BIOPOLE COOKBOOK: PARAMETERS AND ANALYSIS

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Contributors

Kate Hendry (1), Clara Manno (1), Carol Arrowsmith (2), Mike Bowes (3), Nathan Callaghan (3), Alanna Grant (3), Stephen Lofts (3), Rebecca McKenzie (3), Alexandra O'Brien (3), Justyna Olszewska (3), Amy Pickard (3), Bryan Spears (3), Andrew Tye (2), E. Malcolm S. Woodward (4), and the BIOPOLE Programme.

(1) British Antarctic Survey, (2) British Geological Survey, (3) UK Centre for Ecology and Hydrology, (4) Plymouth Marine Laboratory

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Introduction

The aim of this document is to detail the key determinands, sampling preparation, methodology and analyses required for the NERC programme BIOPOLE, with the overall objective of maintaining consistency in different sampling locations and between different Research Institutes.

This document builds on the work carried out in the NERC programme LOCATE, with thanks to Jenny Williamson, Dan Mayor, Adrian Martin, Mark Stinchcombe & the whole LOCATE Team.

Generic sampling protocols

Fieldwork timings, planning, checklists and information regarding the shipping of samples will be outlined separately (contact Amy Pickard: amypic92@ceh.ac.uk).

Some brief information on sampling planning and execution is given below, adapted from the LOCATE sampling protocol document, the GEOTRACES cookbook (https://www.geotraces.org/methods-cookbook/), the GO-SHIP guide (https://www.go-ship.org/HydroMan.html) (see also Becker et al., 2020), the Nansen Legacy guidelines (https://septentrio.uit.no/index.php/nansenlegacy/article/view/5793). These guidelines are also available on the internal BIOPOLE Sharepoint.

Responsible parties and health and safety

It is useful to identify a contact in each institution who will be responsible for ensuring the sampling program is undertaken from each Research Institute. They will need to select the appropriate staff and ensure the smooth planning and execution of the sampling program from their institutes.

Institution	Institution lead	e-mail
BAS	Kate Hendry	kathen@bas.ac.uk
BGS	Melanie Leng	mjl@bgs.ac.uk
NOC	Adrian Martin	adrian.martin@noc.ac.uk
UKCEH	Bryan Spears	spear@ceh.ac.uk
CPOM	Sammie Buzzard	sammie.buzzard@northumbria.ac.uk

Each Institution should be responsible for preparing their own Risk Assessments (RA) and Safe Systems of Work for their staff. It is likely that requirements for sampling are going to be different for each institution with respect to boats, bridges etc and so this makes it easier. It is also likely that each institution will have different Health and Safety (H&S) documents to reference as part of their assessments. However, sharing previous RA to each institute will provide examples of previous RA requirements to save time. Please save a copy of the finished RA to the folder on the BIOPOLE Sharepoint site so it can be stored as part of the project management. Please also consider whether staff require also:

- Extra safety training given the likely cold weather conditions (Fig. 1)
- Vaccinations (Tetanus, COVID-19)
- PPE

Figure 1: Arctic fjord view

Important reminders for all sampling events

Wear powder-free gloves at all times whilst sampling – this is for H&S reasons, as well as to reduce contamination of the samples. Please keep in mind that some sampling methodologies require specific glove materials.

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

Please remember that the bottles and filters need rinsing thoroughly to avoid contamination. If the sample is being filtered the bottle needs rinsing with filtered water. If you are using any sampling tubing, clean well with v/v 10% HCl and Milli-Q between sampling events wherever possible.

For any particulate sampling, make sure to record accurately the volume of water filtered.

Make sure all bottles are labelled. Make sure all pre-labelled bottles still have labels after sample collection.

Throughout the sampling please place all samples in the dark and in either the cool box or freezer as required. This extends to when you get back to base and prior to sending samples out to institutions – samples must be kept in dark and in fridge if not frozen.

For any determinand where fresh analysis is preferred, please also plan on taking some spare bottles for freezing samples if needed. It is recommended that if samples are frozen that an additional sample is also stored in the cold and dark at 4 °C.

Please make sure you're happy with the calibration and use of your salinity meter and other sensors/probes. We are hoping that you will take salinity and temperature samples in the field. However, if you do not have a salinity probe we therefore request that you take a sample in a bottle (125ml), seal it with tape to prevent any loss through evaporation and send it to part of your organisation that has one. **Make a note of all serial numbers and additional information on calibration for T, S and other sensors used.**

Rivers

Key principles:

a) It was decided that all filtering and separating samples into requisite bottles will be undertaken in the field with the exception of POC samples which will be filtered in the lab on return. It is important that the sampler ensures bottles and filters are rinsed as described below.

b) Samplers should carry out a local assessment for potential upstream influences before sampling (e.g., sources of pollution).

c) Sensor measurements will also need to be made (temperature, salinity, conductivity, O_2, pH). Please make sure you're happy with the calibration and use of your salinity meter. We are hoping that you will take salinity and temperature samples in the field. However, if you do not have a salinity probe we therefore request that you take a sample in a bottle (125ml), seal it with tape to prevent any loss through evaporation and send it to part of your organisation that has one.

d) If possible, measure flow velocities - even approximate values could be useful for later calculations.

Sampling methodology:

Note that all sampling equipment and clear instructions must be assembled by the team doing the analysis. The sampling team will be responsible for ensuring that there are fridges/freezers available for immediate use as and when needed.

There are some generalised ideas listed below, but specific sampling strategies will be discussed by the science team prior to deployment.

- Please fill out field data form. Use the comments box to give a little information about the weather whilst sampling (please be as specific as possible e.g., use oktas to describe cloud cover), and in the previous 24 hours. If needed, use this box for any other comments and/or observations. Samplers should transfer the data to an online spreadsheet on the Sharepoint site as soon as possible.
- Photo to be taken both upstream and downstream of the river from the sampling site at time of sampling. This will allow us to assess conditions of sampling later in the project. Ideally the picture will be taken in the same place on each visit to the site.
- Please wear gloves to prevent hand contamination
- Sample will be obtained by the selected procedure for the river and sampling site – hand, rope & non-metallic bucket, or pole (See below) but we think most will be bucket
- Rinse bucket or collecting vessel with sample water x3.
- Fill bucket or collecting vessel with water from which all samples will be taken
- Salinity, Electrical Conductivity (SEC) and temperature need to be collected in the field. Place probe SEC and Temp probe into bucket and record both onto sample sheet. Please measure temperature to 0.1 °C

- Rinse and fill the two 1 L POC bottles to the top for return to lab and filtering. This could be in 1 2L bottle – the intention is to get a sufficient sample to filter so enough carbon is collected. Please make sure that POC does not settle out by quickly putting samples into bottles – mixing the water in the bucket before filling bottles will help.

- Use 500ml wide neck bottle (rinsed 3 times from bucket water) to take sample from which all other bottles will be filled (See Appendix A). Please remember that the bottles and filters need rinsing thoroughly to avoid contamination. If the sample is being filtered the bottle needs rinsing with filtered water.

Estuaries/fjords

Key principles:

a) The most important factor is to sample a range of salinities within each estuary so that linear/non-linear DOC vs salinity relationships can be captured. Thus, sampling the full freshwater – sea transition is desirable. Depth profiles could also be considered if a Niskin bottle is available (see below; Fig. 2).

b) Each estuary/fjord is different and the BIOPOLE sampling teams will undertake a combination of fixed point and chasing salinity to try and obtain a range of salinities on each transect between 0-25 ppt.

c) There should be a bias towards the freshwater end of the estuary. Typically, flocculation of organic carbon occurs at relatively low salinities in what is known as the turbidity maximum. Sampling teams should therefore gain knowledge of where the turbidity maximum exists during different seasons so that sampling plans can be determined.

d) Ideally salinities to be sampled will be 0, 2, 5, 10, 15 and 25 with a +/- of 2 ppt with the exception of the Freshwater end member. However, this will depend on estuaries/fjords and conditions as salinity changes can be very rapid.

e) In general, we will be aiming to sample from the middle of the central channel and boat-based surveys will ideally follow a similar passage for each trip. Obviously, weather and boat skipper may mean other passages are required.

f) Sampling should begin with the freshwater end-member and move downstream on a falling tide.

Field equipment needed

a) Bottles for each site as listed in Appendix A labelled as described in the method.
b) H&S equipment, including mobile phone or satellite phone
c) GPS for taking position
d) 1 4-5 litre plastic bucket and a rope securely tied to the handle
e) 2 x 60 ml syringes (a wear and a spare for water sampling)
f) 1 20 ml all plastic syringe per site
g) 1 salinity / conductivity meter
h) Camera
i) Field sheets and pencils (writing implements)
j) Lab gloves
k) Cool box and cool packs
l) Appropriate sized jerry cans or similar if sampling water and bringing onto land for filtering and bottling.
m) Mercuric chloride, dispensers and associated H&S equipment for safe handling and disposal of dispensers etc for those groups who will collect gas samples.

Sampling methodology:

- Find a location, take GPS co-ordinates where you can safely get to the water. Rinse bucket with river water x3, then fill bucket with river water from which all samples will be taken.
- If required boat crews can sample and collect estuary water and filter on land by filling 10 L jerry cans.
- All bottles, lids and syringes need rinsing x3 to avoid contamination of the sample. If the sample is filtered, the bottle needs rinsing with filtered water. Rinse filter with a volume of sample water that is equal to the volume of the syringe used.
- Fill the bottles as in Appendix A and in the order listed in the table. The same filter can be used for all bottles unless it blocks, in which case rinse new filter as before.
- Take two photos if possible, one upstream and one downstream from the sampling site at time of sampling. This will allow us to assess conditions of sampling later in the project
- Record temperature, salinity/conductivity, date, time, number of filters used, weather conditions and other relevant observations on the field sheet.
- Store samples in cool box in the dark and freeze when needed.

Ocean/marine sampling

Key principles:

Samples will generally be collected using Niskin bottles attached to a CTD rosette. The Niskin bottles will be closed ('fired') at different depths and, usually, the depths can be selected to capture i) a range of depths and water masses and ii) particular features of interest including high turbidity or high chlorophyll layers, or layers of lower salinity.

Sampling methodology:

- The Niskin bottles will be brought up onto the deck. When it is safe to do so, the Niskin bottles can be subsampled. If doing small boat work, it is likely that a manual Niskin bottle operation will be used, where the bottle is fired using a brass "messenger" (Fig. 2)
- To open the bottles, first loosen the spigot at the top of the Niskin to allow air to flow through. The spigot at the bottom of the Niskin bottle can now be opened.
- Dry the spigot with clean absorbent material before opening.
- Samples can either be collected straight from the spigot or, if filtering using an inline (e.g., Acropak) filter, by first attaching a length of silicone tubing. If the water is dripping out too slowly from the filter, then a peristaltic pump can be used to increase the flow rate.

Note: if there are trace metal clean Niskin bottles on board, and a clean laboratory available, then the waters could be sampled for iron parameters. This should only be done by someone who has training in trace metal clean procedures. Unless there is no other Niksin bottle available, prioritise trace metal samples when using a trace metal clean Niksin bottle.

Figure 2: Correct set up for Niskin bottle deployment

Field Blanks

Field blanks will need to be undertaken at least for the first couple of samplings. However, if each group does blanks it will add costs in terms of analysis and filters. This is particularly

critical for the contamination-sensitive determinands i.e., Fe, REEs, and other trace metals, and particularly in the marine environment.

- Please take a supply of Milli-Q water (500ml) with you in a washed-out new bottle (i.e. rinse the bottle 3x) which is kept in the dark and cool.
- This needs only to be undertaken once for each sample type and not at every river/estuary/fjord/ocean station.
- It's important to test each batch of filters and bottles.
- Please filter or fill bottles as per Appendix A (i.e. do whatever you do for the samples).

Labelling of samples

We recognise that labelling systems might need to vary from site-to-site, platform-to-platform etc. As such, it's not going to be possible to use a consistent labelling system throughout the whole BIOPOLE project. However, there are a number of principles that would assist in sample and data management.
Please make sure that your labelling protocol has the following (in order):

1. Project identifier "BIOPOLE"
2. Expedition marker/acronym/cruise number (e.g., KEP, RaTS, SDA)
3. A unique sampling location ID that's workable and consistent (e.g., station number)
4. Sample ID e.g., event number and/or depth
5. Analyte (DOM, TP, $\delta^{18}O$ etc…)

Date can be added if likely to be useful – but must be logged in a sampling spreadsheet too such that events etc. can be matched up with date later on

Pre-label where possible, and make sure to use either good quality sticky labels (not insulation tape that could fail in cold conditions or if the bottles are frozen), or good quality permanent marker pen.

Generic analytical procedures

The analysis of samples will be undertaken across the participating Research Institutes. Rapid dispatch of the samples will be required so that they can be analysed quickly. In addition, it is likely that the analysing Institute will want to analyse samples all together in one run, thus saving time.

- Follow instructions on checklist with respect to checking samples are collected
- Undertake preservation techniques that haven't been done in the field
- Ensure all bottles are labelled
- Samples will need to be distributed to the following Institutes and Contacts asap
- Couriers will need to be notified and used
- Use online datasheets in the field where possible. If hard copies are used in the field, then these sheets will need to be transferred/saved to the Sharepoint ASAP by the samplers (see BIOPOLE Data Management Plan)

The sampling teams will be required to undertake some preservation techniques after collecting the samples. These are outlined below, along with a brief description of the analytical methods.

Summary of determinands

BIOPOLE analytical schematic

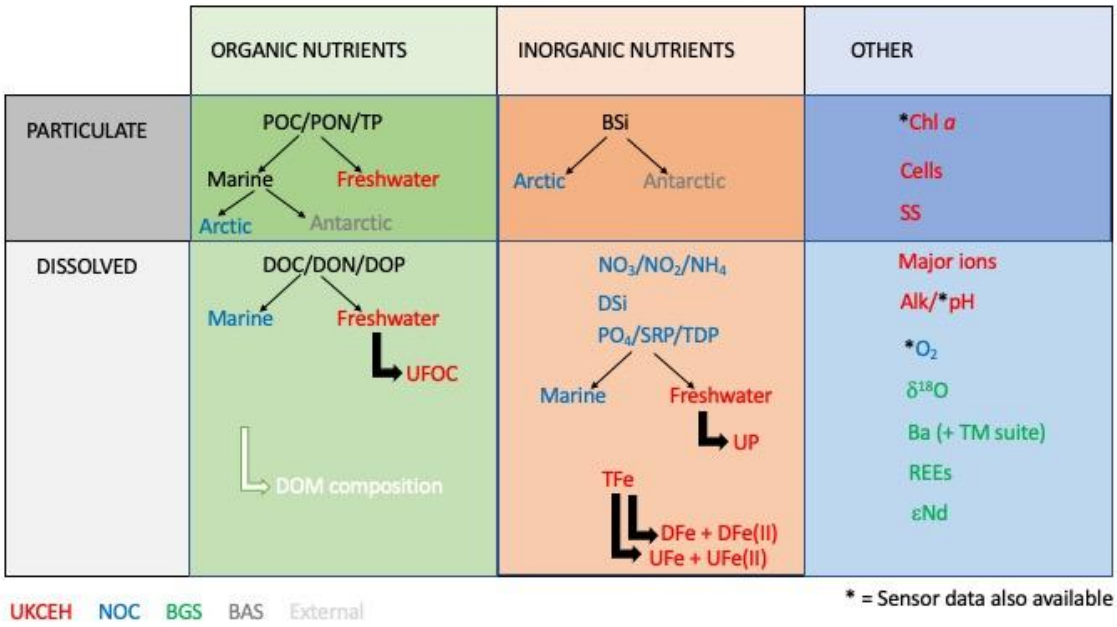


Figure 3: Summary of determinands key to BIOPOLE objectives, and RIs responsible for analysis (see Table 1 for definition of abbreviations and symbols)

Glossary of abbreviations

Acronym/symbol	Definition
POC	Particulate Organic Carbon
PON	Particulate Organic Nitrogen
POP	Particulate Organic Phosphorus
BSi	Biogenic silica (opal)
Chl *a*	Chlorophyll a
DOM	Dissolved Organic Matter
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DOP	Dissolved Organic Phosphorus
UFOC	Ultrafilterable Organic Carbon
NO_3	Nitrate
NO_2	Nitrite
NH_4	Ammonium
DSi	Dissolved silica(te)/silicic acid
PO_4	Orthophosphate
SRP	Soluble Reactive Phosphorus (equivalent to orthophosphate)
TDP	Total Dissolved Phosphorus (equivalent to orthophosphate)
TP	Total Phosphorus
UP	Ultrafilterable Phosphorus

TFe	Total iron
DFe	Dissolved iron
DFe(II)	Dissolved iron (II)
UFe	Ultrafilterable iron
UFe(II)	Ultrafilterable iron (II)
Alk	Alkalinity*
SS	Suspended solids
O2	Oxygen (usually referring to dissolved oxygen)
$\delta^{18}O$	Stable oxygen isotopic composition (usually referring to water)
Ba	Barium
TM	Trace Metal
REE	Rare Earth Element
εNd	Neodymium isotopic composition

*Table 1: Abbreviations, acronyms and symbol definitions; * NOTE ON ALKALINITY*

Carbon

Note that where a single sampling methodology is used for several determinands, the method has been summarised as an Appendix.

Dissolved organic carbon (DOC)

Location of analysis: UKCEH/NOC
Person responsible: Chris Barry/Amy Pickard/Ed Mawji

General comments on freezing of samples for DOC etc.

There are mixed findings in the literature on the potential artefacts of freezing samples on Dissolved Organic Matter (DOM) composition and DOC concentrations, likely related to DOM source and chemical characterisation. There is potential for precipitation of some DOM with freezing (note relevance for polar lake and sea ice DOM), and this transfer from dissolved to particulate fractions can mean that the particulates formed (if any) may not be sampled by typical DOC analysers (unless using stirred auto-samplers and analytical tubing of sufficiently high diameter) leading to potential for underestimation. Some studies employ sonication after thawing.

This is principally an issue for more highly coloured, higher doc samples (e.g., those with SUVA254 > 4 L mg C^{-1} m^{-1}; DOC > 5-10ppm).

As such, samples should be frozen only if needed – analysis of fresh samples should be prioritised if possible. If samples are frozen, it is recommended to also take another set of subsamples that are stored dark refrigerated post filtration (long term studies have shown analytically imperceptible changes to DOC concentrations for certain freshwater samples from the UK stored this way.

Use either acid-cleaned polycarbonate bottles, well pre-rinsed with sample water, or pre-combusted amber borosilicate vials, which are probably more robust if more breakable.

Sampling

Freshwater:

- Attach 0.45 μm cellulose acetate filter onto plastic 20 ml syringe and flush filter with 20 ml of water.
- Then rinse the 30 ml amber glass bottle with 20 ml filtered water, put lid on and shake before emptying rinse water.
- Completely fill the 30ml amber glass bottle with filtered water taking care to exclude as much air as possible.

Marine:

- Prior to sampling the kit needs to be rinsed in a 5-10% acid bath. Make sure the kit does not stay in longer than an hour or so to avoid the kit from getting brittle. Rinse 3 times with Milli-Q and avoid it from getting dusty by keeping it in a contained box. Load the filter unit (Fig. 4) with a pre- combusted GF/F filter.
- Use a pre-acid cleaned polycarbonate or HDPE bottle (Kellerman et al., 2021; Halewood et al., 2022).
- Filter sample directly from the Niskin into the sample bottle. To get the filter going, remove the little white lid and let all air escape by tilting the filter unit accordingly. Put lid back on, rinse bottle 3 times and fill up to bottom of the shoulder of the bottle to allow room for freezing. Store the sample in the -20°C freezer.

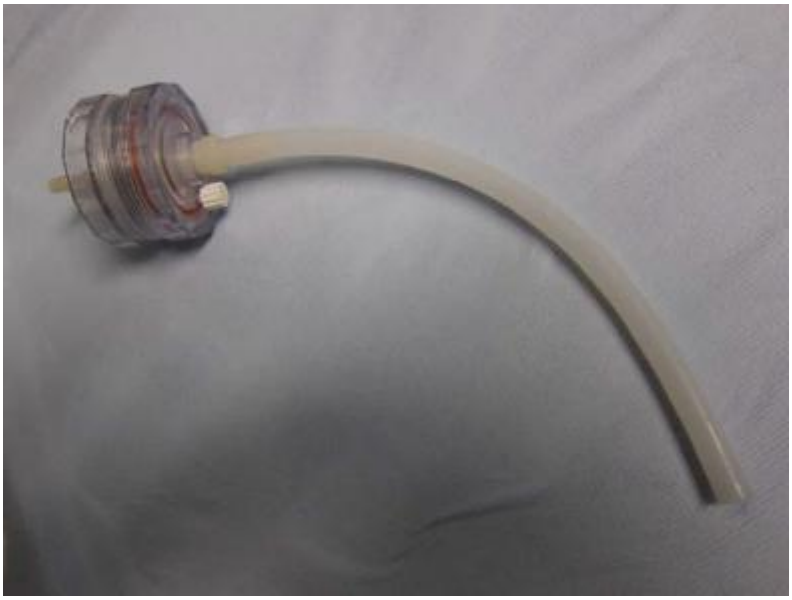


Figure 4: Set up of DOC filtration system for marine sampling, to be connected directly to a Niksin bottle

Analysis

TBD

Ultrafilterable organic carbon (UFOC)

Location of analysis: UKCEH
Person responsible: Stephen Lofts

For sampling and analytical methods see Appendix B-C.

Ultrahigh resolution DOM composition

Location of analysis: Florida State University
Person responsible: Robert Spencer (rgspencer@magnet.fsu.edu)

Samples collected as for marine DOC samples (Kellerman et al., 2021). Frozen samples to be sent to Florida State University (USA) for further analysis.

CDOM/FDOM

TBD

Location of analysis:

Sampling

Analysis

Dissolved Inorganic Carbon (DIC)

Location of analysis:

Sampling

- Prior to sampling, label each bottle
- Remove air bubbles from sample tube (squeeze tube)
- Rinse the bottle with sample (3x)
- Fill the sample bottle making sure:
    - The sample tube is placed fully down to the bottom of the bottle
    - While filling, avoid trapping of air bubbles in the bottle
    - Let water overflow by 2-3 full bottle volumes
    - Remove the sample tube while water is still flowing
- Put the glass stopper back on

Suggest taking one replicate sample per CTD

Processing

Wear gloves and a lab coat and work under the fume hood - Mercuric chloride is a poison!

- Remove the stopper from the sample bottle
- Remove 2.5 mL of sample using a pipette
    - discard the water into a plastic beaker
- Add 50 µL of saturated $HgCl_2$ (Mercury(II) chloride) solution
- using a pipette
- Dry the glass stopper with blue roll / tissue paper
- Put grease on the glass stopper
- Put the glass stopper on the glass bottle
    - turn the stopper to disperse the grease
- Use a rubber band and plastic clip or electrical tape to secure the stopper
- Turn/invert the bottle several times to mix the sample + $HgCl_2$
- Put the bottle back into the crate

Store samples at room temperature.

Particulate organic carbon (POC)

Location of analysis: Marine - NOC (Arctic)/BAS (Antarctic); Freshwater - CEH
Person responsible: Ed Mawji (ezm@noc.nerc.ac.uk)/Geraint Tarling (gant@bas.ac.uk) /Chris Barry

For full SOP, see Appendix E.

Sampling

It is of utmost important that you record the volume of water that you filter. This will enable us to convert the POC we captured on the filter paper to mg L^{-1}. Please do not forget to do this.

- The sampler will be required to filter water for the POC samples instead of sending the 2L of water.
- This should be undertaken on the same or next day
- Responsible persons will distribute ashed filters and filtration kit
- **Ensure samples are well mixed and particulate load hasn't settled out**.

1. Filter each sample onto a 25mm ashed GF/F in lab. Vacuum at 7-10psi (<200mm Hg) only. Put filter into cryo-vial, put cryo-vial in the rack provided.
2. After filtering please fold filter paper in half (dirty side in). This will help keep all the carbon on the filter paper.
3. Dry at 50 degrees for at least 12 hours

Analysis

TBD

Particulate organic nitrogen (PON)

Location of analysis: Marine - NOC (Arctic)/BAS (Antarctic); Freshwater - CEH
Person responsible: Ed Mawji (ezm@noc.nerc.ac.uk)/Geraint Tarling (gant@bas.ac.uk) /Chris Barry

Methodology as per POC. For full SOP, see Appendix E.

It is of utmost important that you record the volume of water that you filter. This will enable us to convert the PON we captured on the filter paper to mg L^{-1}. Please do not forget to do this.

Total Dissolved Phosphorus (TDP) and Total phosphorus (TP) - Freshwater

Location of analysis: CEH

Person responsible: Mike Bowes (mibo@ceh.ac.uk)

Sampling

For freshwaters see Appendix D.

It is of utmost important that you record the volume of water that you filter. This will enable us to convert the POP we captured on the filter paper to mg L^{-1}. Please do not forget to do this.

Analysis

Stable carbon isotopes (DIC)

Collection and poisoning of water samples for stable carbon isotope analysis ($^{13}C/^{12}C$, defined as $\delta^{13}C$).

Location of analysis: BGS
Person responsible: Carol Arrowsmith

Sampling

Samples are collected using 30 ml narrow mouth HDPE bottles (such as Fisher Scientific cat no-10768711), and then poisoned using 8µl of saturated mercuric chloride ($HgCl_2$) solution to inhibit biological activity and reliably preserve the carbon isotope ratios for later analysis.

The following sampling procedure was used to collect, preserve and store the $\delta^{13}C$ samples.

1. Make up the saturated mercuric chloride ($HgCl_2$ CAS: 7487-94-7) solution in an exhausting fume hood. Wear a lab coat and nitrile gloves, take one of the preloaded 30ml bottles containing 2.1g mercuric chloride and make up to 30ml level (the bottom of the neck) with deionised/RO water. Replace lid, invert and then leave to settle before use.
2. Begin bottle rinsing by half filling sample bottle to the top with sample water if available, replace lid, shake sample bottle and discard contents.
3. Continue bottle rinsing by filling sample to the top with sample water and discard contents, while also rinsing lid again.
4. Collect sample. Fill sample bottle as full as possible with water. Surface tension will allow a large dome of water to form in the top of the sample bottle, but a couple of droplets from this were poured away (as otherwise when poisoning, the mercuric chloride solution could potentially overflow the bottle).
5. Screw on sample bottle lid, and try to limit the time when the sample in the bottle does not have a lid on.
6. Label and place sample back in holder.
7. When all samples have been collected, transfer sample bottles to a fridge to keep cold until poisoning can be carried out, or continue immediately with poisoning.
8. When ready to begin poisoning, put on lab coat and nitrile gloves.
9. Transfer 30 ml sample bottles to a fume cupboard, or appropriately ventilated space.

10. Using a spill tray and liner in the fume cupboard, gather mercuric chloride solution and pipette for use, and add new tip to pipette.
11. Remove 30ml sample bottle lid (the lid should stay off for as short a time as possible).
12. Pipette 8µl of mercuric chloride solution into sample (hover the pipette over the top of the water but don't touch it, to avoid cross-contamination between samples).
13. Replace bottle lid, ensure is really tight, hand tight is good but not finger tight.
14. Secure the lid with wrap of electricians tape around base of lid and bottle and indicate closure position with vertical marker pen line.
15. Repeat steps 11-14 for all samples.
16. After poisoning, clear away mercuric chloride solution and pipette tips, and ensure surface working area is wiped down. Any used pipette tips, or tissues used to wipe down the surface or come into contact with the mercuric chloride should be disposed of in a UN approved hazardous waste bin.

Analysis

Samples will be sent to the British Geological Survey (BGS) labs in Nottingham to determine their δ^{13}C values via isotope ratio mass spectroscopy.

Inorganic macronutrients

Note that where a single sampling methodology is used for several determinands, the method has been summarised as an Appendix.

General note on standardisation

Primary standards should be prepared at a minimum of once every three months, and kept in the dark and at room temperature. If they are stored in a refrigerator they must be brought to room temperature before use.

Secondary standards must be used for quality control, specifically Certified Reference Materials (CRMs), and analysed in each run. RMs for freshwaters are available from the USGS (https://bqs.usgs.gov/srs). CRMs for marine waters include SCOR-JAMSTEC CRMs and KANSO CRMs, which are traceable to the International System of Units (SI). Standard solutions with stated uncertainties from the Japan Calibration Service System (JCSS) of the Chemicals Evaluation and Research Institute (CERI), and the National Metrology Institute of Japan (NMIJ) are used to certify nitrate, nitrite and phosphate values. A silicon standard solution produced by Merck KGaA, and a silicon standard solution (SRM3150) of the National Institute of Standards and Technology (NIST) are used to certify silicate values. Each solution has a stated uncertainty value.

CEH analyses are UKAS accredited (for freshwaters), certified reference materials used; employs aquacheck scheme (https://www.lgcstandards.com/GB/en/Proficiency-Testing/Water-and-Environment-Schemes/Water-Chemistry-AQUACHECK-/cat/280827)

All standards must be salinity-matched.

Nitrate/nitrite (NO_3/NO_2) – Marine waters

Location of analysis: NOC
Person responsible: Ed Mawji (ezm@noc.nerc.ac.uk)
Also contact: Mike Bowes (mibo@ceh.ac.uk)/Isabelle Fournier (isafou@ceh.ac.uk)/ Kate Hendry (kathen@bas.ac.uk)

Collection of water samples for macronutrient analysis (nitrate, nitrite, ammonia, silicic acid, phosphate).
Samples are filtered through 0.2μm PES filter capsules and collected into 60 ml narrow mouth HDPE bottles (such as Thermo product code - 20180060).
Samples will be sent to the British Antarctic Survey (BAS) labs in Cambridge for measurement using SEAL AA500 analyser.

Sampling

Wear gloves (powder-free vinyl) throughout.

Cellulose nitrate filters should NOT be used.

1. Remove syringe plunger and rinse syringe and plunger with water from the Niskin bottle.
2. Attach new filter capsule to the end of the syringe.
3. Fill syringe with water and replace plunger. Flush filter through with water (use a volume of water equal to the volume of the syringe, if using a syringe).
4. Remove plunger, fill syringe again, and replace plunger.
5. Rinse the pre-labelled sample bottle three times with filtered water, putting lid on and shaking before emptying rinse water.
6. Fill bottle with water to shoulder (i.e., leave head space for freezing).

Storage

Freeze the samples upright and check that the caps are tightened before and after the samples have frozen. Do not freeze samples in a freezer that has had organic material (fish samples or food) stored in it. **Alternatively, samples may be preserved by pasteurisation.**

Analysis

GO-SHIP document on inorganic nutrient analysis can be found at https://www.go-ship.org/HydroMan.html). These guidelines are also available on the BIOPOLE Sharepoint.

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

Analyze frozen samples as soon as possible after returning to the lab. Thaw frozen samples in a 50˚C water bath for 30-45 minutes and then allowing the samples to come back to room temperature before analysis.

Follow all GO-SHIP guidelines for analyses. The basic steps for sample analysis include:

1) a. Establish a steady baseline with ultrapure water.
   b. Establish a steady baseline with ultrapure water plus reagents.
   c. Check the reagent blank (difference between ultrapure water and ultrapure water plus reagents).
2) Calibration curve determination from standard concentrations and measured peak heights.
3) Measurement of sample peak heights.
4) Corrections for carryover, baseline and sensitivity drift.
5) Determination of initial concentrations of samples based on calibration curve and sample peak heights.

6) Application of other corrections including RIBs, salt effect, etc.

Ammonium – Marine waters

Location of analysis: NOC
Person responsible: Ed Mawji (ezm@noc.nerc.ac.uk)
Also contact: Kate Hendry (kathen@bas.ac.uk)

Sampling

As for nitrate/nitrite. If samples can be analysed immediately without freezing, this is much preferred.

Analysis

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

Analyze frozen samples as soon as possible after returning to the lab. Thaw frozen samples in a 50˚C water bath for 30-45 minutes and then allowing the samples to come back to room temperature before analysis.

Follow all GO-SHIP guidelines for analyses, as for nitrate/nitrite.

Total dissolved (sol.) nitrogen (TDN) - Freshwaters

Location of analysis: UKCEH
Person responsible: Chris Barry (cbarry@ceh.ac.uk)/Mike Bowes (mibo@ceh.ac.uk)

Sampling

For freshwaters see Appendix D.

Analysis

TDN is determined in series on the same sample using standard Pt catalysed TOC combustion analysers. (via reaction of NO produced with O_3 and measurement of chemiluminescence of NO_2; summary here https://www.americanlaboratory.com/913-Technical-Articles/171561-Total-Nitrogen-in-Water-by-High-Temperature-Catalytic-Combustion-and-Chemiluminescence-Detection/). This is broadly the UKCEH method. Kjedahl method rarely used nowadays.

Total nitrogen (TN)

Location of analysis: UKCEH
Person responsible: Chris Barry (cbarry@ceh.ac.uk)

Sampling

Cleanly collect an unfiltered sample.

Analysis

As per TDN method above but on unfiltered sampled, for TOC if analysed. Or as sum of TDN and Particulate Organic N.

Soluble reactive phosphorus – Fresh and marine

Location of analysis: UKCEH (Freshwater/Brackish), NOC (Marine)
Person responsible: Mike Bowes (mibo@ceh.ac.uk)/ Ed Mawji (ezm@noc.nerc.ac.uk)
Also contact: Isabelle Fournier (isafou@ceh.ac.uk)/ Kate Hendry (kathen@bas.ac.uk)

Sampling
As for nitrate/nitrite.

For freshwaters see Appendix D

Analysis

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

Analyze frozen samples as soon as possible after returning to the lab. Thaw frozen samples in a 50˚C water bath for 30-45 minutes and then allowing the samples to come back to room temperature before analysis.

Follow all GO-SHIP guidelines for analyses, as for nitrate/nitrite.

Ultrafilterable phosphorus

Location of analysis: UKCEH
Person responsible: Stephen Lofts (stlo@ceh.ac.uk)

For sampling and analytical methods see Appendix B-C.

Dissolved silicon

Location of analysis: NOC
Person responsible: Ed Mawji (ezm@noc.nerc.ac.uk)
Also contact: Mike Bowes (mibo@ceh.ac.uk)/Isabelle Fournier (isafou@ceh.ac.uk)/ Kate Hendry (kathen@bas.ac.uk)

Sampling
As for nitrate/nitrite. It is preferable, if freezing can be avoided. Glass Fiber filters (GFF) should NOT be used.

For freshwaters, see Appendix D.

Analysis
GO-SHIP document on inorganic nutrient analysis can be found at https://www.go-ship.org/HydroMan.html). These guidelines are also available on the BIOPOLE Sharepoint.

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

Analyze frozen samples as soon as possible after returning to the lab. Thaw frozen samples in a 50˚C water bath for 30-45 minutes and then allowing the samples to come back to room temperature before analysis.

Follow all GO-SHIP guidelines for analyses, as for nitrate/nitrite.

Biogenic silica

Location of analysis: NOC/BAS

Person responsible: Ed Mawji (ezm@noc.nerc.ac.uk)/ Kate Hendry (kathen@bas.ac.uk)

Sampling

It is of utmost important that you record the volume of water that you filter. This will enable us to convert the POC we captured on the filter paper to mg L^{-1}. Please do not forget to do this.

Avoid using any filter housing that has been in contact with GF/F filters.

- The sampler will be required to filter water for the BSi samples
- This should be undertaken on the same day
- Responsible persons will distribute polycarbonate filters and filtration kit
- **Ensure samples are well mixed and particulate load hasn't settled out**.
- Filter each sample onto a 0.8μm 25mm polycarbonate filter in lab
- After filtering please fold filter membrane in half (dirty side in), and then half again
- Dry for at least 12 hours on a small piece of foil
- Seal foil packet and then place in the refrigerator or (preferably) freezer for storage

Analysis

Biogenic silica is digested using an alkaline extraction method, and then analysed for [Si] using spectrophotometry (Krause et al., 2009).

Iron (and other trace metals)

Follow all GEOTRACES guidelines for collection and analyses.

For Iron and all trace metals, secondary standards must be used for quality control, specifically Certified Reference Materials (CRMs), and analysed in each run. SLRS-6 is a river water CRM that has been certified for a range of trace metals (including barium and iron). CASS-6 or NASS-7 are available reference standards for marine waters.

All standards must be salinity-matched.

Note that where a single sampling methodology is used for several determinands, the method has been summarised as an Appendix.

Total iron, Total (filterable) iron, Total (filterable) iron(II)

Location of analysis: UKCEH
Person responsible: Stephen Lofts (stlo@ceh.ac.uk)

For sampling and analytical methods see Appendix B-C.

Ultrafilterable iron (II)

Location of analysis: UKCEH
Person responsible: Stephen Lofts (stlo@ceh.ac.uk)

For sampling and analytical methods see Appendix B-C.

Tracers

Oxygen isotopes

Location of analysis: BGS
Person responsible: Carol Arrowsmith (caar@nigl.nerc.ac.uk)

Sampling

Always wear gloves (vinyl preferably).

- Apply pre-printed label to bottle. Write station and Niskin bottle number on label with an indelible marker, and write sequential label number on sampling log sheet.
- Use absorbent material to dry the outside of the spigot.
- Rinse the bottle by half-filling with sample water, shaking, then discarding.
- Fill the bottle, leaving minimal air gap.
- Screw cap tightly onto bottle. Wipe bottle dry.
- Secure the lid with wrap of electrician's tape around base of lid and bottle.

Analysis

The $\delta^{18}O$ measurements are made with an Isoprime 100 mass spectrometer plus Aquaprep device. Isotope measurements used internal standards calibrated against the international standards VSMOW2 and VSLAP2. Errors are typically < 0.05‰ for $\delta^{18}O$.

Barium

Location of analysis: BGS
Person responsible: Carol Arrowsmith (caar@bgs.ac.uk)
Also contact: Simon Chenery (srch@bgs.ac.uk)

Follow all GEOTRACES guidelines for collection and analyses.

Sampling

Always wear gloves (vinyl preferably).

- The sample bottles need to be either LDPE or HDPE, and acid cleaned before hand, at least with reagent grade HCl and rinsed thoroughly in ultrapure water.
- Rinse the bottles with filtered seawater three times before collecting the sample.
- Filter (0.2 micron through rinsed filters - preferably 0.8/0.2 Supor Acropak filters). Acidify the samples at sea (0.1% v/v concentrated and trace metal clean HCl).
- Seal the bottles and store in sealed plastic bags under cool, dark conditions.

Analysis

Barium is measured by inductively-coupled plasma mass spectrometry, together with a suite of other trace metals.
Ba concentrations will be analysed by direct solution isotope dilution (Ba-135 spike) analysis on an Agilent 8900 ICP-MS/MS. The samples will be subject to a 10:1 dilution to avoid cone-clogging and the instrument will be run in He gas collision cell mode to avoid interferences.

Rare Earth Elements

Location of analysis: BGS
Person responsible: Carol Arrowsmith (caar@bgs.ac.uk)
Also contact: Simon Chenery (srch@bgs.ac.uk)
Follow all GEOTRACES guidelines for collection and analyses.

Sampling

Always wear gloves (vinyl preferably).

- The sample bottles need to be either LDPE or HDPE, and acid cleaned before-hand, at least with reagent grade HCl and rinsed thoroughly in ultrapure water.
- Rinse the bottles with filtered seawater three times before collecting the sample.
- Filter (0.2 micron through rinsed filters - preferably 0.8/0.2 Supor Acropak filters) Acidify the samples at sea (0.1% v/v concentrated and trace metal clean HCl).
- Seal the bottles and store in sealed plastic bags under cool, dark conditions.

Analysis

REEs are measured by inductively-coupled plasma mass spectrometry, together with a suite of other trace metals.REE elements will be analysed on an Agilent 8900 ICP-MS/MS both directly and after sample pre-concentration/matrix separation. The samples will be subject to a 10:1 dilution to avoid cone-clogging for direct analysis and the instrument will be run in He gas collision and O2 reaction gas cell modes to avoid interferences. The sample pre-concentration/matrix separation procedure will use a SPR-IDA (imino-diacetate) ion-exchange resin and the sample will be spiked with Y to monitor and correct for recoveries.

Neodymium

Location of analysis: BGS
Person responsible: Carol Arrowsmith (caar@bgs.ac.uk)
Also contact: Ian Millar (ilm@bgs.ac.uk)

Follow all GEOTRACES guidelines for collection and analyses.

Sampling

Always wear gloves (vinyl preferably).

- The sample bottles need to be either LDPE or HDPE, and acid cleaned beforehand, at least with reagent grade HCl and rinsed thoroughly in ultrapure water.
- Rinse the bottles with filtered seawater three times before collecting the sample. The samples are not to be filtered at sea.
- Acidify the samples at sea (0.1% v/v concentrated and trace metal clean HCl).
- Always wear gloves (vinyl preferably).
- Seal the bottles and store in sealed plastic bags under cool, dark conditions.

Analysis

Samples will be concentrated by coprecipitation prior to separation of a bulk rare-earth element fraction using standard ion-exchange methods using AG50x8 cation resin. Samples will be spiked with 150Nd to allow accurate determination of concentrations in addition to isotope ratios. Nd will be separated from the bulk REE fraction using LN-SPEC ion exchange columns. Nd fractions will be dissolved in 2% HNO3 and analysed on a Thermo Scientific Neptune Plus mass spectrometer operated in static multicollection mode. Data are normalised to 146Nd/144Nd = 0.7219. The JNd-I reference material will be used throughout, with a BCR-2 Nd solution run as a secondary reference. Concentrations will be reported as parts per million, in addition to the 143Nd/144Nd isotope ratio.

Neodymium isotopes are measured by multi-collector inductively-coupled plasma mass spectrometry.

Biological parameters

Note that where a single sampling methodology is used for several determinands, the method has been summarised as an Appendix.

Chlorophyll

Location of analysis: UKCEH
Person responsible: Mike Bowes (mibo@ceh.ac.uk)
Also contact: Isabelle Fournier (isafou@ceh.ac.uk)

For sampling, see Appendix D.

Phytoplankton

Location of analysis: UKCEH
Person responsible: Dan Read (dasr@ceh.ac.uk)
Also contact: Mike Bowes (mibo@ceh.ac.uk)/Isabelle Fournier (isafou@ceh.ac.uk)

For sampling, see Appendix D.

Preparing sediment trap bottles for biogeochemical analysis

Materials Needed:

- Polycarbonate or polyethylene bottles (500 mL)
- 37% Formaldehyde solution (for preservation)
- Sodium tetraborate (borax) and NaCl (to buffer formalin)
- Filtered seawater (0.2 µm filtered)
- Milli-Q or distilled water (for cleaning)
- 70% Ethanol (for cleaning)
- Nitrile gloves, safety goggles, and lab coat
- Measuring cylinders (for preparing the solution)
- Labels and waterproof marker

Cleaning the Bottles: Rinse bottles thoroughly with Milli-Q water. Wash with 70% ethanol to remove contaminants. Rinse three times with filtered seawater. Air dry in a clean environment.

Filling the bottles with Formalin Preservative Solution Prepare a 4% buffered formalin solution: Mix 1L 37% formaldehyde with 19L filtered seawater (0.2 µm). Add 5 g sodium tetraborate (borax) to buffer the solution and prevent acidity. Shake periodically, if possible, to ensure Borax dissolves completely. Add **100 g sodium chloride (NaCl) to create an iper-**

saline solution. Leave the solution the overnight before to use it to fill the bottles. Pull the prepared 4% buffered formalin to the bottles until they are approximately full and seal the bottles tightly with caps.

Recovering and deployment of the bottles: Label each bottle with waterproof marker. For the deployment ensure that bottles are placed correctly in the sediment trap to prevent contamination or leakage. For the recovering gentle remove the bottles containing the collected samples from the sediment trap unit. Store bottles upright in a cool, dark location (4°C recommended). Ensure tight sealing to prevent evaporation or contamination. All bottles must be labelled with appropriate hazard labels, contents and contact names.

Appropriate workwear (lab coat, gloves, and safety glasses) is required for all the steps and all the activities need to be done in a fume hood or outside on the deck.

Other analyses

Note that where a single sampling methodology is used for several determinands, the method has been summarised as an Appendix.

Major anions (e.g., Cl/SO_4)

Location of analysis: UKCEH
Person responsible: Mike Bowes (mibo@ceh.ac.uk)
Also contact: Isabelle Fournier (isafou@ceh.ac.uk)

For sampling, see Appendix D.

Suspended solids

Location of analysis: UKCEH
Person responsible: Mike Bowes (mibo@ceh.ac.uk)
Also contact: Isabelle Fournier (isafou@ceh.ac.uk)

Suspended solids are determined as part of POM/POC via Loss on Ignition (where POC = circa 0.5*POM)

Oxygen

Location of analysis: NOC
Person responsible: Ed Mawji

Note that for most of the BIOPOLE localities, O2 will be measured using sensors. For marine applications of O_2 sensors, please contact Kate Hendry, Hugh Venables or Alex Brearley at BAS for more information.

If possible, O_2 will also be analysed using titrations (also useful for sensor calibration purposes). For sampling and analysis – see Langdon, 2019 (GO-SHIP methods).

APPENDIX A: SUMMARY TABLE FOR SAMPLING

Determinand	**Volume collected**	**Filter/acid used**	**Storage**	**Responsible person**	**Shipping address**
Alkalinity	250ml	Unfiltered	Dark fridge 4°	Chris Barry	UK Centre for Ecology & Hydrology Environment Centre Wales Deiniol Road Bangor Gwynedd LL57 2UW UK
DOC/DIC & TDN	30ml	GF/F (ashed)	Dark fridge 4°	Chris Barry	UK Centre for Ecology & Hydrology Environment Centre Wales Deiniol Road Bangor Gwynedd LL57 2UW UK
SS	Volume dependent on concentration through filter	Precombusted, pre-weighed 47mm diameter GF/F; 2 replicates	Dry at 110deg C or Freezer	Chris Barry	UK Centre for Ecology & Hydrology Environment Centre Wales Deiniol Road Bangor Gwynedd LL57 2UW UK

CDOM, fDOM	30ml	GF/F (ashed)	Dark fridge 4°	Chris Barry	UK Centre for Ecology & Hydrology Environment Centre Wales Deiniol Road Bangor Gwynedd LL57 2UW UK
POC	500ml through filter	GF/F (ashed) 25mm d	Freezer	Chris Barry	UK Centre for Ecology & Hydrology Environment Centre Wales Deiniol Road Bangor Gwynedd LL57 2UW UK
PON	500ml through filter	GF/F (ashed) 25mm d	Freezer	Chris Barry	UK Centre for Ecology & Hydrology Environment Centre Wales Deiniol Road Bangor Gwynedd LL57 2UW UK
SRP	5ml	0.45µm	Dark fridge 4°	Justyna Olszewska	Analysed at UKCEH Edinburgh
TDP	60ml	GF/F (ashed)	Dark fridge 4°	Mike Bowes/Chemistry Labs	UK Centre for Ecology & Hydrology Maclean Building Benson Lane Crowmarsh Gifford Wallingford Oxfordshire OX10 8BB UK

TP	60ml	Unfiltered	Dark fridge 4°	Mike Bowes/Chemistry Labs	UK Centre for Ecology & Hydrology Maclean Building Benson Lane Crowmarsh Gifford Wallingford Oxfordshire OX10 8BB UK
Majors	60ml	GF/F	Dark fridge 4°	Mike Bowes/Chemistry Labs	UK Centre for Ecology & Hydrology Maclean Building Benson Lane Crowmarsh Gifford Wallingford Oxfordshire OX10 8BB UK
Flow Cytometry	~15ml	Unfiltered	Dark fridge 4°	Mike Bowes/Chemistry Labs	UK Centre for Ecology & Hydrology Maclean Building Benson Lane Crowmarsh Gifford Wallingford Oxfordshire OX10 8BB UK
Phytoplankton	~20ml	Unfiltered / lugol	Dark fridge 4°	Mike Bowes/Chemistry Labs	UK Centre for Ecology & Hydrology Maclean Building Benson Lane Crowmarsh Gifford Wallingford Oxfordshire OX10 8BB UK

Chlorophyll	500ml	GF/F (ashed)	Freezer	Mike Bowes/Chemistry Labs	UK Centre for Ecology & Hydrology Maclean Building Benson Lane Crowmarsh Gifford Wallingford Oxfordshire OX10 8BB UK
Inorganic nutrients	125ml	0.45µm acropak	Freezer	Ed Mawji	
Mass Spec DOM	1L	GF/F (ashed)	Dark fridge 4°	Stacey Felgate	
Total metals	50ml	Unfiltered	Dark fridge 4°	Steve Lofts	
Filterable metals	50ml	0.45µm acropak	Dark fridge 4°	Steve Lofts	
Ultrafilterable TC-IC	50ml	GF/F	Freezer	Steve Lofts	
Ultrafilterable Fe	50ml	0.45µm acropak	Dark fridge 4°	Steve Lofts	
εNd	10L	Unfiltered, HCl	Room temp	Carol Arrowsmith	British Geological Survey Nicker Hill Keyworth Nottingham NG12 5GG UK
Oxygen ($\delta^{18}O$)	30ml	Unfiltered	Dark fridge 4°	Carol Arrowsmith	British Geological Survey Nicker Hill Keyworth Nottingham NG12 5GG UK

DIC $\delta^{13}C$	30ml	Unfiltered	Dark fridge 4°	Carol Arrowsmith	British Geological Survey Nicker Hill Keyworth Nottingham NG12 5GG UK
REEs	60ml	0.45µm acropak, HCl	Dark fridge 4°	Carol Arrowsmith	British Geological Survey Nicker Hill Keyworth Nottingham NG12 5GG UK
Barium	60ml	0.45µm acropak, HCl	Dark fridge 4°	Carol Arrowsmith	British Geological Survey Nicker Hill Keyworth Nottingham NG12 5GG UK

APPENDIX B: CENTRIFUGATION-ULTRAFILTRATION OF AQUEOUS SAMPLES

Version table

Version	Date	By	Comments
1	3/8/22	SL	Initial version

Principle

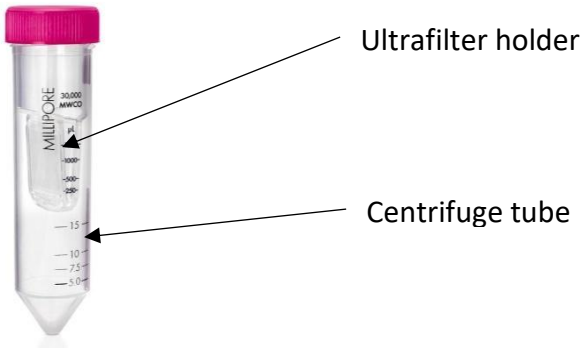
This protocol describes the size separation of aqueous samples by ultrafiltration using a 10kDa pore size membrane and the centrifugation-ultrafiltration approach.
The approach is adaptable to other size separations if suitable ultrafiltration-centrifugation devices are available.

Reagents

1. Ultrapure nitric acid for acidification of ultrafiltered samples.

Apparatus required

1. Amicon® Ultra-15 Centrifugal Filter Units, Ultracel-10 regenerated cellulose membrane, 15 mL sample volume (Merck cat. nos. UFC901008, UFC901024, UFC901096)
2. Centrifuge capable of accepting Amicon units (they are 12.1cm long, 2.97cm diameter).



Procedure

1. Preweigh the centrifuge tube and cap (not including the ultrafilter holder);
2. Place approximately 15ml of raw sample into the ultrafilter holder and place this into the centrifuge tube;
3. Place the centrifuge tubes into the centrifuge and spin for 30 minutes at 4000 *g*.
4. Remove the tubes from the centrifuge;
5. Remove the ultrafilter holders, discard any remaining unfiltered solution and discard the holders;
6. Decant approximately 5ml of sample into a cleaned, acid-washed test tube and store at 4 deg C for DOC analysis;
7. Replace the tube caps and reweigh the tube and cap to obtain the remaining mass of ultrafiltered sample;

8. Acidify the sample using the ultrapure nitric acid, using 17µL of acid per g of sample, and store at 4 deg C for total Fe, Fe(II) and P analysis.

APPENDIX C: COLORIMETRIC DETERMINATION OF TOTAL FE AND FE(II) IN AQUEOUS SAMPLES, USING FERROZINE

Version table

Version	Date	By	Comments
1	3/8/22	SL	Initial version – adapted from protocol of 2007

Principle

Needed

Reagents

2. **0.05M H_2SO_4.** dilute 2.5ml concentrated H_2SO_4 to 1000ml with deionised water.
3. **100 mg/l Fe stock solution.** Dissolve 0.0497g ferrous sulphate.$7H_2O$ in 100ml of 0.05M H_2SO_4. Note the exact mass of salt used on the storage vessel.
4. **100mg/l Fe stock Analytical Quality Control solution.** Dissolve 0.0723g $Fe(NO_3)_3.9H_2O$ in 100ml of 0.05N H_2SO_4. Note the exact mass of salt used on the storage vessel.
5. **10 mg/l Fe intermediate calibration standard solution.** Dilute 10ml of Fe stock solution to 100ml with 0.05M H_2SO_4.
6. **10 mg/l Fe intermediate Analytical Quality Control solution.** Dilute 10ml of Fe stock AQC solution to 100ml with 0.05M H_2SO_4.
7. **100µg/l, 200µg/l, 300µg/l, 400µg/l, 500µg/l working Fe calibration standard solutions.** Dilute 1ml, 2ml, 3ml, 4ml, 5ml respectively of Fe intermediate calibration standard solution to 10ml with 0.05M H_2SO_4.
8. **10 and 500µg/l working Fe AQC solutions.** Dilute 0.1ml and 5ml of Fe intermediate AQC solutions respectively to 10ml with 0.05M H_2SO_4, in duplicate.
9. **5.0M HCl.** Dilute 200ml concentrated HCl to 480ml with deionised water.
10. **Reducing solution.** Dissolve 10g hydroxylamine hydrochloride in 100ml deionised water.
11. **Ferrozine.** Dissolve 0.075g of Ferrozine in 50ml deionised water, add 2ml 5.0M HCl and dilute to 100ml with deionised water. Store in the dark and replace monthly.
12. **Buffer solution.** Dissolve 118.5g sodium acetate trihydrate in 125ml deionised water with gentle warming. Cool, add 29ml glacial acetic acid and dilute to 250ml.

Apparatus required

A note on spectrophotometer cell sizes

The volumes of standard, sample and added reagents listed in the 'Measurements' sections are suitable for measurement either at a path length of 1cm or at 4cm using a small volume cell. Volumes given in brackets are suitable for a conventional 4cm cell.

Measurement of total Fe and Fe(II)

Sample preservation

Fe may adsorb to the walls of sampling vessels. Therefore, as soon as possible after sampling, the acidification step (Step 1 in the measurement procedures below) should be done as soon as possible. If measurements on filtered, ultrafiltered or dialysed samples are required then the acidification should be carried out after filtering/ultrafiltering/dialysis.

Blank absorbances

It is necessary to correct the absorbances measured for the blank absorbance at 562nm (i.e. for the contribution to the absorbance due to the natural colour of the water). Before making iron measurements, measure the absorbance of each unaltered sample at 562nm. Samples may be diluted for this purpose, but if so, the absorbance blank should be corrected for the dilution before use in calculations.

The absorbance blank is identical whether total Fe or (FeII) is being measured; therefore, for samples where both total Fe and Fe(II) are measured, the blank absorbance needs measuring only once.

Calibration

One of two sets of calibration standards may be used:

Standard: 0, 100, 200, 300, 400, 500 ug/l Fe

Low: 0, 5, 10, 20, 50, 100, 250, 500 ug/l Fe

Take 1ml (2ml) of the intermediate calibration standard (reagent no. 4) and add 9ml (18ml) deionised water to make a 1 mg/l Fe solution.

To prepare the standard calibration set, prepare the standards by pipette, in duplicate, using the following volumes:

Standard	Volume of 1 mg/l Fe soln. (µl)	Volume of deionised water (µl)
Calibration blank	0	5000 (10000)
Sample blank*	0	5000 (10000)
100µg/l	500 (1000)	4500 (9000)
200µg/l	1000 (2000)	4000 (8000)
300µg/l	1500 (3000)	3500 (7000)
400µg/l	2000 (4000)	3000 (6000)
500µg/l	2500 (5000)	2500 (5000)

To prepare the low calibration set, prepare the standards by pipette, in duplicate, using the following volumes:

Standard	Volume of 1 mg/l Fe soln. (µl)	Volume of deionised water (µl)
Calibration blank	0	5000 (10000)
Sample blank*	0	5000 (10000)
5µg/l	25 (50)	4975 (9950)
10µg/l	50 (100)	4950 (9900)
20µg/l	100 (200)	4900 (9800)
50µg/l	250 (500)	4750 (9500)
100µg/l	500 (1000)	4500 (9000)
250µg/l	1250 (2500)	3750 (7500)
500µg/l	2500 (5000)	2500 (5000)

* sample blanks are only required if measurement of Fe(II) is being done.

Quality control

In duplicate, mix 50µl (100µl) of the intermediate Analytical Quality Control solution with 4950µl (9900µl) of deionised water. The concentration of the Quality control samples is 100µg/l Fe.

Sample preparation

In duplicate, take 5ml/5000µl (10ml/10000µl) of each sample on which Fe is to be measured. If the analyte concentration (total Fe or Fe(II) depending upon what is being measured) is likely to be greater than 500µg/l, dilute the sample accordingly using deionised water.

Measurement procedure

1. **Calibration blanks, calibration standards and quality control standards**

   NOTE: steps (a) and (b) should be done the day before sample measurement.

   (a) **Acidification step.** To each standard add 100µl (200µl) of 5.0M HCl (reagent no. 8);

   (b) **Reduction step.** Add 250µl (500µl) of reducing solution (reagent no. 9) and leave overnight;

   (c) **Measurement step.** Add 250µl (500µl) of Ferrozine (reagent no. 10) and 625µl (1250µl) of buffer solution (reagent no. 11). Measure the absorbance at 562nm *30 seconds after the addition of the buffer solution*.

2. **Sample blanks and samples for total Fe measurement**

   (a) **Acidification step**. To each sample add 100µl (200µl) of 5.0M HCl (reagent no. 8).

   (b) **Reduction step**. Add 250µl (500µl) of reducing solution (reagent no. 9) and leave overnight.

(d) **Measurement step.** Add 250µl (500µl) of Ferrozine (reagent no. 10) and 625µl (1250µl) of buffer solution (reagent no. 11). Measure the absorbance at 562nm *30 seconds after the addition of the buffer solution*.

3. Sample blanks and samples <u>for Fe(II) measurement</u>

(a) **Dilution step**. To each blank and sample, add 350µl (700µl) of deionised water.

(b) **Measurement step.** Add 250µl (500µl) of Ferrozine (reagent no. 10) and 625µl (1250µl) of buffer solution (reagent no. 11). Measure the absorbance at 562nm *30 seconds after the addition of the buffer solution.*

Calculation of iron concentrations

Fe in calibration blanks, calibration standards and quality control standards is calculated using

$$Fe\ (\mu g/l) = A.\{corrected\ absorbance\}^2 + B.\{corrected\ absorbance\} + C$$

The parameters A, B and C are calculated from the calibration curve.

<u>{corrected absorbance} for calibration standards</u> is given by

```
calibration absorbance - mean of calibration blanks
```

<u>{corrected absorbance} for samples on which total Fe is being measured</u> is given by

```
sample absorbance - (absorbance blank x 0.8032) - mean of calibration
blanks
```

The absorbance blank is the absorbance of the sample at 562nm without the addition of any reagents. The factor 0.8032 corrects this absorbance for dilution by the reagents. (i.e. it gives you the expected blank absorbance of the sample after all the reagents have been added).

<u>{corrected absorbance} for samples on which Fe(II) is being measured</u> is given by

```
sample absorbance - (absorbance blank x 0.8032) - sample blank
```

APPENDIX D: Sampling procedure for freshwater chemistry – Majors (SRP, Si, F, Cl, NO2, NO3, SO4), TDP, TP and chlorophyll

1) Record date and time of sampling in field notebook.
2) Take a water sample using a bucket or large wide-mouthed bottle, and discard, (to wash out bucket/bottle).
3) Refill the bucket with river or loch water, being careful to avoid disturbing the bed sediment.
4) Unscrew the filter unit, and remove the old filter paper if present.
5) Make sure that the red O-ring is still in place in the top of the filter unit (left side of photo below). If it has come loose, push it back into place. If it won't fit, it helps to take it out and stretch it a little before retrying to fit it.

6) Place a GF/F filter onto the filter unit (see photo below) and screw on the top of the filter unit.
7) Fill a 60 ml syringe with water from the bucket and discard (to rinse out the syringe)
8) Half-fill the syringe and place the syringe nozzle into the top of the filter unit (see photo below). Press down on the plunger to wash out the filter unit with the river water sample. Hold the syringe barrel and plunger, rather than the plunger and filter housing, as this can cause the syringe tip to snap off.

9) Filter 60 ml river water samples into the small bottles labelled Majors F and TDP F. Fill to the neck of the bottle, rather than completely fill to the brim.
10) Use the syringe to stir the water in the bucket (to resuspend any sediment or algae) and to take another 60 ml sample, and fill the remaining small bottle labelled TP UF. This sample is NOT filtered. Again, fill to the neck of the bottle.
11) Fill up the large bottle marked chlorophyll with unfiltered water.
12) Add 20ml of unfiltered water sample to the 30 ml brown glass bottle, using the syringe. (Caution, this contains a few ml of Lugol's solution. Avoid skin contact). This sample is for algal microscopy.
13) Half fill the plastic Sterilin tube with white cap with unfiltered sample. This is for algal analysis by flow cytometry
14) Keep the samples in a cool box with ice packs, and transfer to a fridge on return to the laboratory.

If you have any problems or queries, please call Mike Bowes on 07984 706306.

APPENDIX F: Collection and storage of samples for determination of POC, PON, POP

Scope – This SOP is for the collection, preparation and storage of samples for subsequent analysis of water **sample concentrations of particulate organic carbon, nitrogen and phosphorus.** Analysing laboratory to be confirmed but likely to be NOC and/or BAS (via elemental analyser)
Summary – Raw water samples are filtered to concentrate particulate matter onto 25mm diameter glass fibre filters. Pre-ashed (combusted at 550°C for 4 hours) glass-fibre filters are used. Raw water sample volumes (typically 0.5 to 2L depending on visually apparent turbidity) will be collected from field sites and returned to the local laboratory where filtration will be performed to prepare filter-samples for storage and onward transport to the analysing laboratory. Some BioPole samples may be particularly dilute so it is conceivable greater raw water volumes could be required (e.g. 2L).
POC and PON can be determined on the same filter-sample. POP requires a separate filter.

Apparatus

- Blue-nitrile or similar laboratory gloves
- Measuring cylinder
- Beakers; 250ml or 500ml capacity
- pre-combusted 25mm glass fibre F-grade filters (GF/F; nominal pore size = 0.7µm)
    - UKCEH-Bangor have supplied 100 pre-combusted filters.
- Sample log book,
    - with columns for Sample ID & sample volume
- Raw water sample containers
    - 0.5, 1, or 2L; judgement/experience is used to select an appropriate sample container volume based on visual assessment of suspended sediment concentration. UKCEH-Bangor have supplied a number of 2L wide mouth Nalgene sample bottles. The raw water samples can be the same as those that will be employed for preparation of sample-filters for TSS-POM-LoI, subject to available volumes (See SOP for TSS-POM-LoI). Lower volumes are required for POC/N/P analysis as 25mm filters are used and the analytical approach is not gravimetric.
- Cold box + cold packs
- Blunt end Tweezers or forceps
- Aluminium foil
- Suitable containers – 15ml centrifuge tubes – not ideal but will do, pending sourcing cryogenic 2ml vials for subsequent campaigns
- Freezer and/or Oven with 50°C capacity
- Vacuum filtration rig for 25mm filters + associated side arm Erlenmeyer flask and tubing
- Permanent markers x2, (e.g. Sharpie / Pentel)
- Ultra-pure water
- 'squeezy' bottle for ultrapure water
- 20ml - 100ml syringes

Protocol – Water sample acquisition

(as per samples for TSS-POM- the same raw water sample for TSS can be used for POC/N/P subject to sufficient volume)

1. Wear laboratory gloves
2. Label base and cap of raw water sample container with site info/site ID. 2L Nalgene bottles hold permanent marker well on their bases and screw-caps. Check if this is the case for other raw-water sample vessels that may be used. This can be conducted prior to departure for field sampling
3. Triple-rinse sample bottle and cap with unfiltered sample water, loosely closing the screw-cap each time.

4. If sampling surface waters, place the bottle upside down below the surface and invert underwater to completely fill (avoid surface film / neuston), and then close bottle cap underwater, avoiding gas headspace as far as is practical.
5. Check all bottle labelling has remained intact.
6. Check the bottles are closed correctly – note that 2L Nalgene bottles can be closed when screw-threads are not aligned correctly, and can leak if this is the case.
7. Store dark-cold, ideally within a cold or other box for return to the local laboratory (expected within 4-6 hours of collection)

Protocol – Preparation and storage of Glass fibre filters containing sample matter

Notes – read me!

- The aim is to collect as much matter on/in filters as is pragmatic given time constraints, as more matter collected on filters is conducive to improved measurement precision (increasingly so for POC>PON>POP). As a general working principal filtration of an individual sample should take < 15mins. If more than 30mins is required then efforts should be made to reduce volumes added to the filter rig.
- The potential for complete 'clogging' of filters means that if large sample volumes are added to filter rigs in one go, they may clog before all water is filtered. This water cannot be removed to acquire the filter as this introduces bias to the sample, whereby more rapidly sinking particles may sediment out of solution. To circumvent this, it is recommended that small sample volumes are added sequentially until the filtration rate drops considerably. As the aim is to determine suspended matter concentrations, the raw water sample must be mixed before volumes are subsampled and added to the filter rig. To achieve this, the raw water sample container can be gently inverted several times (avoid shaking and bubbles), and then a subsample can then be poured into a 'working' beaker (e.g. 250 – 500ml volume) from which syringe volumes of sample can be taken and added to the filter rig. Where more than one syringe volume is required the working beaker is re-mixed to re-suspend particles that may have settled before taking a syringe volume sample. <u>Care must be taken to accurately record the total volumes filtered when using sequential additions of syringe volumes.</u>
- A level of operator judgement is required to assess what volumes to sequentially filter. For example if a sample is particularly turbid then 20ml syringe volumes could be subsampled from the working beaker, with 5ml volumes added to the filter sequentially. If a sample is apparently clear then perhaps 200ml subsamples can be added sequentially. On-the-job / dynamic assessment is required.
- As total filtration times per sample can be somewhat long it is worthwhile setting up several filter rigs so that several samples can be filtered in tandem. 3 – 6 filter rig manifolds are available, or individual filter rigs can be connected to the same individual gas vacuum line (with on-off valves; note that if the vacuum pump is small then pressures will tend to decrease with increasing numbers of filter rigs used). The specific filter instrumentation to be used for the Etive campaign remains to be clarified- <u>Kate Hendry may be bringing a 3-rig 25mm filter manifold. UKCEH-Bangor are sending an individual filter 25mm rig.</u>

Protocol steps

1. Wear laboratory gloves & follow lab protocols for safe laboratory working practices
2. Set up filter rig(s) and connect to vacuum line
3. Rinse through filter rig with ultrapure water – use squeezy bottle to distribute ultrapure water across all internal sides of the rig that will contain unfiltered raw water sample - empty filtrate collection reservoir if required
4. Set up lab notebook, containing columns to record Sample Ids and filtered volumes (ml)
5. Label containers for final prepared sample-filters with labels corresponding to Sample Ids,. (At present this will be 15ml centrifuge tubes, however small plastic c.4ml 'cryotubes' would be

better, as filters can be placed in these after filtration and then dried within the tube). Ensure separate tubes are used for POC/N filters & POP filters

6. Select first water sample and write the sample ID in the lab notebook sample ID column

   E.g. column names and 1st and 2nd sample example for log-book

SampleID	Vol filtered (ml)
xyz_20221004	100ml
abc_20221004	85ml

7. Open filter manifold and use tweezers to position filter in place. Ensure the fritted section is completely covered by the filter; i.e. position in the centre. Close manifold securely ensuring the filter edges are entirely trapped between the two filter rig sections.

8. Invert raw water sample container several times to re-suspend any material that may have settled out, and pour a subsample into a clean working beaker (e.g. 250 or 500ml beaker; rinse beaker and syringes with ultrapure water and then sample water before use and between samples)
9. Ensure working beaker raw water sample is homogenously mixed, and then subsample a selected volume using a syringe
10. Turn on the vacuum line, and transfer the entire or a partial (but known) syringe volume to the filter
11. Record the volume added with each successive syringe volume.
12. When no more water can be filtered within time constraints (c. 15-30mins), rinse the raw sample section of the filter rig with ultrapure water to move any residual material to the filter.
13. Leave the filter drawing air through it for several seconds (c.30secs to remove excess moisture); unlock the filter manifold and use tweezers or forceps to carefully remove the filter from the manifold. It may be necessary to release the vacuum in order to easily remove the filter.
14. Subject to local lab infrastructure, samples are to be either
    i. Stored frozen and dispatched to the analysing laboratory frozen.
    ii. Stored frozen for duration of field campaign, and later dried (overnight at 50°C) and stored in a desiccator before dispatch to the analysing laboratory. Short term storage of dried filters in sealed containers outside of a desiccator is ok (1-2days), but samples should be re-ovened before longer term storage in a desiccator.
    iii. Immediately dried for >12hrs at 50°C, and stored within a desiccator before dispatch to the analysing laboratory
15. Fold or roll the filter with sample side inwards using forceps, and place within its respective, labelled container. Typically material is embedded within the filter and not likely to fall off, even when dried. If 14.i or 14.ii, seal the container with its cap and place in freezer. If 13.iii,

place the container containing the sample filter in the 50°C oven unopened and with its cap. Seal the containers the following day after >12hrs, and store in a desiccator if available.

16. Take a photo record of the completed hard copy notebook pages containing Sample-Filter Ids and filtered volumes.
17. Update/create electronic spreadsheet copy of the lab-notebook contents; with the sample Ids and volumes filtered.

APPENDIX F: References

Becker, S., Aoyama, M., Woodward, E. M. S., Bakker, K., Coverly, S., Mahaffey, C., & Tanhua, T. (2020). GO-SHIP repeat hydrography nutrient manual: the precise and accurate determination of dissolved inorganic nutrients in seawater, using continuous flow analysis methods. Frontiers in Marine Science, 7, 581790.

Halewood, E., Opalk, K., Custals, L., Carey, M., Hansell, D. A., & Carlson, C. A. (2022). Determination of dissolved organic carbon and total dissolved nitrogen in seawater using high temperature combustion analysis. Frontiers in Marine Science, 9, 1061646.

Kellerman, A. M., Vonk, J., McColaugh, S., Podgorski, D. C., van Winden, E., Hawkings, J. R., et al. (2021). Molecular signatures of glacial dissolved organic matter from Svalbard and Greenland. Global Biogeochemical Cycles, 35, e2020GB006709. https://doi.org/10.1029/2020GB006709

Krause, J. W., Lomas, M. W., & Nelson, D. M. (2009). Biogenic silica at the Bermuda Atlantic Time-series Study site in the Sargasso Sea: Temporal changes and their inferred controls based on a 15-year record. Global Biogeochemical Cycles, 23(3).