Sampling and Sample-handling Protocols for GEOTRACES Cruises

Edited by the 2024 GEOTRACES Standards and Intercalibration Committee:

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Version 4.0; August 2024

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1. 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 2010-2023, 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. Information regarding data management and data submission for inclusion in the GEOTRACES intermediate data product are also included.

Those who contributed to preparing these protocols are listed in Section 15 and are sincerely thanked for their efforts in helping GEOTRACES and the worldwide TEI community.

2. 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 in order to verify proper bottle and rosette operation and sampling depths (i.e., compare to the hydrography established with the conventional CTD/rosette). Experience to date indicates that routine nutrient samples and salinity samples in oceanic settings 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. Analyses at sea are preferable, especially for nitrite and ammonium.

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, or SAFe or GEOTRACES Consensus Intercalibration samples as described in the Trace Element Section (Shipboard Determinations of Selected Dissolved Trace Metals), 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 (Section 3.2). Except where activities are reported (e.g., radionuclides), we recommend concentration units be in fractions of a mole per unit mass (kilogram) or volume (liter; most appropriately when shipboard analyses are used) - µmol l^{-1} or nmol kg^{-1} as examples. Use of capital "M" to indicate moles l^{-1} should not be used because this causes confusion in the GEOTRACES data base.

3. SUBMISSION OF DATA, METADATA AND INTERCALIBRATION REPORTS

3.1. DOOR PORTAL

The GEOTRACES Data for Oceanic Research (DOoR) Portal has been designed in order to help on the task of having the data registered and intercalibrated by the Standards and Intercalibration (S&I) Committee for inclusion in the GEOTRACES Intermediate Data Product (IDP).

The DOoR portal is a user-friendly on-line portal available at the following link: https://geotraces-portal.sedoo.fr/pi/ . Users can login to DOoR directly by using their own ORCID identifiers (https://orcid.org) and no specific registration step is needed.

DOoR is open to accept registrations at any time. Indeed, it is highly recommended to register the datasets **before the departure of the cruise** and not to wait until the official IDP deadlines to start the process (for more details see the flowchart here).

Important preliminary notes are that:

1. DOoR does not accept or store data. Data is not submitted through DOoR but directly to the GEOTRACES Data Assembly Centre (GDAC, geotraces.dac@noc.ac.uk) **or the relevant US/Dutch/French/Chinese national data centre.** This needs to be done concurrently with the registration and intercalibration process through DOoR.

2. Completing the registration and submission of the intercalibration report through DOoR, and submitting the data to the appropriate data centre, do not imply the data will be automatically included in the IDP.

3. Inclusion of data in IDP is only guaranteed once:
(1) the registration and submission of the completed S&I report through DOoR is finalised before the established deadlines,
(2) the S&I Committee has approved the datasets and,
(3) the permission is given for the data to be included in IDP. Without the explicit permission for the data to be included in the IDP (through DOoR), the data will NOT be included and released in the IDP.

The DOoR portal allows users to complete the registration and submission of the S&I report by going through 6 steps, each one having a dedicated page in the portal:

(Step 1) Register datasets for inclusion in the IDP. The main objective of this step is to attribute a barcode that allows uniquely identification and tracking of the dataset along the whole process. To register the datasets, users just need to select the cruise from the list of GEOTRACES cruises available and then select the appropriate name of the parameter (see section 3.2) using a drop-down menu tool.

(Step 2) Provide names of associated scientists (students, postdocs, etc.) with each dataset to ensure they are duly acknowledged. Users need to provide only the ORCID number and e-mail address of the associated scientist. DOoR will then obtain from ORCID the metadata associated with this user. This ensures that DOoR keeps automatically track on any future change of these metadata (e.g. future changes on the name or affiliation of the person). In addition, users are asked to identify who the principal investigator for the dataset is (defined as the scientist who should in principle grant permission for the data to be included in IDP). This allows this person to edit and keep track of the dataset in DOoR in addition to the person who has actually registered the data in DOoR (if a different person).

(Step 3) Generating and downloading of templates needed to (a) submit their datasets for intercalibration, (b) be used for data submission to the appropriate data centre. In the case of multiple datasets being submitted, the users can select the datasets to be included in the intercalibration report or select all the datasets to group all of them in a single report.

(Step 4) Submitting the intercalibration report to the S&I Committee. In this page, users can upload and submit the completed S&I report. Once the report is submitted the S&I committee is informed about it. Once approved, the users receive an e-mail notification from DOoR.

(Step 5) Giving permission for the release in IDP2021 for each dataset registered. This is an important step. Users can select the datasets they give permission to be included in the IDP. If permission is not given the dataset will not be included in the IDP.

(Step 6) Linking publications that must be cited when using the datasets. In this page, users can provide the DOI of any publication that needs to be cited when using their data. This feeds a dynamic database so it can be updated at any time ensuring that any publication added even after the release of the IDP is available in the IDP reference links.

A final summary page allows for users to track the status of their datasets (status of the intercalibration process and of the data processing). In addition, once the GEOTRACES Data Assembly Centre has processed their data, the users receive a notification e-mail from DOoR informing them that proof checks of their data are available on this page giving them the opportunity to review them before the data is being included in the IDP.

More detailed information on the DOoR functions is available on the DOoR landing page, including a *How to document* or a *Video guide* available on *Youtube* and *Youku* *(for Chinese researchers)*.

In addition, DOoR is also the management tool for each specific group that works on assembling the Data Product (the Standards and Intercalibration Committee, the GEOTRACES Data Assembly Centre, the Data Management Committee, the GEOTRACES Parameter Definition Committee and the International Project Office) and gives each of these groups a dedicated interface to help them perform their work.

3.2. Parameter naming conventions

All of the GEOTRACES parameter names that are used in the Intermediate Data Products are sorted into Domains; these are used to separate different types of datasets and to cluster different sets of parameters:

Aerosols - all measurements associated with aerosols, with different collection and analytical methods.

BioGEOTRACES - Biological measurements (including pigments, DNA parameters, cell quotas and proteins)

Dissolved TEIs - dissolved trace metals, ligands, radionuclides, rare earth elements and their isotopes

Hydrography and Biogeochemistry - Including temperature, salinity, oxygen, major nutrients and their isotopes and noble gases

Particulate TEIs - particulate trace metals, ligands, radionuclides and major phases, and their isotopes

Precipitation - all measurements associated with rain and with freshly falling snow, with different collection and analytical methods.

Polar - unique samples from polar expeditions, also cross listed with "Dissolved TEIs," "Precipitation," "Particulate TEIs," "Hydrography and Nutrients," "Ligands" and, with "BioGEOTRACES" to facilitate locating this information.

Ligands - dissolved and particulate ligands also cross listed with "Dissolved TEIs", "Particulate TEIs" and with "Polar" to facilitate locating this information.

GEOTRACES Intermediate Data Products employ the following parameter naming scheme. Standard hydrographic parameters, such as temperature, salinity and oxygen use names as defined in the WOCE/CLIVAR naming convention (CTDTMP, CTDSAL and CTDOXY for temperature, salinity and oxygen from CTD sensors; https://exchange-format.readthedocs.io/en/latest/parameters.html). Other hydrographic and biogeochemistry parameters use names defined intuitively. Examples are CTDPRS for the CTD pressure at the bottle sample depth, SALINITY, PHOSPHATE, NITRATE, and SILICATE for salinity, phosphate, nitrate and silicate measured on bottle samples. Note that NO3 and NO2 are used in the Precipitation Domain parameter names. Biogeochemistry parameters use names defined by SCOR naming conventions (e.g., HPLC pigments; Roy et al., 2011) or names that intuitively define the parameters (e.g., nifHUCYN-ADNAPCONCBOTTLE; concentration of nifH genes from uncultured unicellular cyanobacteria (UCYN-A) particles (P) in a bottle sample).

All other trace elements and isotope names are composed of up to six separate tokens as shown below. Tokens 2 and 3 are optional, while all other tokens are mandatory for trace elements and isotopes, nutrients and biogeochemistry parameters. A few physical parameters that do not align with the convention of 4 to 6 tokens are exempt from the requirement to include Phase, Data Type and Sampling system (see examples below). This is indicated by "NONE" in the examples list.

1	2	3	4	5	6
Element/ Compound	[Oxidation State]	[Atomic Mass]	Phase	DataType	Sampling System

Explanations

#	Explanation	Example
1	Element or compound (mandatory); information about TEI speciation (e.g., methylation of TEIs, such as MeMM, MeDM and free inorganic elements (Me')) is incorporated into the first token.	Fe, Th, DIC, NITRATE, L1Fe
2	Oxidation state as roman number (optional)	II, IV, IIIV where III and V are combined
3	Atomic mass (optional); two entries for isotope ratios	228, 208204
4	Phase on which element or compound was measured (mandatory); may include two components (e.g., RTD refers to the Total Dissolvable concentration of a constituent in Rain). The default is "seawater" when the medium (e.g., R (rain), A (aerosol), SNOW, ICE is not given. Note that "CELL" is	A (aerosol) C (colloidal) D (dissolved) DL (dissolved labile) LPT (large particulate, total (unleached)) R (rain) S (soluble)

	considered a phase where cell quotas are presented.	SMLH2O (soluble mild leach with ultrapure water) SMLSW (soluble mild leach with seawater) SSLNH4AC (soluble strong leach with ammonium acetate) SSLHAC (soluble strong leach with acetic acid) SP (small particulate) SPL (small particulate, labile fraction) SPR (small particulate, refractory fraction) SPT (small particulate, total (unleached)) T (total: dissolved plus particulate) TD (total dissolvable) TP (total particulate) TPL (total particulate, labile fraction) TPR (total particulate, refractory fraction) CELL (specific individual cell) ICE (ice) ICED (dissolved on melted ice) ICETD (total dissolvable on melted ice) ICET (total dissolved plus particulate on melted ice) ICETP (total particulate on melted ice) ICETPL (total particulate, labile fraction, on melted ice) SNOW (snow) SNOWD (dissolved on melted snow) SNOWTD (total dissolvable on melted snow) SNOWT (total dissolved plus particulate on melted snow) SNOWTP (total particulate on melted snow) SNOWTPL (total particulate, labile fraction, on melted snow)
5	DataType (mandatory)	CONC (concentration) DELTA (isotope ratio in delta notation) EPSILON (isotope ratio in epsilon notation) LogK (log of binding constant of ligand) RATIO (atomic abundance ratio of isotopes or molecular ratio of gases)
6	Sampling system (mandatory)	BOTTLE (Niskin or similar water sampling bottle) FISH (trace-metal clean towed surface sampler) PUMP (either *in-situ* pump or on-deck pump) UWAY (ship's underway surface seawater)

		HIVOL (high-volume aerosol sampler) LOWVOL (low-volume aerosol sampler) FINEIMPACTOR (size-fractionated aerosols, small fraction) COARSEIMPACTOR (size-fractionated aerosols, large fraction) AUTO (automated rain or snow sampler) MAN (rain or snow sampler with manual on-off controls) CORER (ICE cores) GRAB (ICE or SNOW grab samples) SUBICEPUMP (seawater collected from an ice floe using a pump) BOATPUMP (seawater collected from a small boat using a pump) MELTPONDPUMP (collected from a meltpond using a pump)

Examples

Parameter Name	Parameter description
Fe_D_CONC_BOTTLE	Concentration of dissolved Fe
Fe_II_D_CONC_BOTTLE	Concentration of dissolved Fe(II)
Fe_II_TP_CONC_BOTTLE	Concentration of total particulate Fe(II) determined by filtration from a water sampling bottle
Fe_TPL_CONC_BOTTLE	Concentration of labile particulate iron determined by filtration from a water sampling bottle
Nd_143144_D_RATIO_BOTTLE	Atom ratio of given isotopes for dissolved Nd
Nd_143144_D_EPSILON_BOTTLE	Atom ratio of dissolved Nd isotopes expressed in conventional EPSILON notation
Cd_114110°D°DELTA_BOTTLE	Atom ratio of dissolved Cd isotopes expressed in conventional DELTA notation
Cu_Cu'_D_CONC_BOTTLE	Concentration of dissolved inorganic Cu
Pb_206204_D_RATIO_BOTTLE	Atom ratio of given isotopes for dissolved Pb
DIC_1312_D_DELTA_BOTTLE	Atom ratio of given isotopes for dissolved C as DIC in delta notation
DIC_1412_D_DELTA_BOTTLE	Atom ratio of radiocarbon as dissolved C in DIC in DELTA notation
NITRATE_1514_D_DELTA_BOTTLE	Atom ratio of given isotopes for dissolved N as nitrate in delta notation
L1Fe_D_CONC_BOTTLE	Concentration of dissolved L1 Fe-binding ligand
L1Fe_D_LogK_BOTTLE	Log of the stability constant of L1 Fe
HOMOCYS_D_CONC_BOTTLE	Concentration of dissolved homocysteine
Chl a_HPLC°TP_CONC_BOTTLE	Concentration of particulate Chlorophyll a measured using HPLC method

nifHUCYN-ADNA_TP_CONC_BOTTLE	Abundance nifH Uncultured unicellular cyanobacteria (UCYN-A)
Al_AT_CONC_HIVOL	Total aerosol Al concentration, high-volume sampler
Al_AS_MLH2O_CONC_HIVOL	Soluble aerosol Al concentration, mild leach with ultrapure water, high-volume sampler
Al_AS_MLSW_CONC_HIVOL	Soluble aerosol Al concentration, mild leach with seawater, high-volume sampler
Al_AS_MLH2O_CONC_COARSEIMPACTOR	Soluble aerosol Al concentration, mild leach with ultrapure water, coarse fraction, impactor sampler
Al_AS_MLH2O_CONC_FINEIMPACTOR	Soluble aerosol Al concentration, mild leach with ultrapure water, fine fraction, impactor sampler
Al_AS_SLNH4AC_CONC_HIVOL	Soluble aerosol Al concentration, strong leach with ammonium acetate, high-volume sampler
Al_AS_SLNH4AC_CONC_COARSEIMPACTOR	Soluble aerosol Al concentration, strong leach with ammonium acetate, coarse fraction, impactor sampler
Al_AS_SLNH4AC_CONC_FINEIMPACTOR	Soluble aerosol Al concentration, strong leach with ammonium acetate, fine fraction, impactor sampler
PH_SWS_BOTTLE	Example of a parameter that is exempt from the requirement to include Phase, Data Type and Sampling System: pH measured on the seawater scale that is measured on a water sample collected with a bottle.
T_MP_ICECORER	Example of a parameter that is exempt from the requirement to include Phase, Data Type and Sampling System: Temperature measured by inserting a probe into a hole drilled into ice.

3.3. Protocols for Metadata acquisition

The metadata of marine chemical measurements includes all the information that describes the context under which a particular dataset was obtained. Without this information, the reusability of the data could be compromised, even if only used by the original data generator (e.g., using temporary, undefined abbreviations to label measured parameters). Therefore, as a best practice for each dataset, essential metadata should be recorded and gathered into one single file once the measured data is in its final version. The idea is to provide essential information about the content of a dataset in order to support its reusability and facilitate potential linking and merging with other datasets that share a similar context. For full in-depth methodology see Swift, J. H. (2010). The following sections detail GEOTRACES metadata and data submission instructions and requirements.

- During Cruise:

Metadata collection begins before the cruise with the preparation of event and CTD log sheets. Once the cruise has begun, these log sheets should be printed off and left in a prominent place in the main laboratory. They should be completed as a matter of routine. The completed forms should be digitized and the originals left accessible for all to refer to. These forms are the definitive metadata records on the ship. If everyone refers to these for basic event information, as opposed to individually noted sampling times/positions, metadata uniformity should be achieved.

- *Activity:*

Most discrete marine chemical data sampling is conducted during research cruises aboard dedicated research vessels, making it a major type of deployment. Therefore, the focus here will be on this type of deployment. Research cruises are assigned a unique ID, which must be provided, along with the name of the Chief Scientist(s), and the start and end dates and ports, including countries. It is highly important to include this information in publications or potentially link it to existing persistent weblinks to the cruise description, such as Cruise Summary Reports (https://csr.seadatanet.org/).

- *Event log:*

An event log sheet is designed to record all scientific activities. This can be generated from a central electronic log from the bridge or via a more manual process. It needs to include the following information:

- Event ID:

A unique reference for each individual scientific activity.
Sampling dates and times should be recorded in UTC, to have a fixed time reference. Both start and end times should be recorded and supplied for all events, except single point samples which only require start times.
The importance of the 'time of sampling' field on metadata forms can never be overstated. This should always be noted from the ship relay screens, not from watches or personal computers - these are invariably less accurate.

- Sampling positions:

Both start and end latitudes and longitudes should be recorded and supplied for all events, except single point samples, which only require start latitudes and longitudes.
Station ID (names/numbers):
Scientists should adopt a consistent "Event log" scheme that includes station numbers (or names) along with event numbers. Station is a rough location identified as an important sampling site as part of the research question or it could be a location designed to be resampled in future independent cruises.

- Sampling equipment:

This represents the sampling device and could be identified as an acronym or abbreviation referenced in detail in the cruise report. e.g. CTD, Trace Metal CTD, FISH, PUMP, etc.

- Gear ID:

A unique reference related to the sampling equipment, separate from event id. Eg. CTD001, CTD002, FISH001, etc.

- *Sampling log:*

A sampling log sheet is designed to keep track of the samples taken from CTD rosette bottles or other water sampling techniques. Information on which samples were collected, from which bottles, and who did the sampling should be recorded.

- Link to unique EVENT ID and/or GEAR ID

Be consistent in linkage to the event logs.

- Actual sample depth

Whilst nominal depth is often recorded, having a record of the actual sample depth can help prevent confusion and mislinkage of data to bottles at later stages.

- Rosette number and firing sequence

Record the position of the sampling bottle on the rosette and the firing sequence if data is from a CTD cast.

- Sampling ID

Use a Sampling ID that is unique for each sample throughout the duration of the cruise or a unique persistent identifier for that sample. The identifiers are critical to accurately resolving data records.

- Additional metadata for specific sensors/samples:

CTD Sampling Data: - Type, manufacturer, number of, and capacity of water bottles.
- Any additional problems relating to each CTD deployment - bottle firing malfunctions or bottle leaks.

CTD Profiles:

- Type, manufacturer, model, and serial number of every sensor deployed on the frame. For light sensors include measured wavelength (in nanometres or stated as red/blue/green light) and path length (10/20/25 cm).
- Sensor calibration information (date of calibration, manufacturer, and equations)
- Full details of any sensor swaps that take place during the cruise (and associated calibration information), i.e. which sensors are used during which CTD deployments.
- Any additional problems relating to each CTD deployment - sensor data quality issues, etc.

Underway system/FISH:

- Full record of any discrete water samples taken from the underway system (noting the time of sampling collection is vital).

Stand-alone pumps (SAPs)

- Pump system type, make, model, and settings.
- Deployment/sample identifier.
- Start and end time of deployment.
- Depth of sample.
- Volume of water pumped.
- Size of filter used.

- <u>Post Cruise:</u>
  - *Sampling Data*

Submit your completed metadata template along with your data in the data template generated by DOoR to GDAC or your National Data Centre.

- *Metadata Template*

Provide information on all sampling and analytical equipment (manufacturer and model number if applicable).
Provide details on the sampling and analytical protocols.

To maintain brevity and coherence, it is acceptable to reference existing method(s) to publications that contain the necessary information, while ensuring that any modifications made by the data samplers and/or analysts are clearly highlighted. The publication references should also be included in a reference list, with full journal references and hyperlinks to the publications, if available.

- *Data Template*

• Populate your sampling metadata.
• Keep all columns representing parameter, parameter standard deviation, and flag in the same format. Do not modify the template structure.
• Preferred uncertainty is 1 Standard deviation (1SD), any other uncertainty may be converted to 1SD during ingestion or not included.
• Provide data preferably without replicates, by performing any calculations, for example calculating the average values and their standard deviation, required before submission.
• Apply data quality control flags. The IDP uses SeaDataNet flags as shown in Table 1.
• Any bottle issues that need to be recorded should be supplied using the bottle flags as shown in Table 2. Other flags are permitted; however, information and mapping must be provided in the metadata template.

- *Data quality flags*

Table 3.1. SeaDataNet measurement qualifier flags.

Flag	Label
0	no quality control
1	good value
2	probably good value
3	probably bad value
4	bad value
5	changed value
6	value below detection
7	value in excess
8	interpolated value
9	missing value
A	value phenomenon uncertain
B	nominal value
Q	value below limit of quantification

Table 3.2. BODC water sampler qualification flags

Flag	Label
0	No problem reported
1	Filter burst
2	Leakage contamination
3	Bottle misfire
4	Bottles fired in incorrect order
5	Bottle leak
6	Partial sample loss
7	No sample
8	Questionable depth
9	Vent left open

- *CTD Profile Data*

Metadata

• Provide all the information listed in the "Additional metadata for specific sensors/samples" section.
• Provide information on post-cruise calibrations (including equations where appropriate, e.g. oxygen, salinity, fluorescence).
• Provide information to link the profile data to the samples data, date/time (UTC) and/or event number.
• Clearly state null data value used within data file.
• Provide list of variables, units, and sensor used to produce the output.

Data

• CTD data for the whole cruise can be submitted as 1 file per CTD event (identify in filename the date/time and/or event number) or 1 file for the whole cruise. If submitting only 1 file, there must be a column/s for date/time and/or event number.
• Data should be binned to 1 or 2 dbars.
• Provide data as a matrix with pressure (preferred) or depth (acceptable) as the uniquely identifying column (primary key).
• Column headers should clearly describe the nature of the data, preferably use the GEOTRACES parameter names. Units must be provided, either in the data file or a separate metadata file.

- *Data Credit:*

The names of the data originator(s) should be listed in the order of their contribution, along with their organizational affiliations, like how it is done in any other scientific publication. This ensures that credit is given to the individuals and organizations responsible for creating the dataset. Additionally, the project(s) that funded the work leading to the dataset should be clearly identified, including its acronyms, grant ID, and funding organization, to serve similar purposes.

References

Swift, J. H. (2010) Reference-Quality Water Sample Data: Notes on Acquisition, Record Keeping, and Evaluation. In, The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. Version 1, (eds Hood, E.M., C.L. Sabine, and B.M. Sloyan), 38pp. (IOCCP Report Number 14; ICPO Publication Series Number 134). DOI: https://doi.org/10.25607/OBP-1346

4. 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; Becker et al. 2020 doi: 10.3389/fmars.2020.581790). 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), 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 is an additional suite of ancillary parameters that are not required for every GEOTRACES cruise, but provide invaluable information on water mass tracing and transport – for example the chlorofluorocarbons, sulfur hexafluoride, and ^{3}He, $d^{13}C$ DIC, etc. 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). 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 (2024).

Table 4.1. 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
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

References

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. Updated in 2019

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 11.

5. RADIOCATIVE ISOTOPES

5.1. 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.

- *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.

- *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.

- *Sample filtration*

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

5.1.1. Sampling for dissolved species

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.

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.

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.

5.1.2. Sampling for 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 230Th and 231Pa 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.

5.1.3. 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.

5.1.4. 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. Labelling of samples should be made with a specific GEOTRACES # for each sample and depth.

5.1.5. Sample volume or weight

A variety of approaches have been used to record sample weight or volume, and the literature should be consulted for the best one to use in a particular cruise (e.g., open water vs. in the

ice). Some labs use an electronic balance to weigh samples at sea, using a simple computer algorithm to average weights on the moving ship until a stable reading is obtained. Other labs weigh samples after they are returned to the home institution.

5.1.6. 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.

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.

5.1.7. Pre-concentration

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

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

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

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

Sample storage: We are not yet sure how long we can store filtered acidified samples for subsequent spiking, pre-concentration and analysis without losing ^{230}Th or ^{231}Pa on the walls of the containers. Samples collected during the first GEOTRACES intercalibration cruise (July, 2008), acidified to pH 1.7, and analyzed over a period of 1.5 years showed no drift in concentrations of dissolved Th or Pa. NOTE: For samples stored this long it is necessary to

make corrections for ingrowth of dissolved ^{230}Th and ^{231}Pa due to radioactive decay of dissolved uranium. The different scavenging methods ($Fe(OH)_3$ vs. $Mg(OH)_2$ vs. MnO_2) still have to be compared.

5.1.8. 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.

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).

References

Anderson, R. F. and others 2012. GEOTRACES intercalibration of ^{230}Th, ^{232}Th, ^{231}Pa, and prospects for ^{10}Be. Limnology and Oceanography: Methods 10: 179-213.

Choi, M.-S., R. Francois, K. Sims, M. P. Bacon, S. Brown-Leger, A. P. Fleer, L. Ball, D. Schneider, and S. Pichat. 2001. Rapid determination of ^{230}Th and ^{231}Pa in seawater by desolvated-micronebulization Inductively-Coupled Magnetic Sector Mass Spectrometry. Mar. Chem., 76, 99-112.

Shen, C.-C., Edwards, R. L., Cheng, H., Dorale, J. A., Thomas, R. B., Moran, S. B., Weinstein, S. E., Edmonds, H. N. 2002. Uranium and thorium isotopic and concentration measurements by magnetic sector inductively coupled plasma mass spectrometry. Chem. Geol., 185, 165-178.

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

5.2. PROTOCOLS FOR ^{234}TH

5.2.1. 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 234Th samples.

5.2.2. 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 1mm, 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 mm) particle collection, 51mm 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.

5.2.3. Pump deployment and handling

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

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

5.2.4. 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.

5.2.5. 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:

A. 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.

B. 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.

C. 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.

References

Buesseler, K.O. et al., 2001. An intercomparison of small- and large-volume techniques for thorium-234 in seawater. Marine Chemistry, 74(1): 15-28.

Buesseler, K.O. et al., 1992. Determination of thorium isotopes in seawater by non-destructive and radiochemical procedures. Deep-Sea Res., 39(7/8): 1103-1114.

Cai, P., Dai, M., Lv, D. and Chen, W., 2006. How accurate are the 234Th measurements in seawater based on MnO_2-impregnated cartridge technique? Geochemistry, Geophysics, Geosystems, 7: Q03020 DOI 10.1029/2005GC001104.

Pike, S.M., Buesseler, K.O., Andrews, J. and Savoye, N., 2005. Quantification of Th-234 recovery in small volume seawater samples by inductively coupled plasma-mass spectrometry. Journal of Radioanalytical and Nuclear Chemistry, 263(2): 355-360.

Rutgers van der Loeff, M. et al., 2006. A review of present techniques and methodological advances in analyzing 234Th in aquatic systems. Marine Chemistry, 100(3-4): 190-212.

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

5.3. 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 reduced considerably the bottle blank (Moore et al., 1985).

During the Atlantic GEOSECS cruise ^{228}Ra was measured by extracting radium from large volume (200-600 L) sea water samples by $Ba(Ra)SO_4$ precipitation followed by sample cleanup and extraction of partially equilibrated ^{228}Th using alpha spectrometry (Li et al., 1980). This large volume sample was used to measure the ^{228}Ra/^{226}Ra activity ratio. This ratio was multiplied by the ^{226}Ra activity to determine ^{228}Ra activity. On Pacific and Indian Ocean GEOSECS cruises, large volume samples were extracted onto Mn-fiber either on deck or *in-situ* followed by sample cleanup and measurement of partially equilibrated ^{228}Th (Moore 1976). On TTO water samples (270 L) were first stripped of CO_2 for ^{14}C measurements and after pH adjustment, radium was extracted onto Mn-fiber (Moore et al., 1985). Workers have since 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.

For the U.S. GEOTRACES program, short (^{223}Ra & ^{224}Ra) and long (^{226}Ra and ^{228}Ra) radium isotopes were simultaneously collected using *in-situ* pumps to concentrate them onto a 5-micron grooved acrylic (phenolic resin) filter cartridge produced by 3M (G80B2) At the time of publication, 3M is no longer producing these cartridges; Lenntech has been used by U.S. and French GEOTRACES participants as an alternative supplier. Cartridges were ordered as 10" (25.4 cm) length and cut down to 4.125" (10.5 cm). The Mn cartridges were loaded in 5" (12.7 cm) cartridge holders and mounted on a McLane Pump Large Volume Water Transfer System (WTS-LV). The cartridges receive seawater that has been prefiltered for particles by Supor and QMA 145mm filter media. Initial coatings of manganese on the cartridges were performed by submerging them in 0.5M of potassium permanganate ($KMnO_4$) solution in large

recirculating baths for 48 hours at room temperature (Henderson et al., 2013). Excess Mn was rinsed off using radium-free water and dried. The method later evolved to vacuum sealing the cartridges in 4mil plastic chamber pouches containing 300 mL of the same concentration Mn solution for a period of 2 weeks. This change in technique allowed for a deeper and more consistent penetration of Mn into the filter media resulting in improved *in-situ* Ra isotope extraction efficiency (typically 65-75% at 6 L/min). Changes were also made to the 5" filter housings, which included a spring plate to help maintain a constant cartridge seal during deployment. Extracting the radium isotopes using the *in-situ* pumps not only allowed for 8 pumps to be deployed on a hydrowire at the same time, but also allowed for large filter volumes (1200-1600L/deployment) over a relatively short sampling time (~4 hours pumping). Due to the high demand for wire time with the GEOTRACES cruises, filtration flow rates of ~6L/min were required for the WTS-LV pumps. This increase in filtration rate requires a small volume sample (~20L) to be collected in parallel and filtered through Mn fiber (<1L/min) on board the research vessel for calibration of each individual cartridge's radium extraction efficiency. The Mn fiber is analysed for ^{226}Ra (alpha scintillation) and the radium extraction efficiency is applied to all radium isotopes assuming similar retention.

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

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

5.3.1. 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 He 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 Mn-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) or RaDeCC (Geibert et al., 2013). The Mn-fiber is sealed in a column for several days to weeks and then connected to a closed loop with the RAD7 or RaDeCC. The circulating air carries ^{222}Rn and ^{220}Rn to the detector chamber where their polonium daughters are measured by alpha-spectrometry. These two methods are much less sensitive than the approach described above and therefore larger volumes are needed for open ocean samples. The ^{226}Ra on Mn cartridges used on U.S. GEOTRACES cruises have been analyzed by the RaDeCC method.

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 ingrowth. 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.

5.3.2. Measurements of ^{226}Ra and ^{228}Ra by precipitation from small volume 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.

Sampling procedure:

- Use a pre-weighed container, note empty weight in logsheet 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 logsheet
- 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; 100ml 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 tubs suitable for gamma spectrometry; samples should be sealed with e.g. Parafilm.

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 prefiltered 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 logsheet.
- 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: 3 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.

5.3.3. Measurement of ^{228}Ra via ^{228}Th ingrowth

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.

With recent advances in the collection of radium samples through Mn coated cartridges, determination of ^{228}Ra was made through sequential measurements on the RaDeCC delayed coincidence detectors without chemical purification. Measurements are performed periodically for 1-3 years after collection to allow enough time for ^{228}Th ingrowth (Moore, 2008). This method was chosen instead of traditional gamma spectroscopy because samples were collected in the South Pacific Ocean where ^{228}Ra levels are typically an order of magnitude lower than average North Atlantic Ocean activities. Being able to measure long lived radium directly from the cartridge significantly reduces the processing time associated with chemical purification of samples.

Procedures for preparing Mn-fiber are detailed in Moore (1976) and Rutgers van der Loeff and Moore (1999). Options for Mn-cartridge preparation are described in Henderson et al. (2013) with updates noted in the introductory section above.

5.3.4. 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 in underground, low-background labs have 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. Alternately, the Ra may be coprecipitated with BaSO4. 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 ingrowth 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. If using the peak at 338 keV for gamma spectroscopy, a correction needs to be made to account for ^{223}Ra also present at this energy through decay of its parent ^{227}Ac. This can be significant for samples collected below 1000 m and account for 20-25% of the total signal. The interference correction can be made based upon peak data from ^{223}Ra at 270 keV. 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. Low background gamma facilities have been used to quantify ^{228}Ra in GEOTRACES samples where expected activities are low (van Beek et al., 2010; Sanial et al., 2018; Hult et al., 2019).

A gamma spectrometer may also be used to quantify ^{228}Ra via ^{228}Th ingrowth using the strong ^{212}Pb gamma line at 239 keV. As with the RaDeCC-based approach, this method requires a measurement of ^{228}Th near the time of collection as well as sufficient time for production of ^{228}Th in the sample (1-3 years). However, at 2-years post collection, the intensity of the 239 keV peak is a factor of 4 times higher than the ^{228}Ac peak at 338 keV. When integrating the area under the 239 keV peak, care must be taken to separate the influence of a much larger peak at 241 keV associated a daughter product of ^{226}Ra.

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).

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

The method of choice for the analysis of ^{223}Ra (half life = 11.4 days) and ^{224}Ra (half life = 3.66 days) is the delayed coincidence technique of Moore and Arnold (1996). Samples are collected in 100-1000 liter tanks. In turbid waters samples are filtered (e.g., 1-µm Hytrex II cartridge). The filtrate is then passed through a column of MnO_2-coated acrylic fiber ("Mn-fiber") at <1 l/min to quantitatively remove radium (Moore et al., 1985; Moore, 2007). 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). 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).

Selzer, et al. (2021) developed a Python program to automaticly read and process data from RaDeCC systems. The program also provides estimates of uncertainties.

During the measurement of samples in RaDeCC, ^{226}Ra on the cartridge produces ^{222}Rn. Although the process is slow (only about 4% of total ^{222}Rn is generated in a 6-hour counting period), the large activity of ^{226}Ra on the cartridge results in a significant increase in the total count rate during the measurement period. As the total count rate increases, the chance coincidence rate increases nonlinearly. Users typically correct for chance coincidence counts using equations in Moore and Arnold (1996); however, these are not accurate if the sample has low activity and the chance coincidence count rate, which is increasing during the measurement, is especially high. Hammond (2023) developed a MATLAB routine for correcting RaDeCC data for chance coincidence counts and other interferences during a measurement period. The routine also assesses leaks and spurious counts and supplies a standard deviation or uncertainty of each measurement.
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).

Similar steps for measuring short lived radium isotopes collected on Mn cartridges and analyzed on RaDeCC delayed coincidence counters are followed. After collection of samples, radium free freshwater is used to rinse off any salt present on the filter media and dried to removed excess moisture. The ideal moisture content range was determined through experimentation with a ratio of 0.1-1.2 {water (g) / cartridge (g)}. Care needs to be taken in-between analysis of cartridge samples due to moisture build up within the recirculation loop of the RaDeCC detection system. Cartridges typically lose of ~1 gram of moisture from the cartridge in roughly 4 hours of analysis. Pumps run for 1 hour should effectively remove 90-95% of the water from the system. In certain circumstances where the cartridge was not sufficiently dried before analysis, a Drierite column was connected to facilitate a faster reduction in moisture.

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

<u>Notes on ^{223}Ra and ^{224}Ra measurements:</u>
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.

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 fiber is very important both before and after sample collection. Since we do not have a very efficient way of rinsing the fiber after cooking, it has some residual Mn

on it that can be washed out before passing the sample through. Ensure that that the fiber is washed especially well before standard preparation.

4. For large volume samples at least 25 g dry weight (~ 250 ml fluffed 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.

References

Bourquin M., van Beek P., Reyss J. L., Souhaut M., Charette M. A., and Jeandel C. (2008) Comparison of techniques for pre-concentrating radium from seawater. Marine Chemistry 109, 226.

Broecker, W. S. (1965) An application of natural radon to problems in ocean circulation., in T. Ichiye, ed., Symposium on Diffusion in Oceans and Fresh Waters, Palisades, New York, Lamont Geological Obs., p. 116-145

Butts, J.L., J.F. Todd, I. Lerche, W.S. Moore, and D.G. Moore (1988) A simplified method for 226Ra determination in natural waters., Marine Chem., 25, 349-357.

Charette, M.A., K.O. Buesseler, and J.E. Andrews. (2001) Utility of radium isotopes for evaluating the input and transport of groundwater-derived nitrogen to a Cape Cod estuary. Limnology and Oceanography, 46, 465-470.

Dimova, N., H. Dulaiova, G. Kim, W. C. Burnett (2008) Uncertainties in the preparation of 224Ra Mn fiber standards. Mar. Chem., 109, 220-225.
Dulaiova, H. and Burnett, W.C. (2004) An efficient method for g-spectrometric determination of radium-226,228 via manganese fibers. Limnol. Oceanogr. Methods, 2, 256–261.

Foster D. A., Staubwasser M., and Henderson G. M. (2004) 226Ra and Ba concentrations in the Ross Sea measured with multicollector ICP mass spectrometry. Marine Chemistry 87, 59-71.

Garcia-Solsona E., Garcia-Orellana J., Masqué P., and Dulaiova H. (2008) Uncertainties associated with 223Ra and 224Ra measurements in water via a Delayed Coincidence Counter (RaDeCC). Marine Chemistry 109, 198.

Geibert, W., Rodellas, V., Annett, A., Van Beek, P., Garcia-Orellana, J., Hsieh, Y. T., & Masque, P. (2013). 226Ra determination via the rate of 222Rn ingrowth with the Radium Delayed Coincidence Counter (RaDeCC). Limnology and Oceanography: Methods, 11(11), 594-603.

Giffin, C., A. Kaufman, and W. S. Broecker (1963) Delayed coincidence counter for the assay of actinon and thoron. J. Geophys. Res., 68, 1749-1757.

Hamond, D.E. (2023) MATLAB program for reducing RaDeCC data. https://github.com/dhammond90290/RadeccMatlab/blob/main/.

Henderson, P.B., Morris, P.J., Moore, W.S., Charette, M.A. (2013) Methodological advances for measuring low-level radium isotopes in seawater. J. Radioanal. Nucl. Chem., 293, 1-6, DOI 10.1007/s10967-012-2047-9.

Hult, M., Charette, M., Lutter, G., Marissens, G., Henderson, P., Sobiech-Matura, K., & Simgen, H. (2019). Underground gamma-ray measurements of radium isotopes from hydrothermal plumes in the deep Pacific Ocean. Applied Radiation and Isotopes, 153, 108831

Key, R. M., R. L. Brewer, J. H. Stockwell, J. N.L. Guinasso, and D. R. Schink (1979) Some improved techniques for measuring radon and radium in marine sediments and in seawater. Mar. Chem., 7, 251-264.

Kim, G., Burnett, W.C., Dulaiova, H., Swarzenski, P.W. and Moore, W.S. (2001) Measurement of 224Ra and 226Ra activities in natural waters using a radon-in-air monitor. Environ. Sci.Technol., 35, 4680-4683.

Li, Y.-H., H.W. Feely, R. Toggweiler (1980) 228Ra and 228Th concentrations in GEOSECS Atlantic surface waters. Deep-Sea Res. 27A, 545.

Lucas, H.F. (1957) Improved low level alpha scintillation cell for radon, Rev. Sci. Inst., 28, 680-683.

Lucas, H.F., and D.A. Woodward (1964) Effect of long decay chains on the counting statistics in the analysis of Ra-224 and Rn-222, J. Appl. Phys., 35.

Mathieu, G. G., P. E. Biscaye, and R. A. Lupton (1988) System for the measurement of Rn-222 at low levels in natural waters: Health Phys., 55, 989-943.

McCurdy, D. E., and R. A. Mellon (1981) Determination of radium-224, radium-226, and radium-228 by coincidence spectrometry. Anal. Chem., 53, 2212-2216.

McKlveen, J.W., and W.J. McDowell (1984) Liquid scintillation alpha spectrometry techniques, Nucl. Instru. Methods Phys. Res., 223, 372-376.

Michel, J., Moore, W.S., King, P.T. (1981) □-ray Spectrometry for Determination of Radium-228 and Radium-226 in Natural Waters. Anal. Chem., 53, 1885-1889.

Moore, W.S. (1976) Sampling 228Ra in the deep ocean. Deep-Sea Res., 23, 647-651.

Moore, W. S. (1984) Radium Isotope Measurements Using Germanium Detectors, Nucl. Inst. Methods 223, 407-41.

Moore, W.S. (2000) Determining coastal mixing rates using radium isotopes. Continental Shelf Research, 20, 1993.

Moore, W.S. (2007) Seasonal Distribution and Flux of Radium Isotopes on the Southeastern U.S. Continental Shelf, J. Geophys. Res. Oceans, 112, C10013, doi:10.1029/2007JC004199

Moore, W.S. (2008) Fifteen years experience in measuring 224Ra and 223Ra by delayed-coincidence counting. Marine Chemistry, 109, 188-197.

Moore, W.S., Key, R.M. and Sarmiento, J.L. (1985) Techniques for precise mapping of 226Ra and 228Ra in the ocean. J. Geophys. Res., 90, 6983-6994.

Moore, W.S. and Arnold, R. (1996) Measurement of 223Ra and 224Ra in coastal waters using a delayed coincidence counter. J. Geophys. Res., 101, 1321-1329.

Ollivier, P., Claude, C., Radakovitch, O., and Hamelin, B. (2008) TIMS measurements of 226Ra and 228Ra in the Gulf of Lion, an attempt to quantify submarine groundwater discharge. Marine Chemistry 109, 337-354.

Orr, J. C. (1988) Evaluation of counting methods for oceanic radium-228. J. Geophys Res., 93, 8265-8278.

Phillips, G.W., and K.W. Marlow (1976) Automatic analysis of gamma-ray spectra from germanium detectors, Nuclear Inst. Methods, 137, 525-536.

Reyss, J.-L., Schmidt, S., Légeleux, F., Bonté, P. (1995) Large, low background well-type detectors for measurements of environmental radioactivity. Nuclear Instruments and Methods in Physics Research, A357, 391-397.

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

Sanial, V., Kipp, L. E., Henderson, P. B., Van Beek, P., Reyss, J. L., Hammond, D. E., ... & Charette, M. A. (2018). Radium-228 as a tracer of dissolved trace element inputs from the Peruvian continental margin. Marine Chemistry, 201, 20-34.

Sarmiento, J. L., D. E. Hammond, and W. S. Broecker (1976) The calculation of the statistical counting error for 222Rn scintillation counting: Earth Plant. Sci. Lett., 32, 351-356.

Schlosser, P., B. Kromer, and W. Roether (1983) Electronics for low-level counting using a microcomputer, Nucl. Instru. Methods, 216, 155-160.

Schlosser, P., M. Rhein, W. Roether, and B. Kromer (1984) High-precision measurement of oceanic 226Ra: Marine Chem., 15, 203-216.

Schmidt, S., and J.-L. Reyss (1996) Radium as internal tracer of Mediterranean outflow water, J. Geophys. Res., 101, 3589-3596.

Scholten J. C., Pham M. K., Blinova O., Charette M., Dulaiova H., and Eriksson M. (2010) Preparation of Mn-fiber standards for the efficiency calibration of the delayed coincidence counting system (RaDeCC). Marine Chemistry, 121, 206-214.

Selzer, S., Annett, A.L., Homoky, W.B. (2021) RaDeCC reader: Fast, accurate and automated data processing for Radium Delayed Coincidence Counting systems, Computers and Geosciences, doi: https://doi.org/10.1016/j.cageo.2021.104699.

Shaw, T.J. and W.S. Moore (2002) Analysis of 227Ac in seawater by delayed coincidence counting, Marine Chemistry, 78, 197-203.

Sun, Y. and Torgersen, T. (1998) The effects of water content and Mn-fiber surface conditions on 224Ra measurement by 220Rn emanation. Mar. Chem., 62, 299-306.

van Beek, P., Souhaut, M., & Reyss, J. L. (2010). Measuring the radium quartet (228Ra, 226Ra, 224Ra, 223Ra) in seawater samples using gamma spectrometry. Journal of Environmental Radioactivity, 101(7), 521-529.

5.4. 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.

- 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).

- Volume requirements

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

5.4.1. Sampling

- **Dissolved 210Po and 210Pb**

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.

- **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.

- **Particulate ^{210}Po and ^{210}Pb**

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.

5.4.2. 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.

5.4.3. 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 millilitres 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 colourless solution and adjusted to pH ~2. Note while plating at lower pH (1M HCl) has been successful, further experiments show that plating solutions with pH of 1.5 has the highest plating efficiencies (Lee et al., 2014). The Po isotopes are separated by spontaneous electroplating onto a polished silver disc, where the reverse side is covered by a neutral cement or plastic film/spray (Flynn, 1968). This residual solution is dried completely and the residue is taken in 5 ml of 9M HCl for the separation of residual Po from the Pb using an anion-exchange column such as AG1-X8 (Sarin et al., 1992). The purified Pb fraction should be spiked again with ^{209}Po and stored in a clean plastic bottle for at least 6 – 12 months after which the ^{210}Pb activity is measured by the ingrown activity of its granddaughter ^{210}Po. One can avoid the column separation of Pb and Po provided another ^{208}Po spike is added at the end of first plating. The correction for residual ^{210}Po is applied from the ^{210}Po/^{209}Po ratios in the first versus second plated counts. The ^{210}Po/^{208}Po ratio is then used to determine the activity of ^{210}Pb from the ingrowth of ^{210}Po in the background corrected second counts (as in Sec. 8.2). Note there is generally some amount of ^{209}Po in the ^{208}Po spike and hence a correction also may have to be applied, as well as possible peak overlap as described above. However, this correction and the ^{209}Po contribution will only increase with time after calibration as the two isotope spikes have very different half-lives (^{208}Po only 2.8 years versus ^{209}Po of 125 years; Colle et al., 2014).

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

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

5.4.4. ^{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.

5.4.5. 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.

5.4.6. Calculations for final activities of ^{210}Po in seawater sample

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

Calculation of the in-situ ^{210}Po activity involves the following specific corrections:
A) Background subtraction of the alpha spectrum for each detector for each ^{208}Po, ^{209}Po and ^{210}Po regions being used;
B) Decay of ^{210}Po from the time of plating on Ag planchets to mid-counting time of the sample;
C) Decay of ^{209}Po (or ^{208}Po) spike from the time of last calibration (or from the time certification for SRMs) to first plating. Note that the half-life has now been revised from 102 to 125 years (Colle et al., 2014).
D) In-growth correction from the decay of assayed in-situ ^{210}Pb via ^{210}Bi; and
E) Decay of ^{210}Po from the time of collection to first plating on Ag planchets.

In principle, a correction factor to the measured ^{210}Po activity from the decay of in-situ ^{210}Bi also needs to be applied. However, only a few labs have reported measurement of in-situ ^{210}Bi on the same sample (Tokieda, et al., 1994; Biggin, et al., 2002). A detailed outline of these steps is presented. A set of model equations are offered that shows the step-by-step calculation. A spread sheet can be constructed with these equations to explicit decay/in-growth corrections, blank/background subtractions and error propagation. These can be confirmed in consultation as presented here (Baskaran et al., 2013) and elsewhere (Rigaud et al., 2013). Either should provide an accurate assay of in-situ ^{210}Po and the ^{210}Pb grandparent.

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

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

$$^{210}A'_{Po\text{-}210}\ (dpm) = (^{210}N_n/^{209}N_n)\ e^{\lambda Po210\ t1}\ e^{-\lambda Pos\ t2}\ A_{spike} \qquad (4.1)$$

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:

$$^{210}A_{in\text{-}growth} = {}^{210}A_{Pb\text{-}in\text{-}situ}\ [\lambda_{Bi}\lambda_{Po}\ e^{-\lambda PbT} / (\lambda_{Bi} - \lambda_{Pb})(\lambda_{Po} - \lambda_{Pb}) + \lambda_{Bi}\lambda_{Po}\ e^{-\lambda BiT} / (\lambda_{Pb} - \lambda_{Bi})(\lambda_{Po} - \lambda_{Bi}) + \lambda_{Bi}\lambda_{Po}\ e^{-\lambda PoT} / (\lambda_{Pb} - \lambda_{Po})(\lambda_{Bi} - \lambda_{Po})] \qquad (4.2)$$

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.

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

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

$$A^{Po\text{-}210}{}_{in\text{-}situ}\ (dpm) = [^{210}A'_{Po\text{-}210}\ (dpm) - {}^{210}A_{in\text{-}growth}]\ e^{-\lambda PoT} \qquad (4.3)$$

5.4.7. Calculations for final activities of ^{210}Pb in seawater samples

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

A) 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;

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

C) 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);

D) 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)

E) Chemical yield for ^{210}Pb

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

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

$$^{210}A^{m}_{Po\text{-}210}\ (dpm) = (^{210}N_n/^{209}N_n)\ e^{\lambda Po210\ t3}\ e^{-\lambda Pos\ t4}\ A_{spike} \qquad (4.4)$$

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

$$^{210}A_{Pb\text{-}210} = {}^{210}A^{m}_{Po\text{-}210}\ /[1- e^{-\lambda Po210\ t5}] \qquad (4.5)$$

The chemical yield of $^{210}A_{Pb\text{-}210}$ is corrected by:

$$^{210}A_{Pb}' = {}^{210}A_{Pb\text{-}210} / \text{ chemical yield} \qquad (4.6)$$

where: the chemical yield (η_c) = amount of stable Pb carrier assayed/amount of stable Pb carrier added

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

$$^{210}A_{Pbin\text{-}situ} = {}^{210}A_{Pb\text{-}210}'\ e^{\lambda Pb\ t6} \qquad (4.7)$$

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:

$$^{210}A_{Pb}\ \textit{in-situ} = (^{210}A^{m}_{Po\text{-}210})\ e^{\lambda Pb\ t6}\ /\eta_c\ [1- e^{-\lambda Po210\ t5}] \qquad (4.8)$$

where $^{210}A^{m}_{Po\text{-}210}$ is calculated using equation (4.4).

5.4.8. Issues for consideration

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.

References

Baskaran, M., Hong, G.-H., Santschi, P.H., 2009. Radionuclide Analysis of Seawater, in: Oliver Wurl (Ed.), Practical Guidelines for the Analysis of Seawater. CRC Press, pp. 259-304.

Baskaran, M., Church, T.M., Hong, G.H., Kumar, A., Qiang, M., Choi, H., Rigaud, S., Maiti, K., 2013. Effects of flow rates and composition of the filter, and decay/ingrowth correction factors involved with the determination of in-situ particulate 210Po and 210Pb in seawater. Limnol. Oceanogr. Methods 11, 126-138.

Benoit, G. and Hemond, H.F., 1988. Improved methods for the measurement of Po-210, Pb-210 and Ra-226. Limnol. Oceanogr. 33(6), 1618-1622.

Biggin, et al., 2002. Time-efficient method for the determination of 210-Pb, 210-Bi, and 210-Po activities in seawater using liquid scintillation spectrometry. Anal. Chem., 74, 671-677.

Boyle, E. A. and Edmond, J.M., 1975. Determination of trace metals in aqueous solution by APDC chelate coprecipitation. In Analytical Methods in Oceanography, Gibb, T. R. P.(Ed), ACS, Adv. Chem. Ser. No. 147: 44-45.

Colle, R., Fitzgerald, R.P., and Laureano-Perez, L., 2014. A new determination of the Po-209 half-life. Jour. Physics G-Nuclear and Particle Physics 41(10), doi:10.1088/0954-3899/41/10/105103.

Fleer, A.P. and Bacon, M.P., 1984. Determination of Pb-210 and Po-210 in seawater and marine particulate matter. Nucl. Instru. Meth. Phys. Res., A, 223, 243-249.

Flynn, W.W., 1968. The determination of low levels of polonium-210 in environmental materials. Anal. Chem. Acta 43, 221-227.

Friedrich, J. and Rutgers van der Loeff, M.M., 2002. A two-tracer (210Po-234Th) approach to distinguish organic carbon and biogenic silica export flux in the Antarctic Circumpolar Current. Deep-Sea Res., I, 49: 101-120.

Hong, G.-H., Park, S-K., Baskaran, M., Kim, S.-H., Chung, C.-S. and Lee, S.-H., 1999. Lead-210 and polonium-210 in the winter well-mixed turbid waters in the mouth of the Yellow Sea. Conti. Shelf Res., 19: 1049-1064.

IAEA (2009). A procedure for the determination of Po-210 in water samples by alpha spectrometry. IAEA Analytical Quality in Nuclear Applications No. IAEA/AQ/12, International Atomic Energy Agency, Vienna.

Kim, G., Hussain, N., Church, T.M. and Yang, H.S., 1999. A practical and accurate method for the determination of 234Th simultaneously with 210Po and 210Pb in seawater. Talanta 49:851-858.

Lee, H.M., G.-H. Hong, M. Baskaran, Kim, S.H., Kim, Y.I., and Cho, K.C., 2014. Evaluation of plating conditions on the recovery of 210Po onto Ag planchets. Appl. Radiat. Isot. 90, 170-176.

Masque, P., Sanchez-Cabeza, J.A., Bruach, J.M., Palacios, E. and Canals, M., 2002. Balance and residence times of 210Pb and 210Po in surface waters of the Northwestern Mediterranean Sea. Conti. Shelf Res. 22: 2127-2146.

Radakovitch, O., Cherry, R.D., Heyraud, M. and Heussner, S., 1998. Unusual Po-210/Pb-210 ratios in the surface water of the Gulf of Lions. Oceanologica Acta 21, 459-468.

Rigaud, S., Puigcorbé, V., Cámara-Mor, P., Casacuberta, N., Roca-Martí, M., Garcia-Orellana, J., Benitez-Nelson, C.R., Masqué, P., Church, T.M., 2013. A methods assessment and recommendations for improving calculations and reducing uncertainties in the determination of 210Po and 210Pb activities in seawater. Limnol. Oceanogr. Methods 11, 561–571.

Sarin, M.M., Bhushan, R., Rengarajan, R., and Yadav, D.N., 1992. The simultaneous determination of 238U series nuclides in seawater: results from the Arabian Sea and Bay of Bengal. Indian Jour. Mar. Sci. 21: 121-127.

Stewart, G., Cochran, J.K., Xue, J., Lee, C., Wakeham, S.G., Armstrong, R.A., Masque, P., and Miquel, J.C., 2007. Exploring the connection between 210Po and organic matter in the northwestern Mediterranean. Deep-Sea Res. I. 54: 415-427.

Tokieda, T., et al., 1994. Sequential and rapid determination of Po-210, Bi-210 and Pb-210 in natural waters. Talanta, 41: 2079-2085.

5.5. 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).

References

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.

5.6. 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).

5.6.1. 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).

5.6.2. 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.

5.6.3. 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.

- 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.

- 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

- *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).

- 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.

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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.

5.6.4. 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.

- Acidification

Although both HCl and HNO_3 are suitable, samples acidified to pH=2 with HCl have fewer 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.

- 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.

- 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.

5.6.5. Spike calibration

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.

References

Aoyama, M., Hirose, K., Miyao, T., and Igarashi, Y., 2000. Low level 137Cs measurements in deep seawater samples. Applied Radiation and Isotopes 53, 159-162.

Baskaran, M., Hong, G. H., and Santschi, P. H., 2009. Radionuclide Analysis in Seawater. In: Wurl, O. (Ed.), Practical Guidelines for the Analysis of Seawater. CRC Press.

Beasley, T. M., Kelley, J. M., Maiti, T. C., and Bond, L. A., 1998. 237Np/239Pu Atom Ratios in Integrated Global Fallout: a Reassessment of the Production of 237Np. Journal of Environmental Radioactivity 38, 133-146.

Bowen, V. T., 1970. Analyses of sea-water for strontium and strontium-90Methods for Marine Radioactivity Studies, Tech. Rept. Ser. No. 118, IAEA, Vienna

Buesseler, K. O., Casso, S. A., Hartman, M. C., and Livingston, H. D., 1990. Determination of fission-products and actinides in the Black Sea following the Chernobyl accident. J. Radioanalyt. Nucl. Chem. 138, 33-47.

Buesseler, K. O. and Halverson, J. E., 1987. The Mass Spectrometric Determination of Fallout 239Pu and 240Pu in Marine Samples. Journal of Environmental Radioactivity 5, 425-444.

Casacuberta, N. and others 2016. First 236U data from the Arctic Ocean and use of 236U/238U and 129I/236U as a new dual tracer. Earth and Planetary Science Letters 440: 127-134.

Kelley, J. M., Bond, L. A., and Beasley, T. M., 1999. Global distribution of Pu isotopes and 237Np. The Science of the Total Environment 237-238, 483-500.

Kenna, T. C., 2002b. Determination of plutonium isotopes and neptunium-237 in environmental samples by inductively coupled plasma mass spectrometry with total sample dissolution. Journal of Analytical Atomic Spectrometry 17, 1471-1479.

Kenna, T. C. and others. 2012. Intercalibration of selected anthropogenic radionuclides for the GEOTRACES Program. Limnology and Oceanography: Methods 10: 590-607.

La Rosa, J., Burnett, W., Lee, S., Levy, I., Gastaud, J., and Povinec, P., 2001. Separation of actinides, cesium and strontium from marine samples using extraction chromatography and sorbents. Journal of Radioanalytical and Nuclear Chemistry 248, 765-770.

Lee, S., Gastaud, J., La Rosa, J., Kwong, L., Povinec, P., Wyse, E., Fifield, L., Hausladen, P., Di Tada, L., and Santos, G., 2001. Analysis of plutonium isotopes in marine samples by radiometric, ICP-MS and AMS techniques. Journal of Radioanalytical and Nuclear Chemistry 248, 757-764.

Lindahl, P., Roos, P., Holm, E., and Dahlgaard, H., 2005. Studies of Np and Pu in the marine environment of Swedish-Danish waters and the North Atlantic Ocean. Journal of Environmental Radioactivity 82, 285-301.

Livingston, H. D., Mann, D. R., and Bowen, V. T., 1975a. Analytical Procedures for Transuranic Elements in Seawater and Marine Sediments. Advances in Chemistry Series, 124-138.

Livingston, H. D., Mann, D. R., Fettis, R. C., and Dempsey, B. L., 1974. Radiochemical Procedures for the Analysis of Strontium, Cesium, Iron, Transuranics and the Rare Earths in Seawater Samples: Laboratory Operations Protocol. Woods Hole Oceanographic Institution, Woods Hole, MA.

Livingston, H. D., Schneider, D. L., and Bowen, V. T., 1975b. Pu-241 in Marine Environment by a Radiochemical Procedure. Earth and Planetary Science Letters 25, 361-367.

Molero, J., Moran, A., Sanchez-Cabeza, J. A., Blanco, M., Mitchell, P. I., and Vidalquadras, A., 1993. Efficiency of radiocaesium concentration from large volume natural water samples by scavenging with ammonium molybdophosphate. Radiochimica Acta 62, 159-162.

Vajda, N. and Kim, C.-K., 2010. Determination of Pu isotopes by alpha spectrometry: a review of analytical methodology. Journal of Radioanalytical and Nuclear Chemistry 283, 203-223.

Wong, K. M., Jokela, T. A., and Noshkin, V. E., 1994. Radiochemical procedures for analysis of Pu, Am, Cs, and Sr in seawater, soil, sediments, and biota samples, Technical Report, UCRL-ID-116497. Lawrence Livermore National Laboratory, Berkley, CA.

Yamada, M., Zheng, J., and Wang, Z., 2006. 137Cs, 239 + 240Pu and 240Pu/ 239Pu atom ratios in the surface waters of the western North Pacific Ocean, eastern Indian Ocean and their adjacent seas. Science of the Total Environment 366, 244-252.

6. RADIOGENIC ISOTOPES

6.1. 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.

6.1.1. Analytical instrument

The two instruments used for analysis of dissolved $^{143}Nd/^{144}Nd$ in seawater are Thermal Ionization Mass Spectrometry, TIMS (Dahlqvist et al., 2005; Lacan and Jeandel, 2005; Shimizu et al., 1994; Piepgras and Wasserburg, 1987), 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 accurante Nd isotope ratios (van de Flierdt et al., 2012; Pahnke et al., 2012).

6.1.2. 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).

6.1.3. 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 7.11.2). 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.

6.1.4. 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. Examples include methods for the combined extraction of Pa, Th and Nd isotopes (Struve et al., 2016, Wang et al., 2021), Th and Nd isotopes (Pérez-Tribouillier et al., 2019) and Rd, Th, Pa, U and Nd isotopes (Jeandel et al., 2011).

- 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.

- Spiking

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

- 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 actid functional groups (see also 4.3.4 below; e.g., Hathorne et al., 2012; Behrens et al., 2016). Another offline preconcentration system (nicknamed '*seaSLOW'*), utilizing Nobias Chelate-PA1 resin, has also been recently developed following protocols in Biller and Bruland, (2012) and Hatje et al (2014) (Pham, 2020).

Fe hydroxide: 2 to 5mg of Fe (as $FeCl_3$, purified by repeated isopropyl ether extraction; Struve et al., 2016) 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 high purity grade ammonium hydroxide (e.g. Suprapur® or Optima®) 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).

C18 cartridge: 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 (van de Flierdt et al., 2012; Pahnke et al., 2012).

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 Loeff 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.

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 and Pr.

6.1.5. Spike calibration 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 or by the use of external calibration curves 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.

6.1.6. Evaluation of 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/ National Research Council of Canada reference materials (for example NOD-A1 or NOD-P1 for Nd isotopes, and SLRS-6 for REE concentrations) 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.

References

Behrens, M.K., Muratli, J., Pradoux, C., Wu, Y., Böning, P., Brumsack, H.-J., Goldstein, S.L., Haley, B., Jeandel, C., Paffrath, R., Pena, L.D., Schnetger, B. and Pahnke, K. (2016) Rapid and precise analysis of rare earth elements in small volumes of seawater - Method and intercomparison. Mar. Chem. 186: 110-120.

Biller, D. V. and Bruland, K. W. (2012) 'Analysis of Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb in seawater using the Nobias-chelate PA1 resin and magnetic sector inductively coupled plasma mass spectrometry (ICP-MS)', Marine Chemistry. Elsevier B.V., 130–131, pp. 12–20. doi:10.1016/j.marchem.2011.12.001

Dahlqvist R., Andersson P.S. and Ingri J. (2005) The concentration and isotopic composition of diffusible Nd in fresh and marine waters. Earth Planet. Sci. Lett. 233: 9-16.

Hathorne, E.C., Haley, B., Stichel, T., Grasse, P., Zieringer, M. and Frank, M. (2012) Online preconcentration ICP-MS analysis of rare earth elements in seawater. Geochem. Geophys. Geosyst. 13, Q01020: doi:10.1029/2011GC003907.

Hatje, V., Bruland, K. W. and Flegal, A. R. (2014) 'Determination of rare earth elements after preconcentration using NOBIAS-chelate PA-1®resin: Method development and application in the San Francisco Bay plume', Marine Chemistry, 160, pp. 34–41. doi:10.1016/j.marchem.2014.01.006

Jeandel C., Bishop J.K. and Zindler A. (1995) Exchange of neodymium and its isotopes between seawater and small and large particles in the Sargasso Sea, Geochim. Cosmochim. Acta, 59: 535-547.

Jeandel C., Thouron D. and Fieux M. (1998) Concentrations and isotopic compositions of neodymium in the eastern Indian Ocean and Indonesian Straits. Geochim. Cosmochim. Acta, 62: 2597-2607.

Jeandel, C., Venchiarutti, C., Bourquin, M., Pradoux, C., Lacan, F., van Beek, P. and Riotte, J. (2011) Using a unique column to sequentially extract Re, Nd, Th, Pa and U from a single natural sample. Geostand. Geoanal. Res. 35: 449-459.

Jeandel, C., Delattre, H., Grenier, M., Pradoux, C. and Lacan, F. (2013) Rare earth element concentrations and Nd isotopes in the Southeast Pacific Ocean. Geochem. Geophys. Geosyst. 14: doi:10.1029/2012GC004309.

Lacan F. and Jeandel C. (2005) Neodymium isotopes as a new tool for quantifying exchange fluxes at the continent-ocean interface. Earth Planet. Sci. Lett. 232: 245-257.

Nozaki Y. (2001) Vertical Profiles of Elements in the North Pacific Ocean EOS Transactions AGU.

Pahnke, K. van de Flierdt T., Jones K.M., Lambelet M., Hemming S.R. and Goldstein S.L. (2012) GEOTRACES intercalibration of neodymium isotopes and rare earth element concentrations in seawater and suspended particles. Part 2: Systematic tests and baseline profiles. Limnol. Oceanogr. Methods, 10(4): 234–251, doi:10.4319/lom.2012.10.234.

Pérez-Tribouillier, H., Noble, T. L., Townsend, A. T., Bowie, A. R., Chase, Z. (2019) Pre-concentration of thorium and neodymium isotopes using Nobias chelating resin: Method development and application to chromatographic separation, Talanta, 202, 600-609

Persson, P-O., Andersson, P.S., Zhang, J. and Porcelli, D. (2011) Determination of Nd isotopes in water: A chemical separation technique for extracting Nd from seawater using a chelating resin. Anal. Chem. 83: 1336-1341.

Pham V. Q. (2020) Traçage de l'empreinte lithogénique en mers de Corail et des Salomon : apport des terres rares et de la composition isotopique du néodyme. PhD. Université de Toulouse III – Paul Sabatier

Piepgras D.J. and Wasserburg G.J. (1987) Rare earth element transport in the western North Atlantic inferred from Nd isotopic observations. Geochim. Cosmochim. Acta, 51: 1257-1271.

Rutgers van der Loeff M. and Moore W.S. (1999) Determination of natural radioactive tracers: In Grasshoff, K., Ehrardet M., Kremling K. (Eds.) Methods of Seawater Analysis. Verlag Chemie, Weinheim, Chap 13.
Shabani M.B., Akagi T. and Masuda A. (1992) Preconcentration of Trace Rare Earth Elements in Seawater by Complexation with Bis(2-ethylexyl) hydrogen phosphate and 2-Ethylexyl Dihydrogen Phosphate Adsorbed on a C18 Cartridge and Determination Inductively Coupled Plasma Mass Spectrometry. Anal Chem. 64: 737-743.
Shimizu H., Tachikawa K., Masuda A. and Nozaki Y. (1994) Cerium and neodymium isotope ratios and REE patterns in seawater from the North Pacific Ocean. Geochim. Cosmochim. Acta, 58: 323-333.

Struve T., van de Flierdt T., Robinson L.F., Bradtmiller L.I., Hines S.K., Adkins J.F., Lambelet M., Crocket K.C., Kreissig K., Coles B. and Auro, M.E. (2016) Neodymium isotope analyses after combined extraction of actinide and lanthanide elements from seawater and deep –sea corals. Geochem. Geophys. Geosyst., 17: 232-240.

Tachikawa K., Arsouze T., Bayon G. et al. (2017) The large-scale evolution of neodymium isotopic composition in the global modern and Holocene ocean revealed from seawater and archive data. Chem. Geol., 457: 131-148, doi: 10.1016/j.chemgeo.2017.03.018.

Vance D., Scrivner A. E., Beney P., Staubwasser M., Henderson G.M. and Slowey N.C. (2004) The use of foraminifera as a record of the past neodymium isotope composition of seawater, Paleoceanography, 19, doi:10.1029/2003PA000957.

van de Flierdt T., Griffiths A.M., Lambelet M., Little S.H., Stichel T. and Wilson D.J (2016). Neodymium in the oceans: a global database, a regional comparison and implications for palaeoceanographic research. Phil. Trans. R. Soc. A 374, 20150293

van de Flierdt T., Pahnke K. and GEOTRACES intercalibration participants (2012) GEOTRACES intercalibration of neodymium isotopes and rare earth element concentrations in seawater and suspended particles. Part 1: Reproducibility of results for the international intercomparison. Limnol. Oceanogr. Methods, 10(4): 234–251,doi:10.4319/lom.2012.10.234.

Wang, R., Clegg, J. A., Scott, P. M., Larkin, C. S., Deng, F., Thomas, A. L., Zheng, X-Y. and Piotrowski, A. M., (2021) Reversible scavenging and advection – resolving the neodymium paradox in the South Atlantic, Geochimica et Cosmochimica Acta, 314, 121-139

6.2. 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).

6.2.1. 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, Griffiths et al., 2020, Benaltabet et al., 2020). Differential Pulse Anodic Stripping Voltammetry (DPASV) has also been employed for measurement of dissolved Pb concentrations (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. For example, single collector ICP-MS measurements can achieve external reproducibilities of ~4800 ppm (relative 2σ) for 206Pb/207Pb ratios, with ~100 to 1000 ppm achieved for the highest precision TIMS and MC-ICP-MS methods (Zurbrick et al., 2003, Pinedo-González et al., 2018, Griffiths et al., 2020).

6.2.2. Sample volume

Lead concentrations in the ocean circa 2010 to 2020 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. 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 laboratory blank levels. The exact volume depends on the analytical equipment used and ratios targeted; 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. Studies reporting high precision data for ^{20x}Pb/^{204}Pb ratios, however, found that these ratios do not offer significantly improved source apportionment constraints over the use of high abundance isotopes (^{208,207,206}Pb) only (Bridgestock et al., 2018).

6.2.3. Sampling

- Sampling system

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 7.4. 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 7.4. 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 7.3), 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.

- Filtration

For many years, clean filtration was sufficiently difficult that "total dissolvable" Pb from unfiltered samples was preferred. This is generally acceptable as >90% of the Pb in most samples is in the dissolved state, although lower proportions occur close to continental margins, or in open ocean waters impacted by atmospheric mineral dust deposition or large river plumes (Bridgestock et al., 2016, Bridgestock et al., 2018, Schlosser et al., 2019, Olivelli et al., 2023. 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 7.5.2; e.g., Acropak Supor capsule filter (0.8/0.2µm) and 0.2 µm Sartobran-300 (Sartorius) filter cartridges (e.g., Noble et al., 2015, Bridgestock et al., 2016, Boyle et al., 2020)). Filter cleaning is discussed in the

contamination-prone trace element sampling section (see section 7.5.2), but typically involves a prolonged acid leach followed by rinsing with ultrapure water (>18.2 MΩ cm) and several liters of clean seawater before being used for samples.

- 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 (see section 7.3), and it is recommended to store them after cleaning in clean containers/laboratory environments inside double Ziplock polyethylene bags. Sample containers should be rinsed with three small aliquots of seawater sample before final collection. To prevent adsorption of Pb to the container walls, samples should be acidified to ~pH 2 with trace metal clean acid (usually 6M HCl). When ultraclean laboratory facilities are available, samples can be acidified at sea. Otherwise, they should be acidified inside a shore-based clean room laboratory and left for several weeks to allow absorbed Pb to be fully released from the container walls prior to processing for analysis. Upon completion of seaboard handling, samples should be stored in clean closed containers inside double Ziplock polyethylene bags.

6.2.4. Sample processing

Analysis for both Pb and Pb isotopes requires Pb separation from the major ion salt and some degree of concentration to smaller volumes.

The two methods are commonly employed for Pb concentration and purification from seawater are $Mg(OH)_2$ co-precipitation (Reuer et al., 2003, Paul et al., 2015), and the use of chelating resins (Lee et al., 2011, Zurbrick et al., 2013, Benaltabet et al., 2020, Griffiths et al., 2020). Following these techniques, Pb is typically further purified from matrix elements by anion exchange chromatography (Boyle et al., 2012, Paul et al., 2015, Jiang and Zhang, 2023). The principal considerations for these techniques are to have the lowest possible Pb blank, high Pb yields and effective removal matrix elements. Minimizing and rigorously characterizing the Pb blank incurred during these procedures is particularly crucial, as it can constitute a significant proportion of the analyzed Pb content. Achieving low Pb blanks requires all sample processing to be conducted within clean room conditions, with acid-cleaned labware and reagents of the highest purity with respect to trace metals.

The $Mg(OH)_2$ co-precipitation typically produces the lowest Pb blank (~0.1 pmol/kg) and is induced by increasing the samples pH by the addition of ammonia (purified by cold vapor equilibration) (Reuer et al., 2003, Boyle et al., 2012, Paul et al., 2015, Griffiths et al., 2020). The precipitate is subsequently concentrated by centrifugation, settling or filtration, and dissolved in acid prior to further purification by anion exchange chromatography for isotope analysis. These techniques can achieve Pb yields of >90%, but yields can decrease to ~40-60 % for higher volume samples (1 to 2 L) due to difficulty in quantitative extraction of the precipitate (Reuer et al., 2003, Boyle et al., 2012, Paul et al., 2015, Griffiths et al., 2020). Non-quantitative Pb yields, although undesirable, are not a critical issue for Pb isotope analysis, or Pb concentration measurements by isotope dilution (ensuring sample-spike equilibration prior to Pb separation steps) (e.g. Paul et al., 2015). 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). Despite these issues, the low blank of this method makes it particularly suitable for measurement of low Pb concentration (<10 pmol/kg) samples (Boyle et al., 2020), while also being relatively inexpensive.

The use of chelating resins to pre-concentration Pb and separate it from major seawater salts has become an increasingly popular alternative to $Mg(OH)_2$ co-precipitation. These methods avoid issues with silica gel precipitation and can provide high Pb yields (~90 to 100%; Griffiths et al., 2020, Jiang and Zhang, 2023). They also have the advantage of being able simultaneously to extract and pre-concentrate a suite of other trace metals (e.g. Cu, Cd, Fe) for concentration measurements and can be automated to improve sample throughput (Sohrin et al., 2008, Lee et al., 2011, Zurbrick et al., 2013, Benaltabet et al., 2020). The blanks of these techniques (~0.1 to 1 pmol/kg) can be slightly higher than those using $Mg(OH)_2$ co-precipitation, but are still suitable for the analyses most seawater samples (Zurbrick et al., 2013, Griffiths et al., 2020, Jiang and Zhang, 2023). To date, methods for Pb and Pb isotope measurements utilizing the following chelating resins have been developed; Nobias Chelate-PA1 (Sohrin et al., 2008, Boyle et al., 2020, Benaltabet et al., 2020, Griffiths et al., 2020), Toyopearl AF-Chelate-650M® (Zurbrick et al., 2013), nitrilotriacetate (NTA)-type Superflow® (Lee et al., 2011) and Presep® PolyChelate (Jiang and Zhang, 2023). They typically involve the adjustment of the seawater samples pH by addition of ammonium acetate prior to loading onto the resin, either within columns (e.g. Benaltabet et al., 2020, Jiang and Zhang, 2023) or by suspension of resin beads within the samples (e.g. Lee et al., 2011, Griffiths et al., 2020). Lead (and other trace metals) is subsequently eluted from the resin in a dilute acid.

6.2.5. 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 (e.g. Benaltabet et al., 2020) or by isotope dilution methods with the isotope spike added to the seawater before Pb pre-concentration (Lee et al., 2011, Paul et al., 2015, Griffiths et al., 2020). 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.

Determination of Pb isotope compositions requires the correction of instrumental mass bias. This can be done by normalization to bracketing measurements of the SRM NIST 981, external normalization to Tl and the use of a Pb double spike (or a combination of the above; e.g. Reuer et al., 2003, Zurbrick et al., 2013, Paul et al., 2015, Griffiths et al., 2020).

As previously noted, Pb blanks are commonly a major limitation on data quality. To minimize systematic errors associated with blank corrections, rigorous characterization of the procedural Pb blank, by processing relatively high ratio of blanks/samples in each measurement batch (e.g. ~1 blank per 5 samples), is recommended. Procedural blanks should be monitored by processing of acidified low-Pb seawater through the entire analytical procedure. Low-Pb seawater for this purpose can be prepared by extracting the Pb from an in-house seawater reference sample by either $Mg(OH)_2$ co-precipitation or the use of chelating resins. Reagent

blanks should be checked before use. Volatile reagents which cannot be run directly by most instrumental methods (such as concentrated acids or NH3 solution) can be evaporated to dryness followed by redissolution in a small volume of dilute acid.

Even with the most carefully handling, spuriously high Pb contamination from laboratory processing can impact the accuracy of individual measurements. Such randomly high Pb contamination will not be accounted for by procedural blank corrections. Low volume samples (~a few mL to 10's mL; volumes typical of Pb concentration analyses) are particularly vulnerable to being adversely impact by spurious Pb contamination, as feature sample Pb contents (~1's to 10's pg) similar in magnitude to typical procedural blanks. To mitigate against this issue, good practice is to run duplicate or triplicate measurements (repeating both the Pb separation and analysis steps) of each sample, and to reject individual measurements that show poor agreement to the other replicates (e.g. Bridgestock et al., 2018, Boyle et al., 2020). Due to constraints on sample volume availability, replicate analyses are not feasible to conduct routinely for larger sample volumes (100s mL to 2L) typically processed for Pb isotope analyses. The larger total Pb content (~100s to 1000s pg) of these samples, make them much less vulnerable to being significantly impacted by spuriously high Pb blanks, but even so, the potential for laboratory Pb contamination should be carefully considered during data interpretation.

6.2.6. Evaluation of analytical uncertainties

A large-volume in-house reference seawater sample(s) should be processed and analyzed with each batch of samples to assess the long-term external reproducibility of the method, and to monitor for any systematic errors that may affect a given analytical batch. For example, abnormally high procedural blanks, poor yields, matrix effects or systematic errors in the production of calibration standards for determining Pb concentrations by standard-addition.

External consistency of the method should ideally be assessed through measurement of GEOTRACES intercalibration samples and comparison to consensus values (Boyle et al., 2012). Given the limited availability of these GEOTRACES intercalibration samples, measuring these samples in just 1 or 2 analytical batches, coupled with longer-term monitoring by repeat analysis of in-house reference seawater sample(s) (as described above) is advisable. Accuracy can also be demonstrated by replicate analysis of individual samples by multiple labs, an option that is particularly expedient in the case that sufficient volumes of GEOTRACES intercalibration sample are unavailable. Finally, processing and analyzing low Pb seawater (after purification by $Mg(OH)_2$ precipitation or with chelating resins) doped with a known amount of SRM NIST 981 can also be used to assess external consistency. However, such measurements are commonly used to correct Pb isotope measurements for drift in instrumental performance (Thirlwall, 2002; Baker et al., 2004), and in such cases would not qualify as an independent constraint on external consistency.

References

Baker, J., Peate, D., Waight, T. and Meyzena C. (2004) Pb isotopic analysis of standards and samples using a 207Pb–204Pb double spike and thallium to correct for mass bias with a double-focusing MC-ICP-MS. Chem. Geol. 211: 275-303.

Bell, J., Betts, J. and Boyle, E.A. (2002) MITESS: A moored in-situ trace element serial sampler for deep-sea moorings. Deep-Sea Research I 49: 2103-2118.

Boyle, E.A., Chapnick, S.D., Shen, G.T. and Bacon, M.P. (1986) Temporal variability of lead in the western North Atlantic. J. Geophys. Res. - Oceans 91: 8573-8593.

Boyle, E.A., John, S., Abouchami, W., Adkins, J.F., Echegoyen-Sanz, Y., Ellwood, M., Flegal, R., Fornace, K., Gallon, C., Galer, S., Gault-Ringold, M., Lacan, F., Radic, A., Rehkamper, M., Rouxel, O., Sohrin, Y., Stirling, C., Thompson, C., Vance, D., Xue, Z. and Zhao, Y. (2012) GEOTRACES IC1 (BATS) Contamination-Prone Trace Element Isotopes Cd, Fe, Pb, Zn, (and Mo) Intercalibration. Limnol. Oceanogr. Methods 10:653-665.

Benaltabet, T., Lapid, G. and Torfstein, A. (2020) Seawater Pb concentration and isotopic composition response to daily time scale dust storms in the Gulf of Aqaba, Red Sea, Marine Chemistry, 227, 103895.

Bridgestock, L., van de Flierdt, T., Rehkämper, M., Paul, M., Middag, R., Milne, A., Lohan, M.C., Baker, A., Chance, R., Khondoker, R., Strekopytov, S., Humphrey-Williams, E., Achterberg, E., Rijkenberg, M., Gerringa, L.J.A. and de Baar, H. (2016). Return of naturally sourced Pb to Atlantic surface waters. Nature Commun. 7: 12921, doi:10.1038/ncomms12921.

Bridgestock, L., Rehkämper, M., van de Flierdt, T., Paul, M., Milne, A., Lohan, M. C., Achterberg, E. P., (2018), The distribution of lead concentrations and isotope compositions in the eastern Tropical Atlantic Ocean, Geochimica et Cosmochimica Acta, 225, 36-51.

Bruland, K.W. (1980) Oceanographic distributions of cadmium, zinc, nickel, and copper in the north Pacific. Earth Planet. Sci. Lett. 47: 176-198.

Griffiths, A., Packman, H., Leung, Y-L., Coles, B. J., Kreissig, K., Little, S. H., van de Flierdt, T., and Rehkämper, M. (2020) Evaluation of optimized procedures for high-precision lead isotope analyses of seawater by multiple collector inductively coupled plasma mass spectrometry, Analytical Chemistry, 92, 11232-11241.

Helmers, E. and Rutgers van der Loeff, M. (1993) Lead and aluminum in Atlantic surface waters (50N to 50S) reflecting anthropogenic and natural sources in the eolian transport. J. Geophys. Res. 98(C11): 20,261-20,273.

Jiang, S. and Zhang, J. (2023) An offline column extraction with Presep PolyChelate resin and AG1-X9 resin for the determination of the isotope compositions of low-concentration lead in natural waters by the multi-collector inductively coupled plasma mass spectrometry, Liminology and Oceanography: Methods, 21, 307-319.

Lee, J.-M., Boyle, E.A., Echegoyen-Sanz, Y., Fitzsimmons, J.N., Zhang, R. and Kayser, R.A. (2011) Analysis of trace metals (Cu, Cd, Pb, and Fe) in seawater using single batch nitrilotriacetate resin extraction and isotope dilution inductively coupled plasma mass spectrometry. Anal. Chim. Acta 686(1-2):93-101.

Noble, A. E., Echegoyen-Sanz, Y., Boyle, E. A., Ohnemus, D. C., Lam, P. J., Kayser, R., Reuer, M., Wu, J. and Smethie, W. (2015) Dynamic variability of dissolved Pb and Pb isotope composition from the U.S. North Atlantic GEOTRACES transect, Deep Sea Research Part ii: Topical Studies in Oceanography, 116, 208-225.

Middag, R., Séférian, R., Conway, T.M., John, S.G., Bruland, K.W.and de Baar H.J.W. (2015) Intercomparison of dissolved trace elements at the Bermuda Atlantic Time Series station. Mar. Chem. 177: 476–489.

Olivelli, A., Murphy, K., Bridgestock, L., Wilson, D. J., Rijkenberg, M., Middag, R., Weiss, D. J., van de Flierdt, T. and Rehkämper, M. (2023) Decline of anthropogenic lead in the South Atlantic Ocean surface waters from 1990 to 2011: New constraints from concentration and isotope data, 189, Marine Pollution Bulletin, 114798.

Paul, M., Bridgestock, L., Rehkämper, M., van de Flierdt, T. and Weiss, D. (2015) High-precision measurements of seawater Pb isotope compositions by double spike thermal ionization mass spectrometry. Analyt. Chim. Acta 863: 59-69.

Pinedo-González, P., West, J. A., Tovar-Sanchez, A., Duarte, C. M., Sanudo-Wilhelmy, S. A., (2018) Concentration and isotopic composition of dissolved Pb in surface waters of the modern global ocean, Geochimica et Cosmochimica Acta, 235, 41-54.

Reuer, M.K., Boyle , E.A. and Grant, B.C. (2003) Lead isotope analysis of marine carbonates and seawater by multiple collector ICP-MS. Chem. Geol. 200:137-153.

Schaule, B. K. and Patterson C.C. (1981) Lead concentrations in the northeast Pacific: evidence for global anthropogenic perturbations. Earth Planet. Sci. Lett. 54: 97-116
Schlosser, C., Karstensen, J., Woodward, E. M. S. (2019) Distribution of dissolved and leachable particulate Pb in the water column along the GEOTRACES section GA10 in the South Atlantic, Deep Sea Research Part 1: Oceanographic Research Papers, 148, 132-142.

Sohrin, Y., Urushihara, S., Nakatsuka, S., Kono, T., Higo, E., Minami, T., Norisuye, K. and Umetani, S. (2008). Multielemental Determination of GEOTRACES Key Trace Metals in Seawater by ICPMS after Preconcentration Using an Ethylenediaminetriacetic Acid Chelating Resin. Anal. Chem 80: 6267-6273.

Thirlwall, M.F. (2002) Multicollector ICP-MS analysis of Pb isotopes using a 207Pb-204Pb double spike demonstrates up to 400 ppm/amu systematic errors in Tl-normalization. Chem. Geol. 284: 255–279.

Vink, S., Boyle, E.A., Measures, C.I. and Yuan, J. (2000) Automated High Resolution Determination of the Trace Elements Iron and Aluminium in the Surface Ocean using a Towed Fish Coupled to Flow Injection Analysis. Deep-Sea Res. I 47:1141-1156.

Wu, J. and Boyle, E.A. (1997) Low blank preconcentration technique for the determination of lead, copper and cadmium in small-volume seawater samples by isotope dilution ICPMS. Anal. Chem. 69:2464-2470.

Zurbrick, C.M., Gallon, C. and Flegal A.R. (2013) A new method for stable lead isotope extraction from seawater. Analyt. Chim. Acta 800: 29-35.

7. 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.

7.1. PRE-CRUISE PREPARATIONS

7.1.1. 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).

- 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).

(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.

The methods and data used in verifying depth should be documented in the metadata for the cruise.

- Cleaning procedures 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.

7.2. Sample bottle types for sample storage

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

Bottles for speciation samples and their cleaning are discussed below in Section 7.3. Polyethylene bottles are not recommended for Hg or metalloids (see Hg Section 5 for bottle types and cleaning).

7.3. 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.

7.3.1. 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.

7.3.2. 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 80°C for 5 hours
9. Rinse 5x with UHPW water inside an ISO Class-5 laminar flow hood. Should be stored doubled bagged.

7.4. Sample collection

7.4.1. 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.

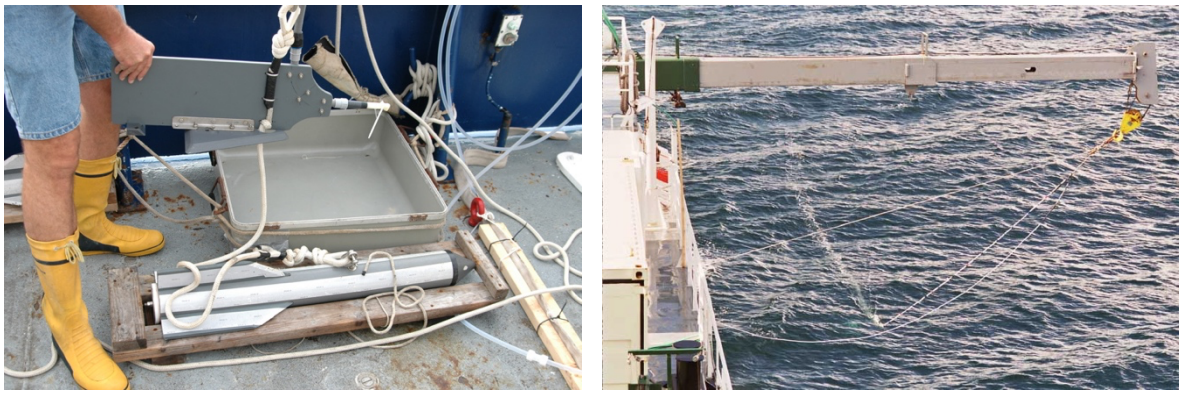


Figure 7.1. Towed fish system and example of a deployment

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.

7.4.2. Depth profiles

See Section 7.1 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.

7.5. SAMPLE HANDLING

All sample handling should take place in a clean area, preferably an ISO Class-5 area (See Table 7.1). 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 mm 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 7.1. 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.

7.5.1. 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.
Note that because absolute concentrations will vary depending on the acidification time, intercalibration is not possible for unfiltered acidified samples

7.5.2. Total Dissolved (filtered) Samples

- 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, but is used successfully by some groups.

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.

- Particle collection

Particle collection from GO-FLO samples is thoroughly discussed in Section 7.10 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.

7.5.3. 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) (e.g. Gonzalez Santana et al., 2021).
(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 and cracking of FPE bottles stored at -80 ºC.

The protocols here apply to the determination of transition metal complexation by organic matter in seawater since the parameter was 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. Furthermore, Fe(II) is an important parameter for understanding the redox state of iron, although the comparison of its sampling protocol could not be verified in the GEOTRACES Intercalibration cruises. 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.

- Speciation 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 sampling 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. The results also indicated that these samples can be collected directly from the pressurized Go-FLOs through capsule filters as for other samples, without a need for specialized plumbing. Therefore, complete integration of speciation sampling with other TM sampling is acceptable and recommended, while recognizing that the filtration protocols (e.g., nominal pore size, filter type, etc.) may impact the speciation in specific ways distinct from total dissolved metal. Some speciation samples may need to be filtered separately, especially if the memory effect from the filter can impact speciation results.

- Sample handling

Two types of containers are recommended for handling speciation samples: Teflon (FEP) and fluorinated linear polyethylene (FLPE). LDPE (low-density polyethylene) 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 until frozen. Rapid freezing in a -80° C freezer is not recommended for FLPE bottles; samples in FLPE gave different organic complexation results and the bottles appeared to be cracked after freezing 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.

- Sampling Protocols for Fe(II)

All of the Fe(II) analyses carried out during the GEOTRACES cruises have in common that samples for Fe(II) can be collected from GO-FLOs/X-Niskins in the same way as other samples, and transferred to another location on the ship for immediate analysis (see Section 3.3.4 below). Acidification to lower pH values is not recommended as it may lead to artificially high values over time. Freezing samples is not an acceptable preservation method for Fe(II). Sample handling for rapid analysis after the recovery of GO-FLOs/X-Niskins is slightly different between research cruises because of the different environments of research vessels. In recent years, there have also been reports of unfiltered analysis in order to shorten the time from collection to analysis and to avoid overestimation due to the effects of artifacts (superoxide and Fe(II) released from cells) due to filtration stress (e.g. Santana-González et al., 2018). The effect of the filtration is still unknown, so it will be necessary to investigate the effect on the redox state of Fe in the future.

- 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 trace metals, especially Fe, Mn, and Co 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/X-Niskin 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).

- Speciation Methodologies

The 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. Note that new parameter names are now available in the DoOr portal for ligand specific parameters, with additional information on the method included in Token 1.

7.5.4. 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. If this is done, care must be taken to ensure samples are acidified for a sufficient time to ensure that elements have been re-released from bottle walls, with specific concerns over acidification time and the need for UV or other oxidation process prior to analysis being element specific – there are a number of papers on this that should be considered when making this decision.

7.6. 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, Janssen and Cullen, 2015).

7.7. 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.

7.8. Analytical Considerations: Precision and Accuracy

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

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

It should easily be apparent from these definitions that precision and accuracy are not necessarily coupled. An analysis may be precise yet inaccurate, whereas the mean of a variable result may be quite accurate. Therefore, precision and accuracy must be evaluated independently. The use of Certified Reference Materials is best for evaluating analytical accuracy, but for most trace elements there none available for seawater at appropriate concentrations as of this writing (2017). For the GEOTRACES Program, consensus intercalibration samples have been created.

GEOTRACES Consensus Samples should be used as a Reference Material (RM) to test of the accuracy of the methods used where available. 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. 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 are available here: https://www.geotraces.org/standards-and-reference-materials/. **Future efforts to collect new reference materials are anticipated to take place in 2025, and news will be displayed on the GEOTRACES website accordingly.**

References

Brown, M.T. and Bruland, K.W. 2008. An improved flow-injection analysis method for the determination of dissolved aluminium in seawater. Limnol. Oceanogr. Methods, 6: 87-95.

Bruland, K.W., Franks, R.P., Knauer, G.A. and Martin, J.H., 1979. Sampling and analytical methods for the determination of copper, cadmium, zinc and nickel at the nanogram per liter level in seawater. Anal. Chim. Acta, 105: 233-245.

Cutter, G.A., Moffett, J.G., Nielsdóttir, M.C., and Sanial, V. 2017. Multiple oxidation state trace elements in suboxic waters off Peru: in-situ redox processes and advective/diffusive horizontal transport. Mar. Chem., in press.

de Baar, H.J.W. et al., 2008. Titan: A new facility for ultraclean sampling of trace elements and isotopes in the deep oceans in the international Geotraces program. Mar. Chem., 111(1-2): 4-21.

González-Santana, D., González-Dávila, M., Lohan, M.C., Artigue, L., Planquette, H., Sarthou, G., Santana-Casiano, M., 2021. Iron (II) oxidation kinetics variability across hydrothermal sites on the Mid Atlantic Ridge. Geochimica et Cosmochimica Acta, 297: 143-

Gosnell, K.J., Landing, W.M., and Milne, A. 2012. Fluorometric detection of total dissolved zinc in the southern Indian Ocean. Mar. Chem., 132-133: 68-76.

Buck, K.N., Moffett, J., Barbeau, K.A., Bundy, R.M., Kondo, Y., Wu, J. 2012. The organic complexation of iron and copper: an intercomparison of competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) techniques. Limnol. Oceanogr. Methods, 10:496-515.

Jakuba, R.W., Moffett, J.W. and Saito, M.A. 2008. Use of a modified, high-sensitivity, anodic stripping voltammetry method for determination of zinc speciation in the North Atlantic Ocean. Anal. Chim. Acta, 614: 143-152.

Janssen, D. J., & Cullen, J. T. (2015). Decoupling of zinc and silicic acid in the subarctic northeast Pacific interior. Marine Chemistry, 177, 124-133.

Johnson, K.S. et al., 2007. Developing standards for dissolved iron in seawater. EOS, Trans., 88: 131-132.

Kondo, Y. and Moffett, J.W. 2013. Dissolved Fe(II) in the Arabian Sea oxygen minimum zone and western tropical Indian Ocean during the inter-monsoon period. Deep Sea Res. I, 73: 73-83.

Lohan, M.C., Aguilar-Islas, A.M. and Bruland, K.W. 2006. Direct determination of iron in acidified (pH 1.7) seawater samples by flow injection analysis with catalytic spectrophotometric detection: Application and intercomparison. Limnol. Oceanogr. Methods, 4: 164-171.

Lohan, M.C, Crawford, D.W., Purdie, D.A. and Statham, P.J., 2003. Iron and zinc enrichments in the northeastern subarctic Pacific: Ligand production and zinc availability in response to phytoplankton growth. Limnol. Oceanogr., 50: 1427-1437.
Measures, C.I., Landing, W.M., Brown, M.T. and Buck, C.S., 2008. A commercially available rosette system for trace metal clean sampling. Limnol. Oceanogr. Methods 6: 384-394.

Measures, C.I., Yuan, J. and Resing, J.A. 2005. Determination of iron in seawater by flow injection analysis using in-line preconcentration and spectrophotometric detection. Mar. Chem., 50: 3-12.

Milne, A., Landing, W., Bizimis, M, and Morton, P. 2010. Determination of Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in seawater using high resolution magnetic sector inductively coupled mass spectrometry (HR-ICP-MS). Anal. Chim. Acta, 665: 200-207.

Obata, H., Karatani, H. and Nakayama, E. 1993. Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection. Anal. Chem., 65: 1524-1528.

Rijkenberg, M.J., de Baar, H.J., Bakker, K., Gerringa, L.J., Keijzer, E., Laan, M., Laan, P., Middag, R., Ober, S., van Ooijen, J. and Ossebaar, S., 2015. “PRISTINE”, a new high-volume sampler for ultraclean sampling of trace metals and isotopes. Mar. Chem., 177: 501-509.

Saito, M.A, Noble, A.E., Tagliabue, A., Goepfert, T.J., Lamborg, C.H., and Jenkins, W.J. 2013. Slow-spreading submarine ridges in the South Atlantic as a significant oceanic iron source. Nature Geosci., 6: 775-779.

Wyatt, N.J., Milne, A., Woodwards, E.M.S., Rees, A.P., Browning, T.J., Bouman, H.A., Worsfold, P.J. and Lohan, M.C. (2014). Biogeochemical cycling of dissolved zinc along the GEOTRACES South Atlantic transect GA10 at 40oS. Global Biogeochm. Cycl. Doi: 10.1002/2013GB004637

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

The intent of this document is to summarize the results of the 2008, 2009 and 2017 GEOTRACES intercalibration cruises, as well as the 2012, 2013, 2014, 2015 GEOTRACES international intercalibration exercises for Hg species in seawater. We describe the accurate and efficacious collection and analysis of open ocean seawater samples for total mercury (Hg) determinations as well as Hg speciation within the context of GEOTRACES cruises. This report is not meant to be a standalone description of all aspects of on-board collection activity during a GEOTRACES cruise, but rather those aspects that we have come to view as the "recommended practice" with regard to Hg determinations. These activities include bottle selection and cleaning, sample collection and handling on board, sample filtration, the recommended analytical procedures for both on board or on shore analyses and the latest view of optimal storage/preservation approaches if immediate analysis is not possible.

7.9.1. Sample Bottle Selection and Cleaning

As part of the Intercalibration Program, we revisited some of the most fundamental analytical considerations regarding bottle selection and cleaning. Particular care was taken to examine the susceptibility of sample bottles to the diffusion of elemental Hg (Hg^o) through the bottle walls. Consideration of this potential contamination pathway is unique to Hg and is particularly important because many GEOTRACES cruises are likely to have large amounts of Hg on board for electrochemical-based speciation analyses, and $HgCl_2$ is often used to preserve samples. The potential for significantly elevated Hg levels in shipboard laboratory spaces may result in airborne Hg concentrations that are highly elevated with respect to ambient air (ca. 1.5 ng m^{-3}). For example, on the two US GEOTRACES Intercalibration cruises, we found Hg^0 concentrations in the Hg Group work spaces that ranged from 20 to 50 ng m^{-3}. Given this range in ship-board air 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 total Hg anticipated in open ocean seawater is below 2.5 pM, the potential impact from airborne contamination is quite significant. While there are methods to fix this contamination (see below), every effort should be made to minimize work space Hg^o concentrations, including the use of activated charcoal scrubbers in laminar flow benches and the requisition of a separate laboratory van so that analyses may be performed outside of ship's lab spaces.

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

Our recommended Teflon and plastic bottle cleaning procedure is shown below, and was found to be effective for the very low-level seawater concentrations, and resulted in low blanks for bottles made of almost any material. The key ingredient seemed to be BrCl, which is the commonly used wet chemical oxidant for digesting aqueous samples prior to total Hg analyses. The BrCl concentration used during cleaning should be greater than that used in subsequent sample digestion to ensure best results. Bottles used for Hg species analyses (Hg^o, $(CH_3)_2Hg$ and $CH_3Hg(I)$) should be have been in contact with BrCl prior to use, to avoid destruction of these forms. In our recommended workflow described below, we also segregate the analysis

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

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

Table 7.1. Recommended cleaning procedure for new bottles for total Hg in seawater.

We recommend that GEOTRACES samples for Hg be collected into those bottles that best fit the individual workflow of the cruise. For example, 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.

7.9.2. Sample Collection and Handling

We found that the collection of Hg is relatively insensitive to the sampling platform used (e.g., CLIVAR clean rosette, GEOTRACES carousel or GO-FLO bottle hung sequentially on a non-metallic hydrographic line, such as Kevlar). Thus, as long as the collection bottle (GO-FLO, X-Niskin, 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 were tested, including the use of pressurized GO-FLOs and in-line capsule filters (Osmonics 0.2 µm Teflon and the Acropak 0.2 µm Polyethersulfone) and as well as vacuum-assisted membrane filtration. The most commonly used membrane (0.45 µm pore size Nuclepore) and the capsule filters all seemed to compare well, suggesting that the particular filtering medium used is not critical, as long it has been previously tested to ensure a low blank. 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. Thus, we should not be surprised that different filtering media, assuming that they do not contribute a Hg blank or absorb Hg, should provide similar "dissolved" Hg results. Colloidal Hg is significant in coastal ocean environments, however, so that near-shore sampling should include a pore size-dependent definition of "dissolved" (e.g., Stordal *et al.*, 1996; Choe et al., 2003).

7.9.3. 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 Intercalibration Program, 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 compared well, while the CVAAS did not exhibit adequate sensitivity to detect total Hg on the Intercalibration samples (250 mL). Thus, we recommend CVAFS or ICP-MS for Hg determinations. The CVAFS approach has the distinct advantage of being field employable allowing rapid determination of 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, we recommend CVAFS for at sea determinations, but feel that either approach is appropriate for on shore analyses.

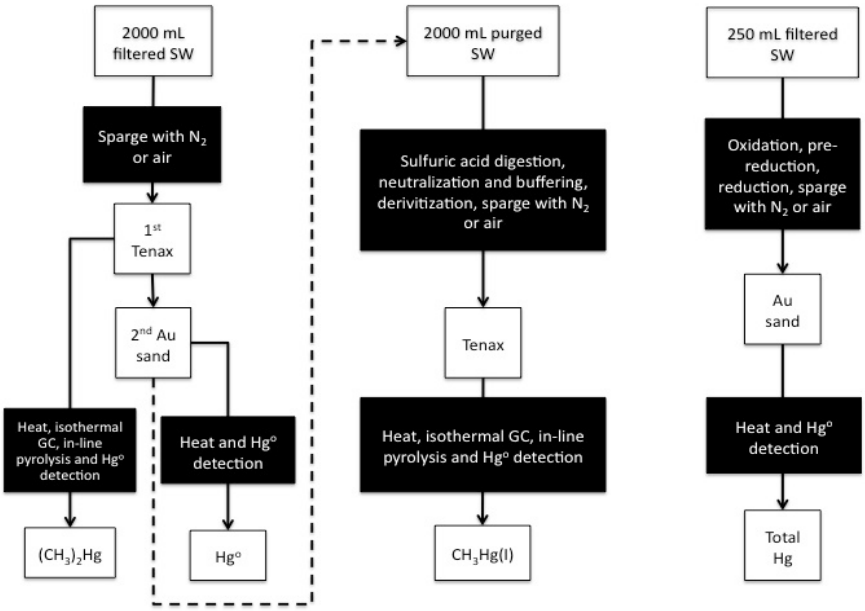
Our recommended workflow is illustrated in Figure 7.2. Details of instrument use are documented elsewhere (e.g., Fitzgerald and Gill, 1979; Gill and Fitzgerald, 1985; Gill and Fitzgerald, 1987; Horvat, 1991; Hintelmann and Wilken, 1993; Horvat et al., 1993; Hintelmann et al., 1997; Hintelmann, 1998; Hintelmann and Simmons, 2003; Bowman and Hammerschmidt, 2011). The workflow presented is oriented toward at-sea, multi-species determinations by CVAFS, but could be easily adapted for use with ICP-MS back on shore. A ready supply of high-quality water (18 MΩ.cm resistivity) will be necessary for at sea or on shore cleaning, standard and reagent making. Most commercially available "ultrapure" water systems are adequate for Hg analyses, but a check of the ship's system should be done immediately, and it may be prudent to bring a back-up system. Though not shown in the workflow, laboratories need to also do a very careful determination of analytical, bottle, and reagent blanks to assure that they are working at levels appropriate to the determination of open ocean seawater. If possible, this should be done on shore prior to a cruise as well as during the cruise. Replicate analyses on several samples to demonstrate precision is also a highly desirable when adequate sample is available. Standard spikes recoveries, especially for the $CH_3Hg(I)$ determination, should also be performed. These QA results should be reported along with the Hg results to demonstrate capability, reproducibility and accuracy.

- Total Hg

During recent cruises, we have documented concentrations of total Hg in surface waters that are often highly depleted due to biological uptake and particle scavenging. Thus, GEOTRACES analysts should be prepared to deal with samples containing as little as 0.1 pM total Hg. As typical CVAFS arrangements have absolute detection limits on the order of 10 fmole, analyses performed on sample volumes of ca. 250 mL is recommended to ensure a resolvable signal. We also propose a method on 40 mL samples allowing 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} (we recommend 0.15 - 0.5 L min^{-1}).

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



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- Hg^0 and $(CH_3)_2Hg$

Although these two dissolved gaseous mercury species are minor components (typically sub-pM concentrations) of the total mercury present in seawater, they are nonetheless highly important to measure as they are involved in air-sea exchange of Hg and probably in the formation of $CH_3Hg(I)$. Given the extremely low concentrations of these species, we recommend using 2 L sample sizes for analysis, with determination of Hg^o, $(CH_3)_2Hg$ and $CH_3Hg(I)$ all performed on the same aliquot. Procedurally, Hg^o and $(CH_3)_2Hg$ are the easiest of the species to measure, requiring only that a volume of stripping gas of at least 15x the volume of liquid be sparged through the fluid without further amendment. We have successfully used two sorption media in series to discriminate between these two gaseous mercury species. The gas exiting the sparger should pass first through a moisture trap (e.g., soda lime), then either Tenax or Carbotrap (or the equivalent) for $(CH_3)_2Hg$ collection, followed by Au or Au-coated sand for Hg^o collection (e.g., Bloom and Fitzgerald, 1988; Tseng

et al., 2004; Conaway *et al.*, 2009). Following sparging, the traps are analyzed separately using a CVAFS system that is equipped with a gas flow train. The Hg^0 collected on the gold trap is liberated for detection by simply heating (600-800 °C) in an argon gas-flow train connected to the CVAFS detector. The $(CH_3)_2Hg$ retained on the chromatography material trap is liberated under low heat (90-250 °C) and is passed first through a low temperature, isothermal chromatographic column (see in $CH_3Hg(I)$ section below) and then through a high temperature (600-800 °C) column packed with quartz wool to pyrolyze the $(CH_3)_2Hg$ to Hg^o and make it available for detection by CVAFS (Bloom and Fitzgerald, 1988). Tenax and Carbotrap columns should be rigorously preconditioned prior to use by sparging and heating them several times. Furthermore, they should be tested to ensure that they do not retain Hg^0 to a large degree. We recommend the use of Tenax rather than Carbotrap as it retains much less moisture and Hg^0. Fresh soda lime drying agent should be used on each sample, and can be recycled through baking.

- $CH_3Hg(I)$

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

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

As noted below, the ethylating agent is made up in small batches, but which often are not completely consumed within one week. After a week, even when kept frozen, the ethylating agent loses its potency and should be discarded. The thawed, working aliquot of 1% (wt:vol) NaTEB will also unavoidably lose potency throughout the course of the day, which can be slowed by keeping the solution cold. We recommend working samples in batches of four, by adding 1.5 mL of NaTEB directly to the buffered 2 L sample, allowing each sample to react for at least 15 minutes, and then sparging the methylethyl mercury ($CH_3CH_2HgCH_3$) from the sample using a bottle top sparging adaptor as mentioned above.

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

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. We make use of the fact that $(CH_3)_2Hg$ converts to $(CH_3)Hg(I)$ upon acidification to preserve samples for total methylated Hg (MeHg = $(CH_3)_2Hg$ + $(CH_3)Hg(I)$). We propose to measure DGM ($(CH_3)_2Hg$ + Hg°) from a larger sample (>250 mL) directly onboard, and preserve then 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.

7.9.4. Calibration and Comparability

One of the findings of the Intercalibration was that interlaboratory comparability was on the order of 50%. This lack of interlaboratory accuracy is unacceptable, as basin-to-basin variation in Hg concentrations (when comparing regions of similar productivity) can be expected to be considerably less. If datasets from cruises where different groups were involved are to be comparable, then overall accuracy must be improved. We therefore recommend that traceable Standard Reference Materials be included at numerous times during analyses. A list of Certified and Standard Reference Materials relevant to marine research is included below in Table 7.2. 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, we have sampled 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 to receive CVRM aliquots.

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

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.

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

7.9.5. Reagents

Hydroxylamine hydrochloride – dissolve 30 g of $NH_2OH \cdot HCl$ in 18 M□-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 M□-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 M□-cm water and shake until dissolved. Test pH, and adjust with acetic acid or sodium acetate to equal 5.5. Add more water to make up to 100 mL.

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

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

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).

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 M□-cm water with a Hg content <0.1 ng/L.

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

References

Bloom, N. and W.F. Fitzgerald (1988) Determination of volatile mercury species at the picogram level by low-temperature gas chromatography with cold-vapor atomic fluorescence detection, Analytica Chimica Acta, 208, 151-161.

Bowman, K. and C.R. Hammerschmidt (2011) Extraction of monomethylmercury from seawater for low-femtomolar determination, Limnol. Oceanogr. Methods, 9, 121-128.

Conaway, C.H., F.J. Black, M. Gault-Ringold, J.T. Pennington, F.P. Chavez and A.R. Flegal (2009) Dimethylmercury in Coastal Upwelling Waters, Monterey Bay, California, Environmental Science & Technology, 43, 1305-1309.

Fitzgerald, W.F. and G.A. Gill (1979) Subnanogram determination of mercury by two-stage gold amalgamation applied to atmospheric analysis, Analytical Chemistry, 51, 1714-1720.

Gill, G.A. and W.F. Fitzgerald (1985) Mercury sampling of open ocean waters at the picomolar level, Deep-Sea Research, 32, 287-297.

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.

Heimbürger, L.E., J. E. Sonke, D. Cossa, D. Point, C. Lagane, L. Laffont, B. T. Galfond, M. Nicolaus, B. Rabe and M. R. van der Loeff (2015) Shallow methylmercury production in the marginal sea ice zone of the central Arctic Ocean, Scientific Reports 5

Hintelmann, H. and R.D. Wilken (1993) The Analysis of Organic Mercury-Compounds Using Liquid-Chromatography with Online Atomic Fluorescence Spectrometric Detection, Applied Organometallic Chemistry, 7, 173-180.

Hintelmann, H., R. Falter, G. Ilgen and R.D. Evans (1997) Determination of artifactual formation of monomethylmercury (CH3Hg+) in environmental samples using stable Hg2+ isotopes with ICP-MS detection: Calculation of contents applying species specific isotope addition, Fresenius Journal of Analytical Chemistry, 358, 363-370.

Hintelmann, H. (1998) Distillation of methylmercury using a microdistillation technique, Canadian Journal of Analytical Sciences and Spectroscopy, 43, 182-188

Hintelmann, H. and D.A. Simmons (2003) Determination of aqueous methylmercury species using electrospray mass spectrometry, Canadian Journal of Analytical Sciences and Spectroscopy, 48, 244-249.

Horvat, M. (1991) Determination of methylmercury in biological certified reference materials, Water Air and Soil Pollution, 56, 95-102.

Horvat, M., L. Liang and N.S. Bloom (1993) Comparison of Distillation with Other Current Isolation Methods for the Determination of Methyl Mercury-Compounds in Low-Level Environmental-Samples. 2. Water, Analytica Chimica Acta, 282, 153-168.

Stordal, M.C., G.A. Gill, L.S. Wen and P.H. Santschi (1996) Mercury phase speciation in the surface waters of three Texas estuaries: Importance of colloidal forms, Limnology and Oceanography, 41, 52-61.

Tseng, C.M., C. Lamborg, W.F. Fitzgerald and D.R. Engstrom (2004) Cycling of dissolved elemental mercury in Arctic Alaskan lakes, Geochimica Et Cosmochimica Acta, 68, 1173-1184.

7.10. 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.

7.10.1. 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.

The US GEOTRACES GP-17 cruises (GP17-OCE and GP17-ANT) have added a 5µm 25mm polycarbonate membrane (Whatman Nuclepore, VWR part no. 76332-608) upstream of the 0.45µm Supor filter at stations characterized by higher phytoplankton biomass. Membranes were contained in separate Swinnex filter holders connected to the Supor filter holders with a short (ca. 3cm) piece of acid-cleaned polypropylene tubing as shown in Figure 7.3 below.

Figure 7.3. Example of collecting size fractionated particles (5 and 0.45 µm) from GO-FLO bottles.

7.10.2. 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 7.3 and 7.4. **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).

Figure 7.4. Advantec-MFS polypropylene 47 mm filter holders.

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 may 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).

7.10.3. Cleaning Filters and filter holders

Filters are cleaned by the following protocol:

1. Pre-clean a 1000 mL LDPE pre-cleaned bottle by filling with 10% (v/v, or 0.12M) of TM Grade HCl, double bagging in 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.

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.

7.10.4. 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 Nordsom 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. Air trapped in the headspace of Swinnex filter holders can also be released by slightly loosening the filter holder while maintaining the Swinnex holder in the upright position. 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.

7.10.5. 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.

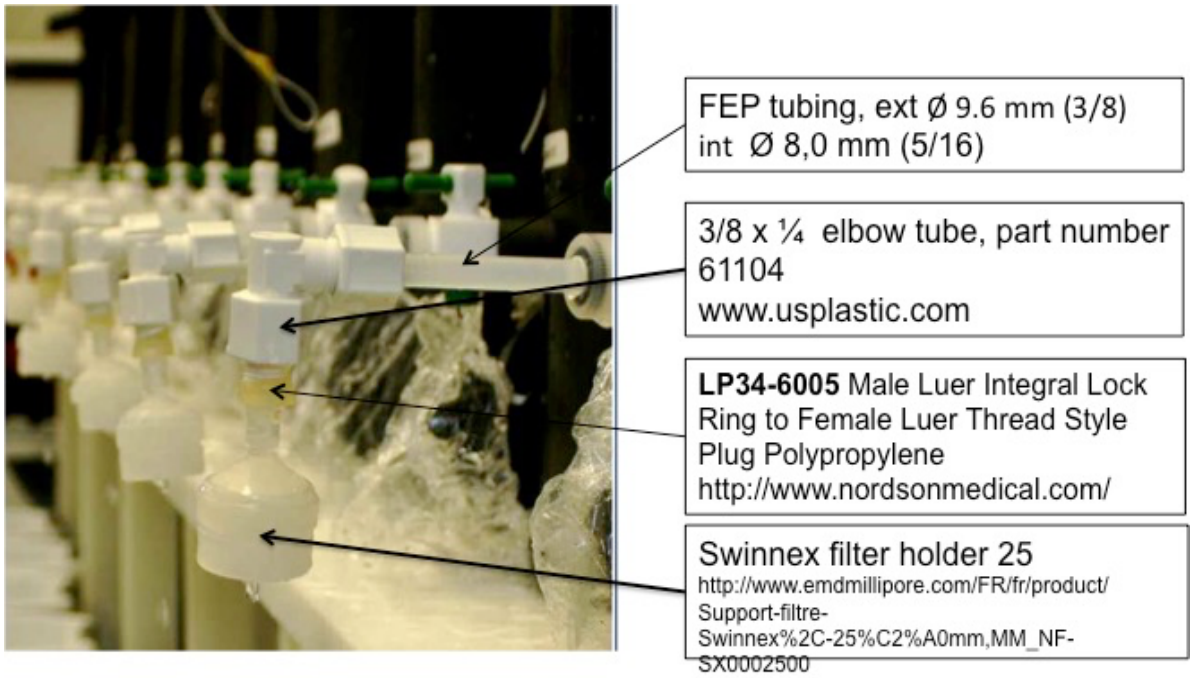

Figure 7.5. (a) Swinnex 25 mm filter holders showing 3/8" OD tubing, 90° compression-NPT adapter, and NPT-Luer lock adapter. Note 11 L waste baskets for filtrate volume measurements; (b) Compact polycarbonate configuration for Luer port filter holders (lock-ring FSLLR-9 is optional).

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. A comparison of particulate TEI concentrations measured with GO-FLO bottles or in-situ pumps on GA-3 found that particulate Fe, Al, and Ti concentrations tend to be 5-15% lower in bottle-collected particles in nearshore environments, likely due to sinking, and hence undersampling, of larger lithogenic particles in GO-FLO bottles during filtration.

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 (e,g., Twining et al. 2015, Lam et al., 2015).

7.10.6. Pressurizing water sampling bottles for filtration

Gas pressure applied to GO-FLO bottles 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.

7.10.7. Process blanks

Filtration process 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 (e.g., via adsorption) 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.**

7.10.8. Storing Sampled Filter

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 (e.g. Figure 7.6). 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.

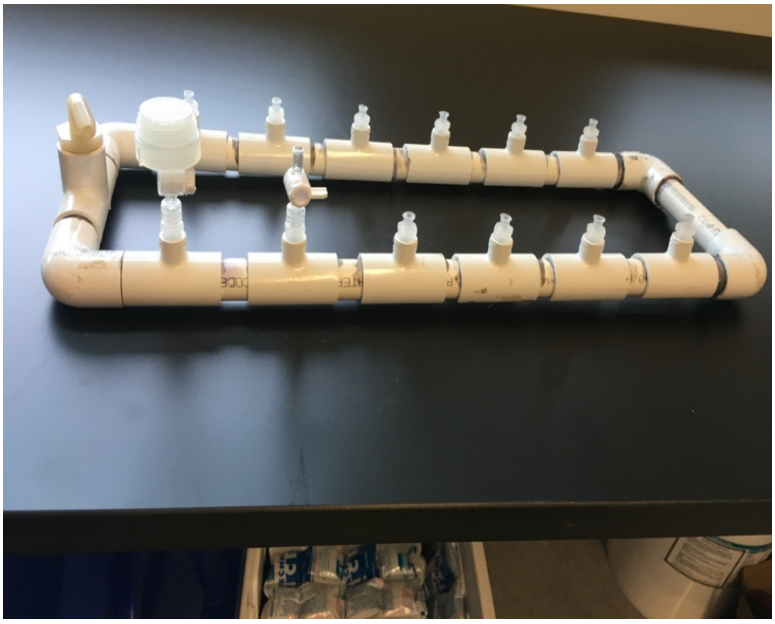

Figure 7.6. Custom-made "Supor sucker" designed to remove residual seawater. More information can be retrieved from Benjamin Twining (Bigelow): btwining@bigelow.org

7.10.9. 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).

7.10.10. 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 is 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 7.7. 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).

7.10.11. 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.

The following three videos were recorded during the 3rd Summer school in Germany in 2022 and are available through regular channels. They cover:

- **the principles and applications of ICP-OES and ICP-MS techniques in the analysis of marine samples:**
https://youtu.be/DDyxY0bk9t4
https://v.youku.com/v_show/id_XNjE3MjM3NjI4MA==.html

- **calibration and validation of analysis of marine samples**:
https://youtu.be/3eSmsUnwrdA
https://v.youku.com/v_show/id_XNjE3NjUxODMzMg==.html

- **the principles of working in a clean room with marine samples**
https://youtu.be/nSB6lIBhxKc
https://v.youku.com/v_show/id_XNjE3NjUyNzIyMA==.html

7.10.12. 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.

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.

- Fill with 1.2M TM grade HCl (this solution can be recycled three times), cap tightly and place in a plastic or polystyrene foam tube rack.
- Double-bag in sturdy plastic zip-lock bags (e.g. 4 mil), then heat in a 60˚ C oven for 4 hours to overnight.
- Turn upside down to cool in fume hood and leach caps.
- Rinse with Milli-Q water 3 times, including careful rinsing of cap and tube threads.
- Shake dry, and allow to dry in laminar flow clean bench.

7.10.13. Filter Digestion procedure

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

- 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.
- 1/16 pie wedges of 142mm filters from in-situ pumps are wrapped against the inner wall of the vial.
- 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 PACS, 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: https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/list/126
  - PACS-3: https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/list/138
  - Arizona Test Dust: available from William Landing (lwlanding@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 than most oceanic samples, but balances what is possible to weigh out reproducibly with what is at the upper limit of plausible concentrations.

- 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 PACS-3) should be processed in parallel of the samples.
- Data from participating labs have been collected by Hélène Planquette (helene.planquette@univ-brest.fr) and consensus data will be available on the GEOTRACES website under "intercalibration".

7.10.14. 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.

7.10.15. 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.

7.10.16. 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.

References

Berger, C. J., Lippiatt, S. M., Lawrence, M. G., & Bruland, K. W. (2008). Application of a chemical leach technique for estimating labile particulate aluminum, iron, and manganese in the Columbia River plume and coastal waters off Oregon and Washington. Journal of Geophysical Research: Oceans, 113(C2)

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

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

Fitzsimmons, J.N., John, S.G., Marsay, C.M., Hoffman, C.L., Nicholas, S.L., Toner, B.M., German, C.R., Sherrell, R.M., 2017. Iron persistence in a distal hydrothermal plume supported by dissolved-particulate exchange. Nature Geosci 10, 195-201. 10.1038/ngeo2900

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

Heller, M.I., Lam, P.J., Moffett, J.W., Till, C.P., Lee, J.-M., Toner, B.M., Marcus, M.A., 2017. Accumulation of Fe oxyhydroxides in the Peruvian oxygen deficient zone implies non-oxygen dependent Fe oxidation. Geoch. Et Cosmoch. Acta. 10.1016/j.gca.2017.05.019.

Lam, P. J., B. S. Twining, C. Jeandel, A. Roychoudhury, J. A. Resing, P. H. Santschi, and R. F. Anderson. 2015. Methods for analyzing the concentration and speciation of major and trace elements in marine particles. Progress in Oceanography 133: 32-42.

Milne, A., Schlosser, C., Wake, B. D., Achterberg, E. P., Chance, R., Baker, A. R., ... & Lohan, M. C. (2017). Particulate phases are key in controlling dissolved iron concentrations in the (sub) tropical North Atlantic. Geophys. Res. Let., 44(5), 2377-2387.

Ohnemus, D.C., Auro, M.E., Sherrell, R.M., Lagerstrom, M., Morton, P.L., Twining, B.S., Rauschenberg, S., Lam, P.J., 2014. Laboratory intercomparison of marine particulate digestions including Piranha: a novel chemical method for dissolution of polyethersulfone filters. Limnol. Oceanogra., Methods 12, 530-547. 10.4319/lom.2014.12.530.

Ohnemus, D.C., Lam, P.J., 2015. Cycling of lithogenic marine particles in the US GEOTRACES North Atlantic transect. Deep Sea Res. Part II: 116, 283-302.

Ohnemus, D.C., Rauschenberg, S., Cutter, G.A., Fitzsimmons, J.N., Sherrell, R.M., Twining, B.S., 2016. Elevated trace metal content of prokaryotic communities associated with marine oxygen deficient zones. Limnol. Oceanogra. 10.1002/lno.10363.

Planquette, H., and Sherrell, R. M. (2012). Sampling for particulate trace element determination using water sampling bottles: methodology and comparison to in-situ pumps. Limnol. Oceanogr., Methods, 10(5), 367-388.

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

Twining, B.S., Rauschenberg, S., Morton, P.L., Ohnemus, D.C., Lam, P.J., 2015. Comparison of particulate trace element concentrations in the North Atlantic Ocean as determined with discrete bottle sampling and in-situ pumping. Deep Sea Research Part II: Topical Studies in Oceanography 116, 273-282

7.11. 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 for further details. For recommendations on best practices for optical detection of particles by transmissometer, please refer to the Particle Optics Protocols section. In the discussion that follows, we focus on experiences from the U.S. GEOTRACES cruises that use modified McLane in-situ pumps.

7.11.1. Configuration of in-situ filtration systems

- Filter holder design to prevent large particle loss

Commercially available (*e.g.* "Standard Radial" McLane WTS-LV holder) and "home-made" single-baffle 142mm filter holders were found to lose major quantities of large particles during the two US GEOTRACES intercalibration cruises (Bishop et al., 2012). 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 a metal-free "vertical intake" 142mm filter holders with multiple baffle systems based on the design tested during the GEOTRACES intercalibration cruises. Contact McLane for details (mclane@mclanelabs.com).

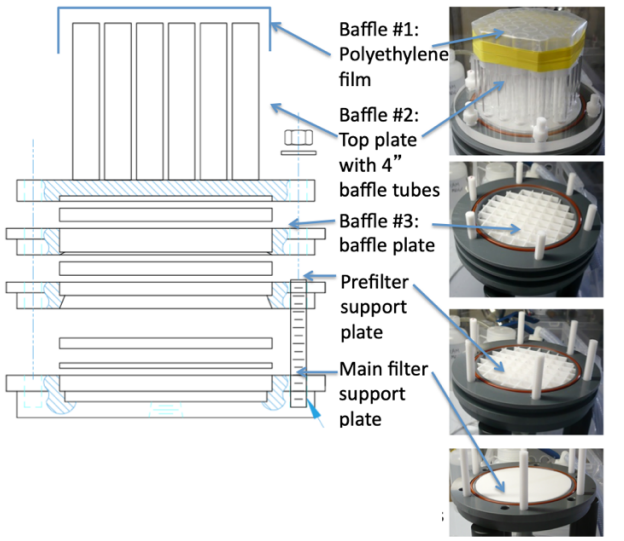

Figure 7.8. Schematic of mini-MULVFS filter holder, with pictures of the baffle and filter support plates. The baffle logic follows that of the main MULVFS filter holder (Bishop and Wood, 2008) with modifications to facilitate

- 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. McLane Research Labs now incorporates a debubbler in their "WTS-LVDF dual filter" in-situ pump model.

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).

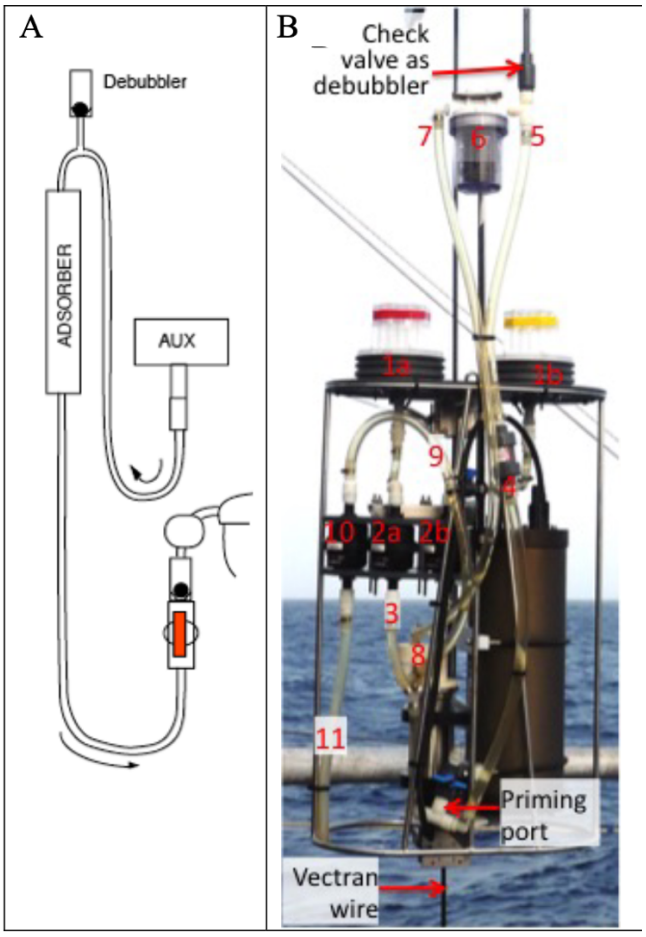

Figure 7.9. A. Schematic of placement of debubbler and Mn-adsorber cartridge relative to filter holder ("AUX") on MULVFS (from Bishop and Wood, 2008). B. WHOI modification of McLane WTS-LV upright pump for U.S. GEOTRACES. Numbers mark the direction of flow during pumping, with flow entering the two "mini-MULVFS" 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

- 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 deployment, seawater is retained in the plumbing lines and subsequent deployments do not require priming. McLane Research Laboratories, Inc., now offer a dual-flow option (WTS-LVDF). Contact McLane for details (mclane@mclanelabs.com).

- 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 backflushing through the Mn cartridge and up into the filter holder. **Placement of the Mn cartridge must be higher than the filter holder to minimize contamination of filters due to backflow (Fig. 2b, #6).**

The placement of the Mn cartridge above the filter holder minimizes the backflushing through the Mn cartridge and into the filter holder as air is forced out of the system through the debubbler. The placement of a debubbler at the highest point in the plumbing and next to the Mn cartridge further allows excess Mn to escape as the plumbing floods with seawater. A one-way check valve is placed just upstream of the Mn cartridge as an additional safeguard from contamination from the Mn cartridge (Fig. 7.9b, #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.

- 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.

7.11.2. 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.

- 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.

- 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.

- 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).

7.11.3. Filter cleaning procedure

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

- 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 or “vertical intake” McLane), but not those that have a recessed stage (e.g., standard radial 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.**
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.

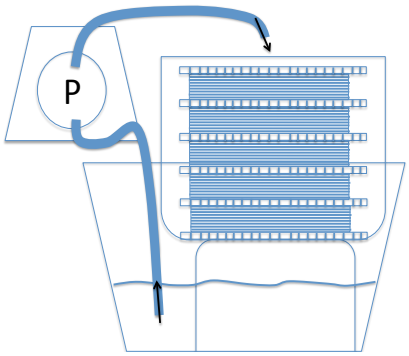

Figure 7.10. Schematic of Milli-Q-drip-rinsing setup for rinsing acid out of filters.

- 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.

- 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.

7.11.4. 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.

7.11.5. 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.**

- 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.

- 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 (Fig. 7.11). 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 polypropylene containers that have had holes punched through them (Fig. 7.11). 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.

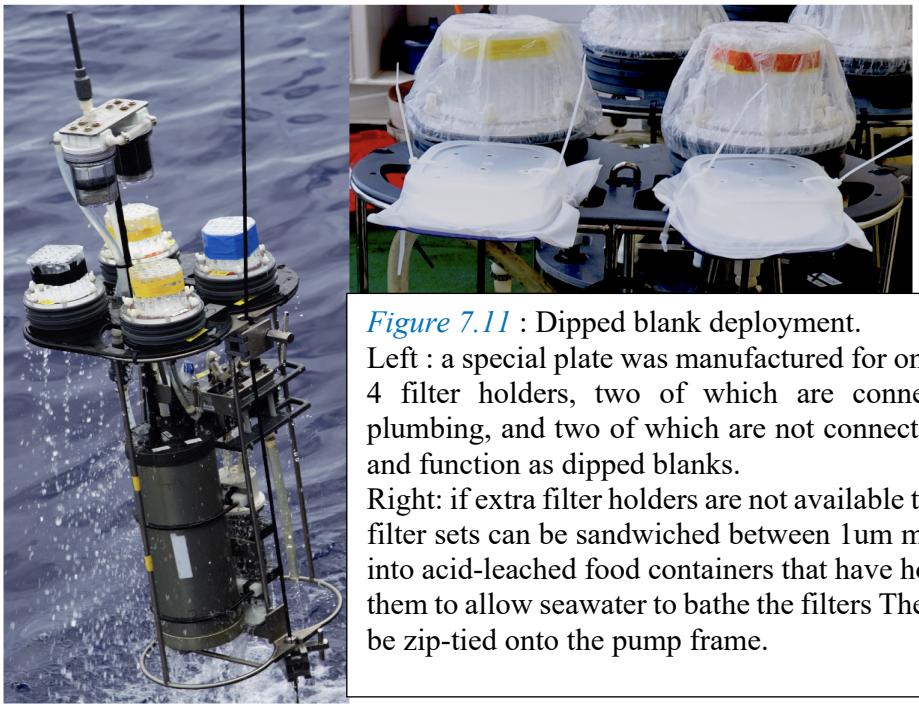

Figure 7.11 : Dipped blank deployment.
Left : a special plate was manufactured for one pump to hold 4 filter holders, two of which are connected to pump plumbing, and two of which are not connected to plumbing and function as dipped blanks.
Right: if extra filter holders are not available to use as blanks, filter sets can be sandwiched between 1um mesh and loaded into acid-leached food containers that have holes punched in them to allow seawater to bathe the filters The containers can be zip-tied onto the pump frame.

- 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 the 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.

- 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.

- 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.

Figure 7.12. 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.

- 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
6)

- Removal of residual seawater

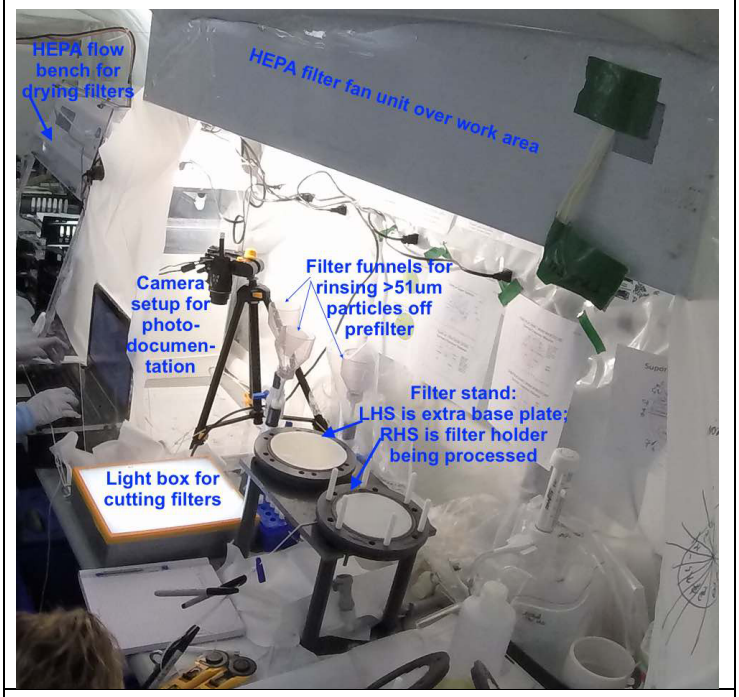

Figure 7.12. In-situ pump filter processing set-up used on U.S. GEOTRACES cruises.

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 seasalt 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.**

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 7.13). 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.

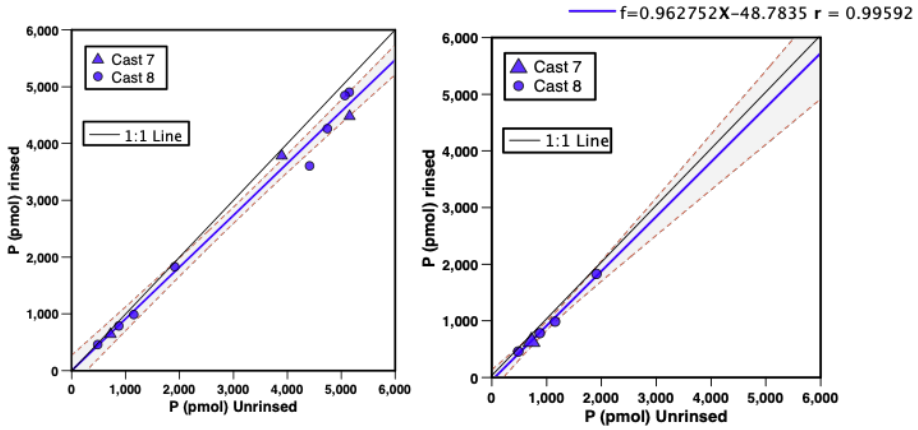

Figure 7.13. Effect of misting with MQ-H_2O on P on samples from SAFe. a) misting leads to a ~9% loss of P (P_{rinsed}=0.912*$P_{unrinsed}$+0.49, r=0.994) P loss is restricted to euphotic zone samples, as B) isted vs. unisted samples deeper than 120 m were not significantly different (H. Bourne and J. Bishop, unpublished).

- 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. 7.14) 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).

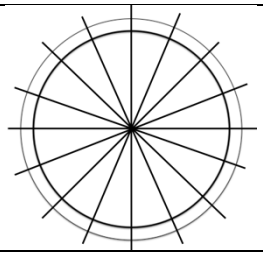

Figure 7.14. template pattern for subsampling plastic filters

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.

- 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.

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. 7.14). 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 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.

- 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.

7.11.6. 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)

References

Anderson, R.F., Fleisher, M.Q., Robinson, L.F., Edwards, L., Hoff, J.A., Moran, S.B., Rutgers vd Loeff, M., Thomas, A., Roy-Barman, M., François, R., 2012. GEOTRACES Intercalibration of 230Th, 232Th, 231Pa and prospects for 10Be. Limnology and Oceanography: Methods, 10, 179-213.

Bishop, J.K.B., Lam, P.J., Wood, T.J., 2012. Getting good particles: accurate sampling of particles by large volume in-situ filtration. Limnology and Oceanography Methods, 10, 681-710.

Bishop, J.K.B., Schupack, D., Sherrell, R.M., Conte, M., 1985. A Multiple-Unit Large-Volume In-situ Filtration System for Sampling Oceanic Particulate Matter in Mesoscale Environments. Advances in Chemistry Series, 155-175.

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, 1684-1706.

Charette, M.A., Moran, S.B., Bishop, J.K.B., 1999. Th-234 as a tracer of particulate organic carbon export in the subarctic northeast Pacific Ocean. Deep-Sea Research Part Ii-Topical Studies in Oceanography, 46, 2833-2861.

Collier, R., Edmond, J., 1984. The Trace-Element Geochemistry of Marine Biogenic Particulate Matter. Progress in Oceanography, 13, 113-199.

Cullen, J.T., Sherrell, R.M., 1999. Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off central California. Marine Chemistry, 67, 233-247.

Lam, P.J., Bishop, J.K.B., 2007. High Biomass Low Export regimes in the Southern Ocean. Deep Sea Research Part II: Topical Studies in Oceanography, 54, 601-638.

Lam, P.J., Bishop, J.K.B., Henning, C.C., Marcus, M.A., Waychunas, G.A., Fung, I.Y., 2006. Wintertime phytoplankton bloom in the subarctic Pacific supported by continental margin iron. Global Biogeochemical Cycles, 20, doi:10.1029/2005GB002557.

Lam, P.J., Ohnemus, D.C., Auro, M.E., 2015. Size-fractionated major particle composition and concentrations from the US GEOTRACES North Atlantic Zonal Transect. Deep Sea Research Part II: Topical Studies in Oceanography, 116, 303-320.

Ohnemus, D.C., Auro, M.E., Sherrell, R.M., Lagerstrom, M., Morton, P.L., Twining, B.S., Rauschenberg, S., Lam, P.J., 2014. Laboratory intercomparison of marine particulate digestions including Piranha: a novel chemical method for dissolution of polyethersulfone filters. Limnology and Oceanography-Methods, 12, 530-547.

8. 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 discussed in Section 8.7.2.

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 of 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.

8.1. AEROSOL SAMPLING EQUIPMENT

High Vol Total Suspended Particulate (TSP) samplers typically filter air at rates greater than about 1 cubic meter (1000 liters) per minute. Low Vol TSP samplers typically filter air at rates of less than 100 liters per minute. Cascade Impactors typically include multiple stages. COARSE refers to the sum of the concentrations on stages greater than or equal to 1 µm (or as close to 1 µm as possible). FINE refers to the sum of the concentrations on stages less than 1 µm (or as close to 1 µm as possible).

8.1.1. High-volume Sampler and control systems

The following sections describe the equipment and methods for high-volume aerosol sampling that is conducted on US GEOTRACES cruises. This type of sampler was chosen because it can provide multiple replicate subsamples for replicate analysis (to test precision) and also to share with other researchers. There are numerous commercially available high and low volume aerosol samplers for collecting TSP (total suspended particulates) as well as size-fractionated aerosols (e.g. PM10 and PM2.5).

Shipboard aerosol collection is conducted using Volumetric Flow Controlled (VFC) high-volume samplers purchased from Tisch Environmental (TE-5170V-BL; Figure 8.1). 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 section 8.1.2 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 8.2). 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 8.3). Both filter holders are interchangeable with a high-volume Sierra-style slotted cascade impactor for particle size distribution studies (Tisch Environmental, TE-235, Figure 8.4). 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.

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).

Figure 8.1. Tisch Environmental (TE-5170V-BL) TSP sampler.

Figure 8.2. Large format (25.4 cm x 20.3 cm) filter holder and filter holder with the cover.

Figure 8.3. 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). http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences/products-and-solutions/lab-filtration)

Figure 8.4. Sierra-style slotted cascade impactor (Tisch PN TE-235).

8.1.2. High-volume 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;
- 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)

8.2. FILTER TYPES

The filter matrix and size depend 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.

8.3. Filter preparation

8.3.1. 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.
8. 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.

8.3.2. 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.

8.4. 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).

8.5. 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.

8.6. 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 8.2), 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 8.2), 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).

8.7. 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.

8.7.1. Total aerosol processing (digestion)

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 3. The size distribution of the ATD is shown in Table 4. Contact William Landing (wlanding@fsu.edu) or Peter Morton (pete.morton@exchange.tamu.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).

- 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 1 and Table 2 below. These are useful for evaluating potential contamination and expected analytical performance for total aerosol trace element determinations.

Table 8.1. 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 8.2. 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† (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

†Detection limit reported as 3σ of the blanks

8.7.2. Soluble aerosol processing (mild and strong leaches)

Assessment of soluble aerosols has been accomplished by various methods. Below are protocols for some of the most commonly used leaches.

- Mild leaching with ultrapure water

The method for this treatment is summarized and available from this link: https://www.bodc.ac.uk/data/documents/nodb/499432/

- Mild leaching with surface filtered seawater

Aerosol leaching methods using surface filtered seawater were described by Aguilar-Islas et al (2010; doi:10.1016/j.marchem.2009.01.011). Their approach was to sequentially filter aliquots of trace-metal clean filtered seawater through a loaded aerosol filter until the trace element concentrations in the initial seawater and the leachate were the same.

- Strong leaching with acetic acid (Berger leach)

Aerosol solubility measured using stronger leaching methods can be conducted sequentially (after a mild leach with ultrapure water) or on a previously un-leached filter. A whole 47mm filter (or filter section) is placed to a 15mL plastic centrifuge tube and the strong leaching (Berger leach) is conducted using 5mL of 25% (4.4 M) ultrapure acetic acid, with 0.02M hydroxylamine hydrochloride as the reducing agent (Berger et al., 2008). After a 10 min heating step (90 C), the leaches were left overnight (12-24 h; for convenience) before being centrifuged for 5 min at 3400 x gravity. The leachate was then carefully decanted into acid-clean LDPE bottles. In order to rinse any residual acetic acid from the filter, 5mL of UHP water was pipetted into the centrifuge tubes, which were then centrifuged again for 5 min at 3400xg. This supernatant was then added to the acetic acid leachate in the LDPE sample bottles. There is a slight risk that the heating step could begin to attack the mineral matrix, resulting in a slight overestimation of the upper limit of solubility, but this risk was shown to be minimal (Berger et al., 2008). The heating temperature and time of exposure as well as the post-heating duration were chosen based on precision and recovery studies conducted with replicate aerosol filters and Arizona Test Dust (Section 11.8). The use of SRMs or CRMs is highly recommended when developing any leaching scheme.

- Strong leaching with ammonium acetate (Baker and Jickells leach)

The method for this treatment is summarized and available from this link: https://www.bodc.ac.uk/data/documents/nodb/497724/

8.8. Reference material: Arizona Test Dust

Iso 12103-1, A1 Ultrafine Test Dust (Powder Technologies, Inc.) is a recommended reference material for assessing accuracy of total digestion methodology for Total aerosol trace element values. Subsamples of this material can be obtained from Peter Morton at Texas A&M University. Elemental composition (%) and particle size distributions are shown in Table 8.3 and Table 8.4.

Table 8.3. % Major element composition of Iso 12103-1, A1 (ultrafine) Arizona Test Dust

	SiO_2	Al_2O_3	Fe_2O_3	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 8.4. Particle size distribution for Iso 12103-1, A1 (ultrafine) Arizona Test Dust

Size (µm)	% less than	Size (µm)	% less than
0.97	3.0 – 5.0	5.50	65.0 – 69.0
1.38	7.0 – 10.0	11.00	95.5 – 97.5
2.75	23.0 – 27.0	22.00	100

8.9. Reporting aerosol data for a GEOTRACES Intermediate Data Product

Inclusion of aerosol data in a GEOTRACES Intermediate Data Product (IDP) requires the registration of each dataset using the GEOTRACES Data for Oceanic Research (DOoR) Portal: https://geotraces-portal.sedoo.fr/pi/.

Detailed explanations are provided in section 3.1.

Results from aerosol samples from a given cruise, or subset of aerosol samples from a given deployment, are defined as a dataset and can include multiple parameters. Each aerosol analyte must have an approved GEOTRACES parameter name (examples for aerosol aluminum are shown in Table 8.5). These names are designed to convey the element name, total or leachable fraction, and the sampler type. The DOoR portal provides template files for the intercalibration of the data as well as spreadsheet template files for registering and reporting the data. When your dataset is registered using the DOoR, each parameter will be assigned a unique barcode that is used to track the dataset through the entire process ultimately to an IDP.

When using the DOoR portal, you may find that your analyte does not have an assigned name. If so, you can click the "Missing parameter" button in Step 1.2 which will pop up an email template to send to the Parameter Definition Committee who will work with you to establish new parameter names.

Table 8.5: Aerosol parameter names used in the GEOTRACES DOoR portal for dataset registration. Aluminum is shown as an example TEI.

Parameter Name	Sampler Type	Preferred Units	Parameter Description
Al_A_T_CONC_HIVOL	High Vol	mol/m^3	Total Al concentration in aerosols (no preliminary leaching)
Al_A_SMLH2O_CONC_HIVOL	High Vol	mol/m^3	Soluble Al concentration in aerosols after a very mild leaching (ultrapure water)
Al_A_SMLSW_CONC_HIVOL	High Vol	mol/m^3	Soluble Al concentration in aerosols after a very mild leaching (fresh filtered seawater)
Al_A_SSLHAC_CONC_HIVOL	High Vol	mol/m^3	Soluble Al concentration in aerosols after a strong leaching (acetic acid “Berger” leach)
Al_A_T_CONC_LOWVOL	Low Vol	mol/m^3	Total Al concentration in aerosols (no preliminary leaching)
Al_A_SMLSW_CONC_LOWVOL	Low Vol	mol/m^3	Soluble Al concentration in aerosols after a very mild leaching (fresh filtered seawater)
Al_A_SMLH2O_CONC_LOWVOL	Low Vol	mol/m^3	Soluble Al concentration in aerosols after a very mild leaching (ultrapure water)
Al_A_SSLHAC_CONC_LOWVOL	Low Vol	mol/m^3	Soluble Al concentration in aerosols after a strong leaching (acetic acid “Berger” leach)
Al_A_SSLNH4AC_CONC_LOWVOL	Low Vol	mol/m^3	Soluble Al concentration in aerosols after a strong leaching (ammonium acetate "Baker and Jickells" leach)
Al_A_T_CONC_COARSE_IMPACTOR	Cascade Impactor	mol/m^3	Larger size fraction of total Al concentration in aerosols (no preliminary leaching) collected with size fractionation (as close as possible to >1um).
Al_A_T_CONC_FINE_IMPACTOR	Cascade Impactor	mol/m^3	Smaller size fraction of total Al concentration in aerosols (no preliminary leaching) collected with size fractionation (as close as possible to <1um).
Al_A_SMLH2O_CONC_COARSE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of larger size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to >1um) using a weak leach (ultrapure water)
Al_A_SMLH2O_CONC_FINE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of smaller size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to <1um) using a weak leach (ultrapure water)
Al_A_SMLSW_CONC_COARSE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of larger size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to >1um) using a weak leach (fresh filtered seawater)
Al_A_SMLSW_CONC_FINE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of smaller size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to <1um) using a weak leach (fresh filtered seawater)
Al_A_SSLHAC_CONC_COARSE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of larger size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to >1um) using a strong leach (acetic acid “Berger” leach)
Al_A_SSLHAC_CONC_FINE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of smaller size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to <1um) using a strong leach (acetic acid “Berger” leach)
Al_A_SSLNH4AC_CONC_COARSE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of larger size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to >1um) using a strong leach (ammonium acetate: "Baker and Jickells" leach)
Al_A_SSLNH4AC_CONC_FINE_IMPACTOR	Cascade Impactor	mol/m^3	Soluble portion of smaller size fraction of total Al concentration in aerosols collected with size fractionation (as close as possible to <1um) using a strong leach (ammonium acetate: "Baker and Jickells" leach)

References:

Berger, J. M., Lippiatt, S. M., Lawrence, M. G., and Bruland, K. W.: Application of a chemical leach technique for estimating labile particulate aluminum, iron, and manganese in the Columbia River plume and coastal waters off Oregon and Washington, J. Geophys. Res., 113, C00B01, https://doi.org/10.1029/2007JC004703, 2008.

Aguilar-Islas, A. et al. (2010). Dissolution of aerosol-derived iron in seawater: Leach solution chemistry, aerosol
type, and colloidal iron fraction. Marine Chemistry 120 (2010) 25–33. doi:10.1016/j.marchem.2009.01.011.

Morton, P.L. et al. (2013). Methods for the sampling and analysis of marine aerosols: results
from the 2008 GEOTRACES aerosol intercalibration experiment. Limnol. Oceanogr.: Methods 11, 2013, 62–78. DOI 10.4319/lom.2013.11.62.

9. NITRATE, SILICON, AND CARBON ISOTOPE RATIOS

9.1. PROTOCOLS FOR NITRATE ISOTOPE RATIOS

9.1.1. 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.

9.1.2. 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.

9.1.3. 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. In addition, we found that backflushing a portion of the GC column between samples kept backgrounds low for m/z 44, 45, and 46 and increased analytical precision (McIlvin and Casciotti, 2011).

9.1.4. Calibration

International reference materials available for nitrate isotopes ratios ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) should be used to calibrate measured $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ (Table 9.1; 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 nmol 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 9.1. 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

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.

9.2. PROTOCOLS FOR SILICON ISOTOPE RATIOS

9.2.1. Sampling

Water samples for silicic acid and biogenic silica isotope analysis should be gravity filtered through 0.45 µm, polycarbonate or polyethersulfone membrane filter cartridges using silicone tubing and then stored at room temperature in the dark. For larger sample volumes a peristaltic pump can be inserted on the silicone tubing between the Rosette sampling bottle and the filter cartridge.

Water volumes of between 1.0 and 4.5 L per depth are required for triplicate analysis, plus bottle rinses. Sample volume will depend upon the needs of the sample preparation and analytical method employed. Triethylamine silico molybdate purification coupled to MC-ICP-MS (Abraham et al., 2008) and IRMS methods (Brzezinski et al. 2006) have higher mass requirements (~2-3 µmol Si) and 4 L samples are recommended in oligotrophic surface waters. The sample mass requirements for cationic chromatography followed by MC-ICPMS (Georg et al. 2006) are lower and a 1 L sample is recommended. For deeper waters with higher $[Si(OH)_4]$ (> 10 µM) a sample volumes of 1.0 L is sufficient for both methods.

Suggested seawater sample containers are HDPE or PP bottles.

Sample containers should be pre-cleaned by soaking overnight in 10% HCl, followed by triple rinsing with high purity water (> 18 MΩ - cm). Bottles should be triple-rinsed with seawater prior to sample collection.

For particulate biogenic silica, samples are collected onto polycarbonate or polyethersulfone filters using in-situ pumping devices. In oligotrophic or deep waters 100-400 L of water should be filtered to obtain sufficient mass for analysis. Membranes should be dried in a clean environment overnight at 60° C.

9.2.2. 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.

9.2.3. 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.

9.2.4. 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.

References

Abraham, K. and others 2008. δ^{30}Si and δ^{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.

9.3. 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.

10. PROTOCOLS FOR OPTICS

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).

10.1. TRANSMISSOMETERS AND SCATTERING SENSORS

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

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

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

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

10.1.1. *Sensor mounting*

Transmissometer sensors are best mounted horizontally with the water path unimpeded to water flow during down and up casts (Figure 10.1). The sensor must be supported, but not stressed by mounting clamps/hardware. Mounting is facilitated by use of all-stainless steel hose clamps and backing the sensor with 2 – 3 mm thick silicone rubber. Use black electrical tape to cover any shiny band material in proximity to the light path of the instrument. The CTD and sensors should be covered to prevent baking in strong sunlight between stations.

Figure 10.1. 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 10.1.3. Methodology from Bishop and Wood (2008).

For Rosette/Carousel Systems: It is not recommended to mount transmissometers vertically clamped to the CTD (Figure 10.2, left). This arrangement makes it extremely difficult to service/clean optical windows and to place or remove plastic caps (to prevent optics contamination) when the rosette is populated with bottles. The use of bulky clamps close to the optical path further results in flow separation during up and down casts and can lead to biased profiles.

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

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

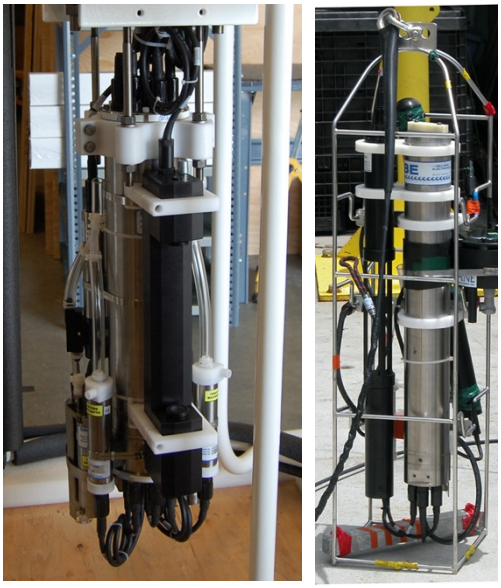

Figure 10.2. Vertical mounting of transmissometers close to the CTD unit (SBE 911 shown) at the center pylon of rosette/carousel frames (left) results in cleaning access difficulty with bottles emplaced and possible flow separation from optics during casts. Vertical mounting of transmissometers on autonomous 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.

10.1.2. 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.

10.1.3. 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.

- **Preinstallation Cleaning and Cap Protocol**

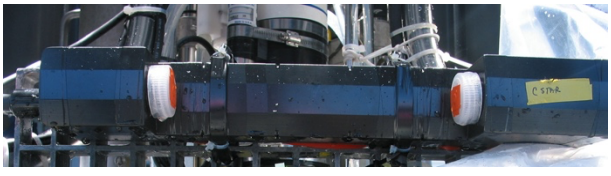

Figure 10.3. CSTAR transmissometer with plastic bottle caps installed on optical windows that are effective at preventing optics contamination while not deployed.

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.

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.

- 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.

10.1.4. 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.

- On CTD Calibration

Assuming that the transmissometer is already cleans and 'lab' calibrated on the ship (section 10.1.2.), '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.

- 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.

$$Vair_{CTD}\text{-n} = Vair_{CTD\text{-cal1}} - R(Vair_{CTD\text{-cal1}} - Vair_{CTD\text{-cal2}}) \quad (10.1)$$

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 $\mathbf{c_p}$ 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 10.2.

$$Vref_{CTD}\text{-n} = (Vair_{CTD}\text{-n} - Vzero_{CTD})/(Vair_{CAL} - Vzero_{CAL})*(Vref_{CAL} - Vzero_{CAL}) + Vzero_{CTD} \quad (10.2)$$

Transmission (T) is calculated using Equation 10.3:

$$T = (Vread\text{-n} - Vzero_{CTD})/(Vref_{CTD}\text{-n} - Vzero_{CTD}) \quad (10.3)$$

where Vread-n is the instantaneous voltage reading of the transmissometer at different depths during the specific cast.

Particle beam attenuation coefficient, $\boldsymbol{c_p}$, is calculated:

$$\boldsymbol{c_p} = -(1/0.25)*\ln(T) \ m^{-1} \quad (10.4)$$

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

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

Other NOTES: Raw data profiles should reproduce on up and down casts by better than 1 mV (the precision of CTD digitization) except when thermal structure of the water column is highly variable (Figure 10.4, below).

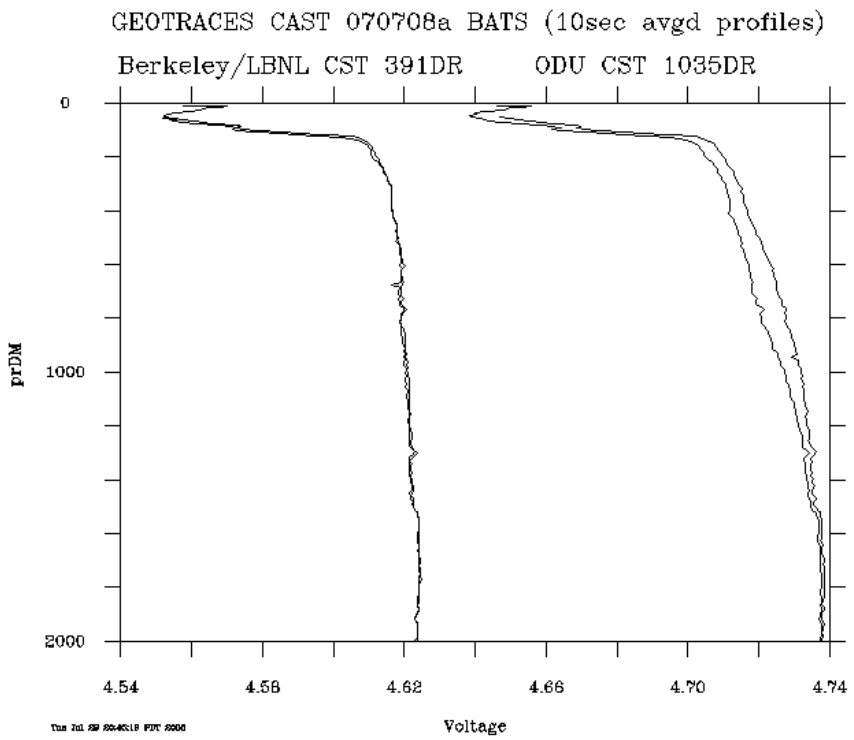

Figure 10.4. Examples of good (left) and poor (right) reproducibility of transmissometer data during GEOTRACES IC1 – Cast 070708a near the Bermuda Time Series Station. The profile on the right shows moderate thermal hysteresis of the C-STAR (1035DR) response during down and up (shifted to higher voltage) 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.

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.

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.

11. BIOGEOTRACES PARAMETERS

11.1. 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).

11.1.1. Analytical Instruments

At present, there are five commercially-available instruments that are commonly used to conduct such analysis:

- 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/chlp700/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/LIFTMarine.php

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 11.1.

Table 11.1. Comprehensive intercomparison of commercially-available instruments

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

11.1.2. Sampling methods

- 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 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).

- 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 taken into account 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).

11.1.3. 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.

11.1.4. 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.

11.1.5. 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).

11.1.6. 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) Instrument and fitting routines (see above).
4. Datasets from external standards (replete versus deplete phytoplankton culture, and species used).
5. 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).
6. 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.

References

(for additional references also see http://www.chelsea.co.uk/technical-papers#frrf and http://www.walz.com/products/chlp700/phyto-pam/publications.html)

Behrenfeld, M. J., and Z. S. Kolber, Widespread limitation of phytoplankton in the South Pacific Ocean, Science, 283, 840–843, 1999.

Boyd, P. W., & Abraham, E. R. (2001). Iron-mediated changes in phytoplankton photosynthetic competence during SOIREE. Deep-Sea Research Part II-Topical Studies in Oceanography, 48(11-12), 2529-2550. doi: 10.1016/s0967-0645(01)00007-8

Boyd, P. W., Strzepek, R., Takeda, S., Jackson, G., Wong, C. S., McKay, R. M., Ramaiah, N. (2005). The evolution and termination of an iron-induced mesoscale bloom in the northeast subarctic Pacific. Limnology and Oceanography, 50(6), 1872-1886.

Cullen J.J. and R. F Davis (2003) The blank can make a big difference in oceanographic measurements Published by the American Society of Linmology and Oceanography, Limnology and Oceanography Bulletin, Volume 12(2) June 2003.

Kolber, ZS, Prasil O and Falkowski PG, 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochimica et Biophysica Acta, 1367, 88-106.

Moore, C.M., Lucas, M.I., Sanders, R. and Davidson, R. (2005) Basin-scale variability of phytoplankton bio-optical characteristics in relation to bloom state and community structure in the Northeast Atlantic. Deep Sea Research Part I: Oceanographic Research Papers, 52, (3), 401-419. (doi:10.1016/j.dsr.2004.09.003).

Morrison, J. R. (2003), In-situ determination of the quantum yield of phytoplankton chlorophyll a fluorescence: A simple algorithm, observations, and a model, Limnol. Oceanogr., 48(2), 618–631, doi:10.4319/lo.2003.48.2.0618.

Olson RJ, HM Sosik, AM Chekalyuk, A Shalapyonok.Effects of Iron enrichment on phytoplankton in the Southern Ocean during late summer: active fluorescence and flow cytometric analyses Deep-Sea Research Part II, 47 (2000) 3181-3200.

Suggett, David J., Moore, C. Mark, Hickman, A and Geider, Richard J. (2009) Interpretation of fast repetition rate (FRR) fluorescence: signatures of phytoplankton community structure versus physiological state. Marine Ecology Progress Series, 376, 1-19. (doi:10.3354/meps07830).

11.2. 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. Intercalibration efforts are currently undertaken and will be available through the Biogeoscapes website.

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.

11.2.1. 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.

11.2.2. 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.
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.

11.2.3. 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 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.

11.2.4. 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).

11.2.5. 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 subquality phred scores removed.
3. Paired end reads are overlapped and merged to generate longer composite sequences.

12. 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.

13. PARAMETER NAMING CONVENTION

All of the GEOTRACES parameter names that are used in the Intermediate Data Products are sorted into Domains; these are used to separate different types of datasets and to cluster different sets of parameters:

1. **Aerosols** - all measurements associated with aerosols, with different collection and analytical methods.
2. **BioGEOTRACES** - Biological measurements (including pigments, DNA parameters, cell quotas and proteins)
3. **Dissolved TEIs** - dissolved trace metals, ligands, radionuclides, rare earth elements and their isotopes,
4. **Hydrography and Biogeochemistry** - Including temperature, salinity, oxygen, major nutrients and their isotopes and noble gases
5. **Particulate TEIs** - particulate trace metals, ligands, radionuclides and major phases, and their isotopes
6. **Precipitation** - all measurements associated with rain and with freshly falling snow, with different collection and analytical methods.
7. **Polar** - unique samples from polar expeditions, also cross listed with "Dissolved TEIs," "Precipitation," "Particulate TEIs," "Hydrography and Nutrients," "Ligands" and, with "BioGEOTRACES" to facilitate locating this information.
8. **Ligands** - dissolved and particulate ligands also cross listed with "Dissolved TEIs", "Particulate TEIs" and with "Polar" to facilitate locating this information.

GEOTRACES Intermediate Data Products employ the following parameter naming scheme. Standard hydrographic parameters, such as temperature, salinity and oxygen use names as defined in the WOCE/CLIVAR naming convention (CTDTMP, CTDSAL and CTDOXY for temperature, salinity and oxygen from CTD sensors; https://exchange-format.readthedocs.io/en/latest/parameters.html). Other hydrographic and biogeochemistry parameters use names defined intuitively. Examples are CTDPRS for the CTD pressure at the bottle sample depth, SALINITY, PHOSPHATE, NITRATE, and SILICATE for salinity, phosphate, nitrate and silicate measured on bottle samples. Note that NO3 and NO2 are used in the Precipitation Domain parameter names. Biogeochemistry parameters use names defined by SCOR naming.conventions (e.g., HPLC pigments; Roy et al., 2011) or names that intuitively define the parameters (e.g., nifHUCYN-ADNAPCONCBOTTLE; concentration of nifH genes from uncultured unicellular cyanobacteria (UCYN-A) particles (P) in a bottle sample).

All other trace elements and isotope names are composed of up to six separate tokens as shown below. Tokens 2 and 3 are optional, while all other tokens are mandatory for trace elements and isotopes, nutrients and biogeochemistry parameters. A few physical parameters that do not align with the convention of 4 to 6 tokens are exempt from the requirement to include Phase, Data Type and Sampling system

14. CONTRIBUTORS

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