

BIOMARKS SAMPLING PROTOCOLS

CHEMISTRY

## Pigments by HPLC

### I- Introduction

Assess the quality and quantity of Chlorophyll-*a* and accessory pigments in sampled water.

### II – Major Peculiarities

If possible do not work under direct sun light, which degrades the pigments.

Filter as quickly as possible when water is available

Freeze samples quickly after filtration

### III – Material and Instruments

2000µm, 20µm and 3µm Pre-filtered SW, plus 20µm (diluted from plankton net) – 180µm or

2000µm Pre-filtered SW, 0.2µm filtered SW

GF/F Filters 25 mm (porosity 0.7µm)

2000µm, 200µm, 180µm and 20µm sieves

2 L bottles

Test-tube 200ml

Filtration device and holder (full system)

Funnels

Tubing

### IV – Chemicals and buffers

### V – Material preparation

Prepare filtration devices: Holders and funnels have to be cleaned with distilled water and protected with aluminium foil until filtration. GF/F filters should be placed with clean tweezers and always with the same side up. Make sure the filter is correctly placed by turning on the pump before to put the top of the filtration holder system.

### VI – Sampling and conditioning

Sub-surface and DCM - 0.7-3 µm; Sub-surface and DCM – 0.7-20 µm; Sub-surface and DCM – 20-2000 µm; Sub-surface and DCM - 0.7-2000 µm; Sub-surface and DCM – 20-180 µm

#### - Sub-surface and DCM – 20-2000 µm

Dilute with 0.2µm filtered SW the sample in the 20µm net box and pour on the 2000µm sieve.

Collect in a clean bottle and filtrate on 25 mm GF/F filter. The exact final volume is reported (this is a quantitative measurement). The volume that has to be filtered depend on the charge of the net box.

As it can be a long process, use 2L bottles with a cap adapted to fit a tube. Turn around the bottle to let the water flow in the filtration unit. Or complete the funnel with the test-tube writing down the volume. Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth. (2x2L)

Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth. (2x2L)

Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth. (2x2L)

#### - Sub-surface and DCM – 20-180 µm

Dilute with 0.2µm filtered SW the sample in the 20µm net box and pour on the 2000µm sieve.

Collect in a clean bottle and filtrate on 25 mm GF/F filter. The exact final volume is reported (this is a quantitative measurement). The volume that has to be filtered depend on the charge of the net box.

As it can be a long process, use 2L bottles with a cap adapted to fit a tube. Turn around the bottle to let the water flow in the filtration unit. Or complete the funnel with the test-tube writing down the volume. Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth. (2x2L)

- Sub-surface and DCM – 0.7-2000  $\mu\text{m}$

Pre-filter on 2000 $\mu\text{m}$  2L of SW and pour on 25 mm GF/F filter. The exact final volume is reported (this is a quantitative measurement).

As it can be a long process, use 2L bottles with a cap adapted to fit a tube. Turn around the bottle to let the water flow in the filtration unit. Or complete the funnel with the test-tube writing down the volume. Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth.

- Sub-surface and DCM – 0.7-20  $\mu\text{m}$

Pre-filter on 20 $\mu\text{m}$  2L of SW and pour on 25 mm GF/F filter. The exact final volume is reported (this is a quantitative measurement).

As it can be a long process, use 2L bottles with a cap adapted to fit a tube. Turn around the bottle to let the water flow in the filtration unit. Or complete the funnel with the test-tube writing down the volume. Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth.

Sub-surface and DCM - 0.7-3  $\mu\text{m}$ :

Pre-filter on 3 $\mu\text{m}$  PC 47mm 2L of SW and pour on 25 mm GF/F filter. The exact final volume is reported (this is a quantitative measurement).

As it can be a long process, use 2L bottles with a cap adapted to fit a tube. Turn around the bottle to let the water flow in the filtration unit. Or complete the funnel with the test-tube writing down the volume. Turn on the vacuum pump (max 0.02 cm Hg). Make sure the filter never gets dry during the filtration process. At the end of the filtration, when the filter gets dry, fold the filter in 2 (color inside) and press it in clean absorbing paper to remove remaining water. Put it in a cryotube. Label the tube. 2 replicates/water depth.

**VII – Storage and shipping conditions**

Cryotubes have to be frozen in liquid nitrogen and subsequently transferred to -80°C. Ship on dry-ice.

**VIII – Chemicals and buffers preparation**

**IX – Disposal of trials and waste**

**X - Bibliography**

**XI - Annexes**

Analysis will be performed at Villefranche sur Mer by Hervé Claustre

## Nutrients

### I- Introduction

The sea water (SW) sampled will be used to measure a range of chemical parameters and particularly macronutrients (nitrate, nitrite, phosphate, silicate)

Part of the samples can be used for stable isotope measurements (Roscoff)

### II – Major Peculiarities

Use gloves to avoid contamination by products on hands and avoid contact with any kinds of material (e.g. Niskin spigot). Do not fill flask too much (volume increase when frozen)

Freeze bottle in upright position.

### III – Material and Instruments

VWR Scintillation Vials, Polyethylene, with Screw Cap VW58500 20 Tray Packed Vials, or similar 20 mL polyethylene scintillations vials with screw cap.

Or Polyethylen flasks 125 ml VWR 215-7506

### IV – Chemicals and buffers

None

### V – Material preparation

Mark the vials with BIOMARKS labeling code prior sampling.

### VI – Sampling and conditioning

Rinse the vials 3 times with the sample. Fill up the vial with approximately 80 mL.

Take 4 replicates from each depth.

### VII – Storage and shipping conditions

Store vials at -20°C in upright position to avoid liquid on the cap.

Ship on dry ice to the University of Oslo for analysis.

Delivery address: Tom Andersen  
Innkjøpskontoret, room 4422 (phone: 48272123)  
University of Oslo  
Department of Biology, Kristine Bonnevis hus  
Blindernveien 31, NO-0316 Oslo, NORWAY

### VIII – Chemicals and buffers preparation

### IX – Disposal of trials and waste

### X - Bibliography

John E. Dore, Terrence Houlihan, Dale V. Hebel, Georgia Tien, Luis Tupas and David M. Karl. 1996. Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. Marine Chemistry, Volume 53, Issues 3-4, August 1996, Pages 173-185.

### XI – Annexes

The nutrient analyses will be performed at University of Oslo, Department of Biology, by Tom Andersen lab using a Bran + Luebbe - AutoAnalyzer 3. For description of the system see:

[http://www.labochema.ee/documents/seadmed/seal\\_analytical/AutoAnalyzer%203%20brochure.pdf](http://www.labochema.ee/documents/seadmed/seal_analytical/AutoAnalyzer%203%20brochure.pdf)

# Pollutants

## **I- Introduction**

e.g. Aims of the Analysis

## **II – Major Peculiarities**

## **III – Material and Instruments**

## **IV – Chemicals and buffers**

## **V – Material preparation**

## **VI – Sampling and conditioning**

At least 2.5 liters for the water samples should be collected on glass that has been sterilized. Perrier bottles are fine.

## **VII – Storage and shipping conditions**

## **VIII – Chemicals and buffers preparation**

## **IX – Disposal of trials and waste**

Among other thing, please mention if it has to be recycled, treated.

## **X - Bibliography**

## **XI - Annexes**

PAH and pesticides (PCB and chlorated pesticides) in sediments	346 Euros
PAH and pesticides (PCB and chlorated pesticides) in sea water	337 Euros
DDT in sea water	24 Euros
DDT in sediments	24 Euros
IPC sea water (Cd, Co, Cu, Ni, Pb, Zn)	228 Euros
IPC sediments (21-30 metals)	158 Euros