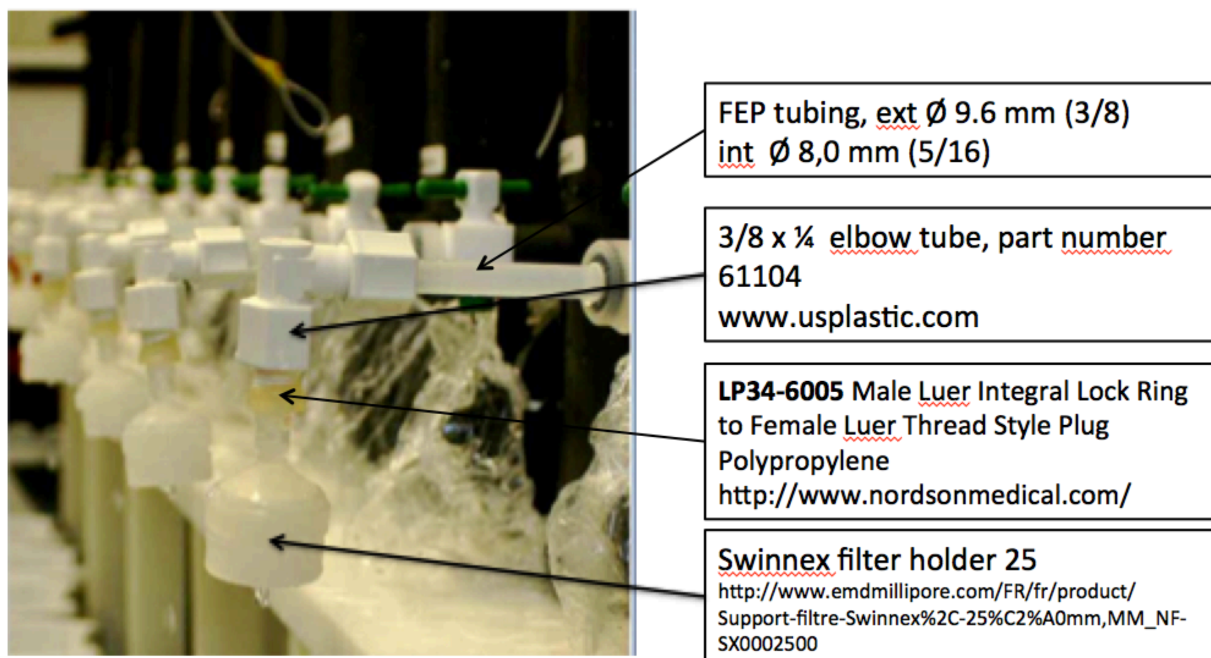

Figure 2a. Swinnex 25 mm filter holders showing 3/8" OD tubing, 90° compression-NPT adapter, and NPT-Luer lock adapter. Note 11 L waste baskets for filtrate volume measurements.

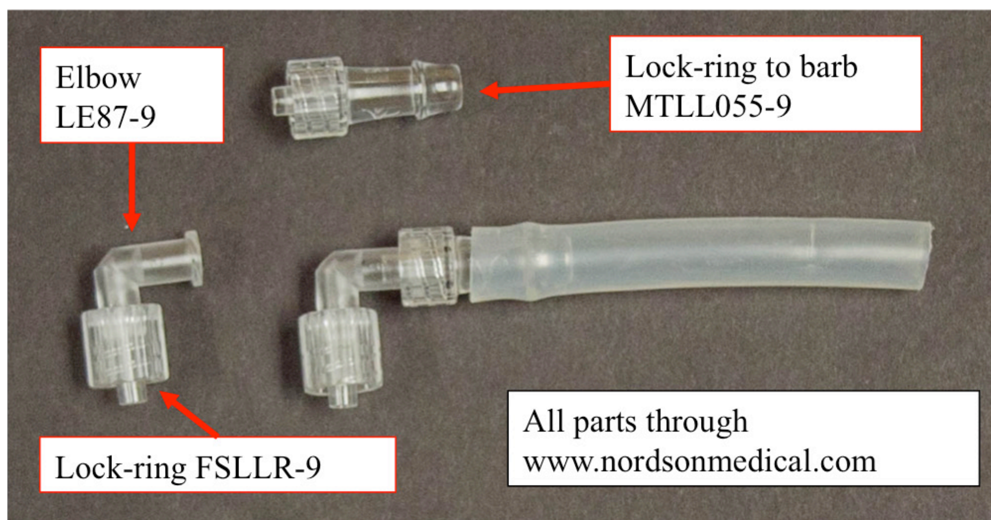

Figure 2b. Compact polycarbonate configuration for Luer port filter holders (lock-ring FSLLR-9 is optional).

However, volumes greater than 10 L were not deemed necessary for sufficient sample/blank ratio when filtering deep particulate matter. When removing filter holders from the GO-FLO bottle connector, unscrew the filter holder from the Luer Lock connector first. Pulling the 3/8'' tube out of the compression fitting on the GO-FLO stopcock with the filter holder still attached (Fig. 2) generates a large negative pressure,

especially if the filter is clogged, causing the filter to bow up away from its support, and to be stretched and distorted such that it will not lie flat for subsequent processing (e.g. subsampling).

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 (Fig. 2b), 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, e.g. Acrovent) at the entrance to each sampling bottle**. We recommend **< 7 psi** (50 kPa) for filtration, a good compromise between a high rate of filtration and the minimization of cell lysis or other pressure-related artifacts. Nitrogen should be considered as a substitute when sampling suboxic waters.

9.7 Process blanks

Filtration process (e.g., adsorption) blanks must be collected 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 (downstream), and filtering normally to a default volume of 2 L, so that TM-"particle-free" 0.2 µm filtered seawater passes through

the particle sampling filter. Alternatively, a second Swinnex filter holder mounted with a clean filter and attached downstream of the main Swinnex with Luer-Lock connection can be utilized to this end. In either method, the filter should be subsequently stored and processed as if it were a genuine sample. 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. Alternatively, a simple plastic bench-mounted manifold can be constructed with 12 male Luer lock connectors, and attached to a variable vacuum source. Filter holders can be removed from the GO-FLO bottles and placed on this manifold for a few minutes, under gentle vacuum, to remove residual seawater. 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 elements, but may not be sufficient to decrease sea salt to a level where salt corrections are small enough for the accurate determination of particulate Ca. 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 each sample-face-up on an acid-cleaned supor or quartz fiber filter for a few seconds, which acts 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. If elements with major salt corrections will be measured, one possibility is to store filters directly in the vials or bottles in which the leach will be conducted.

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, extra rinsing is recommended as particles may

adhere to the filter holder, and to the top headspace surfaces in particular. Then, filter holders can sit in a 1% (v/v) HCl bath for a day before being rinsed thoroughly with TM-clean DI water. O-rings must not be in contact with acid. At very least, filter holders should be rinsed with TM-clean DI water using a squirt bottle. No visible particulate matter should be visible on any surface of filter holder. After shaking the 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 the filter holders are stored in a metal-clean location (e.g., multiple layers of plastic bags or within a plastic 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 as on-line filtration, without large obvious artifacts (experiments by R. Sherrell and J. Bishop; Planquette and Sherrell, 2012). 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 (e.g. tow-fish sampling of surface waters, sea ice, snow).

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. A receiving bottle with 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 the 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 it may prove difficult to integrate a vacuum method that can cleanly collect 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 the 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-L 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 the filter diameter is scaled down (e.g. 13mm) so that particle loading overcomes the 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

For complete digestion of all particle types (e.g. biogenic, lithogenic, authigenic), a strong mineral acid digestion (ultrapure grade, such as Fisher Optima, SeaStar Baseline, or equivalent) that includes some hydrofluoric acid (HF) and nitric acid (HNO_3) at elevated temperature is necessary. Supor® (polyethersulfone) filters are particularly resistant to degradation, so several procedures have been developed that either keep the filter largely intact (e.g., Fitzsimmons et al., 2017; Planquette and Sherrell, 2012), break the filter down partially (e.g., Ohnemus et al., 2016; Twining et al., 2015), or digest the filter completely (e.g., Heller et al., 2017; Ohnemus and Lam, 2015). The above-mentioned digestion procedures have been intercalibrated for most key trace elements (e.g., Ohnemus et al., 2014). It is important to note that the two-step Piranha procedure that digests the Supor filter completely is not suitable for Zn (has very high filter blanks) (Ohnemus et al., 2014), and may not be suitable for Ba in some cases (may lead to precipitation of insoluble BaSO4) (Lee and Lam, unpublished). In section 9.11.3, details for one of the procedures are given (used by Planquette and Sherrell, 2012), making the distinction between methods appropriate for the resistant Supor® (polyethersulfone) and the more soluble MF-Millipore® (mixed cellulose ester) filters. Please refer to the above publications for more details on the other methods. Alternative 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 these methods. 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 (Fisher optima grade or equivalent) 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.

Recently, we have found that new Savillex jars have required much more aggressive cleaning procedures to prepare for TM analyses, including:

1. More concentrated detergent cleaning with manual scrubbing using a paper towel or KimWipe
2. Rinses with hot tap water (not DI)
3. Follow-up with acetone and/or methanol (HPLC grades) to remove detergent and any residual organic coating on the vials.
4. Rinse with tap or DI water
5. At least 24 hours in aqua regia (reagent grade)
6. Rinse with DI water
7. At least 24 hours in hot 50/50 nitric acid (reagent grade) with a small addition of hydrogen peroxide (reagent grade)
8. Rinse with DI water

From this point on, vials can be cleaned using ultrahigh purity acids (quartz distilled, "SeaStar" grades or similar) according to the procedure already listed.

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 can be recycled three times), capped tightly and placed in a plastic or polystyrene foam tube rack.
- Double-bagged in sturdy plastic zip-lock bags (e.g. 4 mil), 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 in laminar flow clean bench.

9.11.3 Filter Digestion procedure
Ultrapure grade acids (e.g., Fisher Optima or equivalent) are recommended in these protocols.

a- Total digestion
- Digestion procedure is presented in Planquette and Sherrell (2012) and Ohnemus et al. (2014), and is based on that developed by Sherrell (1991) and Cullen and Sherrell (1999).
- Ideally, one 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, or rotary cutter 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.
- Since there is no certified reference material (CRM) for suspended oceanic particulate matter, **a combination of CRMs that represent a biogenic-endmember** (such as BCR-414, a freshwater plankton, see below) **and a lithogenic endmember** (such as MESS or HISS, see below) should be processed in parallel of the samples:

  – BCR-414: https://crm.jrc.ec.europa.eu/p/q/BCR+414/BCR-414-PLANKTON-trace-elements/BCR-414

  – MESS-4: http://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/mess_4.html

  – HISS-1: http://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/hiss_1.html:

  – Arizona Test Dust: available from William Landing (wlanding@fsu.edu) or Pete Morton (pmorton@fsu.edu) upon request.

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. For open ocean samples, a reasonable amount might be 15 mg of CRM in 4 mL of acid, this amount being higher that most oceanic samples, but balances what is possible to weigh out reproducibly with what is at the upper limit of plausible concentrations.

b- Leaching

- In order to get access to the labile particulate fraction of trace elements, it is possible to perform a chemical leach. This leaching procedure is presented with great detail in Berger et al. (2008) and should followed as closely as possible.
- It is better to perform this leach in parallel when there is adequate sample available. The filter should be cut in half with a ceramic blade. One half will be dedicated to the total digestion (outlined above), and the other half will be dedicated to the leachable fraction Berger et al. (2008).
- The same combination of CRMs described above (e.g. BCR-414 and HISS-1) should be processed in parallel of the samples.

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 (Corning) and store them at 4° C to minimize evaporative loss.

9.11.6 Analytical procedures

The following is 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 analytical method. However, the ideal procedure should consider the following aspects: reproducibility, precision, accuracy, and drift. We describe below the procedures used in the lab of R. Sherrell (Rutgers University), in order to show an example of the aspects of a successful analytical approach:

- Each sample should be spiked with a drift monitor (e.g. In or 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 stock 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. Also, it is recommended to apply two dilution factors on the same sample digest solution.
- Spike recovery should be also assessed every 10 samples by spiking one 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.
- Several CRMs should be run as well.

9.12 References

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10. In-situ pump 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 WTS-LV, Challenger Stand Alone Pumps (SAPS) can operate at any depth (McLane WTS-LV pumps are rated to 5000-5500 m, depending on the model; Ti pressure housings are available from McLane to allow standard models to reach 6000 m), and although scaled

down in terms of volume filtered, can be used to achieve the same performance goals with modifications as detailed below.

For analytical details of particulate trace metal analysis, please refer to the GO-FLO filtration section (VI.8) for further details. For recommendations on best practices for optical detection of particles by transmissometer, please refer to the Particle Optics Protocols (Section VIII). In the discussion that follows, we focus on experiences from the U.S. GEOTRACES cruises that use modified McLane in-situ pumps.

10.1 Configuration of in-situ filtration systems

10.1.1 Filter holder design to prevent large particle loss

Commercially available (*e.g.* standard McLane WTS-LV holder) and "homemade" 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). While particles are collected during operation of pumps, the loss of large particles occurs from single baffle filter holders after the pumps shut down prior to and during the recovery process, even in near waveless and windless conditions. **We thus strongly recommend use of filter holders that have multiple baffle systems similar to that used in the MULVFS system to eliminate effects of horizontal flows on collected large particle samples when pump is no longer running.** A "mini-MULVFS" design was tested and shown to be effective at retaining large particulates during the 2009 intercalibration cruise (Fig. 1; and is now used exclusively for all U.S. GEOTRACES cruises. 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).

10.1.2 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 (Fig. 2). 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 (Fig. 2) 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 (Fig. 2b, #4). 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).

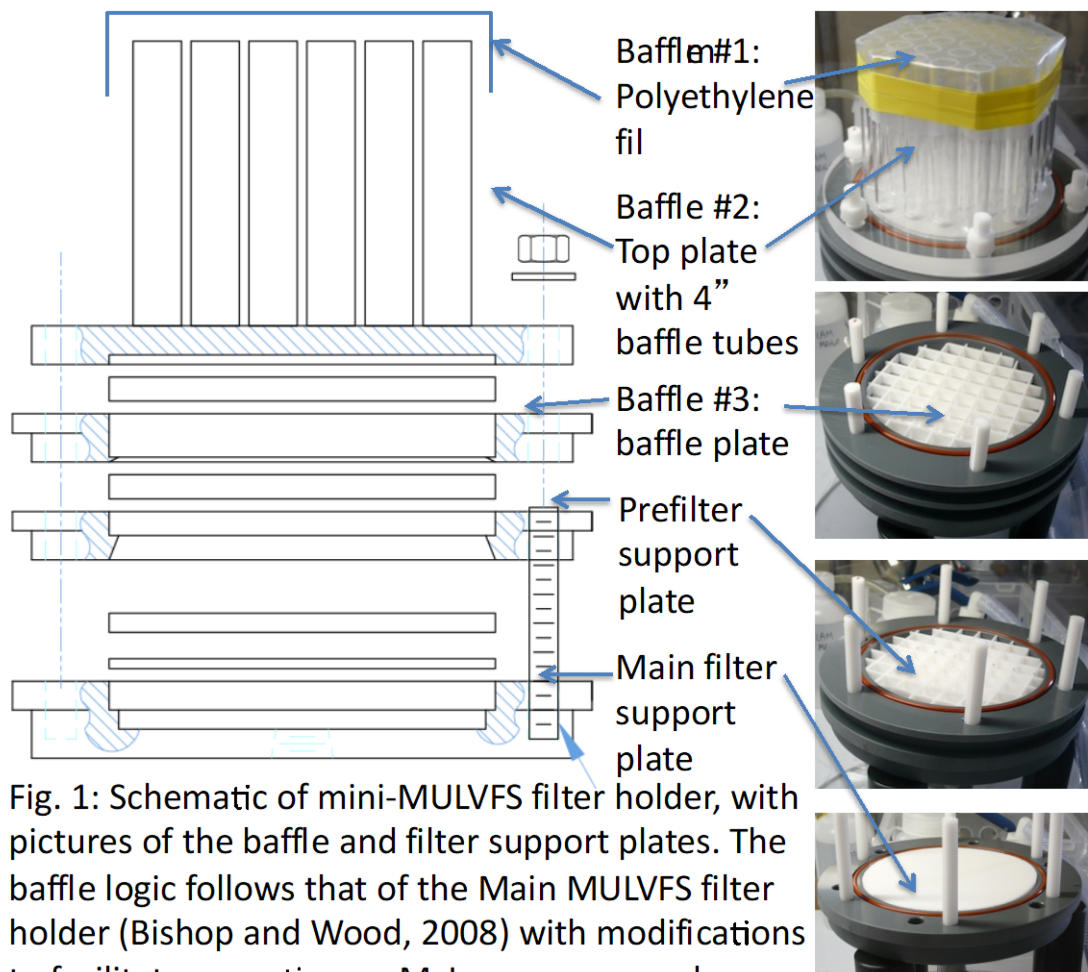

Fig. 1: Schematic of mini-MULVFS filter holder, with pictures of the baffle and filter support plates. The baffle logic follows that of the Main MULVFS filter holder (Bishop and Wood, 2008) with modifications to facilitate mounting on McLane pumps and handling in the laboratory. From Bishop et al. 2012.

10.1.3 Dual-flow modification for McLane pumps

Based on successful multipath filtration achieved by MULVFS, dual flow battery operated pumps were developed and have been used on all U.S. GEOTRACES cruises (GA03, GP16, GN01) to allow the simultaneous use of quartz fiber filters (Whatman QMA) and hydrophilic polyethersulfone (Pall Supor) filters and MnO_2-coated adsorption cartridges (Fig. 2b). Using paired QMA filters in one holder and paired 0.8µm Supor filters in the other holder (see section 10.2) 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. 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 (Fig. 2b). A ball valve below one of the flow paths allows flow to be turned off if a single flow path is desired. The WHOI upright dual-flow version has a priming port (Fig. 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

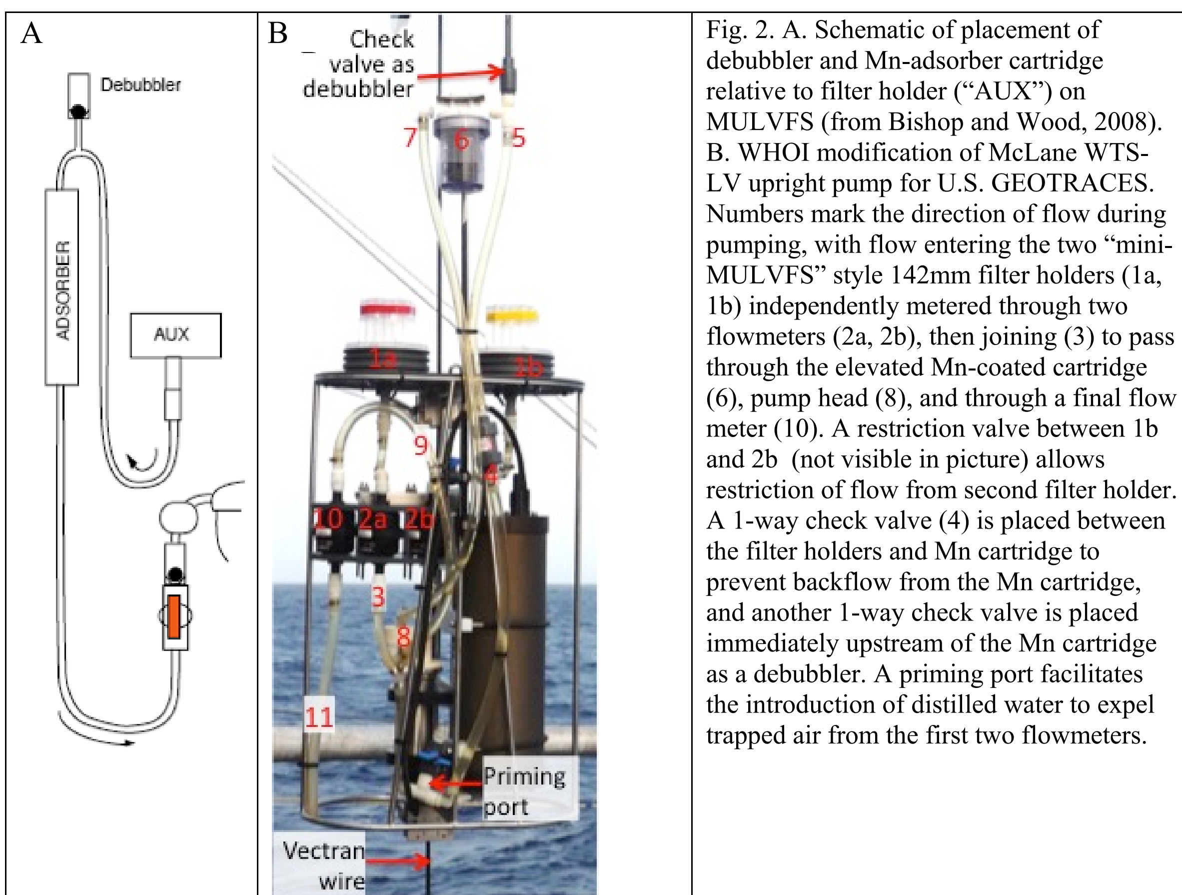

Fig. 2. A. Schematic of placement of debubbler and Mn-adsorber cartridge relative to filter holder ("AUX") on MULVFS (from Bishop and Wood, 2008). B. WHOI modification of McLane WTS-LV upright pump for U.S. GEOTRACES. Numbers mark the direction of flow during pumping, with flow entering the two "mini-MULVFS" style 142mm filter holders (1a, 1b) independently metered through two flowmeters (2a, 2b), then joining (3) to pass through the elevated Mn-coated 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.

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).

10.1.4 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). 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 back flushing 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 (Fig. 2b, #6).**

The placement of the Mn cartridge above the filter holder minimizes the back flushing 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 (Fig. 2b, #4). 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.

10.1.5 Cable for deploying pumps
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.**

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 U.S. GEOTRACES program uses a 0.194" Vectran braid strength member (5700 lbs minimum breaking strength) with Hytrel jacket extruded to 0.322" OD (Cortland Cable Co.) for deploying up to 8 dual-flow upright McLane pumps at once. The Hytrel jacket provides adequate grip and rigidity for clamping the pumps. U.S. winches, blocks, and level winds, which are frequently optimized for 0.322" hydrowire, so 0.322" OD wire improves 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). 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.

10.2 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.

10.2.1 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 weak acid leaches (e.g., hot 0.6M 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 (especially lithogenic elements) are extremely 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 most 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.

10.2.2 Hydrophilic polyethersulfone (PES) membrane filters

Hydrophilic polyethersulfone (PES) membrane filters (e.g., Pall Supor or Sterlitech PES) 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 (also see section VI on GO-FLO filtration). 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. PES filters 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.

The most serious drawbacks to PES filters and plastic filters in general are that they can have poor (heterogeneous) particle distribution, especially in deeper (>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 Pall Supors, as it may have to do with the manufacturing process and be inherent to the filter medium itself. We have not systematically compared different manufacturers of polyethersulfone filters (Pall Supor vs. Sterlitech PES).

For in-situ filtration, we 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 (sum of top plus bottom filters) a particle population somewhat smaller than a single 0.8 µm but slightly larger than 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.** The top and bottom filters should thus by analyzed separately.

10.2.3 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 (most open ocean samples).

For total digestion of the >51 µm size fraction, we recommend at-sea rinsing of freshly collected particles from a pie slice subsample of the prefilter of known area onto a 25 mm Supor filter using trace-metal clean filtered (0.2-0.45 µm) seawater (such as from a towed fish) (see Fig. 6).

10.3 Filter cleaning procedure

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

10.3.1 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 U.S. GEOTRACES IC and section cruises.

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). Precut 150 mm diameter circles are available from Whatman, and fit some 142 mm filter holders (e.g., mini-MULVFS), but not those that have a recessed stage (e.g., standard McLane). 293 mm QMA filters for MULVFS are available by special order from Whatman and have been cut from bulk roll material.

The following protocol is used by the Lam lab for preparation of QMA filters for U.S. GEOTRACES cruises.

A. QMA filters are cleaned in batches of up to 100, in up to 10 stacks of 10 filters, with each stack separated by a polystyrene "eggcrate" grid (see Materials List) and topped with an eggcrate, and the entire stack placed within a perforated, plastic basket, which is placed in a plastic tub (tub 1Q).
B. Plastic tub 1Q is filled with 1.2 M trace-metal grade HCl to submerge the entire filter stack and soaked overnight. This first HCl bath is reused up to four times before being discarded.

C. The basket containing the filter stack is lifted out of the first HCl bath from plastic tub 1Q, drained, and transferred to plastic tub 2Q containing fresh 1.2 M trace-metal grade HCl and soaked overnight.
D. The basket containing the filter stack is lifted out of the second HCl bath (plastic tub 2Q), drained thoroughly, then placed into plastic tub 3Q filled with Milli-Q (or similar ultrapure) water for an initial rinse.
E. The basket containing the filter stack is lifted out of the initial Milli-Q rinse (which is discarded), then transferred to the Milli-Q-drip-rinsing setup (Fig. 3). 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 (e.g., L/S Masterflex PTFE-diaphragm pump) 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 (Fig. 3). 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 several times a day for 2-3 days 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.**

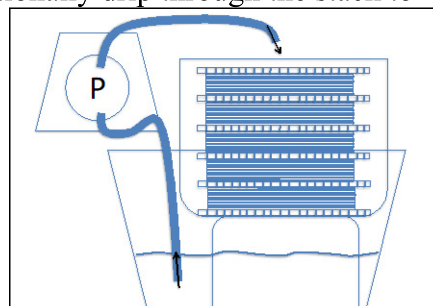

Fig. 3. Schematic of Milli-Q-drip-rinsing setup for rinsing acid out of filters.

F. After rinsing, each filter stack of 10 is removed from the basket by lifting the eggcrate grid beneath each stack and laying out in a laminar flow hood to dry (~2 days).
G. After drying, build a filter stack in a clean Pyrex baking dish: each stack of 10 is separated by 2 Pyrex rods (be sure to remove the eggcrate grids!), and the entire stack is covered with an inverted Pyrex dish to guard against contamination, and combusted at 450° C for 4 hrs in a clean muffle furnace (one that is dedicated to combusting unused filters, glassware, etc., and not used for combusting samples).
H. 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.

10.3.2 Supor (PES) filters

A. Supor filters are cleaned in batches of up to 100, in up to 4 stacks of 25 filters, with each stack separated by an eggcrate grid and topped with an eggcrate. Be sure to remove the blue separator paper that comes in the original packaging. The entire stack placed within a perforated, plastic basket, which is placed in a plastic tub (tub 1S).
B. Plastic tub 1S is filled with 1.2 M trace-metal grade HCl to submerge the entire filter stack. The Supor stack tends to float, so may need to be weighed down (we place a clean Teflon jar that is filled with water on top of the top eggcrate). The entire tub is placed on a 60°C hotplate inside of a clean laminar fume hood and soaked overnight in the warm acid. Be sure that the hotplate used doesn't create

hotspots that could melt the plastic tub (melting points of polyethylene and polypropylene are typically ~105-180°C).

C. The basket containing the filter stack is lifted out of the warm HCl bath (acid reused twice before discarding), drained thoroughly, then placed into plastic tub 2S filled with Milli-Q (or similar ultrapure) water for an initial rinse.
D. Simple soaking of the acid-leached Supors in milli-Q is not always sufficient to get residual acid out, and drip rinsing aids the rinsing process. Follow QMA cleaning step E to rinse acid out of the Supors using a Milli-Q drip rinsing setup (Fig. 3), monitoring pH to assess rinsing completion.
E. After rinsing, each filter stack of 25 is removed from the basket by lifting the eggcrate grid beneath each stack and laying out in a laminar flow hood to dry (~2 days).
F. When dry, stacks of 25 Supor filters are packaged in polyethylene clean room bags.

All plastic tubs, tubing, eggcrate grids, and Pyrex dishes and rods used in cleaning filters should be leached in 10% HCl and rinsed with MQ-water prior to use. If using the same plastic tubs for cleaning both Supor and QMA filters, be sure to clean Supor filters first, since bits of QMA fibers that are shed into the acid and rinse solutions during the cleaning process can easily contaminate Supor filters.

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.

10.3.3 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. Drip rinsing is not necessary. They are air dried in a laminar flow bench and packaged in polyethylene clean room bags.

10.4 Mini-MULVFS Filter Holder Preparation and Cleaning

Filter holder cleaning protocol:

- Step 1: Disassemble filter holders completely before cleaning. Inspect O-ring grooves and any edges for dried on plankton/particles, and work off any stuck-on particles with a clean toothbrush or gloved finger. Place all components EXCEPT for the polyethylene frit in a mild detergent bath (e.g., 1% citranox) overnight. Frit is cleaned separately (see below).
- Step 2: Rinse everything that was in the detergent bath copiously with distilled water. O-rings, nylon wing nuts, Delrin threaded rods should then get a QUICK (~<1 hr) soak in 10% (1.2 M) HCl (reagent grade ok). VERY IMPORTANT NOT TO LEAVE THESE THINGS IN ACID OVERNIGHT as they are not very acid resistant. Rinse well with milli-Q water and dry in laminar flow bench. Everything else (A, B, C, D plates, eggcrate grids, perforated PVC) should be

soaked in the 10% HCl acid bath overnight.

- Step 3: After overnight acid bath, shake off excess acid, and rinse thoroughly with milli-Q water, and lay out the component pieces to dry in a laminar flow bench
- Step 4: Reassemble pieces back into functional filter holder (without frit).

Porous polyethylene frit cleaning:

- Step 1—Porous polyethylene frits should be soaked in 10% HCl (1.2M TM-grade HCl) overnight (this should be separate from the rest of the filter holder components)
- Step 2—After acid soaking, take frits out and rinse in milli-Q. Frits retain acid that is not easily rinsed out. 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 milli-Q onto the frit. Monitor rinse water pH to ensure all acid is removed (pH should be ≥5).
- Step 3—After vacuum rinsing the frits, they should be dried in a laminar flow bench. Frits should be COMPLETELY dry before packaging in clean plastic bags, separately from the filter holders to prevent residual acid fumes from degrading filter holder components in transit.
- During a cruise, filter holder plates should be disassembled and components 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 they are kept damp in the filter holders, they can get moldy and must then be discarded. Stainless steel threaded rods and quick disconnect fittings should be removed from the bottom plate, rinsed in fresh water, dried, and packaged separately for transit.

10.5 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.**

10.5.1 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, 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. As an added backup, photographs of flow-meters can be used, but should not be relied upon exclusively.

10.5.2 Process ("Dipped") Blanks

Filter blanks are determined using 1) cleaned, unused and 2) process ("dipped") 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 filters in the dipped blank 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 (Fig. 4). This filter is processed in an identical way to samples. Process blanks should be obtained at every station and used in the determination of detection limits for analytes of interest (cf. Lam et al., 2015). One unused filter set should be retained for blank purposes at least once every 30 samples.

Fig. 4: 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.

10.5.3 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 at least one 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.

10.5.4 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, wire-time constraints, and particle concentrations. On U.S. GEOTRACES cruises, McLane pumps are typically programmed to pump at an initial rate of 8 L/min for 4 hours (~1500 L), but may be reduced to 2-3 hours in particle-rich coastal waters. 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. Thus far, the dual-flow version of the McLane pump (see section 10.1) 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.

10.5.5 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 while two additional people take the pump off the wire.

Fig. 5. On-deck evacuation of seawater from filter holder headspace using vacuum lines to an aspirator pump on 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. It is important to keep the filter holder upright to prevent particle redistribution on filter surface in the event that residual water remains in the filter holder.

10.6 Particle Sample Handling and Processing

All particle sample handling should be done in a HEPA filtered environment (flow hood or bubble) wearing powder-free nitrile or vinyl gloves. In summary, the steps to in-situ pump sample processing are:

1) Removal of residual seawater from filters by gentle suction
2) Photographing the filter
3) Subsampling the filter for PIs requiring fresh samples, and photographing the subsampled filter
4) Drying the remaining filter
5) Packaging the dried filter

10.6.1 Removal of residual seawater

Filter holders are placed on a filter stand and connected to gentle suction pulling a 0.25 – 0.5 atm vacuum. Prior to disassembling the filter holder, ensure that there is no standing seawater still in the holder. This vacuum suction is important to remove as much residual seawater as possible from filter pores to reduce sea salt on the sample. An extra base plate with frit may be used for additional suction of >51 um prefilters.

In previous versions of this cookbook, we recommended that samples be misted with Milli-Q water under gentle vacuum using a metal-free aerosol mister to further reduce sea salt that may cause matrix effects for ICP-MS analyses. **Isotonic rinses (e.g. ammonium formate) are to be avoided since weakly associated metals are easily lost.**

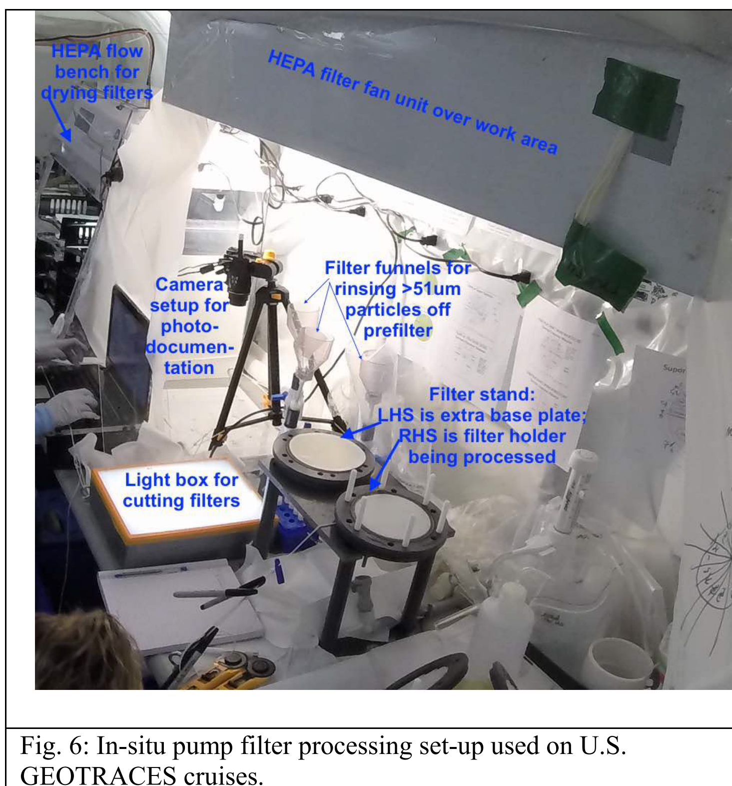

Fig. 6: In-situ pump filter processing set-up used on U.S. GEOTRACES cruises.

Previous reports have suggested the extreme lability of some elements such as P 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 7). 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).

Nalgene no longer makes the metal-free aerosol mister, however, and we haven't found an appropriate metal- free substitute. We have found that gentle suction of residual seawater from filters before drying appears adequate to prevent strong matrix interference for ICP-MS analyses, but we recommend analyzing samples at more than one dilution level to assess this.

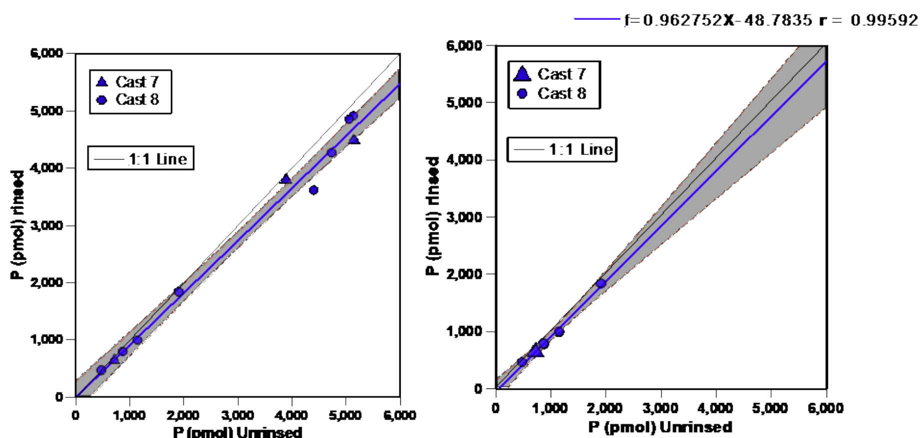

Figure 7: Effect of misting with MQ-H2O on P on samples from SAFe. a) misting leads to a ~9% loss of P (P_{rinsed}=0.912*$P_{unrinsed}$+0.49, r=0.994) P loss is restricted to euphotic zone samples, as B) isted vs. unisted samples deeper than 120 m were not significantly different (H. Bourne and J. Bishop, unpublished).

10.6.2 Photo documentation of filters

Because of sometimes unavoidable heterogeneity in particle distribution on Supor and Polyester mesh filters, we recommend photo documentation of all filters using fixed lighting and camera geometry (Fig. 6) before and after subsampling to document heterogeneity. Details of the procedures are described in Lam and Bishop (2007). 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).

After residual seawater has been removed, the filter is transferred using two forceps from the filter stand onto an acid-leached, clear acrylic plate (referred to below as the "sample plate"). **Separate sample plates should be used for processing QMA filters and for Supor or mesh filters so as not to cross-contaminate the plastic filters with quartz fibers from the QMA.** The sample plate containing the filter is placed beneath the camera for imaging, and is then moved onto the light box for subsampling.

10.6.3 Particle subsampling

If analysts require fresh samples, subsampling can occur prior to drying.
QMA filters are easily subsampled using a sharpened and acid-leached acrylic or polycarbonate tube of any required diameter. A machinist can sharpen stock tubes. Round punches do not work with Supor or mesh filters, which require slicing using a stainless steel scalpel or ceramic blade.

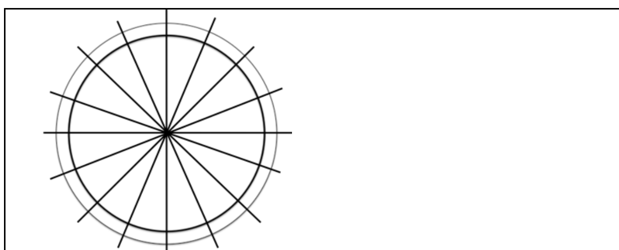
Fig. 8: template pattern for subsampling plastic filters

A rotary ceramic blade held in a fabric cutter works well for cutting straight lines without need for a straight-edge, especially if filters are still damp. We have found that the most representative subsamples are pie-wedges, and equal-area pie wedges can be traced if the sample plate is placed over a "template plate" on a light box. The template plate is made by drawing a circle with a diameter representing the active area of the filter (126 mm diameter for a 142 mm filter in mini-MULVFS holder) split into 16 equal pie wedges using a dark, indelible marker on an acrylic plate (Fig. 8). A protractor and compass should be used for this to ensure that the wedges are equal in area. The template plate is kept on a light box, onto which the sample plate containing the filter can be placed, centering the active area over the template. If the sample filter is not that heavily loaded, the illumination from the light box should make the template lines visible through the filter to aid in cutting. Extending the template lines beyond the diameter of the filter (Fig. 8) helps when filters are heavily loaded. Subsamples of various multiples of 1/16 can then be cut according to analysts' needs. The filter should be re-imaged after subsampling by moving the sampling plate back under the camera.

All subsampling is done directly on the acrylic sampling plates. Plates may be lightly rinsed with milli-Q water in between samples of a cast. After a cast's samples are processed, the sampling plates are placed in a 10% trace-metal grade HCl bath until the next use. Acrylic sampling plates should be discarded when their surfaces are marred by too many cut marks.

10.6.4 Filter drying

PES and 51 µm prefilters are dried on square [~15 cm (for 142 mm) or ~30 cm (for 293 mm)] acid-leached polystyrene grids (see materials list) in a laminar flow bench. 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 in salt, which is important for elements in which salt corrections need to be made (e.g., U, Ca). Stacks of leached, slotted plastic letter trays in a laminar flow bench can be used to efficiently dry a station's worth of samples at once. QMA filters are dried in a clean, 55°C-60°C oven.

Drying is complete in 1-2 days for QMA filters, and ~1 day for prefilter or Supor filters, depending on filter loading. Dried samples can then be stored in polyethylene clean room bags or acid leached plastic containers. To facilitate future subsampling, filters should be stored flat and not be folded. Contact of the filter surface with the inside bag surface has not been a problem.

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.

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 that must be cut manually, or as precut 150 mm circles, that fit mini-MULVFS holders. Larger 293 mm filters for MULVFS must be custom ordered.
- 0.8 µm hydrophilic polyethersulfone (PES) membrane filters: available in 142 and 293 mm diameter from Pall Corporation ("Supor800 PES Membrane Disc Filters") or Sterlitech ("PES")
- 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
- Light box: e.g., McMaster-Carr https://www.mcmaster.com/#light-boxes/=18fgrt9
- Ceramic blades: e.g., Cadence Blades http://cadenceblades.com/parts/sbiz45
- Fabric cutter: e.g., Fiskars 45mm Contour Rotary Cutter (replace steel blades that come with the cutter with ceramic blades)

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11. Shipboard Aerosol Sampling

Aerosols are key parameters in the GEOTRACES Science Plan and this section describes the collection and sample processing procedures for the determination of total aerosol elements; selective leaches and other manipulations of these atmospheric samples are not considered here.

The equipment and operating conditions described here are those used by the US GEOTRACES program, and serve as guidelines and recommendations. Alternative

aerosol sample collection methods can be used, and their accuracy and precision should be tested via an intercalibration effort.

Intercalibration is essential for producing aerosol data that meet the criteria for inclusion in the GEOTRACES Data Products and data base, and should include the following steps:

1. Replicate aerosol collections and distribution of the filters to multiple labs (at least two labs).
2. Multiple labs perform the total and/or leaching experiments for these samples.
3. Multiple labs also conduct leaching experiments on Arizona Test Dust (ATD; see below for details) or other SRMs/CRMs in parallel with the samples.
4. On a frequent basis during analyses, perform analyses of powder (solids), fresh water, or seawater SRMs/CRMs appropriate for your digestion and leach solutions that include all the elements you want to report.
5. Perform the intercalibration with the other lab(s) by quantitatively comparing sample results, ATD results, and SRM/CRM recoveries.

The Standards and Intercalibration Committee can provide advice on setting up an appropriate intercalibration program for aerosol sampling and analysis.

11.1 Aerosol sampling equipment

11.1.1 Sampler and control systems

Shipboard aerosol collection is conducted using Volumetric Flow Controlled (VFC) high volume samplers purchased from Tisch Environmental (TE-5170V-BL; Figure 1). Brushless vacuum motors are used to eliminate the need to vent the exhaust from the motor away from the samplers. For shipboard sampling Tisch Environmental fully encloses the sampler with aluminium walls to minimize the impact of blowing sea spray on the internal components. The sampler components and assembly are listed in *11.1.2* and consist of an aluminium shelter which contains the following:

- Flow Controller Funnel attached to the brushless Blower (vacuum)
- Motor Elapsed Time Indicator (ETI)
- Mechanical (vacuum) Recorder
- Optional ON/OFF timer with switch (not pictured).

The aerosol sampler can be loaded with a filter holder to house large-format filters; 25.4 cm x 20.3 cm (10” x 8”) filters (Figure 2). To make the collection of replicate filters for sharing with the aerosol community more efficient, the standard filter holder can be replaced with a PVC plate modified to hold 12 replicate 47mm open-face filter holders (AdvantecMFS PN PPO-47; Figure 3). Both filter holders are interchangeable with a 5-stage high volume Sierra-style slotted cascade impactor for particle size distribution studies (Tisch Environmental, TE-235, Figure 4). The high-volume cascade impactor is available with up to 6 stages (plus the final backing filter: 25.4 cm x 20.3 cm) for different particle size ranges.

Figure 1. Tisch Environmental (TE-5170V-BL) TSP sampler.

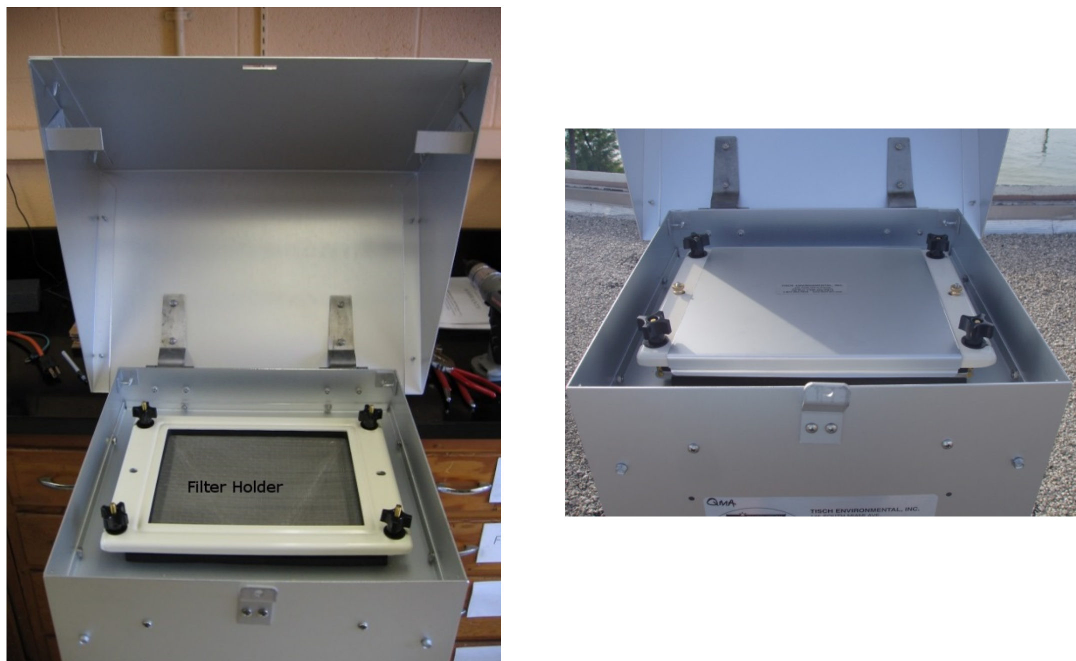

Figure 2. Large format (25.4 cm x 20.3 cm) filter holder and filter holder with the cover.

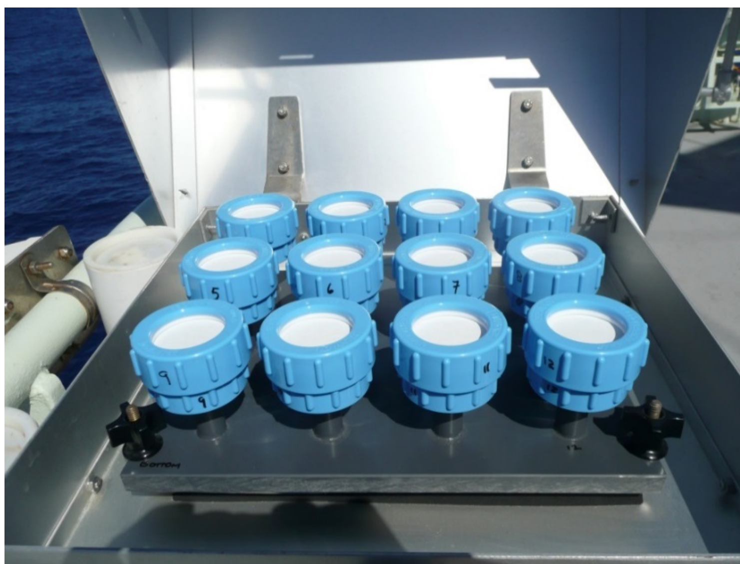

Figure 3. In-house-fabricated PVC adapter plate with 12 replicate 47mm open-face filter holders. Details of fabrication are available on request from William Landing, Florida State University (wlanding@fsu.edu).

Figure 4. Sierra-style slotted cascade impactor (Tisch PN TE-235).

Samplers are powered through individual 1500 watt AC relays connected to a Campbell Scientific Inc. A6REL-12 relay driver and controlled using any of the CSI data loggers and CSI software. The wind speed and wind direction data from a stand-alone anemometer (CSI 03002-L Wind Sentry Set) are collected by the data logger and used to control the operation of the aerosol samplers. Wind speed and sector (wind direction) are user-defined in the data logger program prior to deployment of the samplers (> 0.5 m s^{-1}

and ± 60° from the bow, respectively). If either parameter does not meet these criteria, the samplers are turned off immediately (by cutting power to the AC relays) and they do not re-start until the wind has met both criteria for 5 minutes continuous elapsed time (also user-defined in the data logger software).

11.1.2 Aerosol sampling equipment parts list
Tisch Environmental (https://tisch-env.com/):
TE-5170V-BL Volumetric Flow Controlled Total Suspended Particulate TSP High Volume Air Sampling System. Includes anodized aluminum shelter, 8" x 10" stainless steel filter holder with stagnation pressure tap, BRUSH-LESS blower motor assembly, transformer, continuous flow/pressure recorder, elapsed time indicator, 30" water manometer, volumetric flow controller with look up table less 7-day mechanical timer with extended sides for additional protection from water spray. 120v/60hz.
TE-5028 Calibration Kit for above
TE-5070BL Spare Blower Motor Assy.
TE-3000 Filter Paper Cartridge
TE-235 Five stage impactor
TE-230-WH slotted cellulose filters 100/box

Dwyer digital manometers (http://www.dwyer-inst.com/):
PN 475-3-FM (0-200 in), 475-7-FM (0-100 in), or 475-8-FM (0-150 in)

Aerosol Filters (GE Healthcare Life Sciences;
http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences/products-and-solutions/lab-filtration):
Whatman 41 25.4 cm x 20.3 cm filter sheets PN 1441-866
Whatman 41 47 mm filters PN 1441-047
Whatman QM-A quartz fiber 25.4 cm x 20.3 cm filter sheets PN 1851-865
Whatman QM-A quartz fiber 47 mm filters PN 1851-047
Whatman GF/F 25.4 cm x 20.3 cm filter sheets PN 1882-866
Whatman GF/F 47 mm filters PN 1882-047

Open-face Filter Holders (Advantec MFS; http://www.advantecmfs.com/):
PPO-047 (NOTE: the upper portion of filter holder is not used so that the filter membrane is held by the Teflon O-ring and directly exposed to incoming air)

11.2 Filter types

The filter matrix and size depends on the parameters under investigation, but must have sufficiently high porosity and flow rates (>20-25 cm/s linear face velocity) to avoid burning out the vacuum motors. Five different filter types have been used in high volume aerosol sampling on GEOTRACES cruises:

- Bulk aerosol collection on 12 replicate 47mm disc filters for trace elements and major ions (low ash cellulose esters, Whatman 41, PN 1441-047).
- Bulk collection on 12 replicate 47 mm disc filters for nitrogen isotopes and trace organics (glass-fiber filters, Whatman GF/F PN 1882-047).

- Bulk collection on 12 replicate 47 mm disc filters for nitrogen isotopes and trace organics (quartz microfiber, Whatman QM-A PN 1851-047).
- Bulk collection on large-format quartz-fiber filters for nitrogen isotopes and trace organics (quartz microfiber, Whatman QM-A quartz fiber 25.4 cm x 20.3 cm filter sheets PN 1851-865.
- Aerosol particle size distribution using a five-stage impactor (slotted cellulose esters substrates, Tisch Environmental TE-230WH with Whatman 41 PN1441-866 backing filter).

It has been shown (Chad Hammerschmidt, unpublished data) that it does not make any difference for aerosol Hg whether one uses quartz fiber (QM-A) or glass-fiber (GF/F filters). The organic matter and nitrogen isotope groups also report that GF/F filters are acceptable, and because they are much less expensive, GF/F filters have been routinely used for these analytes.

11.3 Filter preparation

11.3.1 Cellulose ester filter washing procedures

Acid-washing and drying of filters is conducted in a dedicated HEPA (class 100 or better) laminar flow hood. Acid baths are prepared using quartz distilled HCl (q-HCl) or commercially-available ultrapure HCl and all filter handling is performed using acid-cleaned plastic tweezers.

Whatman 41 47mm filter discs:

1. Place discs in a 0.5M q-HCl bath in a closed lid polyethylene bin for 24 h at room temperature.
2. Move discs to a rinsing container and batch rinse 3-5 times with ultra-high purity (UHP) water (>18.2 MΩ*cm).
3. Place discs in second 0.5M q-HCl bath for 24 h.
4. Move discs to a rinsing container and rinse 3-5 times with UHP water.
5. Cover the filters in the rinsing container with ~1L of UHP water and let sit 24 h.
6. Test UHP water with pH strips and replace the rinse water until the water has the same pH as fresh UHP water.
7. Lay the discs out individually on a clean plastic mesh inside a laminar flow hood and let sit for 24 h or until dry.

The acid washes are carried out in separate containers; one for each acid wash. This procedure is strictly adhered to in order to minimize the chance of cross-contamination. Acid baths are remade after each batch of filters is washed. Two hundred 47 mm filters can be washed at a time. Take care when handling the filters as they are easy to tear or puncture when wet.

TE-230WH slotted impactor filters and 25.4 cm x 20.3 cm Whatman-41 filters:

1. Place filters, separated by a sheet of polypropylene or polyethylene mesh, in a 0.5M q-HCl acid bath in a closed lid polyethylene bin and leave for 24 h at room temperature.

2. Remove filters with their underlying plastic mesh very carefully, one at a time (they are very easy to tear) from the acid bath using plastic tweezers. Rinse each filter individually with UHP water from a squirt bottle.
3. After rinsing, place the filters into a ~2 L bath of UHP water. After soaking in UHP water remove all the filters from the UHP water bath, allowing excess water to drain off the filters.
4. Place the filters into a second fresh 0.5 M q-HCl bath and leave for an additional 24h at room temperature.
5. Repeat steps 2 and 3.
6. Continue to rinse the filters with ~2 L of fresh UHP for 24 h periods until the pH of the water reaches that of UHP water—usually at least five washes.
7. When the water has reached the pH of UHP water (pH ~5.6), do a final individual rinse of each filter with a squirt bottle of UHP water and individually place each filter onto a polyethylene or polypropylene drying rack in a laminar flow hood to dry.
8. When dry, place filters (unfolded) into zipper-seal polyethylene bags.

11.3.2 QMA, GF/F filters

QM-A or GF/F filter handling must be done wearing polyethylene gloves (not nitrile gloves) to minimize organic matter contamination. In addition, glass and quartz fiber filters must be kept away from nitric acid, especially the fumes, as QMA and GF/F filters are known to adsorb nitric acid vapor.

1. Tear aluminum foil into approximately 55 cm x 45 cm pieces. One piece of foil per large filter (or for every 12 of the 47 mm filters) is required. As you tear the pieces, stack one on top of the next.
2. Lightly fold the pile of aluminium foil pieces in half.
3. Unstack the QM-A or GF/F filters and place them in a clean baking tray made of aluminium foil or Pyrex glass (so that they are not packed tightly on top of each other; overlapping edges are acceptable.)
4. Place the gently folded stack of aluminium foil sheets on top of the filters in the baking tray and place the tray in a pre-heated muffle furnace (480°C) for 6 hours.
5. While still hot, remove the tray from the muffle furnace, and place the tray in a laminar flow hood to cool.
6. Use plastic tweezers to carefully place each filter (or each set of 12 replicate 47 mm filters, side by side) on an aluminium foil piece. Do not fold the filters.
7. Fold the foil in half and fold the edges over again to wrap the filters.
8. Place foil-wrapped filters in zipper-seal polyethylene bags.

11.4 Calibration check of the aerosol samplers

The calibration of the Volumetric Flow Controlled samplers should be checked prior to and after operation on-board ship to ensure that the flow rates are within the technical specifications provided by Tisch Environmental (flow look-up tables are provided with each sampler). A Variable Orifice Calibrator (Tisch Environmental TE-5028A) is required for calibration along with two handheld digital manometers. Because the vacuum underneath the filters can exceed the capacity of the manometers provided by the manufacturer (0-40 inches of water), digital manometers with a range of at least 0-100

inches of water are required (Dwyer PN 475-3-FM, 475-7-FM, or 475-8-FM) for calibration and also for operation of the cascade impactor (where a backing filter is deployed).

11.5 Deployment of the aerosol samplers

Position the aerosol samplers upwind of potential sources of contamination (e.g., the ship's exhaust stack, incinerator exhaust, or even kitchen vent/fans) and as high on the ship as possible to avoid sea spray. For these reasons, it is best to position them as high and as far forward as possible on the ship. This is often on the deck above the bridge if it is allowed. Mount the anemometer nearby in "free air" to avoid excessive wobbling due to air flowing upwards from the ship. Run the anemometer leads to the data logger, and run the power from the AC relays to the samplers. Filters are typically deployed for ~24 h periods, although if dust loading is very low sampling duration may be increased.

11.6 Loading/recovery of aerosol filters

It is important to wear clean nitrile or polyethylene gloves when directly handing the filters and the filter holders. Filter loading and unloading from the filter holders is done in a "clean air" HEPA laminar flow environment. Clean room nitrile gloves should be worn while handling the W41 filters (polyethylene gloves for QM-A and GF/F filters), though actual touching of the filters should be avoided. Any necessary manipulation should be performed using clean plastic tweezers and limited to the edges of the filter where aerosols are not collected.

When using 47 mm filters, pre-load a set of 12 filters into the open-faced filter holders on a PVC plate using clean plastic tweezers, gently tighten the locking rings, and place the loaded plate into a plastic bin with a tight-fitting lid. When using 25.4 cm x 20.3 cm filters, pre-load each filter into a filter holder cassette, place the aluminium lid on the cassette (see Figure 2), and store in a clean plastic bin or large polyethylene zipper-seal bag. The high-volume cascade impactor is loaded first with a 25.4 cm x 20.3 cm final stage backing filter (Whatman 41) and then 5 slotted filters. Load the slotted filters from finest (stage 5, smallest particle size cut-off) to the coarsest (stage 1). The slotted filters are positioned so that the slots (holes) of the filter are open to the slots of the underlying stage (see manual for further details of the loading procedure). Gently tighten the knurled nuts and place the loaded impactor in a clean plastic bag or bin. Carry the bins out on deck to the samplers in preparation for changing filters.

In addition to the Start and Stop dates and times for each deployment, the reduced pressure underneath the filters, and the ambient temperature and barometric pressure must be recorded at the beginning and end of each deployment interval. These data are essential in order to calculate the air flow rate through the filters.

- If filters have already been deployed and are being recovered, approach the aerosol sampler from downwind to avoid contamination and attach a manometer to the vacuum valve to measure the "final" reduced pressure under the filters.
- Then, turn off the power to the sampler.

- Open the lid and recover the loaded samples into a clean plastic bin, replacing with a new set of filters.
- Gently tighten the knurled nuts to hold the filter holder in place. Each filter holder has a dense foam "gasket" that mates over the screen leading to the vacuum motor (Figure 2), and they are all designed to fit the same way atop the aerosol sampler and to be secured in place using four knurled nuts (Fig. 2, 3, 4).
- Close the sampler lid and restore power to the sampler.
- Again, approach the sampler from downwind and record the "initial" reduced pressure.
- If you are using an Elapsed Time Indicator (ETI), record the elapsed time at the start and end of each deployment interval in order to calculate the amount of time the vacuum motor was running for each deployment.

After recovery, change out the filters in a clean air environment wearing clean nitrile or polyethylene gloves. It is most efficient to re-load each filter holder with new filters for the next deployment after the loaded filters have been removed. The 47 mm filters are removed from the 12-position PVC plate into individual "petri-slides" (EMD Millipore PN PD1504700), then usually stored frozen (-20°C) until analysis. The 25.4 cm x 20.3 cm filters and the slotted impactor filter substrates are folded in half (aerosols into the middle), replaced in their zipper-seal polyethylene bags and stored frozen. Each filter must be associated with the appropriate meta-data (cruise details, deployment and recovery dates, impactor stage, etc.) either by indelible writing on the bags or affixing some unique sample identification number (or bar code label).

11.7 Post collection filter processing

If the filters must be sub-divided prior to analysis, additional handling will be required. For the cellulose filters, trace metal-clean procedures should be followed:

- All manipulations should be carried out in a HEPA laminar flow clean air environment.
- Use clean nitrile (W41 filters) or polyethylene gloves (QM-A filters) and clean plastic tweezers when handling. Similar to the recovery of the filters during on-board sampling, avoid the actual touching of the filter by utilizing the edges of the filters for handling.
- Use ceramic (Zr oxide) knives or scissors for cutting the filters.
- Individual filter pieces should be stored in labelled petri-slides for storage or distribution.

Total digestion of filters for trace element analysis requires the use of hot nitric and hydrofluoric acids with modest pressure (see Morton et al., 2013). A programmable microwave digestion oven is somewhat more efficient than using Teflon "jars" on hot plates. Appropriate Certified Reference Materials must be included when performing total aerosol digestions. A1 Arizona Test Dust (ATD), a very fine aerosol material (<3 µm) from Powder Technologies Inc., is recommended for use as a potential "consensus reference material". It is more like a true aerosol material, and testing has shown that it appears to be homogeneous for total trace elements down to sample sizes as small as 8-10

mg. The major element composition of ATD is shown below in Table 1. The size distribution of the ATD is shown in Table 2. Contact William Landing (wlanding@fsu.edu) or Peter Morton (pmorton@fsu.edu) to obtain a Test Dust sample to use. When analyses are completed, your results for this consensus reference material should be reported to Landing or Morton along with appropriate metadata (e.g., digestion and analytical methods, analytical figures of merit).

11.8 Example analytical figures of merit for aerosol total digestions

Details for aerosol sample collection and analysis from the GEOTRACES Aerosol Intercalibration project are summarized in Morton et al. (2013) and are summarized in Table 3 and Table 4 below. These are useful for evaluating potential contamination and expected analytical performance for total aerosol trace element determinations.

11.9 References

Peter L. Morton, William M. Landing, Angela Milne, et al. 2013. INTERCAL: Results from the 2008 GEOTRACES aerosol intercalibration study. Limnology and Oceanography: Methods 11: 62–78.

Table 1. Major element composition of A1 (ultrafine) Arizona Test Dust (Powder Technologies Inc.).

	SiO_2 %	Al_2O_3 %	Fe_2O_3T %	MnO %	Na_2O %	K_2O %	TiO_2 %	CaO %	MgO %
Average	32.74	12.5	3.5	0.094	3	3.5	0.75	3.5	1.5
+/-	4	2.5	1.5		1	1.5	0.25	1.5	0.5
MWt	60.09	101.96	159.694	70.94	61.98	94.2	79.87	56.08	40.31
AWt	28.09	26.98	55.847	54.94	22.99	39.1	47.87	40.08	24.31
ppm	153048	66153	24480	728	22256	29055	4495	25014	9046

Table 2. Size distribution of A1 (ultrafine) Arizona Test Dust (Powder Technologies Inc.).

Iso 12103-1, A1 Ultrafine Test Dust Particle Size Distribution by Volume %

Size Micrometer	*ISO 12103-1, A1 Ultrafine Test Dust % Less Than*
0.97	3.0 – 5.0
1.38	7.0 – 10.0
2.75	23.0 – 27.0
5.50	65.0 – 69.0
11.00	95.5 – 97.5
22.00	100.0

Table 3. Comparison of unwashed versus washed W41 filters: blanks and detection limits (Morton et al., 2013)

Element	Average (ng cm^{-2})		Average* (ng m^{-3})		Detection Limit† (ng m^{-3})	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Al	5.63	20.7	1.66	6.12	0.79	7.94
Ti	1.63	4.15	0.48	1.23	0.09	0.98
V	0.014	0.014	0.004	0.004	0.007	0.002
Mn	0.600	0.051	0.178	0.015	0.013	0.006
Fe	18.8	4.15	5.56	1.23	0.62	0.46
Co	0.0132	0.00173	0.0039	0.00051	0.00768	0.00069
Ni	0.342	0.055	0.101	0.016	0.053	0.011
Cu	0.864	0.038	0.256	0.011	0.144	0.019
Zn	0.78	1.04	0.23	0.31	0.10	0.35
Cd	0.00369	0.0012	0.0011	0.00035	0.0011	0.00051
Pb	0.055	0.032	0.016	0.010	0.014	0.016

*Filter blanks in units of "ng m^{-3}" were calculated assuming a typical 24 h filtered air volume of 1400 m^3
†Detection limit reported as 3σ of the blanks (n = 5 for unwashed filters, n = 7-8 for washed filters)

Table 4. Size-fractionated W41 (slotted Impactor filter) blanks and detection limits (Morton et al., 2013).

Element	Average ($ng\ cm^{-2}$)	Average* ($ng\ m^{-3}$)	Detection limit (3σ; $ng\ m^{-3}$)
Al	4.81	0.66	1.242
Fe	4.12	0.729	1.928
Mn	0.056		
Ti	1.23	0.149	0.385
Cu			
Cd			
Zn	1.38	0.164	0.28
Co	0.0019		
Ni			
V	0.007	0.005	0.009
Pb	0.013	0.048	0.368

*Filter blanks in units of "$ng\ m^{-3}$" were calculated assuming a typical 24 h filtered air volume of 1400 m^3

VII. Nitrate, Silicon, and Carbon 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, it has been more common to use 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. 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 and Casciotti, 2011). In addition, we found that back flushing a portion of the GC column between samples kept backgrounds low for m/z 44, 45, and 46 and increased analytical precision (McIlvin and Casciotti, 2011).

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.
- If one lab is responsible for the nitrate isotopic analyses, crossover or sample sharing procedures outlined in GEOTRACES documentation should be followed.

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

Böhlke, J. K., S. J. Mroczkowski, and T. B. Coplen. 2003. Oxygen isotopes in nitrate: new reference materials for O-18 : O-17 : O-16 measurements and observations on nitrate-water equilibration. Rapid Communications in Mass Spectrometry 17: 1835-1846.

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.

McIlvin, M.R., and K. L. Casciotti. 2011. Technical updates to the bacterial method for nitrate isotopic analyses. *Analytical Chemistry* 83: 1850-1856.

Sigman, D. M., K. L. Casciotti, M. Andreani, C. Barford, M. Galanter, J. K. Böhlke. 2001. A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Marine and Fresh Waters. *Analytical Chemistry*, **73**: 4145-4153.

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) 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

Abraham, K. and others 2008. $\delta^{30}Si$ and $\delta^{29}Si$ Determinations on USGS BHVO-1 and BHVO-2 Reference Materials with a New Configuration on a Nu Plasma Multi-Collector ICP-MS. Geostandards and Geoanalytical Research **32:** 193-202.

Brzezinski, M. A., J. L. Jones, C. P. Beucher, and M. S. Demarest. 2006. Automated determination of silicon isotope natural abundance by the acid decomposition of cesium hexafluosilicate. Anal. Chem. **78:** 6109-6114.

De La Rocha, C. L., M. A. Brzezinski, and M. J. Deniro. 1996. Purification, recovery, and laser-driven fluorination of silicon from dissolved and particulate silica for measurement of natural stable isotope abundances. Anal. Chem. **68:** 3746-3750.

Georg, R. B., B. C. Reynolds, M. Frank, and A. N. Halliday. 2006. New sample preparation techniques for the determination of Si isotopic compositions using MC-ICPMS. Chemical Geology (Isotope Geoscience Section) **235:** 95-104.

Ragueneau O., N. Savoye, Y. Del Amo, J. Cotten, B. Tardiveau and A. Leynaert, 2005. A new method for the measurement of biogenic silica in suspended matter of coastal waters: using Si:Al ratios to correct for the mineral interference. *Continental Shelf Research*, 25, 697-710.

Reynolds, B. C. and others 2007. An inter-laboratory comparison of Si isotope reference materials. J. Anal. At. Spectrom **22:** 561-568.

Reynolds, B. C., M. Frank, and A. N. Halliday. 2006a. Silicon isotope fractionation during nutrient utilization in the North Pacific. Earth Plan. Sci. Let. **244:** 431-443.

Reynolds, B. C., R. B. Georg, F. Oberli, U. Wiechert, and A. N. Halliday. 2006b. Re-assessment of silicon isotope reference materials using high-resolution multicolletor ICP-MS. J. Anal. At. Spectrom **21:** 266-269.

Varela, D. E., C. J. Pride, and M. A. Brzezinski. 2004. Biological fractionation of silicon isotopes in Southern Ocean surface waters. Global Biogeochem. Cycles **18:** GB1047, doi:1010.1029/2003GB002140.

C. Protocols for Carbon Isotopes in Dissolved Inorganic Carbon (DIC)

For δ^{13}C-DIC analysis, sampling, storage, analysis, and calibration should follow the GO-SHIP Repeat Hydrography Manual (IOCCP Report No. 14) for Collection and Measurement of Carbon Isotopes in Seawater DIC. In particular, an effort should be made to perform an external validation through replicate sample sharing, analysis of consensus materials, or standard seawater samples.

Reference:

McNichol, A.P. P. D. Quay, A.R. Gagnon, and J.R. Burton. 2010. Collection and Measurement of Carbon Isotopes in Seawater DIC. GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. IOCCP Report No. 14, ICPO Publication Series No. 134, Version 1.

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, 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, 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.

For Rosette/Carousel Systems: It is not recommended 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.

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 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.

<u>Scattering sensors.</u> 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.

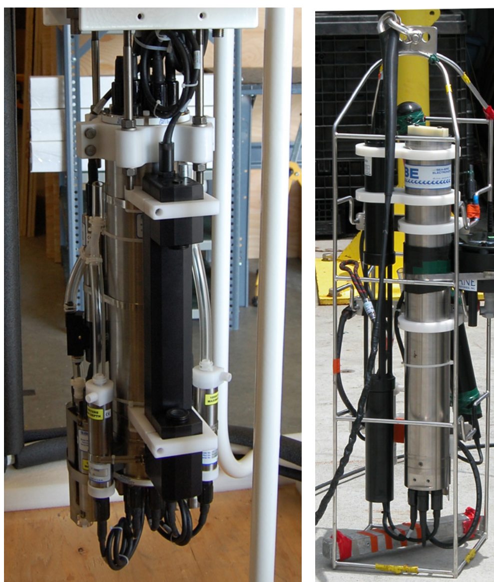

Figure 2. Vertical mounting of transmissometers close to the CTD unit (SBE 911 shown) at the center pylon of rosette/carousel frames (left) results in cleaning access difficulty with bottles emplaced and possible flow separation from optics during casts. Vertical mounting of transmissometers on autonomous 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.

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.

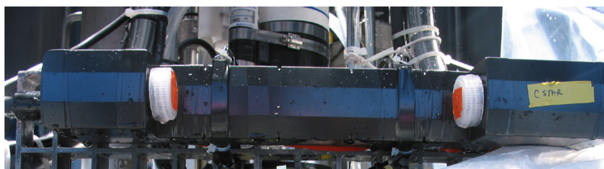

Figure 3. CSTAR transmissometer with plastic bottle caps installed on optical windows that are effective at preventing optics contamination while not deployed.

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., WET Labs, 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 **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, c_p, is calculated:

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

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 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).

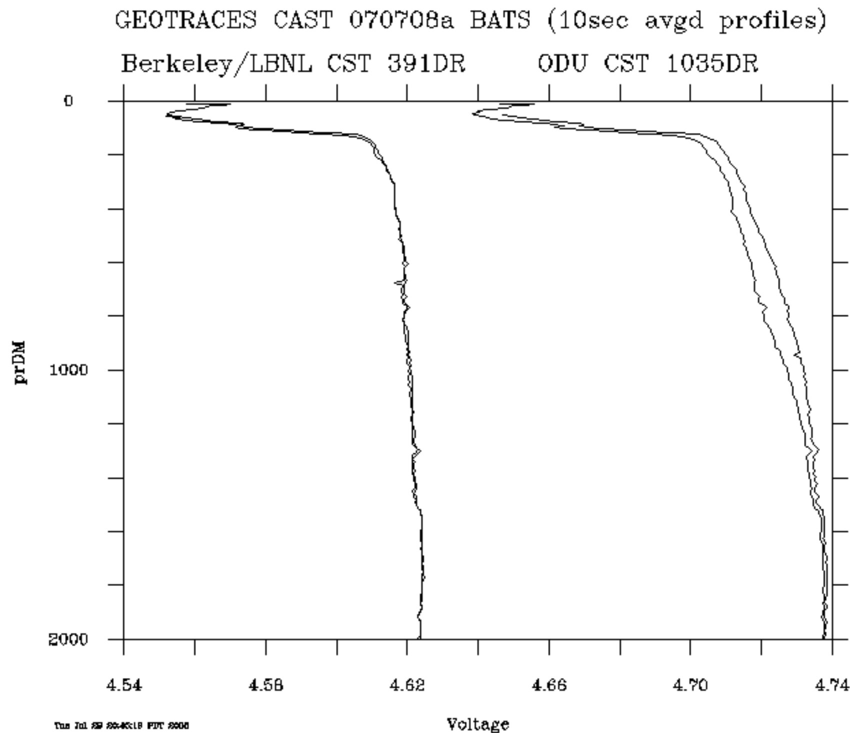

Figure 4. Examples of good (left) and poor (right) reproducibility of transmissometer data during GEOTRACES IC1 – Cast 070708a near the Bermuda Time Series Station. The profile on the right shows moderate thermal hysteresis of the C-STAR (1035DR) response during down and up (shifted to higher voltage) 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

Bishop, J.K.B., Wood, T.J., 2008. Particulate matter chemistry and dynamics in the twilight zone at VERTIGO ALOHA and K2 sites. Deep Sea Research Part I: Oceanographic Research Papers 55 (12), 1684-1706.

Bishop, J.K.B. (1999) Transmissometer Measurement of POC. *Deep-Sea Research I.* 46(2) 353-369.

Boss, E., W.H. Slade, M. Behrenfeld, G. Dall'Olmo (2009) Acceptance angle effects on the beam attenuation in the ocean. *Optics Express* 17 (3) 1535-1550.
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Gardner, W.D., Mishonov, A.V., Richardson, M.J., 2006. Global POC concentrations from in-situ and satellite data. Deep-Sea Research II 53, 718–740.

IX. BioGEOTRACES Parameters

A. Active fluorescence (i.e., F_v/F_m and other biophysical metrics)

Metrics of active fluorescence such as F_v/F_m (photosynthetic competence) have been widely used to assess the relationship between trace metal supply (mainly iron) and the status of resident photosynthetic cells (or phytoplankton lab cultures) (Kolber et al., 1998). Sampling is often conducted underway (Boyd and Abraham, 2001; Olson et al., 2000; Moore et al., 2005), from discrete samples obtained from a trace metal clean rosette or conventional CTD rosette (Boyd et al., 2005), and/or through deployment of instruments in situ (Moore et al., 2005).

1. Analytical Instruments

At present, there are five commercially-available instruments that are commonly used to conduct such analysis. LIFT-FRR is most sensitive, followed by Fastracka/FIRe/Fast Fluorometer, with Phyto-PAM being least sensitive to make biophysical measurements at low chlorophyll concentrations. A comprehensive intercomparison of these approaches is summarized in Table 1.

Light Induced Fluorescence Transients (LIFT) LIFT_FRR (designed by Zbigniew Kolber)

Chelsea Instruments Fastracka
http://www.chelsea.co.uk/allproduct/marine/fluorometers/fast-ocean-system

Satlantic FIRe Fluorescence Induction and Relaxation System
http://satlantic.com/fire

Waltz Phyto-PAM
http://www.walz.com/products/chl_p700/phyto-pam/introduction.html

PSI Fast Fluorometer
http://psi.cz/products/fluorometers/fast-fluorometer-fl-3500-f

Soliense Inc. LIFT-FRR
http://www.soliense.com/LIFT_Marine.php

2. Sampling methods

2.1 Discrete sampling

Requires dark adaptation on replicate samples (or dim light less than 10 µmol quanta) for 30 minutes before running the samples through the dark chamber of the instrument (do not use PTFE tape for any of the plumbing of the underway lines as it has an interference

Table 1

Instruments	FastOcean	PAM (various)	FRR LIFT	FIRe	Fast Fluorometer
Manufacturer	Chelsea Technologies Group, UK	Walz, Germany	Soliense Inc., USA	Satlantic, Canada (Seabird Scientific)	PSI, Czech Republic
General:					
Depth rating	>250m	None (lab only)	None (*in situ* 2017)	None	None (lab only)
Sensitivity (mg/m^3)	0.05	0.1 (WaterPAM only)	<0.02	0.05	0.05
Excitation λ	Multi (450, 530, 620)	Multi (440, 480, 540, 590, 625 MultiPAM only)	Multi (450, 470, 530, 570, 630)	Multi (455, 540)	Multi (455, 630)
Bacterio-chl capability	No	No	Yes	Yes	No
Flow through	Yes (dark chamber)	Yes (cuvette)	Yes (pump/cuvette)	Yes (cuvette)	Yes (cuvette)
Operating software	FastPro (PC)	WinControl (PC)	Script-GUI (PC)	FirePro (DOS) or FiReWORK (Matlab)	Script-GUI (PC)
Operating flexibility	Medium (GUI only, some restrictions to choices of induction and no. of iterations per acquisition; ST and MT)	High (Script-based); ST and MT	High (Script-based); ST and MT	Medium (GUI only, some restrictions to choices of induction); ST and MT	High (Script-based); ST and MT; OJIP
Continuous data collection	Autogain generally good	Limited	Autogain general good; many options can be scripted	Autogain generally good	Autogain general good; many options can be scripted
Parameter retrieval:					
Blanking required (F_0, F_m, F_v/F_m)	Yes	Yes	Yes	Yes	Yes
σ_{PSII}	Yes; instrument response influence minimal	Yes (MultiPAM only); no instrument response influence	Yes; instrument response influence minimal	Yes; instrument response influence very high	Yes; instrument response influence very high
tau	yes	yes	yes	No	Multiple dynamics modeled
Other notes on fitting	Calibrated to also deliver [RCII] for ETRs		'Instantaneous light curve' parameters with each induction; many options		Must be performed in external software
Additional Specs for discrete sampling:					
Light-curve capacity	Yes (FastAct lab unit)	Yes	Yes	Yes (add-on unit)	Yes
Temperature control	Yes (external)	Yes (external)	Yes (external)	No	Yes (external)
Autosampling	Yes	No	Yes	No	No

effect on the instrument due to being fluorescent). Such dark sampling allows the relaxation of a number of light related physiological processes which will alter the fluorescence characteristics of the sampled population. Samples are also required for blanks (0.2 μm filtered, see Cullen and Davis, 2003), for nutrients and trace metals (as macronutrient concentrations can also influence photosynthetic competence), and for floristics (as the maximal values of F_v/F_m can be influenced by the dominant phytoplankton species (0.65 for diatoms, 0.5 for pico-cyanobacteria, Suggett et al., 2009).

2.2 Underway sampling

Requires dark adaptation (or dim light less than 10 μmol quanta) for 30 minutes (unless sampling at night/dusk) before running the samples through the dark chamber of the instrument. Hence, the sample should be run through a reservoir tank, or long length of non-toxic tubing (along with a debubbling system); these processing can introduce some 'smearing' / averaging of the sample, and the time lag must also be considered when comparing the active fluorometry record with other sampling (phytoplankton, flow cytometry, nutrients, trace metals if sampling from a TM fish or clean underway line). Alternatively, data collected during the dark period of the diel cycle might be selected during post processing, although it should be noted that diel variability in photosynthetic physiology during both day and night due to the range of photosynthetic processes operating at different timescales and with different light dependencies (e.g. Behrenfeld and Kolber 1999; Morrison 2003).

Samples are also required periodically for blanks (0.2 μm filtered, and must be run as a batch of discrete samples after the underway sampling is completed).

3. Data Analysis/Curve-fitting

Curve-fitting to obtain biophysical metrics, such as FoD, FmD (i.e., Fm in the dark, or simply Fm, as employed by physiologists, see list of terminology in Kolber et al., 1998), FvD, Fv/FmD, SigD (functional cross section of PS II), TauD (turnover time for electron transport from PS II to PS I), for some of the above four instruments are fundamentally different. All Fast Repetition Rate Fluorometers (FRRF's) use the empirical model of Kolber et al. (1998). Some, like the LIFT and Fastracka, have ways to adjust the parameters of the fit and assumptions of the model (see www sites and manuals for more detailed information). PAM (Pulsed Amplitude Modulation) and FRRF are fundamentally different. PAM has no limited fitting.

4. Confounding factors

The comparability of datasets can be compromised if the following factors are not incorporated into the sampling protocol: fluorescence blanks; dark adaptation, information on floristics, and data on macro- and micro-nutrients to identify the potential environmental control(s) on photosynthetic competence. Most instruments are multi-spectral now and can preferentially excite some taxa more than others using a particular

wavelength of excitation light. Multiple-excitation wavelengths instruments allow to spectrally resolve induced fluorescence measurements, and gain insight into taxonomic differences in photo-physiology between samples/sites.

5. Intercalibration

Given the difficulty in matching the excitation protocols between different instruments as closely as possible (flashet number, duration, and spacing, excitation wavelength), alongside the differences that specific measurement protocols and data fitting techniques can impose on the derived data, the best means of intercalibration is often to run samples from a low cell density Fe replete and Fe deplete culture through the instrument to assess how they scale to the theoretical maximum and some minimum value (usually ~1/3 of the maximum; Kolber et al., 1998).

6. Metadata requirements

Measurement protocols for sampling strategies should be reported. For example, for the Fastracka instrument, the duration and frequency (MHz/KHz) of the train of (usually 100) saturation and subsequent (~20) relaxation flashlets are required along with information about the gain (which is biomass dependent). Additionally, the excitation and measurement wavelengths of the specific instrument and or protocol used should be reported.

Additional, critical parameters that must be reported:

1. Time of day and depth the samples were collected
2. Discrete versus underway samples or in situ measurement
3. Sample preparation (such as blanks, dark adaptation) and/or collection (underway system)
4. Instrument and fitting routines (see above).
5. Datasets from external standards (replete versus deplete phytoplankton culture, and species used).
6. Other ancillary sample data (chlorophyll, nutrients, temperature, salinity, trace metals, surface PAR and K_d(PAR), and/or PAR at the depth and time of sampling).
7. Floristic dominant phytoplankton species and/or phytoplankton community composition. Some recommended methods include HPLC pigments (with CHEMTAX), 16s/18s, flow cytometry and microscopic determination of most common phytoplankton taxa.

7. References

(for additional references also see http://www.chelsea.co.uk/technical-papers#frrf and http://www.walz.com/products/chl_p700/phyto-pam/publications.html)

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B. Metagenomics

Because the technology facilitating DNA extraction, processing, and sequencing is continuously and rapidly evolving, multiple strategies can and should be applied to bioGEOTRACES sequencing samples as long as care is taken that results generated by different pipelines are comparable. Samples should be filtered at the same size fraction i.e. 0.22 µm without prefiltration, and should be collected in triplicate. The volume of water collected for each sample should be recorded and biological samples should be paired with GEOTRACES bottle identifiers.

Ideally, technical sample replicates should be collected at multiple stations during a cruise. For example, water could be collected at two or three "intercalibration" stations agreed upon by bioGEOTRACES scientists and the steering committee before cruise departure. Samples could be collected at specified depths, filtered using a single methodology, and then circulated to different laboratories upon cruise completion. The different laboratories could follow the official bioGEOTRACES DNA extraction, sequencing library preparation, and DNA sequencing protocol outlined below and then compare final results as well as measured parameters at intermediate steps, for example extracted DNA concentrations. Additionally, this procedure could be performed on a set of standard filters that have been prepared from a single marine source (e.g. Sargasso seawater, coastal water from WHOI, etc.) available to all bioGEOTRACES scientists and accepted as the standard source of marine microbes for intercalibration of methodology across laboratories. Again, samples filtered from this single source could be distributed to other participating laboratories and compared as described above.

The most comprehensive calibration strategy would be to spike certain redundant bioGEOTRACES samples with synthetic transcripts or genome equivalents of a known concentration. These synthetic nucleotides could be provided by the bioGEOTRACES intercalibration committee to participating laboratories along with the officially recommended protocol for DNA extraction. After DNA extraction and prior to sequencing library preparation, qPCR measurements targeting the synthetic nucleotide addition could quantify DNA recovery efficiencies, which could then be compared between labs for purposes of intercalibration. Additionally, this strategy could provide a benchmark for substituting newer protocol versions (e.g. those that utilize reagents and other components from a different manufacturer) or comparing largely different extraction methodologies (e.g., phenol chloroform extraction versus silica mini column based extractions) into the bioGEOTRACES "endorsed" protocol library.

For best consistency, all labs should annotate metagenomes using the same algorithms, parameters, and reference database. This reference database could be generated and maintained by members of the bioGEOTRACES steering committee and distributed to labs processing the data. New and updated sequence processing algorithms and reference databases are continuously released so the steering committee should work to ensure that the latest recommended annotation pipeline and the recommended reference database is modern, effective, and comparable between different data product releases. Labs are free to annotate and analyze libraries using the methodologies of their choice, but labs should submit derived data that has been analyzed following the official recommended annotation pipeline and reference database of the bioGEOTRACES steering committee.

1. Sampling methods

1. Prepare 500 mL amber collection bottles by soaking in 0.5% sodium hypochlorite (bleach) for 20 minutes and rinsing six times with distilled water.
2. Collect seawater from Niskin bottles fired at depths of interest. For each Niskin sampled, make a detailed record of the bottle number that was fired, CTD cast,

the GEOTRACES cruise station, the GEOTRACES cruise name, GPS coordinates, date and time in 24 hour format.

3. Collect samples in cleaned (see step one) 500 mL amber bottles that have been pre-rinsed times with the seawater sample. For each sampled depth fill to bottles neck with seawater.

2. Sample Filtration

1. For intercalibration DNA extraction should be on whole seawater samples collected on 0.22 µm filters. Samples should not be pre-filtered.
2. For each sample collected from Niskin bottles, filter 100 mL volume of whole seawater onto 0.22-µm-pore-size polycarbonate filters (diameter, 25 mm; GTTP; Millipore) using a sterile filter rig. Use new, pre-bleached and DDI-rinsed filter funnel and base for each sample depth. Wet filter base with DDI water (squirt bottle). Filter at 0.3 bars maximum pressure.
3. After filtering the sample, load 3 ml Preservation Solution (10 mM Tris, 100 mM EDTA, 500 mM NaCl, solution should be pH 8, stored at room temp, use 1 bottle for each station) onto the membrane. Fold filter over once with sterile tweezers, avoiding touching cells at center, and transfer filter to labeled 2 mL bead beating tube. Store at -80 °C.
4. Record the exact volume filtered and generate and record a unique sample identifier. Perform filtrations in triplicate to enable intercalibration with other laboratories. Replicate filters should be archived at the institution of the laboratory performing biological analysis, and should be made available to other laboratories upon request to facilitate intercalibration.
5. After filtering, clean tubing and filter rig using dilute bleach (0.5% vol/vol sodium hypochlorite) and not HCl. The use of bleach ensures removal of potential residual contaminating DNA.
6. NOTE: If the analyst/laboratory are targeting larger biological size fractions for DNA extraction, larger volumes of water may be needed. Adjust the above sampling protocol accordingly and ensure exact volumes of filtered water are recorded.

3. DNA extraction

The following protocol is a modified version of the protocol reported in: Urakawa, H., W. Martens-Habbena, and D.A. Stahl (2010). High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. Appl Environ Microbiol 76(7):2129–2135.

1. Thaw filters on ice and warm up AMPure XP beads (Agencourt, Beckman Coulter) to room temperature.
2. Add filter to a bead beating tube (lysing matrix E tube; MP Biomedicals).

3. Add 400 µl of Phenol:Chloroform:Isoamyl Alcohol (25:24:1; pH 8.0; TE saturated) and 400 µl of 2x TENS Buffer (100 mM Tris-HCl [pH 8.0], 40 mM EDTA, 200 mM NaCl, 2% SDS) to the tube.
4. Disrupt each filter using a bead beater for 40 seconds at maximum speed.
5. Centrifuge bead-beating tubes at 15,000 RPM for 5 minutes.
6. Transfer the resulting aqueous phase to a 2.0-ml Phase Lock Gel tube (Eppendorf, Westbury, NY) and add an equal volume of chloroform to the tube. Mix thoroughly by repeated gentle inversion. Do not vortex.
7. Centrifuge the Phase Lock Gel tube at 15,000 RPM for 5 minutes and cleanly and carefully transfer supernatant to a 1.5 mL microcentrifuge tube.
8. Add an approximately equal volume of AMPure XP beads to supernatant and incubate at room temperature for 10 minutes.
9. Separate the AMPure XP beads and wash with 75% ethanol. Ensure all residual ethanol has evaporated and resuspend DNA pellet in 20 uL DEPC-treated water.
10. DNA concentrations should be quantified fluorescence based DNA assay or another high sensitivity DNA quantification method.

4. Metagenomic Library Preparation and Sequencing

1. Sequencing libraries should be prepared using Nextera XT kits (Illumina, San Diego CA). Library preparation should include tagmentation, barcoding, and enrichment steps.
2. Throughput of library preparation and sequencing can be dramatically increased by utilizing resources from an institutional sequencing center.
3. Labs should sequence to the degree they can afford. The MIT/Chisholm lab utilizes 150 nucleotide paired end reads on the Illumina NextSeq platform (Illumina, San Diego CA).

5. Sequenced Read Quality Control and Processing

Reads should be processed through quality control pipelines before assembly or annotation. The following is a recommended protocol.

1. Reads should be demultiplexed based on added barcode sequences.
2. Once demultiplexed, residual adapter contamination should be removed and nucleotides with sub quality phred scores removed.
3. Paired end reads are overlapped and merged to generate longer composite sequences.

X. 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.

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Appendix 2, Version 3.0

JGOFS Report 19, Determinations of POC/PON for GEOTRACES Cruises

Preface 1

Chapter 1 Introduction by Dr. A.H. Knap 3

~~Chapter 2 Shipboard Sampling Procedures~~

~~1.0 Introduction 5~~
~~2.0 Hydrocasts 5~~
~~3.0 Water Sampling 6~~
~~4.0 Primary Production 6~~
~~5.0 Sediment Trap Deployment and Recovery 7~~
~~6.0 Shipboard Sample Processing 7~~

~~Chapter 3 CTD and Related Measurements~~

~~1.0 Scope and field of application 9~~
~~2.0 Apparatus 9~~
~~3.0 Data Collection 11~~
~~4.0 Data Processing 12~~
~~5.0 References 16~~

~~Chapter 4 Quality Evaluation and Intercalibration~~

~~1.0 Introduction 19~~

~~Chapter 5 Salinity Determination~~

~~1.0 Scope and field of application 21~~
~~2.0 Definition 21~~
~~3.0 Principle of Analysis 21~~
~~4.0 Apparatus 21~~
~~5.0 Reagents 21~~
~~6.0 Sampling 22~~
~~7.0 Procedures 22~~
~~8.0 Calculation and expression of results 22~~
~~9.0 Quality assurance 23~~
~~10.0 References 23~~

~~Chapter 6 Determination of Dissolved Oxygen by the Winkler Procedure~~

~~1.0 Scope and field of application 25~~
~~2.0 Definition 25~~
~~3.0 Principle of Analysis 25~~
~~4.0 Apparatus 26~~
~~5.0 Reagents 27~~
~~6.0 Sampling 28~~
~~7.0 Tiration Procedures 29~~
~~8.0 Calculation and expression of results 31~~
~~9.0 Quality assurance 32~~
~~10.0 References 33~~

Chapter 7 The Determination of Total Inorganic Carbon by the Coulometeric Procedure

1.0	Scope and field of application	35
2.0	Definition	35
3.0	Principle of Analysis	35
4.0	Apparatus	36
5.0	Reagents	37
6.0	Sampling	38
7.0	Procedures	39
8.0	Calculation and expression of results	41
9.0	Quality assurance	41
10.0	References	42

Chapter 8 The Determination of Nitrite, Nitrate +Nitrite, Orthophosphate and Reactive Silicate in Sewater using continuous Flow Analysis

1.0	Scope and field of application	43
2.0	Definition	43
3.0	Principle of Analysis	45
4.0	Apparatus	46
5.0	Reagents	51
6.0	Sampling	54
7.0	Procedures and Standardization	57
8.0	Analytical Methods	65
9.0	Calculations	81
10.0	Quality Assurance	88
11.0	References	90

Chapter 9 The Determination of Nitrate in Sea Water

1.0	Scope and field of application	93
2.0	Definition	93
3.0	Principle of Analysis	93
4.0	Apparatus	94
5.0	Reagents	94
6.0	Sampling	94
7.0	Procedures	95
8.0	Calculation and expression of results	97
9.0	Notes	97
10.0	References	98

Chapter 10 The Determination of Nitrite in Sea Water

1.0	Scope and field of application	99
2.0	Definition	99
3.0	Principle of Analysis	99
4.0	Apparatus	99
5.0	Reagents	99
6.0	Sampling	100
7.0	Procedures	100
8.0	Calculation and expression of results	101
9.0	References	102

Chapter 11 The Determination of Phosphorus in Sea Water

1.0	Scope and field of application	103
2.0	Definition	103
3.0	Principle of Analysis	103
4.0	Apparatus	103
5.0	Reagents	103
6.0	Sampling	104
7.0	Procedures	104
8.0	Calculation and expression of results	105
9.0	References	106

Chapter 12 The Determination of Reactive Silicate in Sea Water

1.0	Scope and field of application	107
2.0	Definition	107
3.0	Principle of Analysis	107
4.0	Apparatus	107
5.0	Reagents	107
6.0	Sampling	108
7.0	Procedures	108
8.0	Calculation and expression of results	109
9.0	Notes	110
10.0	References	110

Chapter 13 Measurement of Algal Chlorophylls and Carotenoids by HPLC

1.0	Scope and field of application	111
2.0	Definition	111
3.0	Principle of Analysis	111
4.0	Apparatus and Reagents	112
5.0	Eluants	113
6.0	Sample Collection and Storage	113
7.0	Procedure	113
8.0	Calculation and expression of results	115
9.0	References	115

Chapter 14 Measurement of Chlorophyll a and Phaeopigments by Fluorometric Analysis

1.0	Scope and field of application	119
2.0	Definition	119
3.0	Principle of Analysis	119
4.0	Apparatus	119
5.0	Reagents	120
6.0	Sample Collection and Storage	120
7.0	Procedure	120
8.0	Calculation and expression of results	122
9.0	References	122

Chapter 15 Determination of Particulate Organic Carbon and Particulate Nitrogen

1.0 Scope and field of application 123
2.0 Definition 123
3.0 Principle of Analysis 123
4.0 Apparatus 123
5.0 Reagents 124
6.0 Sampling 124
7.0 Procedures 124
8.0 Calculation and expression of results 125
9.0 References 125

~~**Chapter 16 Determination of Dissolved Organic Carbon by a High Temperature Combustion/Direct Injection Technique**~~

~~1.0 Scope and field of application 127~~
~~2.0 Definition 127~~
~~3.0 Principle of Analysis 127~~
~~4.0 Apparatus 128~~
~~5.0 Reagents 129~~
~~6.0 Sampling 130~~
~~7.0 Procedures 131~~
~~8.0 Calculation and expression of results 134~~
~~9.0 Quality control/quality assessment 137~~
~~10.0 Notes 139~~
~~11.0 Intercomparison 141~~
~~12.0 References 142~~

~~**Chapter 17 Determination of New Production by ^{15}N**~~

~~1.0 Scope and field of application 145~~
~~2.0 Definition 145~~
~~3.0 Principle of Analysis 145~~
~~4.0 Apparatus 145~~
~~5.0 Reagents 146~~
~~6.0 Sampling 146~~
~~7.0 Procedures 146~~
~~8.0 Calculation and expression of results 147~~
~~9.0 Quality Control 148~~
~~10.0 Intercomparison 149~~
~~11.0 Parameters 149~~
~~12.0 References 149~~

~~**Chapter 18 Determination of Bacterioplankton Abundance**~~

~~1.0 Scope and field of application 151~~
~~2.0 Definition 151~~
~~3.0 Principle of Analysis 151~~
~~4.0 Apparatus 151~~
~~5.0 Reagents 152~~
~~6.0 Sampling 152~~
~~7.0 Procedures 152~~
~~8.0 Calculation and expression of results 153~~

9.0	Quality Control	153
10.0	References	154

Chapter 19 Primary Production by 14c

1.0	Scope and field of application	155
2.0	Definition	155
3.0	Principle of Analysis	155
4.0	Apparatus	156
5.0	Reagents and Supplies	156
6.0	Sampling	158
7.0	Procedures	160
8.0	Calculation and expression of results	160
9.0	Quality Control	161
10.0	Notes	162
11.0	References	162

Chapter 20 Determination of Bacterial Production using Methyltritiated Thymidine

1.0	Scope and field of application	163
2.0	Definition	163
3.0	Principle of Analysis	163
4.0	Apparatus	163
5.0	Reagents	164
6.0	Sampling and incubation	165
7.0	Procedures	166
8.0	Calculation and expression of results	166
9.0	Quality Control	167
10.0	Interpretation of results	168
11.0	References	168

Chapter 21 Determination of Bacterial Production using Tritiated Leucine

1.0	Scope and field of application	171
2.0	Definition	171
3.0	Principle of Analysis	171
4.0	Apparatus	172
5.0	Reagents	172
6.0	Sampling and incubation	173
7.0	Procedures	173
8.0	Calculation and expression of results	174
9.0	Other Remarks	175
10.0	References	176

Chapter 22 Microzooplankton Biomass

1.0	Scope and field of application	179
2.0	Definition	179
3.0	Principle	179
4.0	Apparatus	179
5.0	Reagents	180
6.0	Sampling	180
7.0	Procedures	180

8.0 Calculation and expression of results 182
9.0 Quality control and Assessment 183
10.0 Notes 183
11.0 Intercomparison 183
12.0 References &JGOFS papers published using these techniques 183

Chapter 23 Microzooplankton Herbivory
1.0 Scope and field of application 185
2.0 Definition 185
3.0 Principle 185
4.0 Apparatus 186
5.0 Reagents 186
6.0 Sampling 186
7.0 Procedures 187
8.0 Calculation and expression of results 189
9.0 Quality control and assessment 190
10.0 Notes 190
11.0 Intercomparison 190
12.0 References & JGOFS papers published using these techniques 190

Chapter 24 JGOFS Sediment Trap Methods
1.0 Introduction 193
2.0 Scope and field of application 193
3.0 Definition 193
4.0 Principle of Analysis 194
5.0 Apparatus 194
6.0 Reagents 194
7.0 Sampling 194
8.0 Post-collection Procedures 196
9.0 Calculation and expression of results 199
10.0 Quality Control/Quality Assessment 199
11.0 Intercomparison 200
12.0 Notes 200
13.0 References 200

Chapter 25 Trap-Collected Particle Flux with Surface-Tethered Traps
1.0 Scope and field of application 203
2.0 Definition 203
3.0 Principle of Analysis 204
4.0 Apparatus 204
5.0 Reagents 205
6.0 Sampling 205
7.0 Sample Processing Procedures 206
8.0 Calculation and expression of results 207
9.0 Quality Control and Assessment 207
10.0 References 208

Preface

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1.0 Scope and field of application

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

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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).

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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

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Appendix 2, Version 3.0

JGOFS Report 19, Determinations of POC/PON for GEOTRACES Cruises

Preface 1

Chapter 1 Introduction by Dr. A.H. Knap 3

~~**Chapter 2 Shipboard Sampling Procedures**~~

~~1.0 Introduction 5~~
~~2.0 Hydrocasts 5~~
~~3.0 Water Sampling 6~~
~~4.0 Primary Production 6~~
~~5.0 Sediment Trap Deployment and Recovery 7~~
~~6.0 Shipboard Sample Processing 7~~

~~**Chapter 3 CTD and Related Measurements**~~

~~1.0 Scope and field of application 9~~
~~2.0 Apparatus 9~~
~~3.0 Data Collection 11~~
~~4.0 Data Processing 12~~
~~5.0 References 16~~

~~**Chapter 4 Quality Evaluation and Intercalibration**~~

~~1.0 Introduction 19~~

~~**Chapter 5 Salinity Determination**~~

~~1.0 Scope and field of application 21~~
~~2.0 Definition 21~~
~~3.0 Principle of Analysis 21~~
~~4.0 Apparatus 21~~
~~5.0 Reagents 21~~
~~6.0 Sampling 22~~
~~7.0 Procedures 22~~
~~8.0 Calculation and expression of results 22~~
~~9.0 Quality assurance 23~~
~~10.0 References 23~~

~~**Chapter 6 Determination of Dissolved Oxygen by the Winkler Procedure**~~

~~1.0 Scope and field of application 25~~
~~2.0 Definition 25~~
~~3.0 Principle of Analysis 25~~
~~4.0 Apparatus 26~~
~~5.0 Reagents 27~~
~~6.0 Sampling 28~~
~~7.0 Tiration Procedures 29~~
~~8.0 Calculation and expression of results 31~~
~~9.0 Quality assurance 32~~
~~10.0 References 33~~

Chapter 7 The Determination of Total Inorganic Carbon by the Coulometeric Procedure

1.0	Scope and field of application	35
2.0	Definition	35
3.0	Principle of Analysis	35
4.0	Apparatus	36
5.0	Reagents	37
6.0	Sampling	38
7.0	Procedures	39
8.0	Calculation and expression of results	41
9.0	Quality assurance	41
10.0	References	42

Chapter 8 The Determination of Nitrite, Nitrate +Nitrite, Orthophosphate and Reactive Silicate in Sewater using continuous Flow Analysis

1.0	Scope and field of application	43
2.0	Definition	43
3.0	Principle of Analysis	45
4.0	Apparatus	46
5.0	Reagents	51
6.0	Sampling	54
7.0	Procedures and Standardization	57
8.0	Analytical Methods	65
9.0	Calculations	81
10.0	Quality Assurance	88
11.0	References	90

Chapter 9 The Determination of Nitrate in Sea Water

1.0	Scope and field of application	93
2.0	Definition	93
3.0	Principle of Analysis	93
4.0	Apparatus	94
5.0	Reagents	94
6.0	Sampling	94
7.0	Procedures	95
8.0	Calculation and expression of results	97
9.0	Notes	97
10.0	References	98

Chapter 10 The Determination of Nitrite in Sea Water

1.0	Scope and field of application	99
2.0	Definition	99
3.0	Principle of Analysis	99
4.0	Apparatus	99
5.0	Reagents	99
6.0	Sampling	100
7.0	Procedures	100
8.0	Calculation and expression of results	101
9.0	References	102

Chapter 11 The Determination of Phosphorus in Sea Water

1.0	Scope and field of application	103
2.0	Definition	103
3.0	Principle of Analysis	103
4.0	Apparatus	103
5.0	Reagents	103
6.0	Sampling	104
7.0	Procedures	104
8.0	Calculation and expression of results	105
9.0	References	106

Chapter 12 The Determination of Reactive Silicate in Sea Water

1.0	Scope and field of application	107
2.0	Definition	107
3.0	Principle of Analysis	107
4.0	Apparatus	107
5.0	Reagents	107
6.0	Sampling	108
7.0	Procedures	108
8.0	Calculation and expression of results	109
9.0	Notes	110
10.0	References	110

Chapter 13 Measurement of Algal Chlorophylls and Carotenoids by HPLC

1.0	Scope and field of application	111
2.0	Definition	111
3.0	Principle of Analysis	111
4.0	Apparatus and Reagents	112
5.0	Eluants	113
6.0	Sample Collection and Storage	113
7.0	Procedure	113
8.0	Calculation and expression of results	115
9.0	References	115

Chapter 14 Measurement of Chlorophyll a and Phaeopigments by Fluorometric Analysis

1.0	Scope and field of application	119
2.0	Definition	119
3.0	Principle of Analysis	119
4.0	Apparatus	119
5.0	Reagents	120
6.0	Sample Collection and Storage	120
7.0	Procedure	120
8.0	Calculation and expression of results	122
9.0	References	122

Chapter 15 Determination of Particulate Organic Carbon and Particulate Nitrogen

1.0	Scope and field of application	123
2.0	Definition	123
3.0	Principle of Analysis	123
4.0	Apparatus	123
5.0	Reagents	124
6.0	Sampling	124
7.0	Procedures	124
8.0	Calculation and expression of results	125
9.0	References	125

~~**Chapter 16 Determination of Dissolved Organic Carbon by a High Temperature Combustion/Direct Injection Technique**~~

~~1.0~~	~~Scope and field of application~~	~~127~~
~~2.0~~	~~Definition~~	~~127~~
~~3.0~~	~~Principle of Analysis~~	~~127~~
~~4.0~~	~~Apparatus~~	~~128~~
~~5.0~~	~~Reagents~~	~~129~~
~~6.0~~	~~Sampling~~	~~130~~
~~7.0~~	~~Procedures~~	~~131~~
~~8.0~~	~~Calculation and expression of results~~	~~134~~
~~9.0~~	~~Quality control/quality assessment~~	~~137~~
~~10.0~~	~~Notes~~	~~139~~
~~11.0~~	~~Intercomparison~~	~~141~~
~~12.0~~	~~References~~	~~142~~

~~**Chapter 17 Determination of New Production by ^{15}N**~~

~~1.0~~	~~Scope and field of application~~	~~145~~
~~2.0~~	~~Definition~~	~~145~~
~~3.0~~	~~Principle of Analysis~~	~~145~~
~~4.0~~	~~Apparatus~~	~~145~~
~~5.0~~	~~Reagents~~	~~146~~
~~6.0~~	~~Sampling~~	~~146~~
~~7.0~~	~~Procedures~~	~~146~~
~~8.0~~	~~Calculation and expression of results~~	~~147~~
~~9.0~~	~~Quality Control~~	~~148~~
~~10.0~~	~~Intercomparison~~	~~149~~
~~11.0~~	~~Parameters~~	~~149~~
~~12.0~~	~~References~~	~~149~~

~~**Chapter 18 Determination of Bacterioplankton Abundance**~~

~~1.0~~	~~Scope and field of application~~	~~151~~
~~2.0~~	~~Definition~~	~~151~~
~~3.0~~	~~Principle of Analysis~~	~~151~~
~~4.0~~	~~Apparatus~~	~~151~~
~~5.0~~	~~Reagents~~	~~152~~
~~6.0~~	~~Sampling~~	~~152~~
~~7.0~~	~~Procedures~~	~~152~~
~~8.0~~	~~Calculation and expression of results~~	~~153~~

9.0	Quality Control	153
10.0	References	154

Chapter 19 Primary Production by 14c

1.0	Scope and field of application	155
2.0	Definition	155
3.0	Principle of Analysis	155
4.0	Apparatus	156
5.0	Reagents and Supplies	156
6.0	Sampling	158
7.0	Procedures	160
8.0	Calculation and expression of results	160
9.0	Quality Control	161
10.0	Notes	162
11.0	References	162

Chapter 20 Determination of Bacterial Production using Methyltritiated Thymidine

1.0	Scope and field of application	163
2.0	Definition	163
3.0	Principle of Analysis	163
4.0	Apparatus	163
5.0	Reagents	164
6.0	Sampling and incubation	165
7.0	Procedures	166
8.0	Calculation and expression of results	166
9.0	Quality Control	167
10.0	Interpretation of results	168
11.0	References	168

Chapter 21 Determination of Bacterial Production using Tritiated Leucine

1.0	Scope and field of application	171
2.0	Definition	171
3.0	Principle of Analysis	171
4.0	Apparatus	172
5.0	Reagents	172
6.0	Sampling and incubation	173
7.0	Procedures	173
8.0	Calculation and expression of results	174
9.0	Other Remarks	175
10.0	References	176

Chapter 22 Microzooplankton Biomass

1.0	Scope and field of application	179
2.0	Definition	179
3.0	Principle	179
4.0	Apparatus	179
5.0	Reagents	180
6.0	Sampling	180
7.0	Procedures	180

8.0 Calculation and expression of results 182
9.0 Quality control and Assessment 183
10.0 Notes 183
11.0 Intercomparison 183
12.0 References &JGOFS papers published using these techniques 183

Chapter 23 Microzooplankton Herbivory
1.0 Scope and field of application 185
2.0 Definition 185
3.0 Principle 185
4.0 Apparatus 186
5.0 Reagents 186
6.0 Sampling 186
7.0 Procedures 187
8.0 Calculation and expression of results 189
9.0 Quality control and assessment 190
10.0 Notes 190
11.0 Intercomparison 190
12.0 References & JGOFS papers published using these techniques 190

Chapter 24 JGOFS Sediment Trap Methods
1.0 Introduction 193
2.0 Scope and field of application 193
3.0 Definition 193
4.0 Principle of Analysis 194
5.0 Apparatus 194
6.0 Reagents 194
7.0 Sampling 194
8.0 Post-collection Procedures 196
9.0 Calculation and expression of results 199
10.0 Quality Control/Quality Assessment 199
11.0 Intercomparison 200
12.0 Notes 200
13.0 References 200

Chapter 25 Trap-Collected Particle Flux with Surface-Tethered Traps
1.0 Scope and field of application 203
2.0 Definition 203
3.0 Principle of Analysis 204
4.0 Apparatus 204
5.0 Reagents 205
6.0 Sampling 205
7.0 Sample Processing Procedures 206
8.0 Calculation and expression of results 207
9.0 Quality Control and Assessment 207
10.0 References 208

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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.

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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:

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

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:

$$\text{Sample (ppm)} \times 71.43$$

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.