

Photosynthetic pigments as biomarkers of phytoplankton populations and processes involved in the transformation of particulate organic matter at the Biotrans site (47°N, 20°W)

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Abstract—Particulate samples were collected throughout the water column (0–4200 m) in June/July 1988 at the Biotrans site and their carotenoids and chlorophylls analysed by HPLC. These photosynthetic pigments were used as biomarkers to characterize the autotrophic populations, their utilization by heterotrophs and sedimentation of particles out of the euphotic zone. In the upper 50 m the pico- and nanophytoplankton accounted for 85% of the chlorophyll *a* biomass. The major pigment of the nanophytoplankton fraction was 19'-hexanoyloxyfucoxanthin (prymnesiophytes), whereas the main pigment in the microphytoplankton was peridinin (dinoflagellates). The peaks in the distributions of phaeophorbide *a* and nanophytoplankton pigments (19'-hexanoyloxyfucoxanthin, 19'-butanoyloxyfucoxanthin, chlorophyll *b*, lutein and/or zeaxanthin) coincided between 75 and 100 m, which pointed to an active grazing of nanophytoplankton by zooplankton. These pigments were detected in particles >20 µm from the Double Longhurst Hardy Plankton Recorder down to 1000 m, probably as a consequence of their incorporation into sedimenting faecal material. In contrast, the vertical distributions of phaeophorbide *a* and peridinin (microphytoplankton pigment) did not coincide, and this carotenoid was not detected below 400 m in particles >20 µm. A vertical profile (0–4200 m) shows, at 2300 m, the presence of nanophytoplanktonic material similar in its pigment pattern and composition to that of surface populations, suggesting fast sedimentation of Prymnesiophyte floc.

INTRODUCTION

CHLOROPHYLLS and carotenoids are powerful chemotaxonomic biomarkers (Foss *et al.*, 1984; GUILLARD *et al.*, 1985; GIESKES and KRAAY, 1986a, 1986b; WRIGHT and JEFFREY, 1987; GIESKES *et al.*, 1988; BJØRNLAND *et al.*, 1988; HOOKS *et al.*, 1988) as well as tracers of the processes involved in the transformation and degradation of phytoplankton populations (JEFFREY, 1974; REPETA and GAGOSIAN, 1982; BIDIGARE *et al.*, 1986; ROY, 1988; HENDRY *et al.*, 1987; NELSON, 1989). These compounds are particularly useful in studies of the fate and sedimentation of phytoplankton populations produced in the euphotic layers of the oceans. This is the case for most of the pico- and nanophytoplankton which are difficult to identify by microscopic studies, especially after they have been grazed or die and are partially degraded by bacteria. Therefore the characteristic molecules (e.g. pigments) in these small size fractions become, for a period of time as they degrade, an efficient means of tracing these particles in the water column.

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Reversed-Phase High-Performance Liquid Chromatography (HPLC) for the analysis of chlorophylls and carotenoids (MANTOURA and LLEWELLYN, 1983; WRIGHT and SHEARER, 1984; ROY, 1987; ZAPATA *et al.*, 1987; GIESKES *et al.*, 1988) makes it possible to identify rapidly and quantify precisely the majority of phytoplankton fat-soluble pigments and their derivatives. Therefore these pigments can be used to characterize the origin and fate of the particulate matter involved in marine biogeochemical cycles.

The Biotrans site is an oligotrophic area in the northeast Atlantic Ocean and a reference site for Joint Global Ocean Flux Studies (JGOFS). An attempt was made to characterize the particulate organic matter at this site and its fate, using photosynthetic pigments as biomarkers.

MATERIALS AND METHODS

The Biotrans site (47°N, 20°W) was occupied from 21 June to 12 July 1988 on board R.R.S. *Discovery*, Cruise 175. The depth of the site varied between 4000 and 4500 m. Samples were collected with a rosette of "Go-Flo" water bottles, 30-l Niskin water bottles and the Double Longhurst Hardy Plankton Recorder (DLHPR) (WILLIAMS *et al.*, 1983) equipped with 20 and 200 μm mesh nets. For this investigation only samples from the 20 μm mesh of the DLHPR were used and were processed immediately on recovery of the sampler. Samples were washed off the gauze and collected on 47 mm GF/C glass fibre filters. Water samples were pre-filtered through 200 μm mesh nylon gauze and collected on 47 mm GF/F filters. A serial filtration of particulates, from water bottle samples, was carried out using 50 and 20 μm nylon gauze (particulates were reconcentrated onto GF/F filters), 5 and 1 μm Millipore and 0.2 μm polycarbonate Nuclepore filters with a Millipore filter stack and hand vacuum pump. All filters collected were stored immediately at -20°C on board ship awaiting analysis, which was carried out within one month of sampling. The analyses were performed on the whole filter or, for fibre filters, punched sub-samples, the assumption being that the particles were evenly distributed on the filter surface. This has been verified from estimation of particulate carbon and nitrogen from punches from filters (WILLIAMS, unpublished work).

All steps of the pigment extraction and analysis were performed under dim light conditions, the samples being maintained in ice. The filters (either polycarbonate or glass fibre) were ground and sonicated in 90% acetone. The extraction volume was adjusted to the type of filter, ranging from 5 ml (47 mm GF/F) to 500 μl (punches of 13 mm diameter corresponding to at least 20 l of seawater filtered by the $>20 \mu\text{m}$ net).

The HPLC system consisted of a LDC constametric pump SM 4000, a LKB spectromonitor (detection at 440 nm) and a LDC fluoromonitor (excitation 400–500 nm, emission 500–700 nm). The column (10 \times 4.6 cm) was filled up with 3 μm C18 Hypersil ODS (Manufacturer: Société Française de Chromato Colonne). The solvent system has been described previously by KNIGHT and MANTOURA (1985). Flow rate was 1.5 ml min⁻¹ and the solvent programme was as follows: start from 100% A (80:10:10; methanol: water: ion-pairing reagent) to 100% B (60:40; methanol: acetone) in 5 min and hold in 100% B for a further 8 min. Pigments were identified by comparison of their retention times with those of pigments of species with well documented composition (*Synechococcus elongatus*, *Phaeodactylum tricornutum*, *Amphidinium carterae*, *Gymnodinium galatheanum*, *Dunaliella tertiolecta*) (F. MANTOURA and C. LLEWELLYN, personal communication). 19'-Hexanoyloxyfucoxanthin (19'-HF), 19'-butanoyloxyfucoxanthin

(19'-BF) and fucoxanthin were identified further by their UV-VIS properties recorded with a Hewlett Packard Diode Array Detector. Carotenoids were quantified on the basis of spectrophotometric detection according to the peak area (registered by a Nelson Analytical system software on a PC compatible) and to those of published extinction coefficients (MANTOURA and LEWELLYN, 1983). Chloropigments were quantified using a fluorometer previously calibrated by injection of known amounts of chloropigments of mussel faeces.

Total particulate volume and particle diameters (1.6–128.0 μm) were measured from water samples taken at each depth using a TA II multi-channel Coulter Counter.

RESULTS AND DISCUSSION

Figure 1 illustrates the distribution of chloropigments and carotenoids at 16 m (0.6 mg m^{-3}) within the five size categories (0.2–1, 1–5, 5–20, 20–50 and 50–200 μm) and Table 1 summarizes the chemotaxonomic relationships of the identified pigments. Pico- and nanophytoplankton contributed to 85% of the chlorophyll *a* biomass and all of the chlorophyll *b* (green algae) (Fig. 1A). Picoplankton was defined in this study as $<1 \mu\text{m}$ and nanoplankton as 1–20 μm . Microphytoplankton ($>20 \mu\text{m}$) was responsible for the presence of 35% of the detected chlorophyll *c* (brown algae). However, because the 0.2 μm polycarbonate Nuclepore filter had a contaminant which co-eluted with chlorophyll *c*, the contribution of picophytoplankton to chlorophyll *c* cannot be estimated. The 19'-HF (Prymnesiophyte) was the main carotenoid at the Biotrans site. The pico- and the nanophytoplankton contributed to 12 and 87% of the presence of this pigment, respectively. The same applies for the 19'-BF (Chrysophytes) which was five times less abundant

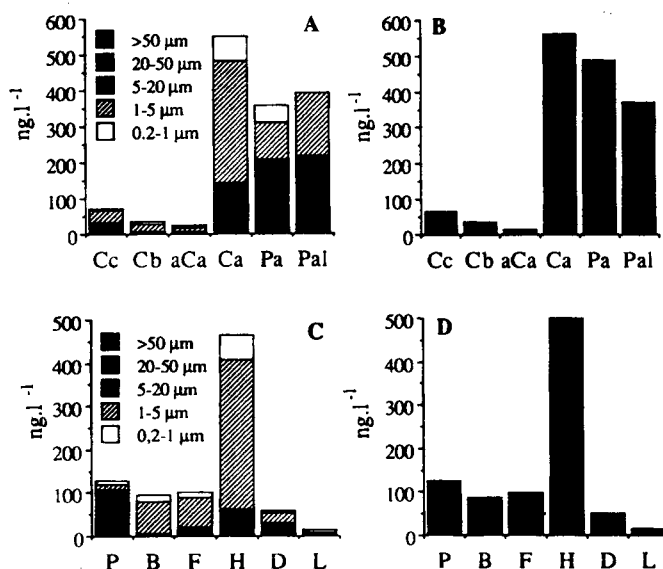


Fig. 1. Pigment composition of water samples collected from 16 m, 5 July 1988, 20.40 h (GMT) at $47^{\circ}17'N$, $19^{\circ}16'W$. Chloropigments (A) and carotenoids (C) in five size categories and chloropigments (B) and carotenoids (D) in 200 μm pre-filtered water samples. Abbreviations: Cc: chlorophyll *c*; Cb: chlorophyll *b*; aCa: chlorophyll *a* allomer; Ca: chlorophyll *a*; Pa: phaeophorbide *a*; Pal: phaeophorbide *a*-like; P: peridinin; B: 19'-butanoyloxyfucoxanthin (19'-BF in the text); F: fucoxanthin; H: 19'-hexanoyloxyfucoxanthin (19'-HF in the text); D: diadinoxanthin; L: lutein and/or zeaxanthin.

Table 1. Chemotaxonomic relationships of some pigments detected at the Biotrans site

Pigment	Organisms	References
19'-Hexanoyloxyfucoxanthin	Prymnesiophytes Chrysophytes*	1, 2, 3, 4
19'-Butanoyloxyfucoxanthin	Chrysophytes Prymnesiophytes†	3, 4, 5, 6, 7
Fucoxanthin	Diatoms Prymnesiophytes Chrysophytes	3, 4, 5, 6, 7, 8
Peridinin	Dinoflagelletes	9
Zeaxanthin	Cyanobacteria	10, 11
Lutein	Green algae	12
Chlorophyll <i>b</i>		

(1) ARPIN *et al.*, 1976; (2) GIESKES and KRAAY, 1986b; (3) WRIGHT and JEFFREY, 1987; (4) BJØRNLAND *et al.*, 1988; (5) HOOKS *et al.*, 1988; (6) LIAAEN-JENSEN, 1985; (7) JEFFREY, 1980; (8) BJØRNLAND *et al.*, 1989; (9) JEFFREY, 1974; (10) GIESKES and KRAAY, 1983; (11) GUILLARD *et al.*, 1985; (12) JEFFREY, 1976.

*As trace only.

†Only described in *Phaeocystis pouchetii*.

than the 19'-HF. Picophytoplankton contributed to 40% of the presence of zeaxanthin/lutein (both pigments co-elute with this method), and this was mainly due to cyanobacteria (zeaxanthin). Fucoxanthin is primarily associated with nanophytoplankton (most likely Chrysophytes and/or Prymnesiophytes), but microphytoplankton (most likely diatoms) contributed to 10% of this pigment. Peridinin is a typical microphytoplankton pigment (70% was due to microphytoplankton) and was the second most abundant carotenoid (Fig. 1C). Diadinoxanthin is present in diatoms, dinoflagellates, Prymnesiophytes and Chrysophytes and is not specific to any particular size category in this study.

In general, the fractionation procedure did not affect the recovery of the pigments (global comparison of Figs 1A–D) except for phaeophorbide *a*. This pigment is the main degradation product of chlorophyll *a* following herbivore grazing (SHUMAN and LORENZEN, 1975). Another phaeophorbide *a* type was observed in this sample (Phaeophorbide *a*-like, Fig. 1B). This pigment (1–5 and 20–50 μm , Fig. 1A) was less polar than the phaeophorbide *a* which was not associated with the same size categories (0.2–20 μm). Many phaeopigment derivatives have been reported in the literature (GIESKES and KRAAY, 1986b, KLEIN and SOURNIA, 1987; NELSON, 1989; CLAUSTRE *et al.*, submitted), but the significance of these observations are still not fully established. Further investigations should be carried out to establish if these phaeopigments can be used as tracers of the different grazing processes responsible for their formation.

A detailed DLHPR profile of selected pigments within the upper 250 m is shown in Fig. 2. The chlorophyll *a* maximum in the size range (20–200 μm) was located at 103 m (16.2 ng l^{-1}). No phaeophorbide *a*-like pigments were observed in the DLHPR profile but phaeophytin *a* was present. Both phaeophytin *a* and phaeophorbide *a* showed similar distributions with chlorophyll *a*. Distinct patterns in the distributions of other pigments

were observed, their maxima occurring at: peridinin (39 m), 19'-HF and chlorophyll *b* (89 m), 19'-BF (100 m) and fucoxanthin (116 m). All pigments were detected in this size range (20–200 μm) down to 1000 m (Fig. 3) except for peridinin, which was not detected below 400 m (Fig. 3B).

Because 19'-HF, 19'-BF and chlorophyll *b* are typical pigments of nanophytoplankton, the coincidence of their distributions with phaeophorbide *a* (Fig. 2B–D) suggests that zooplankton grazed on Prymnesiophytes, Chrysophytes and green algae at this site. Therefore, the characteristic pigments of the phytoplankton groups detected at 1000 m were probably the result of heterotrophic activity producing faecal material that sedimented. Moreover, the pigment maximum observed in this particle size range (20–200 μm) around 100 m could result, in part, from an accumulation of zooplankton faecal pellets. The concentration of chlorophyll *a* at 21 m in this size range from the DLHPR was 4 ng l^{-1} , which was a tenth of the value measured in the water samples at 16 m. This illustrates the large quantitative variability which occurred at this site over the study period. However, the pigment pattern was similar throughout the sampling period at this location (WILLIAMS and CLAUSTRE, unpublished data). We can therefore assume that the relative proportions of the phytoplankton population remained constant at the Biotrans site. Despite the quantitative variability of the phytoplankton biomass over the cruise period, some qualitative conclusions concerning the fate of these phytoplankton populations can be drawn. In the size range which provides potential food for the grazers (seawater pre-filtered through 200 μm), the ratio chlorophyll *b* : 19'-BF : 19'-HF was 0.07 : 0.21 : 1 (Fig. 1B, D) while in the DLHPR profile (size range 20–200 μm) at 100 m it was 0.35 : 0.96 : 1 (Fig. 2B, D). This observation suggests either that chlorophyll *b* and 19'-BF are less degraded by grazing activity than 19'-HF or that green algae and Chrysophytes are preferred to Prymnesiophytes by grazers. As a consequence, there is an increasing contribution of chlorophyll *b* with depth compared to chlorophyll *a* (Fig. 3C). However, the ratio 19'-BF : 19'-HF did not show any particular relationship with depth.

Fucoxanthin was the most abundant pigment after peridinin in the DLHPR $> 20 \mu\text{m}$ samples. The coincidence of the distribution of fucoxanthin and phaeophorbide *a* near

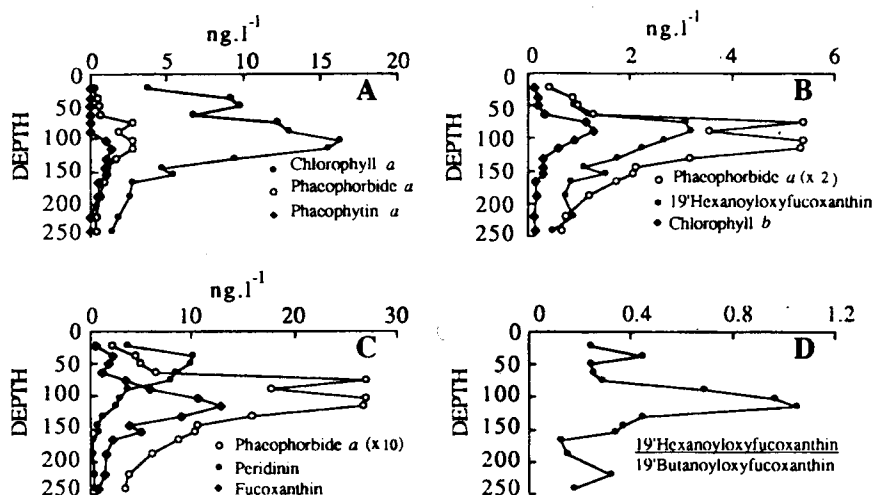


Fig. 2. Pigment distribution, in the size range 20–200 μm , 0–250 m, taken from a DLHPR haul 11.38–14.36 h (GMT) 24 June, 47°15'N, 19°30'W to 47°20'N, 19°17'W at the Biotrans site.

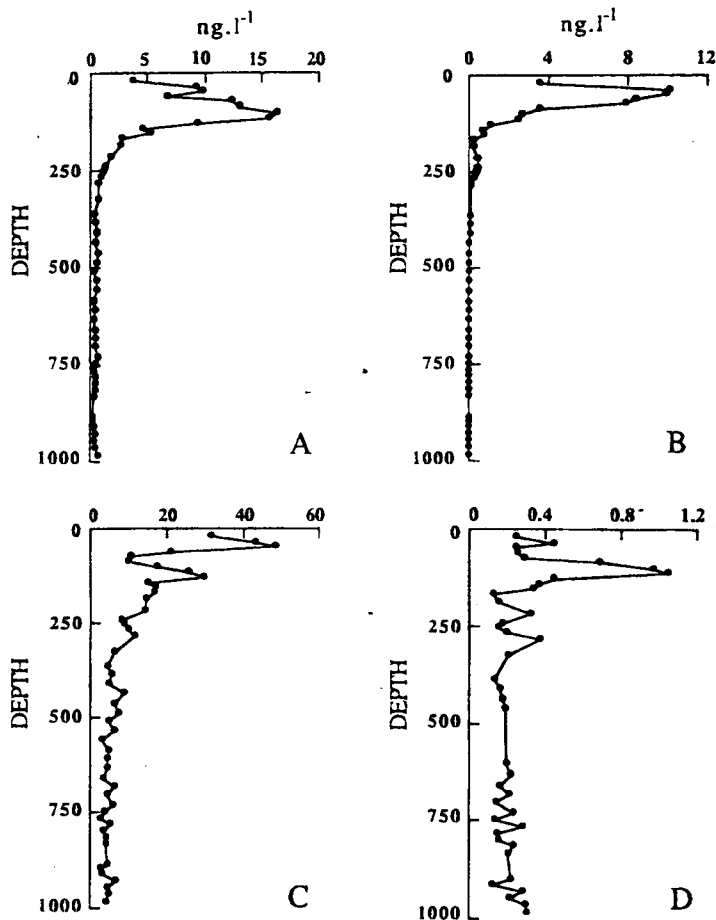


Fig. 3. Pigment distribution in the size range 20–200 μm , 0–1000 m (as in Fig. 2). (A) Chlorophyll *a*; (B) peridinin; (C) chlorophyll *a* : chlorophyll *b* ratio; (D) 19'-BF : 19'-HF ratio.

116 m could be due either to the presence of nanoflagellates (Prymnesiophytes or Chrysophytes whose main carotenoid would be fucoxanthin), diatoms or faecal pellets of fucoxanthin-containing algae (diatom and/or nanoflagellates). Microscopic elucidation may have been helpful in resolving these different assumptions but, due to the size category considered ($>20 \mu\text{m}$), the first assumption can be discarded.

The discrepancy between peridinin and phaeophorbide *a* (Fig. 2C) provides evidence that zooplankton did not graze on dinoflagellates at this site. Peridinin was the second main carotenoid in the upper layers (Fig. 1D) and was not detected below 400 m in the DLHPR sampling (Fig. 3B). It can therefore be assumed that dinoflagellates were not consumed by grazers and incorporated into fast sedimenting faecal pellets. Dinoflagellates sink slowly in comparison to faecal pellets so that their characteristic pigment, peridinin, which is quite labile (R. DAWSON, personal communication), degrades before the dinoflagellates reach deep water. The pigment distribution observed with the DLHPR in the water column reflects, at a given depth, a specific story for each pigment and therefore for each phytoplankton group it traces.

A profile of chlorophyll *a* taken from water samples collected by bottles between the surface and 4200 m is shown in Fig. 4A. Chlorophyll *a* was not detected below 200 m except in the sample at 2300 m where significant amounts were recorded (20 ng l^{-1}). There was

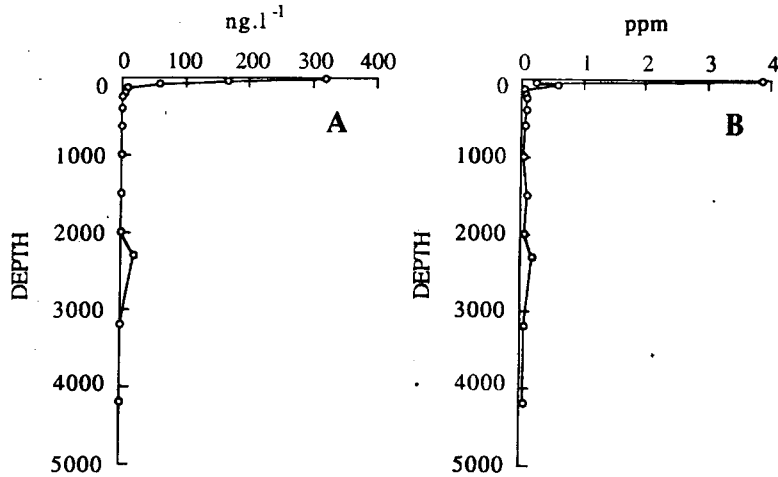


Fig. 4. Chlorophyll *a* (A) in the size range 1.0–200 μm from water bottle samples 10–4200 m. [10–2008 m, 21/22 June 21.49–02.23 h (GMT), 47°13'N, 19°34'W; 2300–4200 m, 27 June, 02.34–06.20 h (GMT), 47°16'N, 19°07'W] filtered through GF/F filters and total particulate volume (B).

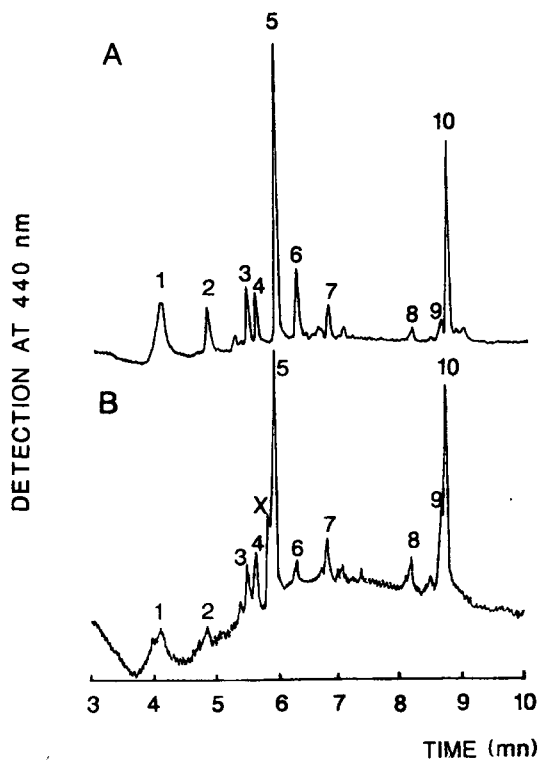


Fig. 5. Absorbance chromatograms of pigments from seawater samples from 10 m (A) and 2300 m (B). Dates and locations as for Fig. 4. 1: chlorophyll *c*; 2: peridinin; 3: 19'-butanoyloxyfucoxanthin (19'-BF in the text); 4: fucoxanthin; 5: 19'-hexanoyloxyfucoxanthin (19'-HF in the text); 6: diadinoxanthin; 7: lutein and/or zeaxanthin; 8: chlorophyll *b*; 9: chlorophyll *a* allomer; 10: chlorophyll *a*; 11: unknown carotenoid.

also an increase in the particulate volume at this depth (Fig. 4B). All surface pigments were detected in the 2300 m sample (Fig. 5). The pigment pattern at 2300 m was similar to that of surface samples and was characterized by the absence of phaeophorbide *a* (for a similar concentration of chlorophyll *a*, a significant amount of phaeophorbide was detected in DLHPR samples, Fig. 2A). These observations suggest a rapid transfer of primary products from the surface to the bottom through an efficient transport mechanism. The absence of phaeopigments in the 2300 m indicate the possibility of a transport mechanism other than faecal pellets. Recent evidence has shown that many types of algae aggregate and sink rapidly (BILLET *et al.*, 1983; TAKAHASHI, 1986; ALLDREDGE and GOTSCHALK, 1989). This is particularly true for Prymnesiophytes, which produce gelatinous colonial stages and consequently sink rapidly (see review in ALLDREDGE and SILVER, 1988). The pigment pattern at 2300 m is typical of Prymnesiophytes, similar to the surface pigment picture and is quite undegraded. This may suggest the rapid sedimentation of aggregated Prymnesiophytes in the form of floc following a surface bloom.

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REFERENCES

- ALLDREDGE A. L. and M. W. SILVER (1988) Characteristics, dynamics and significance of marine snow. *Progress in Oceanography*, **20**, 41–82.
- ALLDREDGE A. L. and C. C. GOTSCHALK (1989) Direct observation of the mass flocculation of diatom blooms: characteristics, settling velocities and formation of diatom aggregates. *Deep-Sea Research*, **36**, 159–171.
- ARPIN N., W. A. SVEC and S. LIAAEN-JENSEN (1976) New fucoxanthin-related carotenoids from *Coccolithus huxleyi*. *Phytochemistry*, **15**, 529–532.
- BIDIGARE R. R., J. T. FRANK, C. ZASTROW and J. M. BROOKS (1986) The distribution of algal chlorophylls and their degradation products in the southern ocean. *Deep-Sea Research*, **33**, 923–937.
- BILLET D. S. M., R. S. LAMPITT, A. L. RICE and R. F. C. MANTOURA (1983) Seasonal sedimentation of phytoplankton to the deep sea benthos. *Nature*, **302**, 520–522.
- BJØRNLAND T., R. R. L. GUILLARD and S. LIAAEN-JENSEN (1988) *Phaeocystis* sp. clone 667-3: a tropical marine planktonic Prymnesiophyte with fucoxanthin and 19-acyloxyfucoxanthins as chemosystematic carotenoid markers. *Biochemical Systematics and Ecology*, **16**, 445–452.
- BJØRNLAND T., S. LIAAEN-JENSEN and J. THRONDSSEN (1989) Carotenoids of the marine chrysophyte *Pelagococcus subviridis*. *Phytochemistry*, **28**, 3347–3353.
- CLAUSTRE H., S. A. POULET, R. WILLIAMS, F. BEN MLIH, A. M. HAPETTE, V. MARTIN-JÉZÈQUEL and J. C. MARTY (submitted) Relation between the qualitative nature of the primary production and its fate in the Irish Sea. *Marine Chemistry*.
- FOSS P., R. R. L. GUILLARD and S. LIAAEN-JENSEN (1984) Prasinolaxanthin—a chemosystematic marker for algae. *Phytochemistry*, **23**, 1629–1633.
- GIESKES W. W. C. and G. W. KRAAY (1983) Unknown chlorophyll *a* derivatives in the North Sea and tropical Atlantic Ocean revealed by HPLC analysis. *Limnology and Oceanography*, **28**, 757–766.
- GIESKES W. W. C. and G. W. KRAAY (1986a) Floristic and physiological differences between the shallow and the deep nanophytoplankton community in the euphotic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. *Marine Biology*, **91**, 567–576.
- GIESKES W. W. C. and G. W. KRAAY (1986b) Analysis of phytoplankton by HPLC before, during and after mass occurrence of the microflagellate *Corymbellus aureus* during the spring bloom in the open northern North Sea in 1983. *Marine Biology*, **92**, 45–52.
- GIESKES W. W. C., G. W. KRAAY, A. NONTJI, D. SETIAPERMANA and SUTOMO (1988) Monsoonal alternation of a mixed and a layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigment fingerprints. *Netherlands Journal of Sea Research*, **22**, 123–137.

- GUILLARD R. R. L., L. S. MURPHY, P. FOSS and S. LIAAEN-JENSEN (1985) *Synechococcus* spp. as likely zeaxanthin-dominant ultraphytoplankton in the North Atlantic. *Limnology and Oceanography*, **30**, 412–414.
- HENDRY G. A. F., J. D. HOUGHTON and S. B. BROWN (1987) The degradation of chlorophyll—a biological enigma. *New Phytologist*, **107**, 255–302.
- HOOKS C. E., R. R. BIDIGARE, M. D. KELLER and R. R. L. GUILLARD (1988) Coccoid eukaryotic marine ultraplankters with four different HPLC pigment signatures. *Journal of Phycology*, **24**, 571–580.
- JEFFREY S. W. (1974) Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. *Marine Biology*, **26**, 101–110.
- JEFFREY S. W. (1976) A report of green algae pigments in the central North Pacific Ocean. *Marine Biology*, **37**, 33–37.
- JEFFREY S. W. (1980) Algal pigment system. In: *Primary productivity in the sea*, P. FALKOWSKI, editor, Plenum Press, New York, pp. 33–58.
- KLEIN B. and A. SOURNIA (1987) A daily study of the diatom spring bloom at Roscoff (France) in 1985. II. Phytoplankton pigments composition studied by HPLC analysis. *Marine Ecology Progress Series*, **37**, 265–275.
- KNIGHT R. and R. F. C. MANTOURA (1985) Chlorophyll and carotenoid pigments in foraminifera and their symbiotic algae: analysis by high performance liquid chromatography. *Marine Ecology Progress Series*, **23**, 241–249.
- LIAAEN-JENSEN S. (1985) Carotenoids of lower plants—recent progress. *Pure and Applied Chemistry*, **57**, 649–658.
- MANTOURA R. F. C. and C. A. LLEWELLYN (1983) The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Analytica Chimica Acta*, **151**, 297–314.
- NELSON J. R. (1989) Phytoplankton pigments in macrozooplankton faeces: variability in carotenoid alterations. *Marine Ecology Progress Series*, **52**, 129–144.
- REPETA D. J. and R. B. GAGOSIAN (1982) Carotenoid transformations in coastal marine waters. *Nature*, **295**, 51–54.
- ROY S. (1987) High-performance liquid chromatography analysis of chloropigments. *Journal of Chromatography*, **391**, 19–34.
- ROY S. (1988) Effects of changes in physiological condition on HPLC-defined chloropigment composition of *Phaeodactylum tricornutum* (Bohlin) in batch and turbidostat cultures. *Journal of Experimental Marine Biology and Ecology*, **118**, 137–149.
- SHUMAN F. R. and C. J. LORENZEN (1975) Quantitative degradation of chlorophyll by a marine herbivore. *Limnology and Oceanography*, **20**, 580–586.
- TAKAHASHI K. (1986) Seasonal fluxes of pelagic diatoms in the subarctic Pacific, 1982–1983. *Deep-Sea Research*, **33**, 1225–1251.
- WILLIAMS R., N. R. COLLINS and D. V. P. CONWAY (1983) The double LHPR system, a high speed micro- and macrosampler. *Deep-Sea Research*, **30**, 331–342.
- WRIGHT S. W. and S. W. JEFFREY (1987) Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. *Marine Ecology Progress Series*, **38**, 259–266.
- WRIGHT S. W. and J. D. SHEARER (1984) Rapid extraction and high-performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. *Journal of Chromatography*, **294**, 281–285.
- ZAPATA M., A. M. AYLA, J. M. FRANCO and J. L. GARRIDO (1987) Separation of chlorophylls and their degradation products in marine phytoplankton by Reversed-Phase High-Performance Liquid Chromatography. *Chromatographia*, **23**, 26–30.