

FIELD AND LABORATORY METHODS FOR BIOGEOCHEMICAL ANALYSES OF SEA ICE, SEAWATER AND PARTICLE INTERCEPTOR TRAP SAMPLES

C. Michel and A. Niemi

Central and Arctic Region
Fisheries and Oceans Canada
501 University Crescent
Winnipeg, MB R3T 2N6

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C. Michel and A. Niemi

Fisheries and Oceans Canada
Central and Arctic Region
501 University Crescent
Winnipeg, MB R3T 2N6

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ABSTRACT

Michel, C., and Niemi, A. 2009. Field and laboratory methods for biogeochemical analyses of sea ice, seawater and short-term particle interceptor trap samples. Can. Tech. Rep. Fish. Aquat. Sci. 2852: v + 24 p.

This report presents a synthesis of biogeochemical methods used in our laboratory (Marine Productivity, Freshwater Institute, Fisheries and Oceans Canada) for the analysis of sea ice, seawater, and particle interceptor trap samples. These methods are used to assess and monitor Arctic marine ecosystems given climate variability and changing sea-ice conditions. The analytical methods presented are derived from current methods in biological and chemical oceanography, whereas some field methods for sea ice and particle interceptor trap sampling have been developed through years of oceanographic sampling in high-latitude environments. Detailed methods are presented when the published information is scattered throughout the literature, or when methods have been adapted from the literature by our laboratory. This document provides guidelines and procedures for the collection and analysis of oceanographic samples, with the intent that they can be reproduced by other investigators.

RÉSUMÉ

Michel, C., and Niemi, A. 2009. Field and laboratory methods for biogeochemical analyses of sea ice, seawater and short-term particle interceptor trap samples. Can. Tech. Rep. Fish. Aquat. Sci. 2852: v + 24 p.

Ce rapport présente une synthèse des méthodes biogéochimiques utilisées dans notre laboratoire (Productivité marine, Institut des Eaux Douces, Pêches et Océans Canada), pour l'analyse d'échantillons de glace de mer, d'eau de mer, ainsi que de pièges à particules. Ces méthodes sont utilisées pour évaluer et faire le suivi des écosystèmes marins arctiques, dans le contexte de la variabilité climatique actuelle et des changements observés dans les conditions de glace. Les méthodes d'analyse présentées sont dérivées de méthodes couramment utilisées en océanographie chimique et biologique, alors que les méthodes d'échantillonnage de la glace de mer et des pièges à particules ont été développées au cours de nombreuses années d'échantillonnage océanographique en milieu nordique. Des méthodes détaillées sont présentées lorsque l'information publiée est dispersée ou lorsque les méthodes publiées ont été modifiées par notre laboratoire. Ce document présente des directives et procédures pour la collecte et l'analyse d'échantillons océanographiques, avec l'intention que celles-ci puissent être reproduites par d'autres investigateurs.

INTRODUCTION

This report brings together a compilation of methods for basic biogeochemical analysis of sea ice, seawater, and particle interceptor trap samples. The analytical methods presented are currently used in our laboratory (Marine Productivity) at the Freshwater Institute (Fisheries and Oceans Canada) and are largely based on current methods in biological oceanography, as described in the classic manual of seawater analysis by Parsons et al. (1989), the JGOFS protocols for core measurements (Knap et al. 1996), and best practices for CO₂ measurements (Dickson et al. 2007). Similar protocols are used in other laboratories dealing with the biochemical analysis of seawater samples. The methods presented are used for assessment and process studies in Arctic marine ecosystems, as well as for monitoring ecosystem changes in view of current climate variability and changing sea-ice conditions.

Protocols for the sampling and handling of sea ice samples have been developed through many years of research on first-year sea ice in the Arctic, using ships or ice camps as sampling platforms. Methods for the deployment of short-term particle interceptor traps have also been developed through first-hand experience, with moorings installed attached to the ice or deployed from small ships and research vessels. Methods for particle interceptor trap moorings and analyses have been developed based on abundant literature on sediment trap methods (e.g. Gundersen 1991; Lee 1991; Knap et al. 1996; O'Neill et al. 2005), and recommendations by Gardner (2000).

The aim of this report is not to provide detailed step-by-step procedures for methods that are commonly used (e.g. chlorophyll *a* determination) and well documented in marine sciences (Parsons et al. 1989). For these well-established methods, the report provides general protocols used in our laboratory. However, the reader will find more detailed step-by-step procedures for existing methods for which the published information is scattered throughout the literature, or for methods that have been adapted from the literature by the Marine Productivity Laboratory. Overall, this report aims at providing solid descriptions of methods and protocols, in a manner that can be reproduced by other investigators. It is suggested that these methods be used for continued monitoring of high-latitude ecosystems, as the harmonization of sampling and analytical methods constitutes an essential step towards the integration of datasets collected over large temporal and spatial scales.

SAMPLE COLLECTION

WATER SAMPLES

Seawater samples are collected using Niskin bottles installed on a stainless steel line attached to a deck winch (KC Denmark), or with a Seabird Carousel Rosette sampler equipped with 12 L Niskin bottles. Samples for nutrients and dissolved organic carbon and nitrogen (DOC and DN) analyses are taken first and put in their respective containers (see detailed methods for DOC and DN below). Samples for biological analyses are transferred into acid-cleaned amber Nalgene bottles or isothermal containers and are normally processed immediately after collection. When sampling from the Rosette, a maximum of 12 depths, including seven depths in the euphotic zone, is typically sampled for biological analyses.

ICE SAMPLES

Ice samples are collected with a manual ice corer, 9 cm internal diameter (Kovacs Enterprises). The ice cores are cut into sections using a stainless steel handsaw and each core section is put in a clean isothermal container, or a sterile Whirl-Pak (NASCO) bag, for further analysis. Sterile gloves are worn at all times during core handling and care is taken to avoid exposing core sections to direct sunlight. Figure 1 shows a first-year ice bottom core section containing abundant algal biomass. Snow depth, ice thickness and freeboard are measured at each coring site at the time of sampling.

According to the sampling plans and objectives, multiple core sections from the same depth-horizon are pooled together in an isothermal container in order to obtain enough material for the analyses to be performed. Separate core sections are also kept in sterile Whirl-Pak bags for the analysis of dissolved constituents (e.g. DOC, DN and nutrients) and bulk salinity determination.

After collection, cores are transported back to the laboratory, either ship, camp, or shore-based. In the laboratory, surface seawater collected at the time of coring is filtered through 0.22 μm polyvinylidene fluoride (PVDF) Millipore Durapore membrane filters. Typically, 500 mL of 0.22 μm filtered seawater is added per 5 cm bottom core section to help prevent osmotic stress of sea-ice organisms during melting (Garrison and Buck 1986). The volume of water added to the cores is carefully measured and noted and, after melting, the total volume of the ice + seawater sample is measured. A dilution factor is then calculated (see equation 1) and used to correct concentrations of all variables measured on subsamples from the diluted cores.

$$D_f = \frac{V_{tot}}{(V_{tot} - V_{FSW})} \quad (1)$$

where:

D_f is the dimensionless core dilution factor (no unit),

V_{tot} is the total volume of the cores and added filtered seawater (L), and

V_{FSW} is the volume of added filtered seawater (L).

Seawater is not added to cores in the Whirl-Pak bags to avoid contamination of dissolved nutrient and DOC/DN samples. A schematic flow chart showing procedures and analyses for diluted and non-diluted cores is presented in Figure 2. Note that when sub-sampling ice core samples for biochemical analyses, care is taken to systematically mix the sample as ice algae can sediment rapidly.

PARTICLE INTERCEPTOR TRAP SAMPLES

Free-drifting, short-term particle interceptor traps are deployed from a research vessel and left adrift for ca. 24 h. The particle interceptor trap deployments are done in accordance with JGOFS protocols (Knap et al. 1996) with recommendations by Gardner (2000).

At each station, cylindrical particle interceptor traps made of PVC (Polyvinyl Chloride), with an aspect ratio (height:/diameter) of 7 and internal diameter of 10 cm, are installed at target depths on a braided nylon line. In order to collect enough material for various analyses, it is often necessary to install a series of two to five particle interceptor traps, centered at each target depth on the mooring line. The free-drifting particle interceptor trap array is surface-tethered with a series of small buoys to minimize vertical motion on the line and it is weighted at bottom. A current meter (Aanderaa, RCM9) that records current speed, direction, and tilt is also attached to the mooring line. At surface, the line is attached to a 10 m braided nylon line connected to an aluminum mast with a radio beacon (Novatech designs Ltd., RF-700C1) for short-range positioning. The mast is connected to another 10 m braided nylon line attached to an ARGOS drifter (Seimac Smart Cap PTT/GPS transmitter) for long-range satellite tracking. A schematic drawing of the particle interceptor trap drifting mooring array is presented in Figure 3.

Alternatively, particle interceptor traps can be deployed from the ice. The particle interceptor traps are attached to a braided nylon or polypropylene line secured to a tripod anchored to the ice. The underice traps are typically moored at shallow depths (e.g. 1, 5 and 25 m) to collect the material sinking from the ice matrix and to avoid excessive drag on the trap line. Similar to free-drifting moorings, underice trap moorings are equipped with a current meter. Prior to deploying and recovering the particle interceptor traps, care must be taken to ensure that the sampling hole is cleared of ice and slush. Note that during periods of melt, when abundant ice algal biomass can be released from the ice, it may be necessary to cap the particle interceptor traps to avoid collection of suspended material during recovery.

Prior to deployment of drifting and underice particle interceptor traps, each trap is thoroughly cleaned and rinsed with seawater filtered through 0.22 μm PVDF Millipore Durapore membrane filters. Each trap is then filled with 0.22 μm filtered seawater (PVDF Millipore Durapore membrane filters) that was collected at depth at a previous sampling station. No poison or preservative is added to the traps for the short-term deployments. Subsamples for determination of salinity, analysis of DOC and DN, and bacterial counts are collected from the 0.22 μm filtered seawater that is used to fill the particle interceptor traps.

Upon recovery, the traps are covered with a tight clean lid and the collected material is allowed to settle for 8 h in a temperature-controlled room (at a temperature ranging between 0°C and 4°C for Arctic studies). After the sedimentation period, the supernatant of each particle interceptor trap

is carefully released from a drainage port on the side of the particle interceptor trap. The remaining volume, which contains the settled material, is then sieved through a 450 μm mesh to remove large swimmers and kept in a clean isothermal container for further analyses. The presence of swimmers and potential debris on the 450 μm is carefully recorded. The settled material, collected from the series of traps deployed in the same depth horizon, is pooled together to obtain one sample per depth, which is used for subsequent analyses. For each variable, sinking fluxes are calculated as follows (Juul-Pedersen et al. 2008):

$$S = \frac{C_{trap} \times V_{trap}}{A_{trap} \times t} \quad (2)$$

where:

S is the sinking flux for each variable ($\text{mg m}^{-2} \text{d}^{-1}$),

C_{trap} is the concentration of the measured variable in the particle interceptor trap sample (mg m^{-3}),

V_{trap} is the volume of the particle interceptor trap sample (m^3),

A_{trap} is the particle interceptor trap surface area (m^2), and

t is the deployment time (d).

SAMPLE PREPARATION AND ANALYSIS

GENERAL PROCEDURES

All reagents and solutions are prepared using ultrapure water (Barnstead Easypure UV ultrapure system). All glassware and polyethylene bottles are acid-washed (10% HCl; reagent grade) prior to laboratory use. Our filtration systems and sampling bottles are carefully washed and rinsed with either ultrapure water when working in the laboratory, or 0.22 μm (PVDF Millipore Durapore membrane filters) filtered seawater when working at sea or in ice camps. A summary of the analytical methods used in our laboratory is presented in Table 1.

CHLOROPHYLL AND PHEOPIGMENTS

This method is directly adapted from Parsons et al. (1989). Fluorometric determination of chlorophyll a (chl a) and pheopigments is carried out on fresh pigment extracts in the laboratory onboard the ship or at the field camp.

For total chl a and pheopigments, duplicate subsamples are filtered onto Whatman 25 mm GF/F filters (nominal pore size of 0.7 μm), whereas size-fractionated chl a and pheopigments are measured after filtration of duplicate subsamples on 5 μm Nuclepore membrane filters (size fraction $>5 \mu\text{m}$) and Osmonics 20 μm membranes (size fraction $>20 \mu\text{m}$). Total pigment concentrations are calculated as the sum of chl a and pheopigment concentrations. Size fractions $<5 \mu\text{m}$ and $<20 \mu\text{m}$ are obtained by subtraction of pigments retained on 5 μm filters and 20 μm membranes, respectively, from total concentrations (GF/F filters). The pigments are extracted in 10 mL of 90% acetone at 5°C in the dark for 24 h.

Chl *a* and pheopigments are determined fluorometrically using a 10AU Turner Designs fluorometer calibrated against known concentrations of chl *a*, as determined spectrophotometrically, using pure chl *a* extract (*Anacystis nidulans*, Sigma Chemicals). A first fluorescence reading is taken against a 90% acetone blank, after letting the samples acclimatize to room temperature. A second fluorescence reading is taken after adding 3 drops of 5% HCl to the sample and carefully mixing. Chl *a* and pheopigment concentrations are calculated according to Parsons et al. (1989).

Reagents

90% Acetone (v/v): Dilute 900 mL of 100% acetone (reagent grade) in ultrapure water, for a final volume of 1 L.

5% Hydrochloric acid (HCl) (v/v): Dilute 5 mL of concentrated HCl (12N HCl; reagent grade) in ultrapure water, for a final volume of 100 mL.

Chl *a* standard solution: Mix 1 mg *Anacystis nidulans* (Sigma) in 200 mL of 90% acetone; extract for 18-24 h at 5°C in the dark.

BIOGENIC AND LITHOGENIC SILICA

Biogenic silica (BioSi), and lithogenic silica (LithoSi) when appropriate (e.g. coastal waters), are determined on duplicate subsamples filtered onto 0.6 µm Nuclepore polycarbonate membrane filters. The filtrations are performed using all plastic filtration apparatus and reagents are prepared and stored in high density polyethylene (HDPE) bottles to avoid any Si contamination. The filtered samples are placed in cryovials, dried at 60°C for 24 h and stored until analysis. The material retained on filters is analyzed according to Conley (1998) and Ragueneau and Tréguer (1994), with hydrolysis using a 0.2N NaOH solution and spectrophotometric determination of a silico-molybdate complex (Parsons et al. 1989).

As the presence of LithoSi interferes with BioSi determination, sequential (time-series) extraction is required when the presence of LithoSi is suspected (see method below for sequential extraction). In our laboratory, we routinely do a few sequential extractions on the first day of analysis, in order to verify the presence of LithoSi and to determine an appropriate extraction time. In the absence of LithoSi, single extractions are performed. Below, separate protocols are presented for samples with and without LithoSi. A schematic showing the main steps to follow throughout the protocol is shown in Figure 4.

Samples with LithoSi

Step 1: Preparation for extraction: Each sample (filter) is placed open in a 15 mL conical polypropylene tube (Falcon). Each water or trap sample is covered with 5 mL 0.2N NaOH (10 mL for ice algae samples); the sample is then vortexed and the tube is loosely capped.

For each series of analysis, a set of 3 blanks with a blank filter and 3 blanks with no filter is analyzed. The blanks are processed in the same manner as the samples.

Step 2A: Single extraction: Samples are placed in a water bath at 95°C for 45 min. The extraction time should be determined by performing extraction kinetics (sequential extraction Step 2B) on some samples.

After the extraction period the sample is cooled in an ice bath, 1 mL of 1N HCl is added for water and trap samples (2 mL for ice algae samples) and then the sample is vortexed. The sample is then centrifuged for 10 min at 3,000 rpm and 4 mL of the supernatant is withdrawn and placed in a 50 mL polypropylene tube for BioSi analysis. This 4 mL sample extract is diluted to a total volume of 10 mL by adding 6 mL of ultrapure water. A higher dilution may be required for ice algae samples. After this dilution step, the sample is processed for dissolved Si analysis (Step 3). Note that 15 mL polypropylene tubes containing the blank and sample filters are kept for LithoSi analysis (Step 5).

Step 2B: Sequential extraction: Samples in 15 mL polypropylene tubes are placed in a water bath at 95°C. To characterize extraction kinetics (see Conley 1998), a subsample of 0.5 mL is removed from the water bath after 10, 20, 30, 45, 60, and 90 min, and placed in a 50 mL conical polypropylene tube. The subsample is diluted to 10 mL by adding ultrapure water and is then processed for dissolved Si analysis (Step 3). Mineral interference with BioSi extraction is evaluated as per Rageneau and Tréguer (1994) and BioSi concentrations are determined using the extrapolated intercept on the y-axis of the linear regression of BioSi concentrations *versus* extraction time, according to De Master (1981).

Step 3: Dissolved Si analysis: For each 10 mL sample, 4 mL ammonium molybdate solution is added. The reaction is allowed to develop for 10 min, but no more than 30 min, after which 6 mL of reducing reagent is rapidly added. The colour is allowed to develop for 2.5 to 3 h and the absorbance is read at 810 nm on a spectrophotometer using 1 cm cuvettes for concentrations $>15 \mu\text{mol L}^{-1}$. After reading absorbance, we use microscopic observation of the filter to verify that there is no residual biogenic silica remaining in the sample (i.e. complete extraction).

The concentration of silica in the sample is calculated according to the following equation:

$$Si = \frac{(Si_e \times V_e \times F_d)}{V_f} \quad (3)$$

where:

Si is the concentration of silica in the sample ($\mu\text{mol L}^{-1}$),

Si_e is the concentration of SiOH_4 in the sample extract, estimated from equation 5 ($\mu\text{mol L}^{-1}$),

V_e is the volume of the sample extract (mL),

F_d is the aliquot factor calculated when a subsample of the extracted volume is used for the colorimetric reaction (no unit), and

V_f is the sample filtration volume (mL).

Step 4: Preparation for LithoSi: For the LithoSi extraction, the 15 mL tube containing the remaining filter and 2 mL of solution from the single or sequential extraction is used. The following steps are then followed:

10 mL ultrapure water is added to the sample which is vortexed and centrifuged for 10 min at 3,000 rpm at room temperature (Biofuge Stratos). The supernatant is removed leaving 1 mL. This procedure is repeated if the BioSi concentration in the 10 mL sample is $>64 \mu\text{mol L}^{-1}$ (BioSi

concentration on filter is $>2.0 \mu\text{mol L}^{-1}$). Note that the dilution/centrifugation procedure has always been repeated for analyses in our laboratory. Next, the samples are uncapped and placed in the drying oven (Yamato DX400) at 60°C until dry. When dry, the filters can be stored in their tubes at room temperature until analysis.

Step 5: LithoSi analysis: After drying, each filter is covered with 0.2 mL of 2.5N HF, ensuring that air bubbles are removed. Note that HF is extremely toxic and corrosive and extreme caution must be taken when using this chemical. Never use glass or metal with HF.

For each series of LithoSi samples, 3 blanks containing 0.2 mL of 2.5N HF only are prepared in addition to the 6 blanks from the BioSi extraction which are carried through all steps of the LithoSi analysis. Samples and blanks are extracted in HF for 48 h, after which 9.8 mL of filtered saturated H_3BO_3 solution is added to samples and blanks. Each sample is vortexed and the complete volume + filter is transferred into 30 mL polycarbonate bottles. The rest of the procedure follows Step 3, for dissolved silicate analysis using colorimetric determination.

The concentration of LithoSi in the sample is calculated as per equation 4, taking into account silicate remaining in the solution from the BioSi digestion (second term of equation 4).

$$\text{LithoSi} = \left(\frac{V_e \times Si_e}{V_f} \right) - (D_{BSi} \times \text{BioSi}) \quad (4)$$

where:

LithoSi is the concentration of LithoSi in the sample ($\mu\text{mol L}^{-1}$),

V_e , Si_e , and V_f are as defined above (equation 3),

D_{BSi} is the dilution factor calculated for the one-step or two step dilution of the sample (no unit), and *BioSi* is the biogenic silica concentration in the sample ($\mu\text{mol L}^{-1}$).

Samples without LithoSi

Step 1: Preparation for extraction: Each sample (filter) is placed open in a 50 mL conical polypropylene tube (Falcon). Each sample is covered with 4 mL 0.2N NaOH (8 mL for ice algae samples), the sample is vortexed and the tube is loosely capped.

For each series of analysis, a set of 3 blanks with a blank filter, and 3 blanks with no filter is analyzed. The blanks are processed in the same manner as the samples.

Step 2A: Single extraction: Samples are placed in a water bath at 95°C for 45 min. The extraction time should be determined by extraction kinetics (sequential extraction) on some samples.

After the extraction period the sample is cooled in an ice bath, 1 mL of 1N HCl is added (2 mL for ice algae samples) and the sample is vortexed.

Step 2B: Sequential extraction: Samples are placed in a water bath at 95°C . To characterize extraction kinetics (see Conley 1998), a subsample of 0.5 mL is removed from the water bath after 10, 20, 30, 45, 60, and 90 min and placed in a 50 mL conical polypropylene

tube. The subsample is diluted to 5 or 10 mL by adding ultrapure water and is then processed for dissolved BioSi analysis (Step 3).

Step 3: Dissolved Si analysis: For each 10 mL sample, 4 mL ammonium molybdate solution is added. The reaction is allowed to develop for 10 min, but no more than 30 min, after which 6 mL of reducing reagent is rapidly added. The colour is allowed to develop for 2.5 to 3 h and the absorbance is read at 810 nm on a spectrophotometer using 1 cm cuvettes for concentrations $>15 \mu\text{mol L}^{-1}$. After reading the absorbance, we use microscopic observation to verify that there is no residual biogenic silica remaining in the sample, (i.e. complete extraction). Note that the volume of reagents is adjusted depending on the sample extraction volume. For example, extractions for ice algae samples are usually done with a final extraction volume of 20 to 40 mL while for suspended phytoplankton, extractions are usually with a final extraction volume of 10 to 20 mL.

The concentration of silica in the sample is calculated according to equation 3.

Calibration curve for Si analysis

A calibration curve has to be prepared for each analytical day. For BioSi, a concentrated stock solution of Na_2SiF_6 , (5 mmol Si L^{-1}) is used to prepare a series of standards with Si concentrations ranging from 0 (no stock solution added) to $200 \mu\text{mol Si L}^{-1}$. Each standard is prepared using only plastic pipettes and volumetric flasks and is stored in Nalgene amber HDPE bottles. The standards are treated in the same way as the samples after NaOH extraction, i.e. ammonium molybdate and reducing reagents are added for the colorimetric reaction and absorbance is read at 810 nm on a spectrophotometer. The slope of the calibration curve of Si concentrations *versus* absorbance is used to estimate Si concentrations in the sample extracts, according to the following equation:

$$Si_e = (Abs_s - Abs_b) \times F \quad (5)$$

where:

Si_e is the concentration of silica in the sample (in $\mu\text{mol L}^{-1}$),

Abs_s and Abs_b are the absorbance of the sample and blank, respectively, read at 810 nm (abs unit),

F is the slope of the calibration curve of Si concentrations versus absorbance ($\mu\text{mol L}^{-1} \text{ absorbance unit}^{-1}$).

As for BioSi analysis, a calibration curve must be made for each series of LithoSi analyses. The calibration curve is prepared using hydrofluoric acid (HF) mixed with a filtered solution of saturated boric acid (H_3BO_3), instead of ultrapure water, for dilution of silica standards. For each standard curve, a bulk mixture of HF and saturated filtered H_3BO_3 is prepared, in a proportion of 0.2 mL 2.5N HF and 9.8 mL saturated filtered H_3BO_3 . The HF/ H_3BO_3 solution must be filtered daily using $0.6 \mu\text{m}$, 47 mm polycarbonate membrane filters.

Reagents

Ammonium molybdate: Dissolve 4 g of ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O; EMD Chemicals) in ca. 400 mL ultrapure water. When dissolved, add 12 mL of concentrated hydrochloric acid (12N HCl), mix and complete volume to 500 mL. Store in amber HDPE bottle. This reagent is stable for 1 month.

Metol sulfite solution: Dissolve 6 g of anhydrous sodium sulfite (Na₂SO₃; EMD Chemicals) in 500 mL ultrapure water. When dissolved add 10 g paramethylaminophenol sulfate ((CH₃NHC₆H₄OH)₂·H₂SO₄; EMD Chemicals). Filter the solution through a 0.6 μm Nuclepore membrane filter and store in a glass bottle tightly stoppered. This solution should be prepared at least once a month.

Saturated oxalic acid solution: Dissolve >10 g oxalic acid dihydrate ((COOH)₂·2H₂O; EMD Chemicals) in 100 mL ultrapure water. Saturation is ca. 10 g for 100 mL. Decant the solution from the crystals for use. Store in amber HDPE bottle. This solution is stable indefinitely.

Reducing reagent: Mix 100 mL metol sulfite solution with 60 mL saturated oxalic acid solution. Add slowly, while mixing, 60 mL of 50% sulfuric acid solution and complete volume to 300 mL with ultrapure water. This reagent has to be prepared daily.

50% Sulfuric acid solution (v/v): Pour 500 mL ultrapure water in a clean thick-walled plastic bottle. Place in an ice bath. Slowly add 500 mL concentrated sulfuric acid (H₂SO₄; EMD Chemicals). A high amount of heat is generated when adding H₂SO₄, so proceed very slowly and be extra careful. Let cool in ice bath and complete volume to 1 L with ultrapure water. This solution is stable indefinitely.

0.2N NaOH: Dissolve 8 g of NaOH in 1 L of ultrapure water. Store in amber HDPE bottle. This solution is stable for long periods.

1N HCl: Dilute 83.3 mL of concentrated HCl in a volume of ca. 900 mL ultrapure water. Complete volume, with ultrapure water, to 1 L. Store in amber HDPE bottle. This solution is stable for long periods.

2.5N Hydrofluoric acid solution (HF; EMD Chemicals)

Saturated boric acid (H₃BO₃; EMD Chemicals) ~1 M solution: Dissolve 60 g H₃BO₃ in 1 L of ultrapure water. This solution must be filtered each time prior to use.

Na₂SiF₆ stock solution (5 mmol Si L⁻¹): Dilute 0.960 g of Na₂SiF₆ in 1 L of ultrapure water.

PARTICULATE ORGANIC AND INORGANIC CARBON AND NITROGEN

For all manipulations associated with the determination of particulate organic carbon and nitrogen, special care is taken to avoid contamination. Filtration units are kept clean by thorough rinsing with 0.2 μm filtered seawater or ultrapure water prior to and after filtration, and forceps used to manipulate the filters are kept in concentrated ethanol.

Total particulate carbon (TPC) and particulate organic carbon (POC) are determined on two separate subsamples filtered onto 21 mm Whatman GF/F filters that have been pre-combusted at 450°C for 24 h. The filtered subsamples are each placed in a Petri dish and dried at 60°C for 24 h prior to analysis. In the laboratory, we acidify filters dedicated for POC measurement by fuming

with concentrated HCl for 24 h to remove inorganic carbon. All samples are analyzed on a Perkin Elmer Model 2400 Elemental Analyzer. Particulate inorganic carbon (PIC) is determined by subtracting POC from TPC values. Blank filters are kept for each series of filtrations and are analyzed similarly as samples. Blank carbon and nitrogen values measured on the blank filters are subtracted from samples values to obtain final carbon and nitrogen concentrations.

Reagents

95% Ethanol

12N HCl (Reagent grade)

DISSOLVED ORGANIC CARBON AND NITROGEN

DOC/DN sampling bottles (Amber EPA glass vials with polypropylene open-top caps and PTFE silicone septas) are thoroughly washed prior to use. The washing procedure involves soaking in 10% HCl for 12-24 h, four subsequent rinses ultrapure water baths, and two final rinses using ultrapure water directly from the ultrapure unit. The bottles are dried at 60°C in a clean oven (Yamato DX400) and then combusted at 550°C for 5-8 h. After cooling, each bottle is sealed/capped with an acid washed Teflon-lined septa and cap.

For water column sampling, samples are collected directly from the Rosette, after nutrients. An acid-washed syringe is mounted directly on the Rosette port to collect the sample. The sample is then passed through an acid-washed Swinnex filter holder containing a 25 mm combusted GF/F filter (450°C for 24 h). The first 5-10 mL of sample are discarded and the rest is poured in the ultra-clean sampling bottle. Sea ice samples for DOC and DN analysis are taken directly from sterile Whirl-Pak bags, to which no seawater has been added (see Figure 2) using a sterile syringe. Subsamples are similarly collected from the settled particle interceptor trap samples and from the filtered seawater used to fill the traps prior to deployment. This allows for an evaluation of the dissolution of organic material in the particle interceptor traps during deployment, assuming no or limited exchange with surrounding waters.

Each sample is acidified by the addition of 200 μL of 50% H_3PO_4 for a 40 mL sampling bottle. Samples are kept in the refrigerator in the dark until analysis. These samples can be stored up to a few months (Knap et al. 1994). DOC and DN is measured using high-temperature catalytic combustion on a Shimadzu TOC-VCPH analyzer with an ASI-V auto sampler and TNM-1 Total Nitrogen module. Procedures follow Dickson et al. (2007). Ultrapure water is systematically run as rinses and results indicate that the ultrapure water DOC concentration is $<2 \mu\text{mol L}^{-1}$. DOC and DN analyses are systematically checked against low carbon water (ca. $2 \mu\text{mol L}^{-1}$ DOC) and deep Sargasso Sea reference water ($44\text{-}47 \mu\text{mol L}^{-1}$ DOC) obtained from Hansell's Certified Reference Materials (CRM) program (rsmas.miami.edu/groups/biogeochem/CRM.html).

Reagents

50% Phosphoric acid (H_3PO_4) (v/v): Dilute 100 mL of concentrated H_3PO_4 (HPLC grade) in ultrapure water, for a final volume of 200 mL.

10% Hydrochloric acid (HCl) (v/v): Dilute 100 mL of concentrated HCl (12N HCl; reagent grade) in 900 mL ultrapure water, for a final volume of 1 L.

EXOPOLYMERIC SUBSTANCES

This method is based on the colorimetric determination of transparent exopolymers (TEP) from Alcian Blue staining, as developed by Passow and Alldredge (1995). As the staining capacity of different Alcian Blue solutions may change, a calibration has to be performed before using the stain (see Alcian Blue calibration below).

For all filtrations, it is important to ensure that the filtration apparatus is clean, i.e. we perform abundant rinsing with ultrapure water prior to processing any sample. Each sample is filtered in triplicate (for water samples, the volume may range between ca. 100-500 mL) onto 47 mm 0.4 μm Nuclepore membrane filters. Filters are stained for <2 sec with 1.5 mL of the pre-filtered Alcian Blue solution, keeping the filtration pressure at ca. 5 psi. The Alcian Blue solution is filtered through a Whatman 0.22 μm syringe filter before use. When staining the samples, it is important to ensure that the Alcian Blue completely covers the entire surface of the filter. After staining, the filters are rinsed once with ultrapure water before they are removed from the filtration unit and each placed in a 30 mL glass beaker. Exopolymeric substances (EPS) extraction is done by adding 6 mL of 80% sulfuric acid (H_2SO_4) to each beaker and letting the filters + acid sit under the fumehood for 2 h, gently agitating the samples at regular intervals during the extraction period. After the 2 h extraction, the absorbance of each sample extract is read on a spectrophotometer at 787 nm using a 1 cm cuvette and using ultrapure water as a blank. For each series of filters, 3 sample blanks are made using blank filters treated the same way as the samples. EPS concentrations measured on the blank filters are subtracted from samples values to obtain final EPS concentrations.

Concentrations of EPS in gum xanthan equivalents are estimated using the following equation:

$$C_{EPS} = \frac{(E_{787} - B_{787}) \times F}{V} \quad (6)$$

where:

C_{EPS} is the concentration of gum xanthan ($\mu\text{g Xeq L}^{-1}$),

E_{787} is the absorbance reading of the sample (1 cm cuvette) (abs unit),

B_{787} is the absorbance reading of the blank (1 cm cuvette) (abs unit),

F is the calibration factor from the slope of the calibration curve ($\mu\text{g Xeq abs unit}^{-1}$), and

V is the volume filtered (L).

Note that stained samples can be stored in acid-washed cryovials and frozen for later analysis.

Alcian Blue calibration

The Alcian blue calibration involves two separate steps. The first step establishes a relationship between the dry weights and respective filtration volumes of a gum xanthan standard solution of known concentration. The second step involves calibration of the staining capacity of the Alcian Blue with gum xanthan standards of known volumes (and therefore weights, as per step 1).

A gum xanthan solution is prepared by mixing 30 mg of gum xanthan powder in 200 mL ultrapure water at room temperature. It is important to perform the following steps when preparing the gum xanthan standard solution as this breaks down the gum xanthan into particles small enough to become fully hydrated.

- After mixing the gum xanthan with ultrapure water, the solution is vigorously shaken for 1-2 min.
- Next, 10-15 mL aliquots of the solution are ground in a glass tissue grinder, 10 times each, and poured into a clean container.
- When grinding is completed (i.e. all 200 mL), the solution is vigorously shaken for 1-2 min and left to sit for 25 min.
- The grinding step is then repeated before the solution is ready for the calibration.

Dry weight calibration for gum xanthan: For this part of the calibration, 47 mm 0.4 μm Nuclepore membrane filters are used. Each filter is dried and weighed on a high-precision balance (Mettler Toledo MX5, precision of 0.1 μg). The filters are stored in an individual clean Petri dish and its weight is noted. Five replicates of 0.5, 1, 2 and 3 mL aliquots of the gum xanthan solution are filtered onto the preweighed filters. Note that volumes of 0.5, 1.0, 1.5, 2.0 and 2.5 mL have also been used in our laboratory. The filters are removed from the filtration unit (without rinsing) and are dried at 80°C for 2 h in a clean drying oven (Yamato DX400). Filters are let to cool in a desiccator at room temperature before re-weighing.

The weight of gum xanthan on each filter is obtained by subtracting the initial weight of each filter from the final weight. Prior to all weighing, the filters are passed below a Sartorius ionizer to neutralize static electricity which can interfere with the measurements. This method allows us to obtain linear relationships between the gum xanthan weight and the filtration volume.

Staining capacity of the Alcian Blue solution: For the second part of the calibration, the same gum xanthan solution and filtration volumes as for the weights are used. Five replicate gum xanthan aliquots are filtered onto new 0.4 μm Nuclepore membrane filters and are stained with 1.5 mL of the pre-filtered Alcian Blue solution for <2 s. The filters are rinsed once with distilled water to remove excess dye. Blank filters are also prepared using the same procedure but without the addition of gum xanthan. The remaining steps are the same as for sample determination, with a 2 h extraction using 80% sulfuric acid, and spectrophotometric absorbance reading at 787 nm.

A calibration curve is made by plotting the gum xanthan dry weights against the xanthan staining capacity. The slope of the regression is the calibration factor (F) for the Alcian Blue solution, as per equation 6. Note that the average blank absorbance is subtracted from each absorbance value prior to proceeding with the calibration curve.

Reagents

Alcian Blue Solution: Mix 0.1 g of Alcian Blue powder (8GX, Sigma) in 500 mL of 0.06% acetic acid solution. The Alcian Blue solution is stable for ca. 2 months.

0.06% Acetic acid (CH_3COOH) (v/v): Dilute 6 mL of glacial acetic acid (CH_3COOH , EMD Chemicals) in ultrapure water, for a final volume of 100 mL.

80% Sulfuric acid (H₂SO₄) (v/v): Pour 200 mL ultrapure water in a clean thick-walled plastic bottle. Place in an ice bath. Slowly add 800 mL concentrated sulfuric acid (H₂SO₄; reagent grade). A high amount of heat is generated when adding H₂SO₄, so proceed very slowly and be extra careful. Let cool in ice bath and complete volume to 1L with ultrapure water. This solution is stable indefinitely.

Gum xanthan (Sigma)

BACTERIAL AND PROTIST ABUNDANCE USING EPIFLUORESCENCE MICROSCOPY

All filtration units are cleaned with 0.22 µm filtered seawater prior to sample filtration. A pipette with sterile pipette tip, or an acid-washed syringe, is used to transfer the sample volume (between 2 and 20 mL) into a dark scintillation vial. If using a syringe, the syringe is rinsed three times with the sample before pouring the sample volume into the scintillation vial. Formaldehyde is added to the sample, for a 1% v/v final concentration, and the sample is mixed. The preserved samples can be immediately stained with DAPI or stored at 4°C in the dark for later processing. Samples are stained with DAPI to a final concentration of 1 µg mL⁻¹ and mixed (Sherr et al. 1993). The samples should be allowed to stain for >15 min, but no more than 2 h, in the dark.

After staining, samples are filtered onto a 0.2 or 0.8 µm black filter, for bacteria and protists, respectively, at low pressure (<5 psi), using a backing membrane filter. Filters are removed from the filtration unit, without rinsing, and placed on a glass slide with one drop of Cargille DF immersion oil. A cover slip is secured with nail polish, using only a small dab on each corner as the presence of nail polish interferes with counting. Slides are labeled clearly and stored in slide boxes at -80°C until analysis by epifluorescence microscopy (Leica DM-LR). Blanks are regularly done for bacteria counts using 0.22 µm filtered seawater processed the same way as samples.

For epifluorescence microscopy, our laboratory is equipped with a Leica DM-LS epifluorescence microscope with a digital Olympus DP70 camera and an image-analysis system (Image Pro plus). Bacteria are counted at 1000X and protists are counted at 400X or 1000X magnification, using an UV excitation filter (Leica filter cube A, BP 340-380, LP 425, dichromatic mirror 400). A blue excitation filter (Leica filter cube I3, BP 450-490, LP 515, dichromatic mirror 510) is used to verify chlorophyll autofluorescence. A minimum of 200 bacteria and 10 fields of view is counted for each sample. For protists, a minimum of 200 cells and 3 transects is counted.

Reagents

DAPI (4,6-diamidino-2-phenylindole; Sigma) working solution (0.1 mg mL⁻¹) This solution is prepared from a stock solution of DAPI 1 mg mL⁻¹. The DAPI solution should be kept in the refrigerator in the dark.

37% Formaldehyde solution (reagent grade)

PHYTOPLANKTON TAXONOMY AND CELL COUNTS USING INVERTED MICROSCOPY

Subsamples from particle interceptor traps (100 to 250 mL), Niskin bottles (100 to 250 mL), or sea ice (20 to 100 mL) are preserved with acidic Lugol's solution (Parsons et al. 1989) for later identification and enumeration of phytoplankton cells with an inverted microscope (Leica DM IRB) according to Lund et al. (1958).

For each sample, a minimum of 400 cells and three transects are counted. The abundance of each taxon is calculated according to the equation of Horner (2002). Average cell sizes are obtained by measuring 30 individual cells from the most abundant species and average cell biovolumes are estimated using appropriate geometric equations (Hillebrand et al. 1999). For the least abundant taxa, average cell sizes are obtained from the literature (Tomas 1997; Bérard-Therriault et al. 1999). Protist carbon biomass is estimated using the conversion factors of Menden-Deuer and Lessard (2000), except for ciliates for which we use the specific conversion factor from Putt and Stoecker (1989). All protist cells, except spores and empty diatom cells, are included in the total protist community carbon estimate. Note that preservation of samples in acidic Lugol's solution can result in underestimates of fragile protist species (e.g. flagellates and ciliates).

Reagents

Acidic Lugol's solution: Mix 200 g potassium iodide (KI; Alfa Aesar), 100 g Iodine (I₂; EMD Chemicals) and 190 mL glacial acetic acid (CH₃COOH; EMD Chemicals) in 2 L of ultrapure water.

FECAL PELLETT ABUNDANCE

The first sub-sample that is drawn from particle interceptor trap samples is for fecal pellet counts, as the fragile fecal pellets are susceptible to breakage during repeated mixing of the particle interceptor samples. Fecal pellets are preserved by adding buffered formaldehyde to particle interceptor trap or water samples, to a final concentration of 1%. Fecal pellets are counted using an inverted microscope (Leica DM IRB) or a stereomicroscope (Leica MZ6) at 100X magnification.

Each fecal pellet is measured and the biovolume of entire and broken fecal pellets is estimated using appropriate geometric equations. The volume of intact cylindrical pellets is calculated using the volume of a cylinder with half-spherical ends, while the volume of broken cylindrical fecal pellets is calculated as cylinders. The volume of intact elliptical pellets is calculated using the equation for an ellipsoid.

Reagents

Borate-buffered formaldehyde (37%): Add 30 g of sodium tetraborate (Na₂B₄O₇·10H₂O) to 1 L of concentrated formaldehyde.

PRIMARY PRODUCTION

Primary production is measured on duplicate samples using simulated *in situ* incubations, or *in situ* incubations. For each set of duplicate samples, a dark bottle is prepared. For each sampling depth (Niskin or particle interceptor trap) or site (ice algae), duplicate samples are poured into polycarbonate bottles and an additional sample is poured into a dark polycarbonate bottle. Incubation volumes are typically 250 mL for ice algae and 500 mL for Niskins and particle interceptor trap samples. The bottles are spiked with 10 μCi (ice algae) or 20 μCi (Niskins and particle interceptor traps) of ^{14}C . These bottles are then ready to incubate *in situ*, or in the simulated *in situ* incubator. Duration of *in situ* incubations vary depending on specific objectives of the study. In the past, we have performed 24 h *in situ* incubations for ice algae, Niskin and particle interceptor trap samples.

Initial activity (T_0) is determined for each sample and dark bottle, as follow: A 50 μL subsample is taken from each sample/dark bottle spiked with ^{14}C , placed in a scintillation vial to which 50 μL of 6N NaOH and 15 mL ecolume are added. The scintillation vial is thoroughly mixed and stored until analysis.

After the incubation period, the samples and dark bottles are processed as follows: For total production, a 3 mL subsample is placed in a scintillation vial and acidified with 50 μL 6N HCl. The sample is left to evaporate on a shaking table in a fume hood for at least 4 h, after which the sample is neutralized with 50 μL 6N NaOH. Ecolume (15 mL) is then added to the sample which is thoroughly mixed and stored until analysis.

Total particulate production is measured on subsamples filtered onto 25 mm Whatman GF/F filters and size-fractionate particulate production is measured on filters of various pore sizes, typically Nuclepore 5 μm membrane filters. After filtration of subsamples on appropriate pore size filters, the filters are placed in a scintillation vial and 200 μL 0.5N HCl is added. The vials are placed on a shaking table in a fume hood for 12 to 24 h, until the filter is dry. After that period, ecolume (15 mL) is added to the sample which is thoroughly mixed and stored until analysis.

Activity in each sample is determined with a Packard Tri-carb 2100TR liquid scintillation analyzer and primary production is calculated according to the following equation:

$$P = \frac{(R_s - R_b) \times W}{R \times t} \quad (7)$$

where:

P is the primary production ($\text{mg C m}^{-3} \text{ h}^{-1}$),

R_s is the sample count corrected for quenching (dpm),

R_b is the dark bottle count corrected for quenching (dpm),

W is the weight of total carbon dioxide present in the water (mg C m^{-3}),

R is the total activity of $\text{NaH}^{14}\text{CO}_3$ added to the sample (dpm), and

t is the incubation period (h).

Reagents

^{14}C bicarbonate ($\text{NaH}^{14}\text{CO}_3$; ICN Biomedicals) stock solution, $100 \mu\text{Ci mL}^{-1}$

6N NaOH (EMD Chemicals)

Ecolume (ICN Biomedicals)

6N HCl: Dilute 500 mL of concentrated HCl (12N; reagent grade) in a volume of 500 mL ultrapure water.

0.5N HCl: Dilute 41.67 mL of concentrated HCl (12N; reagent grade) in a volume of ca. 900 mL ultrapure water. Complete volume, with ultrapure water, to a final volume of 1 L.

SEDIMENTATION COLUMNS (SETCOLS)

Sedimentation columns (Setcols) are used to estimate potential sinking rates of ice algae and phytoplankton in the absence of turbulence. This method was originally developed by Bienfang (1981). The Setcols used in our laboratory have an internal diameter of 7.75 cm and a height of ~50 cm. The Setcols have two ports on the side and one at the bottom, allowing the removal of sample from the upper ~7 cm (V_b), and lower ~7 cm (V_s) fractions of the column. Each Setcol is made of plexiglass and is contained in a plexiglass jacket through which ambient seawater is circulated in order to maintain *in situ* temperature. During the sedimentation period, Setcols are kept in the dark.

The Setcols are filled with a homogeneously mixed sample (Niskin or ice algae), and the material is allowed to sediment for a period typically ranging between 2 h for ice algae and 6 h for phytoplankton. A subsample is set aside to measure initial (time 0) and end (time t) concentrations of all variables measured in the Setcols. This allows us to evaluate any change in biomass over time, due to processes other than sedimentation and buoyancy (e.g. cell growth), during the sedimentation period.

After the sedimentation period, three fractions, bottom, middle, and top, are drained from the Setcol using the ports at the side and bottom, and the volume of each fraction is carefully measured and noted. Only the bottom and top fractions are used for further analyses. Analyses performed can include any biomass index and typically include chl a , POC, and cell counts. The sinking rate of each variable is calculated as follows:

$$S = \frac{B_s}{B_t} \times \frac{h}{t} \quad (8)$$

where:

S is the sinking rate of a given variable (m d^{-1}),

B_s represents the change in biomass in the bottom section of the Setcol, as per equation 9 (mg m^{-3} , or other biomass unit),

B_t is the total biomass in the Setcol, as per equation 10 (mg m^{-3} , or other biomass unit),

h is the height of the Setcol (m), and

t is the duration of the sedimentation experiment (d).

$$B_s = V_s b_s - \left(\left(\frac{b_{0,0} + b_{0,t}}{2} \right) \times V_s \right) \quad (9)$$

where:

V_s is the volume of the bottom fraction of the Setcol (m^3),

b_s is biomass concentration in the bottom fraction of the Setcol at the end of the experiment (mg m^{-3} , or other biomass unit),

$b_{0,0}$ is the initial biomass concentration in the Setcol, as measured from the subsample set aside (mg m^{-3} , or other biomass unit), and

$b_{0,t}$ is the biomass concentration in the same subsample at the end of the experiment (time t) (mg m^{-3} , or other biomass unit).

$$B_t = \left(\frac{b_{0,0} + b_{0,t}}{2} \right) \times V_t \quad (10)$$

where:

$b_{0,0}$ and $b_{0,t}$ are as described above (equation 9), and

V_t is the total volume of the Setcol (m^3).

The ascent rate (A) for each variable can also be calculated according to the following equation:

$$A = \frac{B_b}{B_t} \times \frac{h}{t} \quad (11)$$

where:

B_b represents the change in biomass in the top section of the Setcol, as per equation 12 (mg m^{-3} , or other biomass unit),

B_t , h , and t are as defined above.

$$B_b = V_b b_b - \left[\left(1 - \frac{tS}{h_b} \right) \times \left(V_b \times \frac{(b_{0,0} + b_{0,t})}{2} \right) \right] \quad (12)$$

where:

h_b is the height of the top fraction of the Setcol (m),

V_b is the volume of the top fraction of the Setcol (m^3)

b_b is the biomass concentration in the top fraction of the Setcol at the end of the experiment (mg m^{-3} , or other biomass unit), and

B_b , t , S , $b_{0,0}$ and $b_{0,t}$ are as defined above.

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Table 1. Summary of methods used by the Marine Productivity Laboratory for sea ice, water column and particle interceptor trap samples. Diam = filter diameter.

Method	Filtration	Standard	Analyzer	References
Chlorophyll <i>a</i> & pheopigments	GF/F, 20 μm , 5 μm Diam: 25 mm	<i>Anacystis nidulans</i>	10AU Turner Designs fluorometer	Parsons et al. 1989
Biogenic & lithogenic silica	0.6 μm Diam: 47 mm	Na_2SiF_6	Varian Cary 100 spectrophotometer	Conley 1998, Ragueneau & Tréguer 1994, Parsons et al. 1989
Particulate organic/inorganic carbon & nitrogen	Combusted GF/F Diam: 21 mm	Acetanilide	Perkin-Elmer Model 2400 CHN analyzer	Knap et al. 1996
DOC & DN	Combusted GF/F Diam: 25 mm	KHP (carbon), KNO_3 (nitrogen)	Shimadzu TOC-VCPH	Knap et al. 1996, Dickson et al. 2007
EPS	Nuclepore 0.4 μm Diam: 47 mm	Gum Xanthan	Jenway model 6300 spectrophotometer	Passow & Alldredge 1995
Bacteria & protist counts	Black 0.2 μm , 0.8 μm Diam: 25 mm	NA	Epifluorescence microscope, Olympus DP70, Image Pro	Sherr et al. 1993
Primary production	GF/F, 5 μm Diam: 25 mm	$\text{NaH}^{14}\text{CO}_3$	Packard Tri-carb 2100TR liquid scintillation analyzer	Knap et al. 1996



Figure 1. Photograph of bottom ice core sample containing high biomass of ice algae, as shown by the dark colour of bottom ice. Photo credits: Marine Productivity Laboratory.

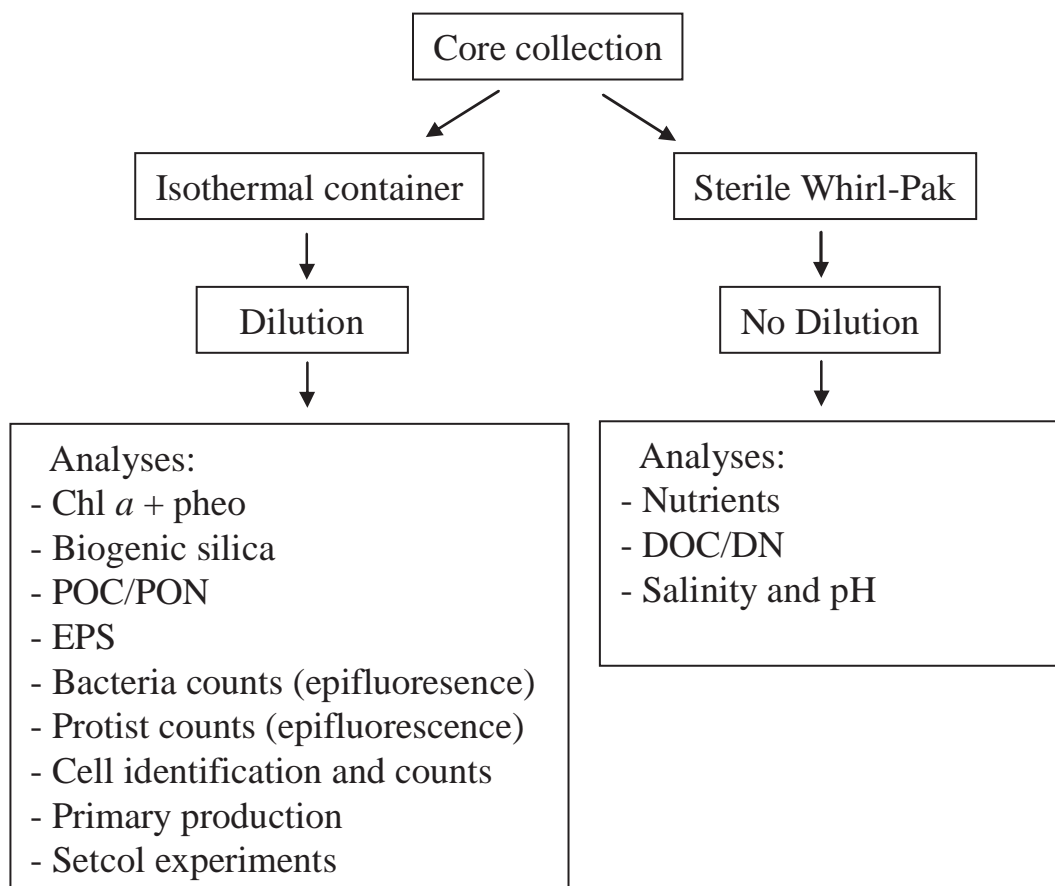


Figure 2. Schematic flow chart for analytical procedures following ice core sampling.

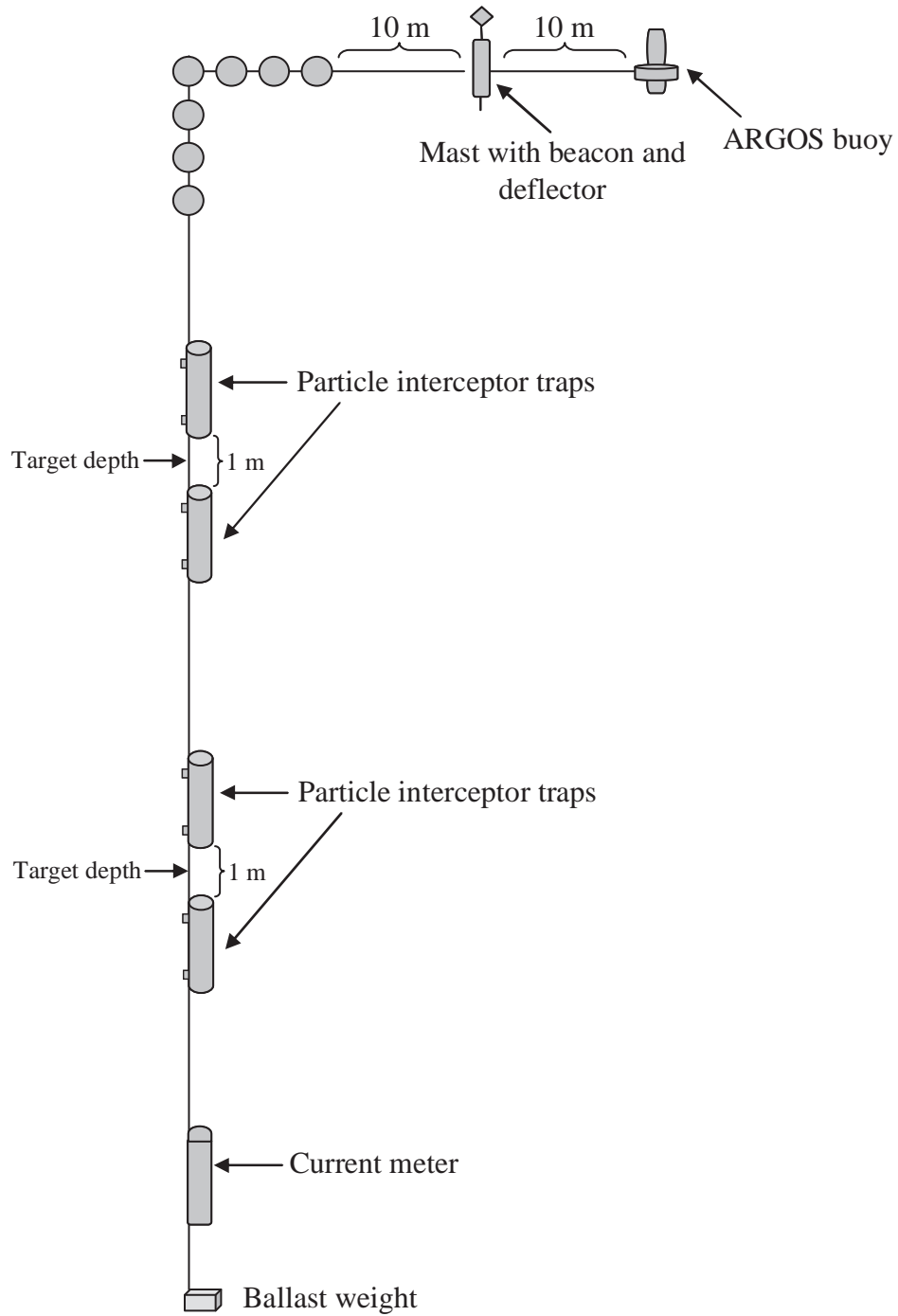


Figure 3. Schematic representation of a short-term drifting particle interceptor trap array.

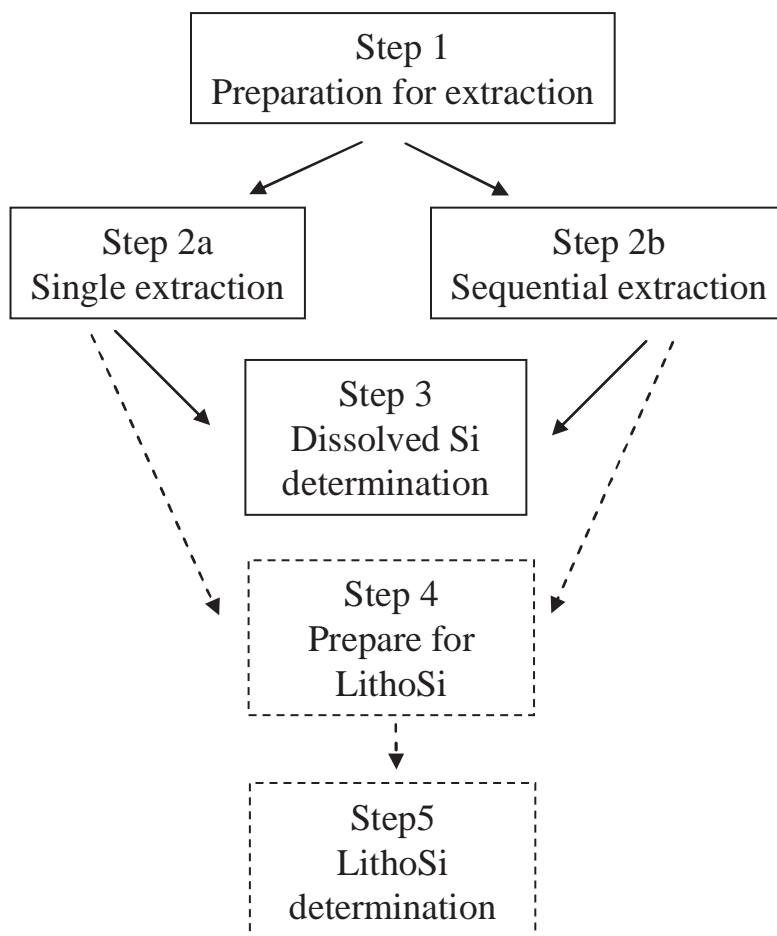


Figure 4. Schematic flow chart of the main steps for the determination of biogenic (BioSi) and lithogenic silica (LithoSi) in sea ice, seawater and particle interceptor trap samples. Dashed boxes and arrows identify steps applicable only for LithoSi determination.