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Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments

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Abstract

Microscopic observations of Lugol-preserved samples collected near a subsurface drogue during the spring bloom of 1981 in the central North Sea suggested that the phytoplankton crop consisted mainly of diatoms. However, the relative abundance of alloxanthin among the carotenoids measured by reversed-phase, high-performance liquid chromatography indicated that in most samples Cryptophyceae were at least as abundant. On the basis of a multiple regression analysis of pigment concentrations to obtain pigment ratios, the contribution of Cryptophycean chlorophyll to total chlorophyll was calculated. The Cryptophyceae:diatom ratio appeared to be variable during the period of observations, ranging between 0 at the beginning to 1.0 ten days later. It is recommended that the classical method of counting phytoplankton for crop estimates be supplemented by chemotaxonomical studies with modern quantitative chromatographic methods such as HPLC for the measurement of algal pigments.

Introduction

It is a widely accepted concept that in the open North Sea the spring bloom of phytoplankton consists mainly of Bacillariophyceae, i.e. diatoms (e.g. Hardy, 1959). Brief outbursts of *Phaeocystis* (Prymnesiophyceae) are apparently considered to be of no more than local importance. However, several years ago Reid (1975, 1977) convincingly refuted this notion of diatom dominance during the spring in the North Sea. On the basis of an analysis of samples collected during many years in the framework of the Continuous Plankton Recorder Survey, Reid (1977) showed that, in the central and northern North Sea, algal species not classifiable either as diatoms or as *Phaeocystis* contributed most to the green colour of the Recorder silks during the first half of the growing season. He was not able to identify any of the species but he suggested that they must have been "microflagellates".

The reason why the importance of this nanoplankton was not appreciated earlier by microscopists is that small cells are not recognized easily in natural samples containing other small particles and particle aggregates. Moreover, the structure of delicate algal cells is often altered beyond recognition due to sample treatment and preservation in Lugol's solution, formaldehyde, glutar aldehyde, and similar fixatives. In the present paper we report that, during the 1981 spring bloom in the central North Sea, Cryptophyceae became dominant in the plankton. Their abundance was not detected by microscopic observation but only after measurement of the concentration of characteristic carotenoid pigments.

Chromatography has been used earlier as a chemotaxonomical supplement to the classical but more subjective method of microscopy for the analysis of the composition of natural phytoplankton populations. Several years ago Jeffrey (1976), observing a relatively large amount of chlorophyll *b* in open ocean samples after thin-layer chromatographic analysis, discovered therewith the abundance of "green algae" in the ocean (Euglenophyceae, Prasinophyceae and Chlorophyceae typically contain chlorophyll *b*). The high-performance (or high-pressure) liquid chromatographic method (HPLC; Robinson, 1979) that we used proved to be highly successful not only as a chemotaxonomical tool but also as a means of quantifying the relative importance of different algal groups in natural phytoplankton populations.

Material and methods

Between 30 April and 10 May, 1981, samples were taken in the central North Sea from R.V. "Tyro" near a buoy attached to a subsurface drogue at 14-m depth. This drifting system was launched on 30 April at Lat. 54°30′N, Long. 4°30′E. Samples were taken with a rosette sampler

with 30-l bottles at depths selected in accordance with the shape of VarioSens profiles (showing *in-situ* chlorophyll fluorescence) recorded daily at noon. Properties of the VarioSens fluorometer are discussed in detail by Herman and Denman (1977).

Phytoplankton cells were counted, and the volumes of the cells estimated, within two months after the end of the cruise in Lugol-preserved samples, using a Zeiss inverted microscope. Cell volumes were converted to carbon with formulae based on studies of Hagmeier (1961) and conversions of Helgoland plankton data 1962–1975 (Hagmeier, unpublished data): for flagellates, C (picograms) = 0.075 Volume (μm^3); for diatoms, log C (picograms) = $-0.864 + 0.857$ log Volume (μm^3).

For the chromatographic analysis of chlorophylls and carotenoids, 10 l of seawater were filtered through Whatman GF/C filters. The filters were stored at −30 °C. All samples were analysed within 2 months after the end of the cruise. The high-performance liquid chromatography was reversed-phase. Filters were extracted in 10 or 20 ml 90% acetone; 100 μl of the pigment extracts (obtained after 30 s shaking in a Braun-Melsungen CO_2-cooled Homogeniser followed by centrifugation) was directly injected in the HPLC system with an Injector U6K of Waters Associates. The column, 30 cm long with an inner diameter of 3.9 mm, was packed with Lichrosorp 10RP18. Two solvents were used: no. I (30% water in methanol) and no. II (20% ethylacetate in methanol). These solvents were pumped through the column in a linear gradient: from 25% of solvent II to 95% of solvent II in 40 min, 1.5 ml min^{-1}, programmed with a Waters Associates gradient mixer (Model 660 Solvent Programmer).

Detection was performed with an Absorbance Detector (Waters model 440) at 436 nm, and with a Perkin-Elmer 2000 fluorescence spectrophotometer set at an excitation wavelength of 436 nm and an emission wavelength of 660 nm; the detectors were attached to an Omniscribe Recorder (Houston Instruments). Peak areas were automatically integrated with a Hewlett-Packard 3390 A Integrator.

Pigments were identified after evaporation of the solvent containing an eluted pigment under vacuum and redissolution in ethanol (fucoxanthin, diadinoxanthin, diatoxanthin, peridinin, alloxanthin), methanol (chlorophyll *c*), or acetone (chlorophyll *a*). An absorption spectrum was then obtained with a Perkin-Elmer Hitachi spectrophotometer. The spectra (Fig. 1) and the retention times of the various pigments were compared with absorption and chromatographic characteristics of pigments isolated from *Phaeodactylum* sp., *Dunaliella* sp., *Isochrysis* sp. and *Rhodomonas* sp. cultures, from a culture of a benthic diatom (*Navicula* sp.), and from natural phytoplankton. Confirmation of the identification of carotenoids was obtained by comparison with published maxima of absorption spectra (Foppen, 1971).

Calibration curves were made after isolating a pigment and diluting it in acetone (90%) to a series of different concentrations before injecting it into the system and recording the resulting peak areas. Concentrations in the dilution series were quantified after measuring the maximum absorption in the solution and calculating the concentration with Lamber-Beer's law, using extinction coefficients listed by Foppen (1971) and Abaychi and Riley (1979). The coefficient for alloxanthin was assumed to be close to that of zeaxanthin and diatoxanthin, which it resembles (Haxo and Fork, 1959; Allen *et al.*, 1964).

Results

The "pigment fingerprint" of the samples was rather simple: besides a number of minor peaks in the chromatograms indicating the presence of small amounts of diadinoxanthin and peridinin, and a number of chlorophyll

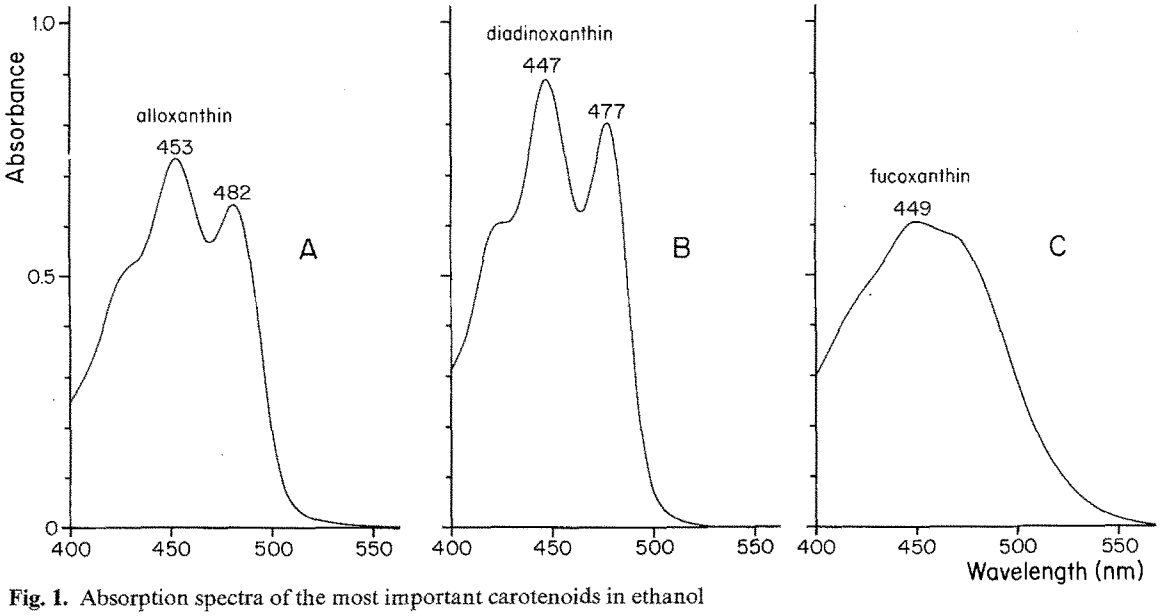

Fig. 1. Absorption spectra of the most important carotenoids in ethanol

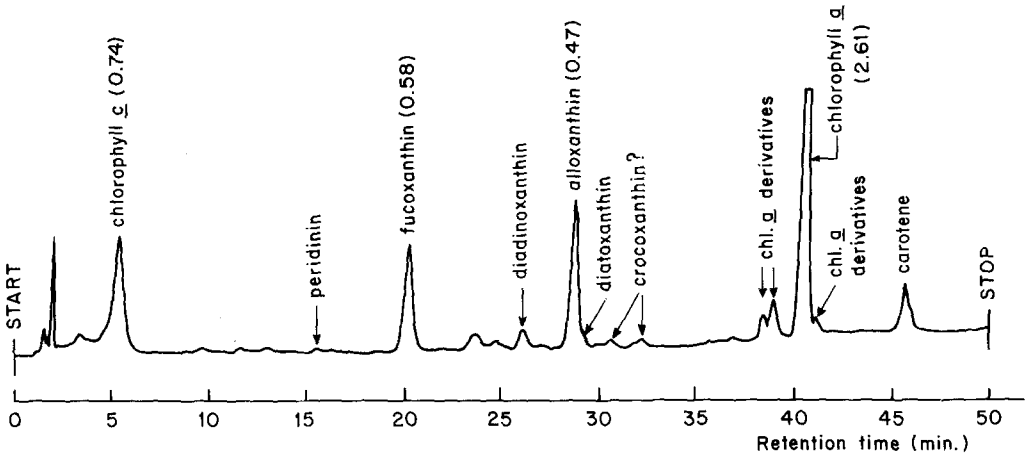

Fig. 2. Chromatogram of a sample taken on 5 May 1981, 19.30 hrs, at a depth of 40 m. Concentrations in μg per liter seawater. Detection at 436 nm (absorbance)

a derivatives, only five major pigments were found: carotene, chlorophylls *a* and *c*, and the carotenoids fucoxanthin and alloxanthin (Fig. 2). It is of interest to note that with a conventional "acidification" method for the measurement of chlorophyll *a* (Holm-Hansen *et al.*, 1965), the concentration was 30% higher than the chlorophyll *a* concentration measured after chromatographic separation. This discrepancy may be due to the presence of the chlorophyll *a* derivatives (Fig. 2), compounds that are measured as "chlorophyll *a*" with conventional methods (cf. Jensen and Sakshaug, 1973). These derivatives were not the result of sample treatment: tests have shown that they were never present in healthy cultures of algae or in fresh leaf fragments (Gieskes and Kraay, 1983).

The "pigment fingerprint" was variable from sample to sample due to short-term concentration changes of all pigments, especially between 3 and 7 May. In Fig. 3 the concentrations of fucoxanthin and alloxanthin in surface samples and in samples taken near the bottom are presented; chlorophyll *a* concentrations are given for three sampling depths, chlorophyll *c* for two depths. In general, the lowest chlorophyll *a* concentrations were recorded at mid-depth. Near the surface, chlorophyll *a* concentrations tended to be highest towards the end of the day (cf. Baars and Oosterhuis, 1982). The pattern of changes of chlorophyll *a* concentration appeared to be different at each depth (Fig. 3).

The number of algal cells varied from day to day (Table 1); coincident with the maximum in chlorophyll *a* there was a tendency towards the highest algal cell numbers between 3 and 7 May. Most of the cells that were counted in the Lugol-preserved samples belonged to the diatoms; dinoflagellate numbers (*Ceratium* spp., *Gymnodinium* spp., *Dinophysis* spp., *Peridinium* spp.) were low (Table 1).

The cell numbers, summarized in Table 1, have been converted (via cell volumes, see "Material and methods") to phytoplankton carbon. M. A. Baars (personal communication) has pointed out that Hagmeier's formulae give lower carbon values than estimates based on formulae for conversion of cell volume to carbon presented by Mullin *et al.* (1966) and Strathmann (1967), the discrepancy being greatest when small cells dominate. However, since most of the volume of cells that we registered by

Table 1. Numbers (per liter seawater) of cells of species contributing more than 90% of total volume of algal cells counted in lugol-preserved samples. Phytoplankton carbon of samples (estimates based on these cell counts) in μg per liter

Surface samples 0–10 m	30 April	1 May	2 May	3 May	4 May	5 May	6 May	8 May	9 May	10 May
Rhizosolenia setigera	8 000	3 000	1 800	7 000	2 200	2 000	1 800	1 500	650	300
Rhizosolenia alata	–	–	250	300	250	400	400	480	650	300
Chaetoceros spp.	4 000	1 100	1 440	1 200	4 800	6 400	4 200	2 200	2 500	1 600
Nitzschia seriata	4 500	1 800	1 800	1 200	2 800	13 000	6 500	650	1 600	1 500
Dinoflagellates	1 580	1 450	850	350	1 200	1 100	800	2 470	1 410	2 000
Phytoplankton carbon	26.6	10.7	6.8	22.0	9.1	10.0	7.2	7.6	4.4	3.4
Phyto-C : chl *a*	33	12	6	18	6	6	4	8	6	3
Near bottom 40–45 m										
Rhizosolenia setigera			10 000	18 000	15 000	1 200	2 100	500	500	400
Chaetoceros spp.			600	2 800	1 000	2 500	800	2 800	500	300
Nitzschia seriata			6 000	4 000	9 000	15 000	2 250	80	100	100
Dinoflagellates			1 800	300	1 650	250	320	800	500	200
Phytoplankton carbon			32.5	55.0	47.8	6.1	7.0	2.7	1.9	1.8
Phyto-C : chl *a*			32	27	18	2	9	5	4	4

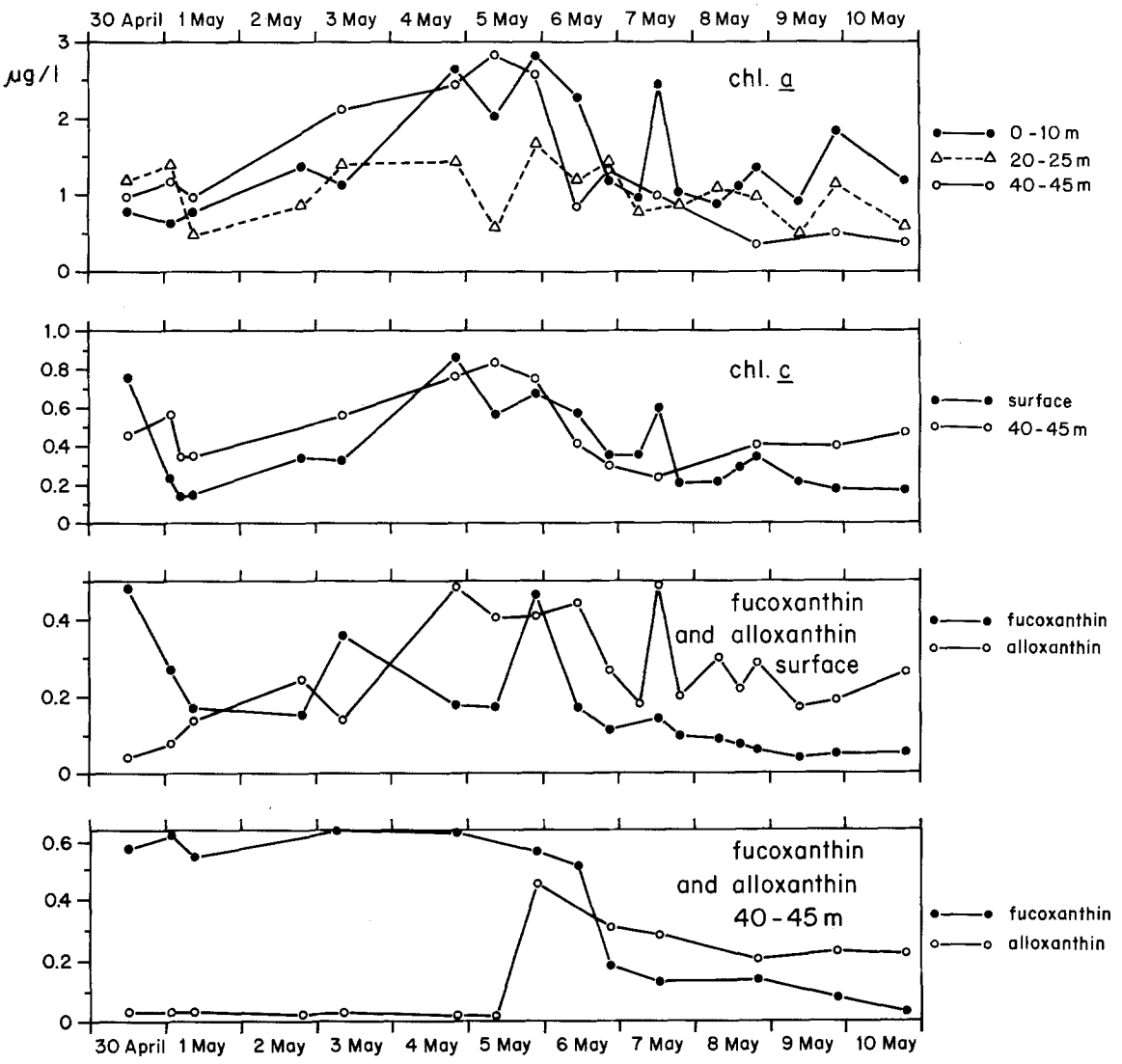

Fig. 3. Concentration changes of chlorophyll *a*, chlorophyll *c*, and fucoxanthin and alloxanthin (μg per liter seawater)

microscope was contributed by large-celled species (see Table 1), the choice of Hagmeier's formulae cannot have influenced our results. Comparison of the carbon values with the concentration of chlorophyll *a* measured in the same samples resulted in carbon to chlorophyll ratios that were below 10 in most cases (Table 1). In surface samples the ratio decreased from 33 on 30 April to no more than 3 on 10 May; in samples near the bottom the ratio was above 10 until 5 May, then decreased to less than 5 for the rest of the observation period.

Discussion

The ratio of phytoplankton carbon to chlorophyll *a*, which we estimated on the basis of cell counts (see Table 1), was in most samples far lower than the lowest estimate of the C:chlorophyll *a* ratio in natural phytoplankton populations (25; Parsons *et al.*, 1977). During the extensive Fladen Ground experiment in the northern North Sea in 1976, estimated C:chlorophyll *a* ratios were also unrealistically low: less than 10 (Gieskes and Kraay, 1980; also compare the C data in Gassmann and Gillbricht (1982) with the chlorophyll concentrations in Radach *et al.*, 1980). Since all these carbon estimates were in fact based on numbers of algal cells, one is tempted to conclude that the counted numbers were underestimates of the numbers that were actually present, in other words: part of the phytoplankton crop may have been overlooked during microscopic analysis.

In the material analysed for the present study, a comparison of the observations made by microscope with the pigments present in the samples reveals that a whole class of algae was missing in the microscopy record: no Cryptophyceae were registered, yet alloxanthin, a pigment that is typical for this family (Chapman, 1966; Riley and Wilson, 1967; Hager and Stransky, 1970) was usually the most abundant carotenoid in the samples (Figs. 2 and 3; it was not possible to decide which one of the small peaks between Rt (retention time) 30 and 40 min was crocoxan-

thin, another typical Cryptophycean pigment; both had a similar, diadinoxanthin-like absorption spectrum). Precisely on the days when the C:chlorophyll *a* ratio was suspiciously low (less than 20, cf. Parsons *et al.*, 1977), suggesting that part of the crop was overlooked during counting, the relative abundance of alloxanthin among carotenoids was high (Fig. 3).

We have calculated the contribution of Cryptophycean chlorophyll *a* to the total chlorophyll *a* stock on the assumption that the chlorophyll *a*:fucoxanthin and the chlorophyll *a*:alloxanthin ratios were constant throughout the sampling period. The ratios were treated as dimensionless constants "x" and "y" in the equation:

$$\text{Total chl } a \text{ in sample} = C + x\ [\text{fucoxanthin}] + y\ [\text{alloxanthin}].$$

C is a constant term that is equal to the amount of chlorophyll *a* that is left when the other terms are zero. Multiple regression analysis of 49 samples from all depths gave the following solution:

$$[\text{Chl } a] = -0.06 + 1.47\ [\text{fucoxanthin}] + 4.28\ [\text{alloxanthin}].$$

The goodness of fit was 0.86, the correlation coefficient 0.93.

This means that the mean ratio of chl *a*:fucoxanthin was 1.47; the mean ratio of chl *a*:alloxanthin 4.28. The very low value of C indicates that no species other than those containing fucoxanthin and alloxanthin contributed significantly to chlorophyll *a* in our samples. Besides Bacillariophyceae (diatoms), Chrysophyceae and Prymnesiophyceae also contain fucoxanthin. However, the chl *a*: fucoxanthin ratio that we found (1.47) is typical for diatoms (Vesk and Jeffrey, 1977), so diatoms were probably the most important fucoxanthin carriers in our samples. The high correlation ($r = 0.76$) between diatom cell volume (Table 1) and fucoxanthin concentrations (Fig. 3) confirms this.

The equations valid for each separate sampling depth are presented in Table 2. The contribution of bacillariophyceae (diatoms) and Cryptophyceae to the chlorophyll *a* crop has been calculated for each depth on the basis of these ratios. The contribution is graphically shown in Fig. 4. Most striking is the persistent dominance of diatoms near the bottom until 6 May. Relatively high concentrations of chlorophyll *c* during diatom dominance (chlorophyll *a*:chlorophyll *c* ratios between 1 and 2, see Fig. 3) suggest the presence of detrital material (Jeffrey, 1974; in diatom cultures the ratio is at least 4, see Vesk and Jeffrey, 1977). The *a:c* ratio was between 4 and 10 when Cryptophyceae dominated. Apparently, detrital algal matter was relatively scarce in such samples.

Re-examination of the Lugol-preserved plankton samples revealed the abundance of a ciliate of 30–80 μm diameter. In samples with an alloxanthin content of more than 0.3 μg (per liter seawater), the number of this ciliate

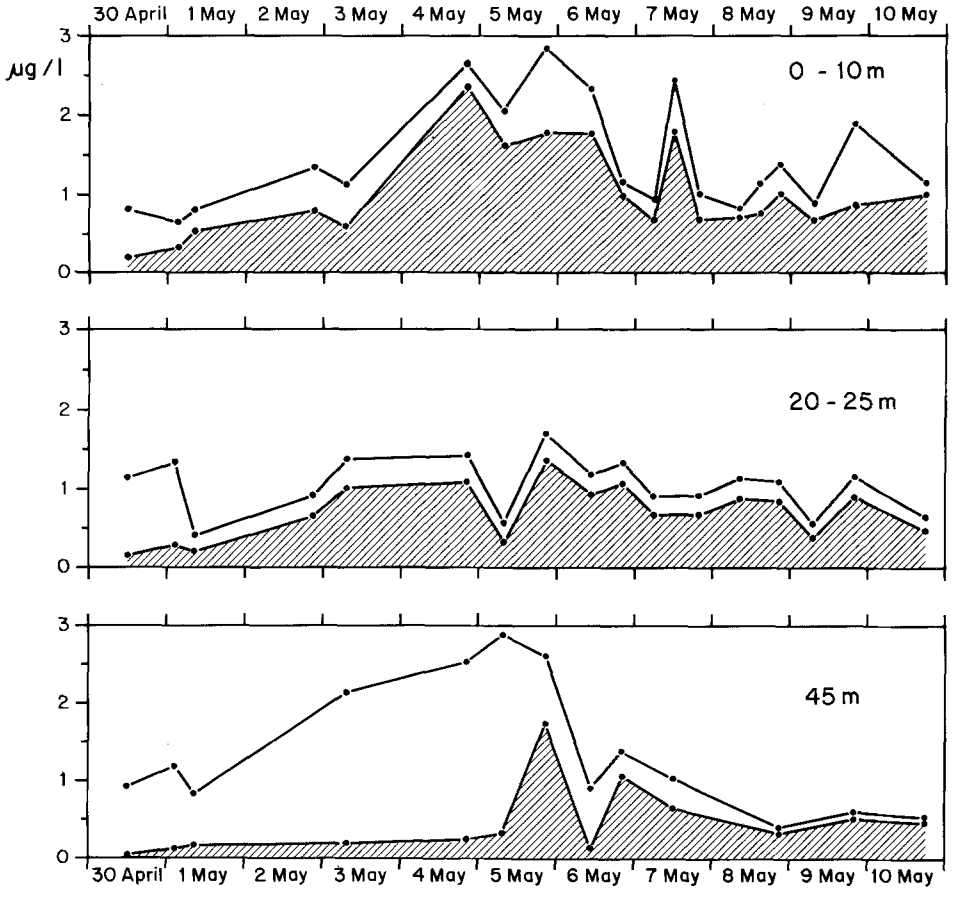

Fig. 4. Contribution of Cryptophycean chlorophyll *a* to total chlorophyll *a* at different depths. Hatched area: Cryptophycean chlorophyll, white area: diatom chlorophyll. Upper curve: total chlorophyll *a*

Table 2. Relation between pigment concentrations (in μg per liter) at three depths, calculated on the basis of a multiple regression analysis of major pigments

Depth (m)	No. of samples	Equation	Goodness of fit	Corr. coefficient	chl *a* / Fucoxanthin	chl *a* / Alloxanthin
0–10	19	[chl a] = 0.08 + 1.37 [fucoxanthin] + 4.19 [alloxanthin]	0.82	0.91	1.4	4.2
20–25	17	[chl a] = 0.004 + 1.32 [fucoxanthin] + 3.86 [alloxanthin]	0.94	0.97	1.3	3.9
40–45	13	[chl a] = −0.07 + 1.89 [fucoxanthin] + 3.92 [alloxanthin]	0.98	0.99	1.9	3.9

was between 25 000 and 50 000 per liter. Inspection of the best-preserved specimens (Lugol's solution is not the ideal fixative for ciliates: Bakker, 1966) and comparison under high magnification (12.5×40, phase contrast) with the detailed description of Bakker (1966) and Taylor *et al.* (1971) revealed the identity of the ciliate *Mesodinium rubrum* (Lohmann). This ciliate is known to contain a Cryptophycean endosymbiont (Bakker, 1966; Parsons and Blackbourn, 1968). Since the correlation coefficient between the calculated Cryptophycean abundance and counted *M. rubrum* numbers was only 0.55, implying that only 30% of the variability is "explained", we conclude that much, but not necessarily all, Cryptophycean chlorophyll was enclosed in *M. rubrum*. The rest must have been in free-living Cryptophyceae.

Chemotaxonomical analysis of the kind presented in this paper can be recommended as a useful supplement to microscopic observations when estimating the total phytoplankton crop in a sample. In our case, much of the crop appeared to be overlooked partly because is was not recognized as algal matter, partly because it was present in symbiosis with a ciliate – a type of organism that may easily escape the attention of a microscopist trained in counting algal cells. In the sea, algal species are often symbiotic with heterotrophs: from Cryptophyceae in *Mesodinium rubrum, Cyclotrichium meunieri* and Radiolaria (Powers, 1932; Ryther, 1967; Parsons and Blackbourn, 1968; Smith and Barber, 1979), to green monads in flatworms (Keeble and Gamble, 1907). Even algae are known to carry symbiotic algae (Tomas and Cox, 1973; Jeffrey and Vesk, 1976). Of course, such algae can also be detected by other means: by autoradiography, or with epifluorescence microscopy (e.g. the bright-red fluorescing zooxanthellae of planktonic tropical Radiolaria; unpublished observations in the Caribbean near Curaçao). However, in the case of Cryptophyceae, the fluorescence of chlorophyll may be quenched by phyco-erythrin, a pink water-soluble biliprotein pigment of Cryptophyceae (Haxo and Fork, 1959; Riley and Wilson, 1967; Vesk and Jeffrey, 1977).

But even when groups of more easily recognizable algae are present, chemotaxonomical data can be a useful supplement to cell counts. The imprecision of Utermöhl's method for counting algal cells is well-known (Hobro and Willén, 1977). Therefore, an independent method for estimating the abundance of algal groups is often necessary. HPLC methodology now serves in our laboratory as a control of counting results. For example, peridinin concentrations often vary with armoured dinoflagellate numbers. We have also been able to relate numbers of *Emiliana huxleyi* with the concentration of a pigment tentatively identified as hexanoyloxyfucoxanthin (see Norgård *et al.*, 1974; retention time of this pigment with our HPLC reversed-phase method is 22 min). On the other hand, a good correlation between fucoxanthin and diatom counts (as found during the present study) implies scarcity of other fucoxanthin-containing algal classes (Chrysophyceae, Prymnesiophyceae). Characteristic pigment combinations are of course even more useful for the detection of algal groups than the measurement of single pigments. For example, Gieskes and Kraay (1983) have presented evidence that the relative abundance of zeaxanthin in open-ocean samples in which chlorophyll *b* was absent or scarce is indicative of the importance of cyanobacteria in oceanic phytoplankton.

However, one must bear in mind that the classification of algae is not only based on colour criteria. Morphology, phytochemistry, ultrastructure and cytology are other important characteristics. A relation between the concentration of any algal pigment with cell counts may further be obscured by variability in the pigment concentration of algal cells.

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Literature cited

Abaychi, J. K. and J. P. Riley: The determination of phytoplankton pigments by high-performance liquid chromatography. Anal. chim. Acta *107,* 1–11 (1979)

Allen, M. B., L. Fries, T. W. Goodwin and T. M. Thomas: The carotenoids of algae. Pigments from some Cryptomonads, a heterokont and some Rhodophyta. J. gen. Microbiol. *34,* 259–267 (1964)

Baars, M. A. and S. S. Oosterhuis: Diurnal rhythms in grazing on labeled food, gut fluorescence and digestive enzyme activities of North Sea copepods. ICES, C.M., *L17,* 1–11 (1982)

Bakker, C.: Een protozo in symbiose met algen in het Veerse Meer. Levende Natuur *69,* 180–187 (1966)

Chapman, D. J.: Three new carotenoids isolated from algae. Phytochem. *5,* 1331–1333 (1966)

Foppen, F. H.: Tables for the identification of carotenoid pigments. Chromatogr. Rev. *14,* 133–298 (1971)

Gassmann, G. and M. Gillbricht: Correlations between phytoplankton, organic detritus and carbon in North Sea waters during the Fladenground Experiment (FLEX'76). Helgoländer Meeresunters. *35,* 253–262 (1982)

Gieskes, W. W. C. and G. W. Kraay: Primary productivity and phytoplankton pigment measurements in the northern North Sea during FLEX'76. "Meteor" Forsch-Ergebnisse A *22,* 105–112 (1980)

Gieskes, W. W. C. and G. W. Kraay: Unknown chlorophyll *a* derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis. Limnol. Oceanogr. *28,* 757–766 (1983)

Hager, A. and H. Stransky: Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. Arch. Mikrobiol. *73,* 77–89 (1970)

Hagmeier, E.: Plankton-Aequivalente. Kieler Meeresf. *17,* 32–47 (1961)

Hardy, A.: The open sea: its natural history. Part I: the world of plankton. London: Collins 1959

Haxo, F. T. and D. C. Fork: Photosynthetically active accessory pigments in Cryptomonads. Nature, Lond. *184,* 1051–1052 (1959)

Herman, A. W. and K. L. Denman: Rapid underwater profiling of chlorophyll with an *in situ* fluorometer mounted on a 'Batfish' vehicle. Deep-Sea Res. *24,* 385–397 (1977)

Hobro, R. and E. Willén: Phytoplankton countings. Intercalibration results and recommendations. Int. Rev. ges. Hydrobiol. *62,* 805–811 (1977)

Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes and J. D. H. Strickland: Fluorometric determination of chlorophyll. J. Cons. int. Explor. Mer *30,* 3–15 (1965)

Jeffrey, S. W.: Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar. Biol. *26,* 101–110 (1974)

Jeffrey, S. W.: A report of green algal pigments in the central North Pacific Ocean. Mar. Biol. *37,* 33–37 (1976)

Jeffrey, S. W. and M. Vesk: Further evidence for a membrane-bound endosymbiont within the dinoflagellate *Peridinium foloaceum.* J. Phycol. *12,* 450–455 (1976)

Jensen, A. and E. Sakshaug: Studies on the phytoplankton ecology of the Trondheimfjord III. Chloroplast pigments in relation to abundance and physiological stage of the phytoplankton. J. exp. mar. Biol. Ecol. *11,* 137–155 (1973)

Keeble, F. and F. W. Gamble: The origin and nature of the green cells of *Convoluta roscoffensis.* Q. J. microsc. Sci. *51,* 167–219 (1907)

Mullin, M. M., P. R. Sloan and R. W. Eppley: Relationship between carbon content, cell volume, and area in phytoplankton. Limnol. Oceanogr. *11,* 307–311 (1966)

Norgård, S., W. A. Svec, S. Liaanen-Jensen, A. Jensen and R. R. L. Guillard: Algal carotenoids and chemotaxonomy. Biochem. Syst. Ecol. *2,* 7–9 (1974)

Parsons, T. R. and D. J. Blackbourn: Pigments of the ciliate *Mesodinium rubrum* (Lohmann). Neth. J. Sea Res. *4,* 27–31 (1968)

Parsons, T. R., M. Takahashi and B. Hargrave: Biological oceanographic processes, 332 pp. Oxford: Pergamon Press 1977

Powers, P. B. A.: *Cyclotrichium meunieri* sp. nov. (Protozoa, ciliata): cause of redwater in the Gulf of Maine. Biol. Bull. mar. biol. Lab., Woods Hole *63,* 74–80 (1932)

Radach, G., J. Trahms and A. Weber: The chlorophyll development at the Central Station during FLEX'76. Two data sets. Proc. ICES/JONSIS Workshop on JONSDAP'76. ICES, C.M., *C3,* 3–21 (1980)

Reid, P. C.: Large-scale changes in North Sea phytoplankton. Nature, Lond. *257,* 217–219 (1975)

Reid, P. C.: Continuous plankton records: changes in the composition and abundance of the phytoplankton of the north-eastern Atlantic Ocean and North Sea, 1958–1975. Mar. Biol. *40,* 337–339 (1977)

Riley, J. P. and T. R. S. Wilson: The pigments of some marine phytoplankton species. J. mar. biol. Ass. U.K. *47,* 351–362 (1967)

Robinson, A. L.: HPLC: the new king of analytical chemistry; Science, N.Y. *203,* 1329–1332 (1979)

Ryther, J. H.: Occurrence of red water off Peru. Nature, Lond. *214,* 1318–1319 (1967)

Smith, W. O. and R. T. Barber: A carbon budget for the autotrophic ciliate *Mesodinium rubrum.* J. Phycol. *15,* 27–33 (1979)

Strathmann, R. R. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. *12,* 411–419 (1967)

Taylor, F. J. R., D. J. Blackbourn and J. Blackbourn: The red-water ciliate *Mesodinium rubrum* and its "incomplete symbionts": a review including new ultrastructural observations. J. Fish. Res. Bd Can. *28,* 391–407 (1971)

Tomas, R. N. and E. R. Cox: Observations on the symbionts of *Peridinium balticum* and its intracellular alga. I. Ultrastructure. J. Phycol. *9,* 304–323 (1973)

Vesk, M. and S. W. Jeffrey: Effects of blue-green light on photosynthetic pigments and chloroplast structure in unicellular marine algae from six classes. J. Phycol. *13,* 280–288 (1977)

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