Pigments

Introduction

This document covers the pigment data held in file PIGMENT. A range of protocols was used as described below. Water samples were obtained for pigment measurements from CTD rosette bottles, the Go-Flo bottles used to collect the water for productivity experiments and from the surface pumped non-toxic supply. Many of the samples were taken for the calibration of fluorometers.

Size fractionated chlorophyll data are treated as component of the primary productivity data set and consequently are documented in the primary production documentation.

Chlorophyll and Phaeopigment

The most commonly used protocol was as follows. 100ml samples were filtered through 47mm Whatman GF/F filters which were then folded and placed in centrifuge tubes containing 10ml Analar grade acetone. These were refrigerated for a minimum of 18 hours and maximum of 72 hours to allow extraction of chlorophyll, then shaken and centrifuged at 5000 rpm for 5-8 minutes. The procedure was then repeated.

Samples were analysed using a Turner Designs type 125 bench-mounted fluorometer. Due to difficulties in adjusting the fluorometer to zero on each window setting, a window of high sensitivity was used. Consequently, it was necessary to dilute each of the samples in the cuvette so that a reading could be taken. The bench fluorometer was calibrated using a primary marine standard, which had been previously assayed by spectrophotometry.

Phaeopigments were determined by acidifying the sample and redetermining the fluorescence.

This procedure was undertaken on board ship in the majority of cases. However, on Discovery 190 and 192 the bench fluorometer on board was discovered to be faulty. Duplicate water samples had been filtered and the filters frozen. For these cruises, the frozen samples were extracted and analysed using the procedures described above back in the laboratory.

Pigments obtained following the protocols described above are stored as the parameters 'fluorometric chlorophyll' and 'fluorometric phaeopigment'. Pigments obtained from the protocols described in the remainder of this section are stored as the parameters 'spectrophotometric chlorophyll' and 'spectrophotometric phaeopigment'.

On Discovery 191, the above extraction protocol was followed. However, the problem with the Turner Designs was known and a visiting scientist from abroad made available a JASCO spectrofluorometer. Consequently, this was used to assay the samples from this cruise.

On cruises Discovery 183 and Charles Darwin 47 a number of samples, usually associated with a pre-dawn CTD cast, were analysed using the following protocol.

Up to 2 litres of water for each sample were filtered through glass fibre filters (GF/F) and frozen quickly on board ship. The samples were returned frozen to the laboratory where they were extracted with 90% acetone and assayed in a scanning spectrophotometer. The concentrations of chlorophyll and phaeopigments were calculated using the SCOR-UNESCO algorithms (Strickland and Parsons (1968)).

Pigment Determinations by HPLC

Water samples (0.5-2l) were filtered through 25 or 47 mm Whatman GF/F filters and frozen until analysed. The frozen filters were extracted in 2-5ml 90% acetone using sonification, and centrifuged to remove cellular debris.

A 300 µl aliquot of clarified extract was mixed with 300 µl of 1M ammonium acetate and 100 µl injected into a Shimadzu HPLC system (dual LC-6A pumps, SPD-6AV spectrophotometric detector, SCL-6B system controller) incorporating a 3-µm Pecosphere column (3.5x0.45 cm, Perkin-Elmer).

Pigments were separated by a modification of the reversed-phase method of Mantoura and Llewellyn (1983): solvent A consisted of 80% methanol and 20% 1M ammonium acetate and solvent B contained 60% methanol and 40% acetone.

A linear gradient from 0% B to 100% B for 10 min followed by an isocratic hold at 100% B for 7.5 minutes was used at a flow rate of 1 ml per minute.

Chlorophylls and carotenoids were detected by absorbance at 440 nm, and a detection of phaeopigments was performed with a Perkin-Elmer LS1 fluorescence detector using an excitation wavelength of 400 (±20) nm and emission at >600nm. Dual channel data collection and integration utilised the Philips PU6000 software running on a Dell PC.

Pigments were identified by comparison of retention times of various pigments isolated from well documented microalgal species in the Plymouth Culture Collection. Peak identity was further confirmed on selected samples by on-line diode array spectroscopy using a Waters 990 diode array spectrophotometric detector.

Quantification of pigments was based on peak areas and extinction coefficients update from those reported by Mantoura and Llewellyn (1983) using their extension of Beer's Law. Details of the extinction coefficients used are given in Barlow et al (1993). Chlorophylls a and b were calibrated using authentic standards (Sigma Chemical Company) in acetone and quantified spectrophotometrically.

References

Barlow, R.G., Mantoura, R.F.C., Gough, M.A. and Fileman, T.W. (1993). Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. Deep Sea Res. 40: 459-477.

Mantoura, R.F.C and Llewellyn, C.A. (1983). The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse phase high performance liquid chromatography. Anal. Chim. Acta 151: 297-314.

Strickland, J.D.H. and Parsons, T.R. (1968). A practical handbook of sea water analysis. Bull. Fish. Res. Bd. Can.: 167.