Vol. 133: 253–262, 1996 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published March 28

Evidence for a novel pigment with *in vivo* absorption maximum at 708 nm associated with *Phaeocystis* cf. *pouchetii* blooms

María Vernet[1,*], B. Gregory Mitchell[1], Egil Sakshaug[2], Geir Johnsen[2], Rodolfo Iturriaga[3], Paul Wassmann[4]

[1] Marine Research Division, Scripps Institution of Oceanography, La Jolla, California 92093-0218, USA
[2] The Biological Station, University of Trondheim, Bynesveien 46, N-7018 Trondheim, Norway
[3] Department of Biological Sciences, University of Southern California, University Park, Los Angeles, California 90089-0371, USA
[4] Norwegian School of Fishery Sciences, University of Tromsø, Dramsveien 201, N-9001 Tromsø, Norway

ABSTRACT: The presence of a chlorophyll *a*-like (chl *a*) pigment, with an *in vivo* absorption maximum in the near-infrared region at 708 to 712 nm, was observed mainly in sedimenting material and *Calanus hyperboreus* fecal pellets associated with *Phaeocystis* cf. *pouchetii* Hariot blooms. (*In vivo* absorption is attributed to natural absorption found in naturally occurring particulate matter and seston. *In vitro* absorption refers to extracts in organic solvents.) This absorption peak was observed in conjunction with the absorption peak at 674 to 676 nm, commonly attributed to chl *a* and its derivatives. The *in vivo* absorption maximum in the near infrared, centered around 708 nm, was observed only in particulate matter and not in methanolic and aqueous acetonic extracts. Absorption efficiency (Q_a) of individual particles measured by microphotometry revealed particles 3 to 4 µm in diameter with an *in vivo* absorption maximum at 708 to 713 nm and no *in vivo* absorption peak at 676 nm, as expected for chlorophylls and its phaeopigments, indicating a different type of particle in the sample. The visible spectrum also had a broad absorption peak in the blue region, between 420 and 450 nm, suggesting a chlorophyll-like spectrum. The main chl degradation product analyzed by high-performance liquid chromatography of sedimenting matter was identified as a phaeophorbide *a*-like pigment, with absorption maximum in the red at 665 to 666 nm in organic solvents, with no indication of a pigment with *in vitro* absorption properties in the near infrared. The accumulation of the 708 nm *in vivo* absorption peak in particles associated with *P.* cf. *pouchetii* seems to be a widespread feature as it was observed during 3 different cruises to the Barents Sea, Fram Strait and the Kattegat. We propose 3 different hypotheses on the origin of this novel peak in *in vivo* absorption not previously observed in marine environments: (1) *P.* cf. *pouchetii* has a chl *a* breakdown pathway which promotes the accumulation of a known chl degradation product with an *in vivo* absorption at 708 nm; (2) there is a new chl degradation product produced by grazing of *C. hyperboreus* on *P.* cf. *pouchetii*, as yet to be isolated; and (3) there is a new pigment, synthesized by either *P.* cf. *pouchetii* or another organism associated with this alga, during mature blooms of *Phaeocystis*. These hypotheses are discussed in view of the available evidence.

KEY WORDS: Chlorophyll · Pigment degradation · Zooplankton grazing · Sedimentation

INTRODUCTION

Studies on the degradation pathway of chlorophyll *a* (chl *a*) are of interest for ecological and geochemical studies of phytoplankton pigments. Understanding the relationship between chl *a* and the distribution of its degradation products can provide a mechanism to trace the sinking of organic particles and newly formed primary production exported out of the euphotic zone (Lorenzen et al. 1983, Downs & Lorenzen 1985). In addition, the abundance and distribution of chl *a* degradation products have been proposed as a tracer of zooplankton grazing in aquatic environments (Shuman

*E-mail: mvernet@ucsd.edu

& Lorenzen 1975, SooHoo & Kiefer 1982, Welschmeyer & Lorenzen 1985). The quantitative degradation of chl *a* to a Mg-free degradation product is being questioned based on the large variance associated with grazing (Conover et al. 1986, Lopez et al. 1988) which might be attributed to degradation of the Mg-free products. Chl *a* degradation in the presence of light is known to form molecules of low molecular weight such as glycerol (Llewellyn et al. 1990). It is not known if grazing would produce similar products.

Chl *a* degradation products that are readily measured with chromatographic analysis in marine systems include phytol-free and/or Mg-free derivatives (chlorophyllide, phaeophytin and phaeophorbide). Oxidation products such as chlorophyll allomers (Gieskes et al. 1978) and epimers (Mantoura & Llewellyn 1983) and other unknown derivatives (Hallegraeff & Jeffrey 1975) have also been cited although there is no systematic knowledge of their composition, distribution and abundance. More recently, non-polar chl *a* degradation products have been described for marine sediments (Furlong & Carpenter 1988, King & Repeta 1991). Other degradation products, such as the pink pigments described in higher plants (Matile et al. 1989), are yet to be reported for marine systems. In this study, we describe the presence of a chlorophyll-like pigment found associated with the prymnesiophyte *Phaeocystis* cf. *pouchetii* Hariot in Arctic and temperate coastal waters. We also present light absorption and pigment composition data of particulates associated with phytoplankton, zooplankton fecal pellets, and sedimenting matter. We discuss 3 different hypotheses which could explain the origin of this peak: (1) *P.* cf. *pouchetii* has a chl *a* breakdown pathway which promotes the accumulation of a known chl degradation product with an *in vivo* absorption at 708 nm; (2) the existence of a new chl degradation product associated with grazing by *Calanus hyperboreus* on *P.* cf. *pouchetii*, yet to be isolated; and (3) there is a new pigment synthesized by either *P.* cf. *pouchetii* or another organism associated with mature blooms.

MATERIALS AND METHODS

Samples were taken at 3 different regions: a cruise to the Barents Sea on the RV 'G.O. Sars' between 17 and 30 May 1987 as part of the ProMare program; a cruise to the Fram Strait on board the RV 'Polar Bjørn' on 9 April to 17 May 1989 as part of the CEAREX Project; and a cruise to the Skagerrak and Kattegat on board the RV 'G.O. Sars' on 15 to 29 April 1990. Samples were collected from the water column with Niskin bottles attached to a CTD rosette. Details on the stations sampled are given in Table 1. Samples of sedimenting matter were taken with 2 types of traps, as explained in Wassmann et al. (1990) for the Barents Sea, and an automatic Mark VII trap (0.5 m^2 surface area) in the Kattegat.

Table 1 Description of stations visited and samples taken during the course of this study

Location	Latitude	Longitude	Depth (m)	Sample type
Barents Sea (1987)				
Stns 941 and 994	74° 29' N	31° 31' E	50, 100	Sediment trap, and fecal pellets
Stns 947 and 987	75° 11' N	28° 37' E	50, 100	Sediment trap
Fram Strait (1989)				
Stn 127	79° 04' N	05° 0.5' E	13, 25	Water column, and fecal pellets
Kattegat (1990)				
Stn 477	57° 58' N	10° 35' E	120	Water column
Stn 486	57° 49' N	10° 21' E	68	Water column
Stn 490	58° 02' N	10° 01' E	120	Water column
Stn 493	57° 57' N	10° 36' E	15, 138	Water column
			120	Sediment trap

In vivo absorption spectra were determined following the technique described in Mitchell (1990). Water column (500 to 1000 ml) and sediment trap (20 to 150 ml) samples were filtered onto Whatman GF/F filters and scanned in a dual-beam spectrophotometer. A blank filter moistened with seawater was used as reference. Two spectra were obtained on each filter, before and after extraction with 100% methanol, allowing estimate of phytoplankton and detrital absorption (Kishino et al. 1985). All determinations were done on board ship with fresh material. During cruises on board the RV 'G.O. Sars', *in vivo* absorption spectra (350 to 750 nm) were measured with a Hitachi Model U-2000 double beam spectrophotometer. In the Fram Strait, spectra were measured with a Bausch and Lomb Spectronic 2000. *In vivo* absorption is presented as chlorophyll-specific absorption [m^2 (mg chl $a)^{-1}$] where total chl *a* concentration was determined. Similarly, *in vitro* absorption spectra of the same material were obtained from the same filters after the *in vivo* measurement on board ship.

Microphotometric analyses of individual particle absorption spectra were performed on particles observed in zooplankton fecal pellets (Iturriaga et al. 1988). Fecal pellets of *Calanus hyperboreus*, collected in the Fram Strait and preserved in 2% buffered formalin, were transferred after filtration to a microscope slide covered with gelatin. Spectral transmission of the

particles within the fecal pellet was measured in a photomicroscope consisting of a Zeiss Universal microscope equipped with a photometer option O3 and interfaced with a high-intensity tungsten light and scanning monochromator. As blanks we used spectra of particle-free fields close to the particles of interest.

Chromatographic analyses were done as described in Vernet (1991). Samples were filtered onto Whatman GF/F filters for water column samples and GF/C filters for sediment trap samples. Filters were immediately frozen with liquid nitrogen and stored at −70°C until analysis in the laboratory. Samples were transported from the ship to the laboratory in dry ice. Pigments were extracted with 100% methanol or 90% acetone for 24 h and analyzed after clearing the extract by filtration. Pigments were analyzed by high-performance liquid chromatography on a reverse-phase C-18 column (Brownlee 25 cm × 4.6 mm, 5 µm particles). Pigments were eluted in a low-pressure gradient system consisting of a linear gradient from 100% A to 100% B for the first 10 min and followed by 100% B for another 15 min. Solvent A consisted of 80:20 methanol:water (v:v) where 100 ml of water was prepared with 1.5 g of tetrabutylammonium acetate and 0.96 g of ammonium acetate (Mantoura & Llewellyn 1983). Solvent B consisted of 60:40 (v:v) methanol:ethyl acetate. Pigments were monitored by absorption at 440 nm and quantified by calibration of the column with pigments isolated by thin-layer chromatography from a culture of *Thalassiosira nordenskioeldii*. Chlorophyllide *a* was prepared after incubation of *Dunaliella tertiolecta* in acetone:water (50:50, v:v) for 1 h (Jeffrey 1968), extracted in 90% acetone and further purified by chromatography. Me-phaeophorbide *a* and Mephaeophytin *a* were prepared by acidification of chlorophyllide *a* and chl *a* in methanol respectively with subsequent return to pH 7 by addition of $MgCO_3$. Absorption and fluorescence excitation spectra of individual pigments were recorded in line in a Hitachi spectrophotometer Model U-2000 fitted with a flow-through cell and a Hitachi spectrofluorometer Model F-3000 at an emission wavelength of 730 nm in a stop flow mode.

Total chl and phaeopigments were determined on board ship using a Turner 111 or a Turner Designs filter fluorometer calibrated with chl *a* from Sigma following the method of Holm-Hansen et al. (1965). Samples were filtered onto 25 mm GF/F filters and extracted immediately in 90% acetone. After 24 h, extracts were centrifuged and cleared.

RESULTS

An *in vivo* absorption peak at 706 to 712 nm was first observed associated with the material collected by the sediment traps in the Barents Sea at Stn 941 visited on 28 May 1987 and at Stn 994, when the same station was revisited on 8 June 1987 (see Wassmann et al. 1990 for details). This absorption peak was in addition to the 674 to 676 nm *in vivo* absorption peak, commonly attributed to chl *a* and derivatives (i.e. chlorophyllide *a*, phaeophorbide *a*, phaeophytin *a*) (Fig. 1). Similar to the chl *a* peak at 676 nm, the peak at 708 nm was extractable with methanol, indicating that the peak involved in near-infrared absorption *in vivo* can be attributed to 'phytoplankton absorption' sensu Kishino et al. (1985).

The sedimenting matter exhibiting the 708 nm absorption peak was sampled at 50 and 100 m in the marginal ice zone. Waters were strongly stratified with a well-developed chl *a* maximum around 43 m depth. Another sediment trap sample from the marginal ice zone where traps were deployed (Stn 947 and 987; Wassmann et al. 1990) did not show a strong 708 nm *in vivo* absorption although a shoulder at the same wavelength was evident (Table 2). *In vivo* absorption spectra of water column samples exhibited the usual chl *a* absorption peak at 674 to 676 nm; of all the samples collected in the Barents Sea there was only 1 station in which seston collected from 150 and 225 m had a shoulder in the absorption spectrum in the

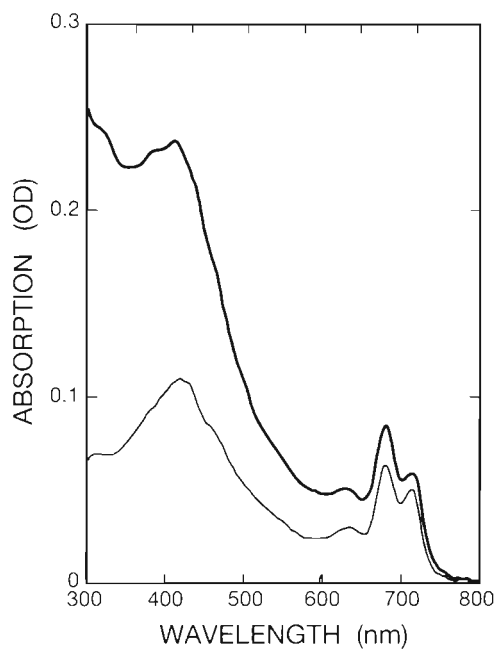

Fig. 1. Absorption spectra of sediment trap material collected at Stn 994, 100 m, in May/June 1987 in the Barents Sea during a *Phaeocystis* cf. *pouchetii* bloom, showing the *in vivo* absorption peak in the near-infrared (708 nm) in addition to the chl *a* peak (676 nm): total absorption (thick line) and phytoplankton absorption (thin line) calculated as the difference between total and detrital absorption, the latter measured after methanol extraction (Kishino et al. 1985)

Table 2. Absorption maxima attributed to chlorophylls and chlorophyll derivatives in the range 650 to 750 nm of *in vivo* and extracted samples collected from water column and sediment traps. All locations had *Phaeocystis* cf. *pouchetii* as a dominant species in the phytoplankton assemblage. In the Barents Sea (1987) the maximum of absorption in the near infrared was observed mainly in sediment traps and fecal pellets of *Calanus hyperboreus*. No absorption at 708 nm was observed at Stn 941 in the water column at 0, 30, 70, 90 m nor at Stn 994 at 0, 10, 20, 30, 43, 60 m. In the Fram Strait and Kattegat the absorption at 708 nm was observed also in particles collected from the water column

Location	Absorption (O.D.) (nm)			
	In vivo	100% methanol	90% acetone	100% ethanol
Barents Sea (1987)				
Water column				
Stn 994 (150 + 225 m)	674, 706–713[a]			
Sediment traps				
Stn 941 (50 m)	676, 712	na	na	na
(100 m)	677, 710			
Stn 947 (50 m)	674, 706–713[a]	na	na	na
(100 m)	676, 706–713[a]			
Stn 987 (50 m)	675, 706–713[a]			
(100 m)	674, 706–713[a]	664	na	na
Stn 994 (50 m)	674, 708			
(100 m)	675, 708	665	665	666
Fram Strait (1989)				
Water column				
Stn 127	676, 713 nm			
Kattegat (1990)				
Water column				
Stn 477	674, 706–713[a]			
Stn 486	674, 706–713[a]			
Stn 490	674, 706–713[a]			
Stn 493	674, 708			

[a]Denotes the presence of a small peak, not fully resolved, in the 706 to 713 nm region of the spectrum

708 nm region. *Phaeocystis* cf. *pouchetii* was the dominant phytoplankton species at all these stations.

Unlike the Barents Sea, seston collected in 1989 within the mixed layer (26 m depth) in the Fram Strait showed a clear *in vivo* absorption at 715 nm (Fig. 2a, thick line), although samples closer to the surface did not show the same properties (Fig. 2a, thin line). Similarly, seston collected at 15 m depth in the Kattegat in spring 1990 exhibited a near-infrared *in vivo* absorption peak (708 nm) (Fig 2b). Deeper in the water column (138 m) the feature remained and was seen as a shoulder (Fig. 2c). The latter sample has a detrital-like absorption typical of samples from below the photic zone. Samples collected by a sediment trap deployed at 120 m depth at the same station showed the same shoulder at 708 nm (data not shown). Neighboring stations had similar *in vivo* absorption around 708 nm (Table 2). Phytoplankton in other areas in the Kattegat or Skagerrak did not show this feature.

Further analysis of zooplankton from the Barents Sea revealed that *Calanus hyperboreus* fecal matter had *in vivo* absorption at 708 nm in addition to the absorption at 674 nm (Fig. 3a). Fecal matter from other zooplankton, including the copepods *Calanus finmarchicus*, *Metridia longa* and krill *Thyssanoessa inermis*, did not (Table 3, Fig. 3b). Fecal pellets of *C. hyperboreus* from the Fram Strait also showed the novel absorption peak.

Absorption efficiency of individual fecal pellets from *Calanus hyperboreus* (Fig. 4a) collected in the Fram Strait indicated strong absorption centered at 708 nm, similar to the mixture of pellets measured with a spectrophotometer (Fig. 3a) or sedimenting matter in general (Fig. 1). After disruption of individual fecal pellets we observed several types of discrete particles under the microscope. Absorption efficiency of individual particles (at a magnification of 1000×) indicated that the absorption at 708 nm was in fact due to small cells (or particles) where all the absorption in the red region of the spectrum was located in the near infrared (Fig. 4b). Other peaks of absorption were located at 625 nm, and there was a broad peak in the blue (410 to 430 nm). The spectrum resembles strongly a chl *a*-like pigment with characteristic maxima in the blue and red regions of the spectrum. In contrast, other cells in the fecal pellet had a classical *in vivo* absorption spectrum attributed to photosynthetic pigments and chl *a* degradation products, with

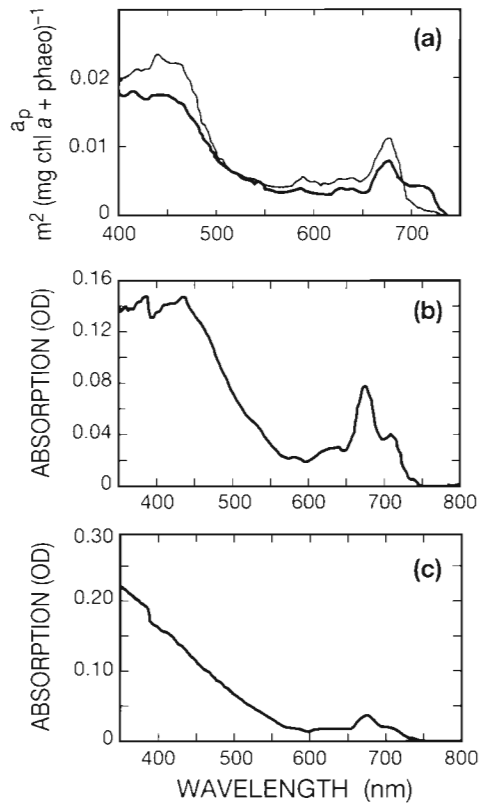

Fig. 2. (a) Absorption spectral coefficients of phytoplankton collected in the Fram Strait in April 1989 during a *Phaeocystis* cf. *pouchetii* bloom at 13 m (thin line) and at 26 m (thick line). The absorption peak in the red at 713 nm is observed only in the deeper sample. (b) Absorption of a sample collected at 15 m in the Kattegat, showing the absorption peak at 708 nm in addition to the 676 nm peak of chl *a*. Chl concentration in the sample was 2.05 µg l^{-1} and phaeopigments were 1.88 µg l^{-1} indicating pigment degradation. (c) Sample collected at the same station as in (b) at a depth of 138 m with 0.4 µg chl *a* l^{-1} and 0.37 µg l^{-1} phaeopigments. The high blue to red ratio is indicative of a high proportion of detrital matter. The 708 nm peak is seen as a shoulder only

Table 3. Absorption maxima observed in fecal pellets of copepods collected in the Barents Sea in May to June 1987 at the stations where the 708 nm absorption peak was observed in the sediment traps

Species	Absorption (O.D.) (nm) *in vivo*	Date
Calanus hyperboreus	674, 709	
	676, 708	10 June
	675, 709	10 June
Calanus hyperboreus[a]	675, 708	28 May
Calanus finmarchicus	674	4 June
	674	10 June
Metridia longa	674	10 June
Thyssanoessa inermis	674	2 June
	674	10 June

[a]Experiment of fecal pellet production after feeding on *Phaeocystis* cf. *pouchetii*

peaks at 420 and 676 nm and a shoulder at 480 nm (Fig. 4c). The combined *in vivo* absorption of these 2 particle types results in the bulk absorption spectrum seen in fecal pellets (Fig. 4a) or the sedimenting matter (Fig. 1), where both the 674 nm and the 708 nm peaks are present. When comparing the *in vivo* absorption spectra of the 2 types of cells, it is clear that the ratio of blue:red absorption maxima is lower in the particles with absorption maximum at 708 nm (blue:red ratio = 1) than in the other phytoplankton particles (blue:red = 1.45) (Fig. 4b, c respectively). The particles in the *C. hyperboreus* fecal pellets which exhibited the near-infrared absorption peak *in vivo* were oblong, 3 to 4 µm diameter. Highly refractive inclusions were noted positioned alongside the outer membrane (Fig. 5).

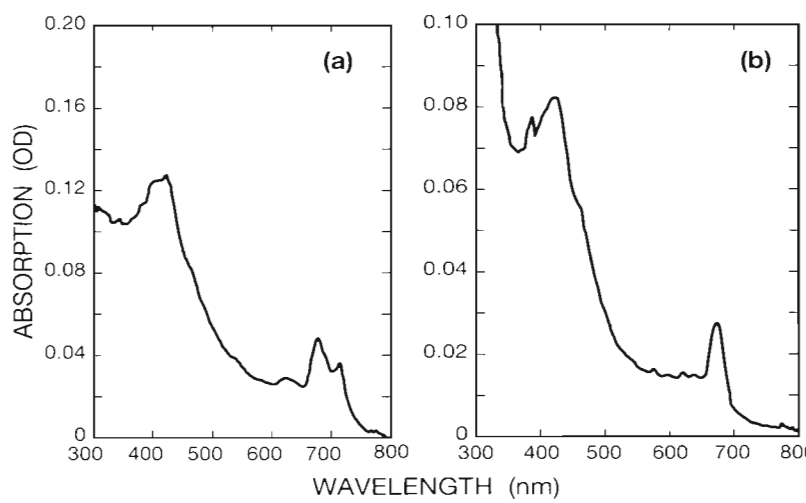

Fig. 3. (a) Absorption spectrum of *Calanus hyperboreus* fecal pellets. *C. hyperboreus* was collected from 75 m to surface on a vertical tow in the Barents Sea, sorted in the lab and left to defecate in filtered seawater for 24 h. Fecal pellets were collected by filtration onto GF/F filters. The peaks at 676 and 708 nm observed here are very similar to the spectra of the material collected by the sediment traps. (b) Absorption spectrum of krill *Thyssanoesa inermis* fecal pellets collected in the same location as above. All spectra with fresh material run on board

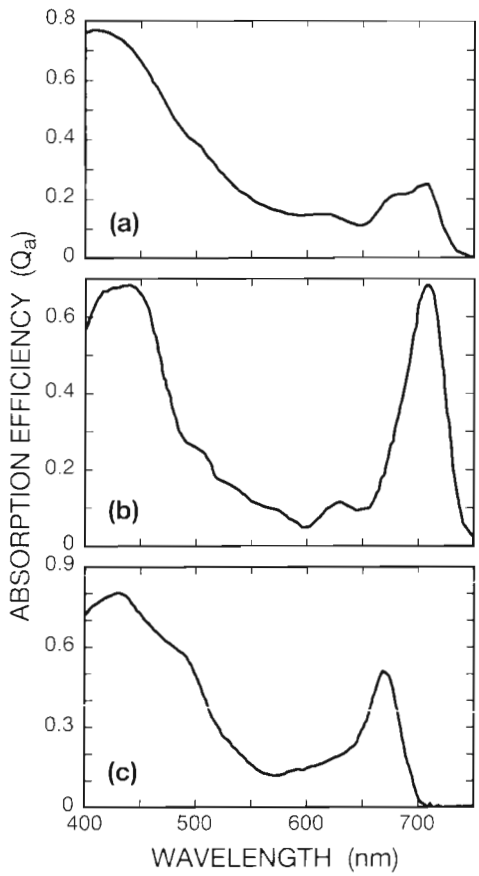

Fig. 4. *Calanus hyperboreus*. Microphotometric analysis of the absorption (Q_a) of particulate matter from fecal pellets of *C. hyperboreus* collected in the Fram Strait in April/May 1989 during a *Phaeocystis* cf. *pouchetii* bloom and stored in 2% buffered formalin. (a) Absorption efficiency spectrum of an individual fecal pellet, measured at a magnification of 100×. (b) Absorption efficiency spectrum of an individual particle (magnification 1000×) contained inside a *C. hyperboreus* fecal pellet, showing that the 708 to 711 nm absorption is associated with a specific type of particle. (c) Absorption efficiency spectrum of a small diatom (magnification 1000×) sampled from the same fecal pellet as panel b, with absorption peak in the red at 676 nm

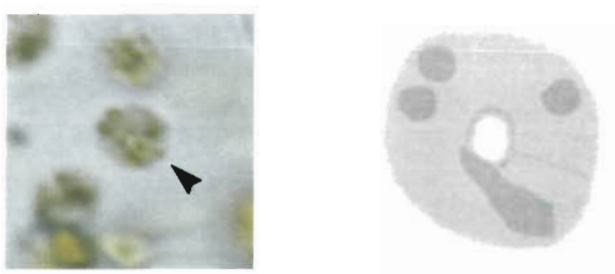

Fig. 5. Photograph of the individual particles with visible absorption properties shown in Fig. 4b (arrow). These particles are approximately 3 to 4 µm in diameter with highly refractive inclusions positioned near the cell membrane

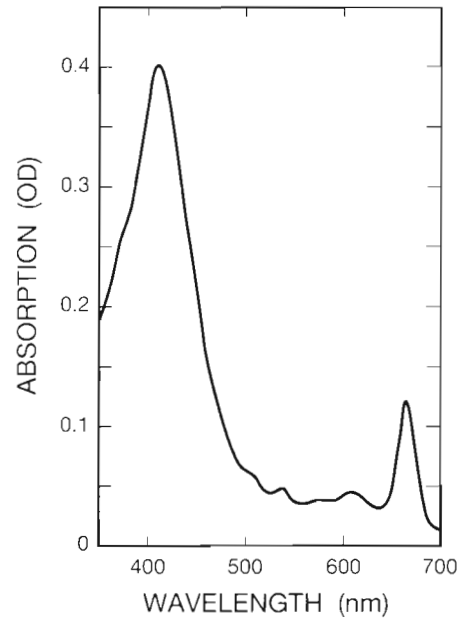

Fig. 6. *Calanus hyperboreus*. *In vitro* absorption spectrum of a methanol extract of fecal pellets from *C. hyperboreus* showing only 1 absorption peak in the red (665 nm). Before extraction, this material had an *in vivo* absorption with peaks at 667 and 708 nm, as shown in Fig. 3a. Spectrum run on board ship with fresh material

The same sediment trap material collected in the Barents Sea at 100 m depth at Stn 994 showed a single peak of *in vitro* absorption at 665 nm in extracts of 100% methanol or 90% acetone:water (Fig. 6). Pigment analysis of the same sample showed the chlorophylls are c_1+c_2, c_3, and *a* (Fig. 7, Table 4), as expected for prymnesiophytes and diatoms in the region (Wassmann et al. 1990). The most abundant carotenoids were fucoxanthin, diadinoxanthin, and β,β-carotene, also characteristic of the same taxa. However, *Phaeocystis* cf. *pouchetii* in the Barents Sea seems to lack the fucoxanthin derivatives present in the Antarctic strains (Wassmann et al. 1990, I. Peeken, University of Kiel, pers. comm.) and in other Arctic prymnesiophytes (Trees et al. 1992). Following Jeffrey & Wright (1994) this alga is classified as having a type 2 pigment complement within the prymnesiophytes. Chl *a* degradation products are represented by chlorophyllide *a*, chl *a* allomer and chl *a* epimer, 2 main forms of phaeophorbide *a*, 2 main forms of phaeophytin *a*, and several other minor peaks identified as chl degradation products. None of these peaks exhibited *in vitro* absorption >700 nm, as observed *in vivo* (Fig. 4b).

DISCUSSION

The evidence gathered so far indicates that (1) the *in vivo* absorption at 708 nm is present when *Phaeo-*

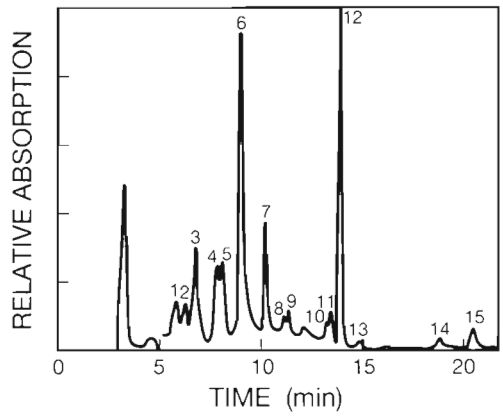

Fig. 7. Chromatogram of the chlorophylls and degradation products collected in sediment traps at Stn 994, 100 m, in the Barents Sea. Separation performed by high-performance liquid chromatography on a reverse-phase C-18 column with a fluorometric detector with excitation at 417 nm and emission at 667 nm. Spectral properties and peak identification are shown in Table 4

Table 4. Spectral properties of pigments analyzed by high-performance liquid chromatography (see Fig. 7) of material collected by sediment traps deployed at Stn 994 in the Barents Sea in May 1987. Spectra taken on eluent with solvent A (80:20, MeOH:water+IP) and solvent B (60:40, MeOH:ethyl acetate) in varying proportions. R_t: retardation factor; na: not available

Peak no.	Pigment	R_t (min)	Absorption maxima (nm)
1	Chlorophyll c_3	5.8	454, 580
2	Chlorophyllide *a*	6.3	437, 624, 666
3	Chlorophyll c_1+c_2	6.9	445, 586, 634
4	Unknown	7.6	433, 530
5	Phaeophorbide a_1	8.6	425, 667.5
6	Phaeophorbide a_2	9.1	416, 666
7	Chlorophyll derivative	10.1	414, 614, 666
8	Chlorophyll derivative	11.6	408, 624, 667
9	Chlorophyll derivative	11.9	409, 623, 667
10	Chlorophyll allomer	13.3	425, 667
11	Unknown	13.5	na
12	Chlorophyll *a*	13.8	433, 664
13	Chlorophyll epimer	14.7	na
14	Phaeophytin a_1	18.4	410, 667
15	Phaeophytin a_2	21.1	410, 667

cystis cf. *pouchetii* is abundant in the water column, (2) there is a specific cell (or particle), a few micrometers in diameter, which is responsible for the 708 nm absorption, (3) the pigment responsible for the absorption at 708 nm is extractable in organic solvents, and (4) extracts in organic solvents have a dominant *in vitro* absorption in the red at 665 nm with no near-infrared features. We lack a pigment whose *in vitro* absorption spectrum coincides with the *in vivo* absorption observed at 708 nm. Although the first 3 pieces of evidence are unambiguous, the fourth observation could be due to limitations in the chromatographic system used and it is therefore not conclusive.

The *in vivo* absorption in the near infrared, centered at around 708 nm, was distinct in seston and fecal pellets of *Calanus hyperboreus* associated with mature or senescent *Phaeocystis* cf. *pouchetii* blooms. At the ice edge, *Phaeocystis* sp. dominated the phytoplankton assemblage in the Barents Sea (Wassmann et al. 1990) and in the Fram Strait (Smith et al. 1991), and was an important component, although not dominant, in the Kattegat (W Eikrem, University of Oslo, pers. comm.) Chl *a* concentrations in the upper water column were moderate to high (4 to 8 mg m^{-3} in the Barents Sea; 2.2 to 3.1 mg m^{-3} in the Fram Strait; 1 to 2.5 mg m^{-3} in the Kattegat). Nitrate was low in the mixed layer at Stn 994 in the Barents Sea (0.1 µM) and in the Fram Strait (0.06 to 0.2 µM). Samples where the 708 nm peak was observed were collected at medium to low irradiances, in the mixed layer (>13 m depth), in sediment traps (50 and 100 m depth), or in copepod fecal pellets.

By comparing Fig. 4b and 4c we see that the *in vivo* absorption spectra (and hence we assume the pigment composition) within these fecal pellets were not homogeneous. From this we conclude that the *in vivo* 708 nm absorption is associated with a certain type of particle (Fig. 4b) which seems to accumulate this pigment to the exclusion of the (phaeo)pigments which absorb at 674 nm. It is not clear from our sampling if the accumulation of the 708 nm pigment in the particles occurs first in the water column and is further accumulated in the gut of *Calanus hyperboreus* by ingestion or if it happens mostly as a result of grazing. Our initial sampling in the Barents Sea does not indicate that the seston exhibits the novel pigment since the 708 nm peak was observed mostly in the fecal pellets and sediment traps. Nevertheless, water-column samples in the Fram Strait and the Kattegat did exhibit the 708 nm peak in the seston, indicating possible formation of this peak before zooplankton ingestion. However, it is known that seston may contain significant detrital pigments resulting from grazing. Large fecal pellets were observed and collected with Niskin bottles in the Kattegat (T. Noji, Institute of Marine Research, Bergen, pers. comm.), as seen in other environments (Alldredge et al. 1987).

The relative contribution of chl *a* degradation products in copepod fecal pellets, in particular the phaeophorbide:chl *a* ratio, was similar to that observed in sedimenting material collected in the 100 m sediment trap at Stn 994, as shown previously (Wassmann et al. 1990). In *Calanus hyperboreus* fecal pellets the more

abundant phaeophorbide eluted just before fucoxanthin (peak 6 at 9.1 min in Table 4, Fig. 7). This phaeophorbide-like molecule, present in both water-column and sediment trap samples, had been previously named phaeophorbide a_3 in the Barents Sea (Vernet 1991), following the nomenclature in Vernet & Lorenzen (1987) and Downs (1989). A pigment with similar chromatographic properties has also been reported for sediment trap samples in the North Atlantic in association with *Phaeocystis* cf. *pouchetii* blooms. Head et al. (1994) attributed the pigment to sedimenting phytoplankton cells. Different from our observations, they did not observe this pigment in zooplankton fecal pellets.

Chlorophyll degradation hypothesis

If the 708 nm peak is a result of chl *a* degradation, 2 possibilities can be considered: (1) the presence of a phaeopigment which has a different *in vivo* absorption spectrum due to factors such as a change in the pigment-protein complex or the deposition of crystalline phaeopigments; (2) the formation of a previously unreported chl *a* degradation product. An increase in *in vivo* absorption at 710 to 715 nm in a mutant of *Chlorella fusca* Shihira et Kraus was observed to coincide with the accumulation of phaeophorbide *a* and pyrophaeophorbide *a* by enzymatic activity (Ziegler et al. 1988) under conditions of nutrient starvation and darkness. Identification of pyrophaeophorbide as the most abundant pigment was done by chromatographic analysis and infrared spectrophotometry. The authors concluded that the shift in *in vivo* absorption from 675 to 715 nm in this alga was due to the precipitation of pyrophaeophorbide due to the lower solubility of this pigment in the aqueous milieu, as compared to chl *a*. In our samples we also observed that the most abundant (phaeo)pigment was phaeophorbide (form a_3 eluting at 9.6 min in our chromatographic system) and that the 708 nm peak was present only in the *in vivo* spectra. We do not have at this point experimental data to correlate an increase in 708 nm absorption with increased phaeopigment concentration (as available for *C. fusca*) which would give further substance to this hypothesis. Preliminary results from *Phaeocystis* cf. *pouchetii* cultures kept in the dark for up to 6 wk, both in exponential and stationary growth stages, did not show a shift in absorption from 667 to 708 nm (Vernet unpubl. data). Similarly, several studies have suggested that a change in the pigment-protein bond will change the absorption properties of the pigments in question (Downs 1989). These 2 types of processes, or similar ones, imply that the *in vivo* absorption of the (phaeo)pigment is red-shifted 45 nm of the *in vitro* peak. Although this phenomenon is common in the blue peak of some carotenoids (i.e. peridinin and fucoxanthin), it has not been reported for the red peak of algal chlorophylls or their derivatives in natural samples except for symbionts of the coral *Favia* (Halldal 1968). On the other hand, a shift to shorter wavelengths from *in vivo* to *in vitro* absorption has been observed in bacteriochlorophylls (Brock & Madigan 1991).

If the 708 nm peak is due to phaeopigment accumulation and/or precipitation, we could ask why would this phenomenon happen in association with *Phaeocystis* cf. *pouchetii* cells and not in other species? It might be that *P.* cf. *pouchetii*, similar to *Chlorella fusca* mutant, has an unknown degradation pathway different from other cells. The conditions for the pyrophaeophorbide to accumulate in *C. fusca* were a period of darkness and nutrient limitation. For our samples, darkness can be provided in the 50 and 100 m sediment trap and/or gut passage in grazers. In addition, both at Stn 994 in the Barents Sea and at the station in the Fram Strait, water-column nitrate concentrations were low so the phytoplankton populations would be expected to be in stationary phase or to have shifted to an NH_4-based system (Wassmann et al. 1990).

If the *in vivo* absorption at 708 nm is related to the formation of a new type of chl *a* degradation product, chromatographic analysis of the sedimenting matter collected at Stn 994 in the Barents Sea failed to show it. If the pigment has *in vitro* absorption properties different from those known for phaeopigments or chls, it was not eluted by the system used, nor others tested (Baumann et al. 1986, Hurley & Armstrong 1990). Given that this pigment is readily extractable by organic solvents (see 'phytoplankton absorption' sensu Kishino et al. 1985 in Fig. 1) it is to be expected that the analytical methods used for chl *a* and phaeopigments should be also applicable. On the other hand, elution time could be longer than usual (see King & Repeta 1991 for non-polar chl degradation products), so that procedures used may have failed to resolve the pigment.

Other hypotheses

The data described for our samples which do not support the hypotheses previously discussed include the low blue:red ratio (~1) in the particle absorption efficiency shown in Fig. 4b, which is not characteristic of chl *a* degradation products in organic solvents (Table 4; Hallegraeff & Jeffrey 1975, King & Repeta 1991) where the blue:red ratio is ≥ 2. Although the *in vivo* absorption properties of phaeopigment in par-

ticles is not known, the high blue:red ratio in detrital particles in general suggests also a high ratio for phaeopigments *in vivo*. In addition, direct observation of absorption by individual particles clearly indicates that *Calanus hyperboreus* fecal pellets have infrared absorption associated with discrete particles. Finally, the absorption efficiency of these individual particles (Q_a), albeit for limited samples, did not show a mixture of both absorption peaks in the same particle, as might be expect if chl *a* (*in vivo* absorption 674 nm) is being progressively degraded to a particular phaeopigment (*in vivo* absorption at 708 nm), as observed in the *Chlorella fusca* mutant (Ziegler et al. 1988).

The low blue:red ratio and the position of the near-infrared peak observed in our particles (Fig. 4b) resemble the *in vivo* absorption due to some bacteriochlorophylls (bchl), for example, bchl *d* and *e*, which have *in vivo* absorption maximum at 705 to 740 nm and 713 to 726 nm (Puchkova & Gorlenko 1982, Oelze 1985, Brock & Madigan 1991). As mentioned above, these bchls have a large shift in organic solvents, to 654 and 647 nm in methanol for bchl *d* and bchl *e*, respectively, too large a shift for our sample which shows a maximum at 665 nm (Fig. 6). Bchl *c* has an *in vitro* absorption maximum in methanol at 667 nm (Jensen et al. 1964) but its characteristic absorption *in vivo* is at longer wavelengths (745 to 755 nm). Organisms with bchls grow largely in anaerobic conditions or under decreased oxygen tension, conditions not readily available in the upper water column. On the other hand, low oxygen tension might be found in microenvironments such as old colonies of *Phaeocystis* cf. *pouchetii*, heavily colonized by bacteria, or in the gut and fecal pellets of *Calanus hyperboreus*.

Further experimental work on the formation and fate of the *in vivo* absorption at 708 nm is necessary to ascertain if this peak is due to a specific compound. Independent of its origin, the characteristic *in vivo* absorption in the near infrared described here is a novel feature not previously reported for aquatic environments. The association of the 708 nm absorption *in vivo* with a discrete particle indicates a process not known yet. Its potential use as an indicator of *Phaeocystis* cf. *pouchetii*, particularly in sedimenting matter, is a new step in the study of this ecologically and biogeochemically important species.

Acknowledgements. We thank the Norwegian ProMare program for ship time during the Barents Sea cruise and to the Institute for Marine Resources at Bergen for ship time to the Kattegat. We thank K. Andreasen for absorption spectra determinations of the Kattegat samples. This study was partially funded by the National Science Foundation (OPP-92-00436 to M.V., OPP-85-20848 to O. Holm-Hansen, and OCE-88-11511 to M.V.), the Office of Naval Research grants N00014-89-J-1639 to B.G.M. and N00014-89-J-1047 to R.I., the Norwegian Research Council (to E.S. and P.W.), the Norwegian Fishery Research Council (to P.W.) and the Norwegian Marshall Fund (to M.V.).

LITERATURE CITED

Alldredge AL, Gotschalk CC, McIntyre S (1987) Evidence for sustained residence of macrocrustaceans fecal pellets in surface waters off Southern California. Deep Sea Res 34: 1641–1652

Baumann T, Vasmel H, Grimme LH, Amesz J (1986) Pigment composition of the photosynthetic membrane and reaction center of the green bacterium *Prosthecochloris aestuarii*. Biochim Biophys Acta 848:83–91

Brock TD, Madigan MT (1991) Biology of microorganisms, 6th edn. Prentice Hall, Inc, Englewood Cliffs

Conover RJ, Durvasula R, Roy S, Wang R (1986) Probable loss of chlorophyll-derived pigments during passage through the gut of zooplankton, and some of the consequences. Limnol Oceanogr 31:878–887

Downs JN (1989) Implications of the phaeopigments, carbon and nitrogen content of sinking particles for the origin of export production. PhD dissertation, Univ Washington, Seattle

Downs JN, Lorenzen CJ (1985) Carbon:phaeopigment ratios of zooplankton fecal pellets as an index of herbivorous feeding. Limnol Oceanogr 30:1024–1036

Furlong ET, Carpenter R (1988) Pigment preservation and remineralization in oxic coastal marine sediments. Geochim Cosmochim Acta 52:87–99

Gieskes WWC, Kraay GW, Tijssen SB (1978) Chlorophyll and their degradation products in the deep pigment maximum layer of the tropical North Atlantic. Neth J Sea Res 12: 195–204

Halldal P (1968) Photosynthetic capacities and photosynthetic action spectra of endozoic algae of the massive coral *Favia*. Biol Bull 134:411–424

Hallegraeff GM, Jeffrey SW (1975) Description of new chlorophyll *a* alteration products in marine phytoplankton. Deep Sea Res 32:697–705

Head EJH, Hargrave BT, Subba Rao DV (1994) Accumulation of a phaeophorbide-*a* like pigment in sediment traps during late stages of a spring bloom: a product of dying algae? Limnol Oceanogr 39:176–181

Holm-Hansen O, Lorenzen CJ, Holmes RW, Strickland JDH (1965) Fluorimetric determination of chlorophyll. J Cons Perm int Explor Mer 30:3–15

Hurley JP, Armstrong DE (1990) Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin. Limnol Oceanogr 35:384–398

Iturriaga R, Mitchell BG, Kiefer DA (1988) Microphotometric analysis of individual particle absorption spectra. Limnol Oceanogr 33:128–135

Jeffrey SW (1968) Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. Biochim Biophys Acta 162:271–285

Jeffrey SW, Wright SW (1994) Photosynthetic pigments in Haptophyta. In: Green JC, Leadbeater BSC (eds) The haptophyte algae. Clarendon Press, Oxford

Jensen A, Aasmundrud O, Eimhjellen KE (1964) Chlorophylls of photosynthetic bacteria. Biochim Biophys Acta 88: 466–479

King LL, Repeta DJ (1991). Novel pyrophaeophorbide steryl esters in Black Sea sediments. Geochim Cosmochim Acta 55:2067–2074

Kishino M, Takahashi N, Okami N, Ichimura S (1985) Esti-

mation of the spectral absorption coefficients of phytoplankton in the sea. Bull mar Sci 37:634–642

Llewellyn CA, Mantoura RFC, Brereton RG (1990) Products of chlorophyll photodegradation. 1. Detection and separation. Photochem Photobiol 52:1033–1041

Lopez MDG, Sykes PF, Huntley ME (1988) Pigment destruction by *Calanus pacificus*: impact on estimates of water column fluxes. J Plankton Res 10:715–734

Lorenzen CJ, Welschmeyer NA, Copping AE, Vernet M (1983) Sinking rates of organic particles. Limnol Oceanogr 28:766–769

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

Matile P, Duggelin T, Schellenberg M, Rentsch D, Bortlik K, Peisker C, Thomas H (1989) How and why is chlorophyll broken down in senescent leaves? Plant Physiol Biochem 27:595–604

Mitchell BG (1990) Algorithms using the absorption coefficient of aquatic particulates using the Quantitative Filter Technique (QFT). In: Spinrad R (ed) Ocean optics X, Vol. 1302. Society of Photo-optical Instrumentation Engineers, Bellinghausen, Washington, p 137–148

Oelze J (1985) Analysis of bacteriochlorophylls. In: Gottschalk G (ed) Methods in microbiology, Vol 18. Academic Press, London, p 257–284

Puchkova NN, Gorlenko VM (1982) A new green sulfur bacterium, *Chlorobium chlorovibrioides* nov. sp. Mikrobiologiya 51:118–124

Shuman FR, Lorenzen CJ (1975) Quantitative degradation of chlorophyll by a marine herbivore. Limnol Oceanogr 20: 580–586

Smith WO Jr, Codispoti LA, Nelson DM, Manley T, Buskey EJ, Niebauer HJ, Cota GF (1991) Importance of *Phaeocystis* blooms in the high-latitude ocean carbon cycle. Nature 352:514–516

SooHoo JB, Kiefer DA (1982) Vertical distribution of phaeopigments. 1. A simple grazing and photo-oxidative scheme for small particles. Deep Sea Res 29:1539–1551

Trees CC, Aiken J, Hirche HJ, Groom SB (1992) Bio-optical variability across the Arctic Front. Polar Biol 12:455–461

Vernet M (1991) Phytoplankton dynamics in the Barents Sea estimated from pigment budget models. In: Sakshaug E, Hopkins CCE, Oeristland NA (eds) Proceedings of the ProMare Symposium on Polar Marine Ecology. Polar Res 10:129–145

Vernet M, Lorenzen CJ (1987) The relative abundance of phaeophorbide *a* and phaeophytin *a* in temperate marine waters. Limnol Oceanogr 32:352–358

Wassmann P, Vernet M, Mitchell BG, Rey F (1990) Mass sedimentation of *Phaeocystis pouchetii* in the Barents Sea. Mar Ecol Prog Ser 66:183–195

Welschmeyer NA, Lorenzen CJ (1985) Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central North Pacific Gyres. Limnol Oceanogr 30:1–21

Ziegler R, Blaheta A, Guha N, Schonegge B (1988) Enzymatic formation of phaeophorbide and pyrophaeophorbide during chlorophyll degradation in a mutant of *Chlorella fusca* Shihira et Kraus. J Plant Physiol 132:327–332

This article was submitted to the editor

Manuscript first received: April 19, 1995
Revised version accepted: September 27, 1995