

This document includes:

Biology/Pigments/Chlorophyll pigment concentrations in the water column

Biology/Pigments/Carotenoid pigment concentrations in the water column

Biogeochemical optical properties

at L4 (biogeochemical optical properties and pigments) and E1 (pigments).

Sampling collection

- 1) 10L carboys are provided in a large cool box (6 carboys in each)
- 2) Each carboy will clearly display the depth of the required water sample
- 3) Using a small amount of water from the required sample rinse each carboy twice.
- 4) Then fill each carboy slowly with the placing the tube provided into the bottom of the carboy and care should be taken not to agitate the sample any more than necessary.
- 5) Place all carboys in the cool box close lid and ideally keep the cool box in the shade. It is important that the water samples remain in the dark as cool as possible and brought to the lab as soon as possible for analysis.
- 6) Also there will be one or more brown glass bottles either in the large cool box or in a smaller one by themselves
- 7) Again the appropriate depths will be marked on the bottles.
- 8) For the surface bottle please collect using the stainless steel bucket a surface water sample.
- 9) Wearing the latex gloves provided submerge the bottle in the water filling with a small amount of water.
- 10) Swirl the water around inside the bottle and then empty.
- 11) Repeat steps 9 and 10 twice.
- 12) Then fill the bottle by slowly submerging into the water on its side.
- 13) When the sample required is at depth use the bottle rosette and rinse the bottle three times with the water sample
- 14) Then fill bottle by allowing the water to run down the inside of the bottle to reduce any agitation of the sample.
- 15) Again return bottle to cool box and store in a shaded area.

Calculations and analysis

1. Pigments

Upon arrival in the laboratory approximately 1-2 L of seawater is filtered onto a GF/F and stored in liquid nitrogen until analysis. Pigments were extracted from GF/F filtered phytoplankton into 2 mL acetone containing an internal standard apo-carotenoate (Sigma-Aldrich Company Ltd.) using an ultrasonic probe (30 S, 50 W) following the methods outlined in Llewellyn et al (2005). Extracts were centrifuged to remove filter and cell debris (5 min at 4000 r.p.m.) and analysed using reversed-phase HPLC (Hypersil 3 mm C8 MOS-2) with gradient elution, as described in Barlow *et al.* (1997), using Thermo-Separations instrumentation with photo-diode array spectroscopy (PDA) and Chrom-Quest software. Pigments were identified using retention time and spectral match using PDA (Jeffrey et al., 1997)), and pigment concentrations were calculated using response

factors generated from calibration using a suite of pigment standards (DHI Water and Environment, Denmark).

2. Particulate absorption

The absorption coefficients of total particulate and detrital material retained on GF/F filters were measured before and after pigment extraction using NaClO 1% active chloride from 350-750 nm at a 1 nm bandwidth using a dual beam Perkin Elmer spectrophotometer retro-fitted with a spectralon coated integrating sphere, following the methods of Tassan and Ferrari (1995). One replicates of each sample were filtered in the laboratory and preserved in liquid Nitrogen for analysis within 3 months after collection. The spectrophotometer was calibrated using Holium Oxide filters every year. Correction for pathlength amplification on the filters was carried out using the methods of Tassan and Ferrari (Tassan and Ferrari, 1998) on both the particulate and detrital fractions. Phytoplankton absorption coefficients were derived from the difference between total particulate and detrital fractions. Further details of the measurement protocol can be found in (Blondeau-Patissier et al., 2004) and Tilstone et al. (2004). Non algal pigment (NAP) slopes (SNAP) were calculated using an offset exponential fit from 350 to 550 nm as in Babin et al. (2003).

3. Coloured Dissolved Organic Material

Replicate seawater samples were filtered through 0.2 μm Whatman Nuclepore membrane filters using pre-ashed glassware. The first two 0.25 L of the filtered seawater were discarded. The absorption properties of the third sample were determined immediately on a Perkin-Elmer Lambda-2 spectrophotometer and a 10 cm quartz cuvette from 350 to 750 nm, relative to a bi-distilled MilliQ reference blank. $a_{\text{CDOM}}(\lambda)$ was calculated from the optical density and the cuvette path length and baseline offset was subtracted from a_{CDOM} . CDOM slopes (S_{CDOM}) were calculated using an offset exponential fit which corrects for water absorption effects >700 nm following the methods outlined in Babin et al. (2003).

4. Total Suspended Material

47 mm, 0.7 μm GF/F filters were pre-ashed at 450 °C and then pre-washed for 5 minutes in 0.5 L of MilliQ to remove friable fractions that can be dislodged during filtration. The filters were then dried in a hot air oven at 75 °C for one hour, pre-weighed and stored in a desiccator (Tilstone et al., 2004; Van der Linde, 1998). Seawater samples were filtered in triplicate onto the filters and then washed three times with 0.05 L MilliQ to remove residual salt. Blank filters were also washed with MilliQ. The filters were then dried at 75 °C for 24 hours and weighed on a Sartorius R-200D semi-microbalance (detection limit 10 μg).

Data stored

In WCO database – new data delivered every three months.