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19′-hexanoyloxyfucoxanthin may not be the appropriate pigment to trace occurrence and fate of *Phaeocystis*: the case of *P. globosa* in Belgian coastal waters

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Abstract

Two haptophycean strains were isolated from field samples collected in 2001 in Belgian coastal waters (southern North Sea) during the *Phaeocystis* monitoring program of the AMORE Project. The morphology and pigment composition of these two strains, one identified as *Phaeocystis globosa* and the other as *Imantonia rotunda*, were carefully examined. The comparative analysis of their pigment signature revealed the presence of two fucoxanthin derivatives, 19′-butanoyloxyfucoxanthin and 19′-hexanoyloxyfucoxanthin (but-fuco and hex-fuco) in *I. rotunda*, which were undetectable in *P. globosa*. A further comparison of pigments and phytoplankton from field samples showed no significant correlation between hex-fuco concentration and *P. globosa* biomass in the water column. Low concentrations of this pigment were, however, detectable before and at the end of the *Phaeocystis* bloom. The presence of *I. rotunda* in the area, overlooked by light microscopy, but isolated in pure culture from field samples, might explain the presence of this pigment. We conclude that hex-fuco is not the appropriate pigment to estimate *Phaeocystis* abundance and trace its trophic fate in Belgian coastal waters. These results also indicate that pigment analysis should be coupled with a precise identification of phytoplankton taxa present in field samples.

Keywords: *Phaeocystis globosa*; *Imantonia rotunda*; SEM; HPLC pigments

1. Introduction

The colony-forming haptophyte *Phaeocystis* recurrently forms massive blooms in the eastern Channel and Southern Bight of the North Sea during spring (e.g. Bätje and Michaelis, 1986; Cadée and Hegeman, 1986, 2002; Veldhuis et al., 1986; Lancelot et al., 1987; Lancelot, 1995; Breton et al., 2000). The uniqueness of *Phaeocystis* rests not only on the extremely high carbon biomass values reached by its blooms (up to 10 mg C l^{-1}) but primarily on its exceptional physiology which impacts the food-web

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structures and hence global biogeochemical cycles (Lancelot et al., 1994). Of particular importance is the existence of a complex polymorphic life cycle exhibiting phase alternation between different types of free-living cells (vegetative non-motile, vegetative flagellate and microzoospore) of 3–8 μm in diameter and colonies usually reaching several mm (Rousseau et al., 1994; Peperzak et al., 2000). This feature associated with a taxonomic controversy on the species status (Sournia, 1988; Baumann et al., 1994) may explain the confusion existing in the literature concerning the taxonomy of *Phaeocystis* in the North Sea. For some years, most researchers referred to *P. pouchetii* (Bätje and Michaelis, 1986; Cadée and Hegeman, 1986; Veldhuis et al., 1986; Lancelot et al., 1987). However, based on temperature tolerance and colony morphology, Baumann et al. (1994) reappraised the *Phaeocystis* literature dealing with the species status and concluded that *P. globosa* was the dominant blooming species in temperate waters. Apart from the colonies, *Phaeocystis* flagellated cells are difficult to identify with the light microscope. In addition to being small (3–5 μm), they lack distinctive morphological features since the haptonema, a characteristic organelle of the class, is not easy to detect under the light microscope. Moreover, the various fixatives used for preservation may damage the cells, rendering their enumeration somewhat imprecise. An accurate identification of the motile stage requires transmission or scanning electron microscopy for an examination of the scale covering (Puigserver et al., 2003) or RNA gene analysis (Zingone et al., 1999). In this direction Lange and Medlin (2002) recommended the development of oligonucleotide probes for a rapid and accurate identification of the single cell stage. On the other hand, there have been some attempts to quantify *Phaeocystis* from its pigment signature (Wright et al., 1996). The accessory pigment 19′-hexanoyloxyfucoxanthin (hex-fuco) has been reported as typical for *Phaeocystis* and its content relative to the more common fucoxanthin has been suggested as a proxy to estimate *Phaeocystis* abundance (Wright and Jeffrey, 1987; Bjørnland et al., 1988; Jeffrey and Wright, 1994; Jeffrey et al., 1997b; Llewellyn and Gibb, 2000). This approach has been challenged by observations pointing out the apparent lack or extremely low concentration of hex-fuco in some strains (Jeffrey and Wright, 1994; Vaulot et al., 1994) as well as a distinct variation in pigment pattern for colony and free-living cells (Buma et al., 1991). In general, the hex-fuco content of *P. globosa* cells is much lower, if not absent, compared to the two cold-water species *P. antarctica* and *P. pouchetii* (Buma et al., 1991; Vaulot et al., 1994). Furthermore, recent studies are reporting significant variation in the relative abundance of hex-fuco and fucoxanthin to chlorophyll-a in response to changing environmental factors (Van Leeuwe and Stefels, 1998; Llewellyn and Gibb, 2000; Schlüter et al., 2000). Altogether these data suggest that the use of marker pigments as 'fingerprint' of the *Phaeocystis* genus in field samples requires a prior examination of the pigment composition of the species occurring in a given area.

In this study we compare the pigment composition of 2 strains of haptophyte species, *P. globosa* and *Imantonia rotunda*, isolated from Belgian coastal waters during a spring phytoplankton bloom. The suitability of pigments as a tool to detect *P. globosa* occurrence in the Belgian coastal waters is further discussed based on a crossed comparison of pigment and taxonomic dominance observed in field samples with the pigment signature of cultivated species.

2. Materials and methods

2.1. Sample collection

Sampling was carried out at station 330 (51°26′N, 2°48′50E; depth 20 m) and station 435 (51°35′N, 2°47′50E; depth 35 m) in the Belgian coastal waters during AMORE 2001 cruises aboard RVs 'Belgica' and 'Zeeleeuw' (Fig. 1). These stations were sampled fortnightly during spring and monthly for the rest of the year, weather permitting. Samples were collected using a 10 L Niskin bottle, at subsurface, middle and near the bottom of the water column. Seawater samples (250–1500 ml) from the different depths were immediately filtered in triplicate using GF/F glass fibre filters (47 mm diameter, 0.7 μm nominal pore size) for HPLC pigment analysis. All filters were folded in aluminium foil and immediately stored in liquid nitrogen throughout the cruise until the return to

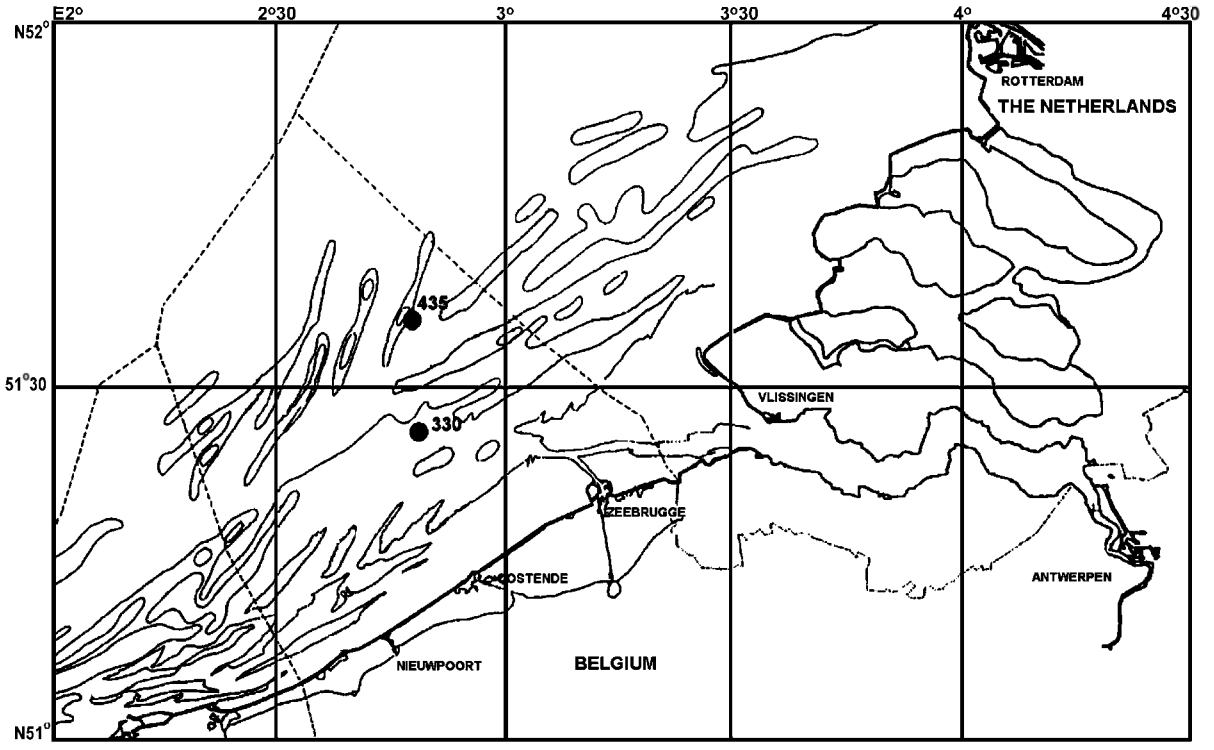


Fig. 1. Map of the Belgian coastal zone showing the locations of the two stations sampled during the AMORE 2001 cruises.

the laboratory where they were stored at −85 °C. Additional water samples (250 ml) were taken in triplicate, after mixing of the three depths, and were fixed with 1% (final concentration) glutaraldehyde for algal cell counting.

2.2. Isolation and maintenance of algal cultures

Cultures of *Phaeocystis* and *Imantonia* were prepared from surface seawater samples collected at station 330 during the *Phaeocystis* bloom in April 2001 (17 and 25 April). *Phaeocystis* colonies were collected under a binocular microscope with an open-end Pasteur pipette. They were inoculated in culture medium F20, prepared with sterile filtered seawater enriched as in Veldhuis and Admiraal (1987) and supplemented with antibiotics (sulphated streptomycin and penicillin) at a final concentration each of 35 mg per litre of culture medium. Cultures were grown at 10 °C under a 12h light:12h dark cycle at 100 μmole quanta m^{-2} s^{-1}. Culture maintenance was secured by weekly diluting an aliquot of the culture in fresh medium. At the same time another aliquot was carefully filtered under sterile conditions, on a Nuclepore filter (10 μm nominal porosity) before transfer in fresh medium. After three repetitions of this purification procedure (one per week), the axenic cultures were completely dominated by small flagellated cells, which SEM examination showed to be *Imantonia*. Samples of *Phaeocystis* colonies and *Imantonia* cells were collected for taxonomic identification (epifluorescence and scanning electron microscopy) and pigment analysis (HPLC). Samples for epifluorescence microscopy were fixed with 1% (final concentration) glutaraldehyde and stained with DAPI (Porter and Feig, 1980) at a final concentration of 2.5 μg ml^{-1}. Samples for scanning electron microscopy were preserved with 1% (final concentration) lugol-glutaraldehyde solution and stored at 4 °C in the dark until analysis. Aliquot of each culture was filtered on Whatman GF/F (0.7 μm) glass-fibre filters and stored in an ultra-cold freezer at −85 °C for 2 days prior to HPLC analyses.

2.3. Scanning electron microscope (SEM) analysis

Preserved sub-cultures were centrifuged on a plastic cell culture coverslip (Thermanox) coated with L-polylysine for better adhesion of material before being critical point dried, mounted on stubs and coated with gold. Observations of cell morphology and scales were made on a Hitachi S 4500 scanning electron microscope in Perpignan, France.

2.4. High performance liquid chromotography (HPLC) pigment analysis

The frozen filters were cut into small pieces (several mm × 1 cm) and sonicated in centrifuge tubes (on crushed ice), with 2 ml of 100% cold acetone for 2 × 15 s at 50 W using a Labsonic sonicator equipped with a 4 mm diameter probe inserted directly into the solvent. After sonication, samples were kept at 4 °C for 2 h before centrifugation for 3 min at about 700 g. Supernatants were filtered through a Millex SR 0.5 μm filter, transferred to 1 ml vials and placed in the autosampler (kept at 4 °C) prior to injection in the HPLC. Reversed-phase HPLC analyses were conducted based on the ternary gradient method of Wright et al. (1991) as described in Gasparini et al. (2000). Pigments were detected by absorption at 436 nm and identified by comparison of their retention time and spectra with standards. High-purity HPLC standards for chlorophyll-a, pheophytin-a, chlorophyll-c_3, fucoxanthin, alloxanthin, peridinin and 19′-hexanoyloxyfucoxanthin were obtained from the International Agency for ^{14}C determination (H¢rsholm, Denmark). Pheophorbide-a was obtained from the ICN Biomedicals, Inc. Other carotenoids were identified using retention time and spectra of well-characterised pigments (Jeffrey et al., 1997a). Our HPLC technique did not allow us to separate chlorophylls c_1 and c_2.

2.5. Phytoplankton enumeration and biomass estimate

Phytoplankton was analysed by an inverted microscope provided with phase contrast (ZEISS Sedival) in 5 ml sedimentation chambers after pre-concentration following the method of Colijn et al. (1990). Depending on cell density, optical fields or selected transects of the entire sedimentation chamber were counted at 200x and 400x magnification. Colonial *Phaeocystis* cells were free in our samples due to the dissolution of the colony matrix by the preserving agent. A conversion factor of 14.2 pgC per cell for colonial cells and of 10.8 pgC per cell for flagellated cells (microzoospores) was used to estimate *Phaeocystis* carbon biomass as recommended by Rousseau et al. (1990).

3. Results

Fig. 2 shows photographs of a *Phaeocystis* colony from a field sample (Fig. 2A), the *Phaeocystis* culture, with colonial forms and free-living cells (Fig. 2B–D) and the culture containing microflagellates, found later to be *Imantonia* (Fig. 2E–F), obtained under inverted and epifluorescence microscopy. Clearly the *Phaeocystis* colonies isolated from field samples (Fig. 2A; bright field, inverted microscopy) hold characteristics of *P. globosa*, i.e. spherical forms with cells evenly distributed (Baumann et al., 1994; Rousseau et al., 1994). Epifluorescence microscopy of the *Phaeocystis* colonial culture (Fig. 2B, C and D) shows typical colony forms of *P. globosa* as well (Fig. 2B) and flagellated and non-flagellated free-living cells of 3–4 μm in diameter (Fig. 2C and D). The microflagellate culture was dominated by very small flagellated cells of 2–3 μm in diameter (Fig. 2E and F) which need further scanning electron microscopy for identification.

3.1. Species identification: scanning electron microscopy (SEM)

SEM showed two different monospecific cultures. One was clearly of the *Phaeocystis*-type, with five ray star-like structures closely surrounding the cells (Fig. 3A). The stars and their attached filaments are of chitin (Chrétiennot-Dinet et al., 1997) and are characteristic of the genus. The small rounded cells observed in the *Phaeocystis* culture have two smooth flagella and a short haptonema (Fig. 3A, B and C). They are 2.5 to 4.2 μm in diameter and correspond to the so-called microzoospores cited by Kornmann (1955). Their scale morphology has been described in details by Parke et al. (1971) based on transmission

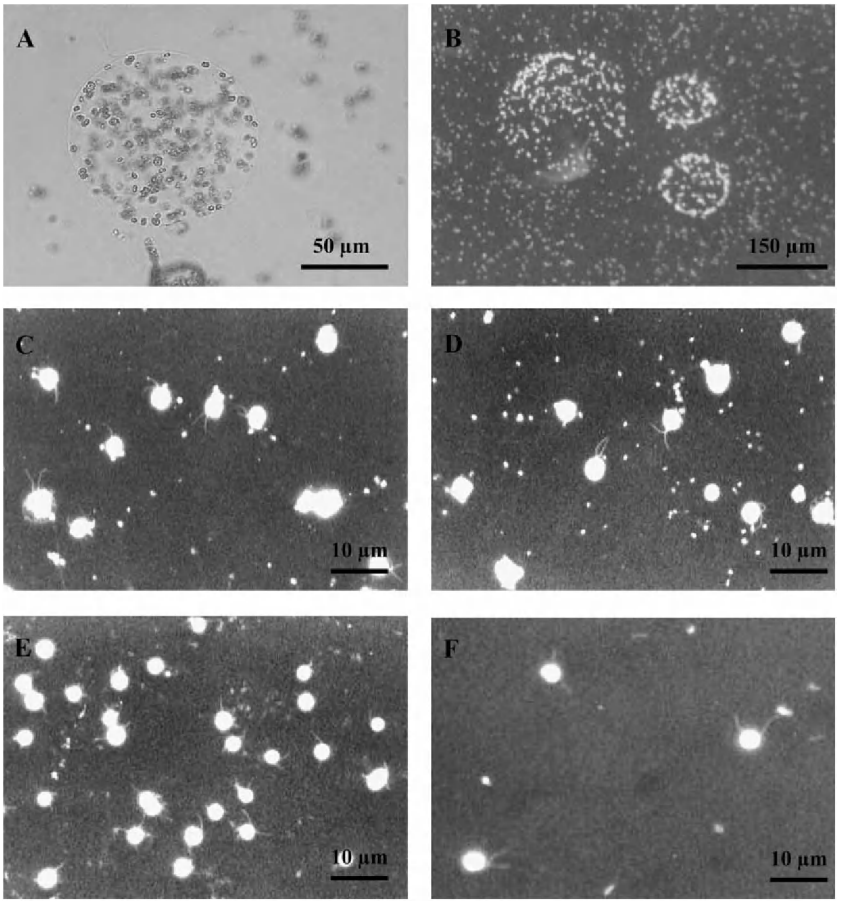
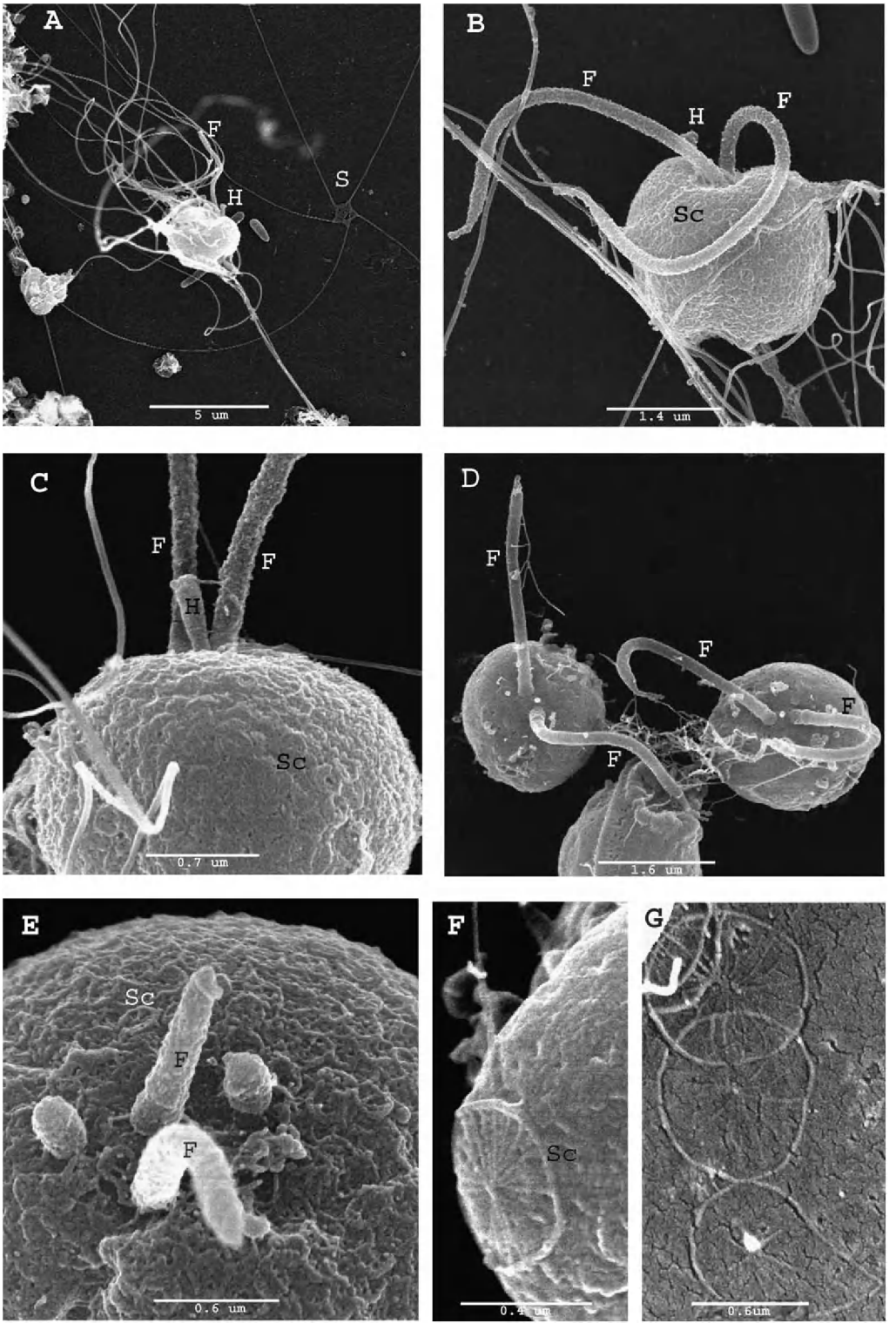


Fig. 2. (A) Inverted microscope photograph of a *Phaeocystis globosa* colony collected at station 330 in the Belgian coastal zone. (B-F) epifluorescence microscopy photographs of (B) *P. globosa* colonies, (C and D) free-living flagellated and non-flagellated cells from a *Phaeocystis* colonial culture, and (E and F) free-living flagellated cells from the microflagellate culture.

electron microscopy. In our culture, the length of the flagella was 4–5 µm (Fig. 3B), in accordance with the $1^{1/2}$ times the cell diameter mentioned in Parke et al. (1971). The haptonema (Fig. 3A, B and C) seems to be somewhat shorter (0.5 µm) than in the strains kept in the Plymouth collection and may have lost its distal swelling. The cell body is covered by small rounded scales varying from 0.13 to 0.18 µm in diameter (Fig. 3C and E) which, according to Zingone et al. (1999), correspond to the species *P. globosa* Scherffel. Parke et al. (1971) regarded the strains they examined and described as belonging to *P. pouchetii*, but a first analysis of the genome size and pigments placed them in a cluster of North European strains representing the true *P. globosa* (Vaulot et al., 1994). Molecular analyses of different *Phaeocystis* strains have shown that *P. pouchetii* and *P. globosa* are two distinct species (Medlin et al., 1994; Edvardsen et al., 2000) and that the description of the motile stages by Parke et al. (1971) is probably that of *P. globosa* (Edvardsen et al., 2000). It is now accepted that truly colonial forms belong to three different species: *P. pouchetii*, *P. antarctica* and *P. globosa* (Lange et al., 2002). Because of the shape of the colony, the distribution of the cells in the colony and the geographical occurrence of the strain isolated, whose motile form definitely belongs to the genus *Phaeocystis*, we identify it as *P. globosa*.

The cell-type identified in the microflagellate culture is very similar in shape and size (2–2.5 µm), but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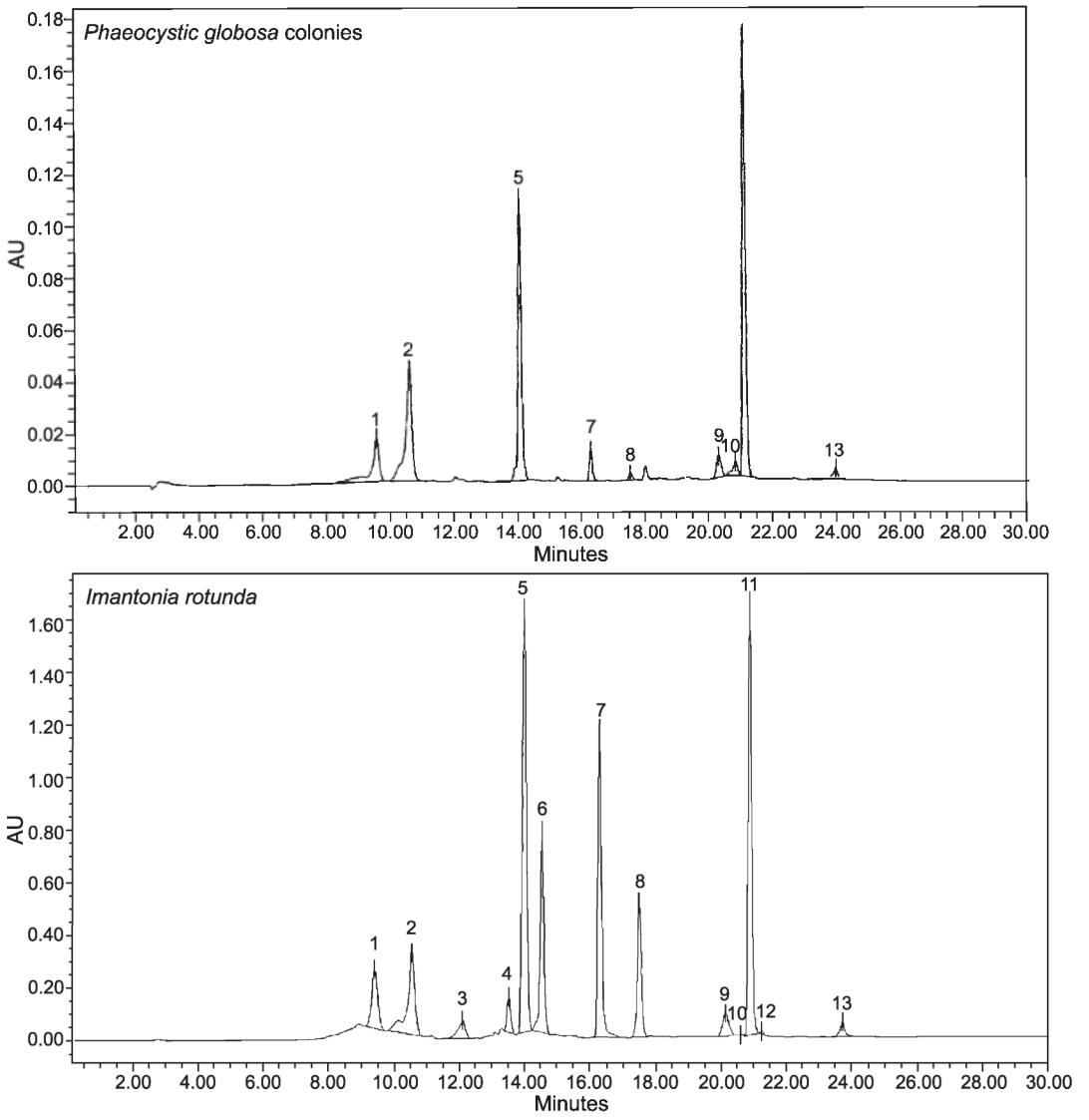

Fig. 4. HPLC absorbance chromatograms of cultures of *Phaeocystis globosa* colonies and *Imantonia rotunda* made at 436 nm. Retention time is given in minutes. AU = Absorption Units. Pigments: 1, chlorophyll-c_3; 2, chlorophyll-$c_1 + c_2$; 3, cis-fucoxanthin; 4, 19′-butanoxyloxyfucoxanthin; 5, fucoxanthin; 6, 19′-hexanoxyloxyfucoxanthin; 7, diadinoxanthin; 8, diatoxanthin; 9, phytylated chlorophyll-c-like; 10, chlorophyll-a allomer; 11, chlorophyll-a ; 12, chlorophyll-a epimer; 13, β,β-carotene.

the haptonema is missing (Fig. 3D). Moreover scales, when present, are completely different (Fig. 3F and G) and correspond to those described for *Imantonia rotunda* Reynolds (Reynolds, 1974; Green and Pienaar, 1977). The two flagella are smooth, distally tapered, slightly unequal, with the bases widely diver-

Fig. 3. Scanning electron micrographs of motile cells of (A-C and E) *Phaeocystis globosa* and (D and F-G) *Imantonia rotunda*. *Phaeocystis globosa*: (A) a cell surrounded by many filaments and a star-like structure (S); (B) a cell with two flagella (F) a haptonema (H) and its scale covering (Sc); (C) detail of another cell showing the flagella departure, the haptonema and scales; (E) detail of scales (Sc) on a cell with 4 growing flagella (F); this cell is probably undergoing a division. *Imantonia rotunda*: (D) two cells with their two sub-equal flagella widely divergent and no haptonema; (F) a scale still on the body cell; (G) three loose scales. All the scales show the typical pattern of radiating ridges.

gent (Fig. 3D) as in the revised diagnosis by Green and Pienaar (1977). The body scales are 0.52 to 0.8 μm in diameter and show the typical pattern of radiating ridges (Fig. 3F and G). This scale pattern is particularly informative and leaves no doubt about the identity of this species, considered as *Imantonia rotunda*.

3.2. Pigment signature

Absorbance chromatograms of the two cultures are shown in Fig. 4. In both cases fucoxanthin (fuco) was the dominant carotenoid pigment. Both cultures contained chlorophyll-a (chl-a), chlorophyll-c_3 (chl-c_3), chlorophyll-c_1+c_2, diadinoxanthin, diatoxanthin, phytylated chlorophyll-c-like and β,β-carotene. The main difference between the two haptophyte species is the presence in *I. rotunda* of two fucoxanthin derivatives, 19′-butanoyl and 19′-hexanoyloxyfucoxanthins (but-fuco and hex-fuco), whereas in *P. globosa* they were both undetectable. Table 1 compares, for each species, values of carotenoids to chlorophyll-a ratios which are commonly used as pigment signature for haptophytes.

3.3. In situ phaeocystis globosa biomass and biomarker pigments

Microscopic observations of field samples revealed the presence of *P. globosa* at both locations, whereas *I. rotunda* was not identified.

P. globosa bloomed impressively in spring (Fig. 5). *Phaeocystis* cells appeared in March and their biomass increased exponentially until reaching a peak in mid-April, which was higher at station 330 than at station 435 (382 and 249 μgC l^{-1}, respectively). At the end of June, *Phaeocystis* disappeared from the water column until September, when a low biomass of *Phaeocystis* was observed (16 and 25 μgC l^{-1} at stations 330 and 435, respectively).

Table 1
Haptophyte biomarker pigments of the two strains isolated from Belgian coastal waters expressed as ratios to chlorophyll-a

Strain	Ratio to Chl-a			
	Chl-c_3	But-fuco	Fuco	Hex-fuco
Phaeocystis globosa	0.208	0.000	0.350	0.000
Imantonia rotunda	0.269	0.060	0.746	0.296

As expected from the pigment analysis of *P. globosa* strain (Fig. 4; Table 1), the field biomass of *P. globosa* was well correlated with concomitant measurements of chlorophyll-c_3 and fucoxanthin concentrations but not with hex-fuco (Table 2). Concentration of but-fuco was under the detection limit in all field samples.

Chlorophyll-c_3 was always detected in our samples except in November (Fig. 5A–B). The concentration of this pigment was relatively high during *Phaeocystis* blooming (over 0.20 μg l^{-1} and up to 1.90 μg l^{-1} at the maximum biomass; Fig. 5A and B) as compared to periods when *P. globosa* was absent (below 0.07 μg l^{-1}; Fig. 5A and B).

Fucoxanthin (Fig. 5C–D) was measured throughout the year as it is the dominant pigment of brown algae (e.g. diatoms, haptophytes and chrysophytes). Fucoxanthin concentration increased from mid-February onwards, with the early spring diatom bloom, and reached its maximum concentration (5.70 μg l^{-1}) in April at the time of maximum *Phaeocystis* biomass (Fig. 5C and D). In mid-June we observed a second peak of fucoxanthin at station 330 (4.22 μg l^{-1}; Fig. 5C), which corresponded with the occurrence of the diatoms *Guinardia delicatula* and *Rhizosolenia imbricata* (not shown).

Hex-fuco (Fig. 5E–F) was not detected during the maximum *Phaeocystis* bloom. Significant concentrations, although relatively low (0.02 – 0.06 μg l^{-1}; Fig. 5E–F), were recorded outside the *Phaeocystis* spring bloom period, in January, May (after the *Phaeocystis* bloom) and in early September.

4. Discussion

Light microscopy and scanning electron micrographs of *Phaeocystis* cells isolated from the Belgian coastal waters during the spring bloom suggest that the main *Phaeocystis* species occurring in these waters is *P. globosa*. This gives support to Baumann et al. (1994)'s reappraisal of the species status of *Phaeocystis* in the North Sea (sometimes wrongly reported in the older literature as *P. pouchetii*) based on colony morphology and temperature tolerance.

The pigment composition of the cultured strain of *P. globosa* examined in the present work is charac-

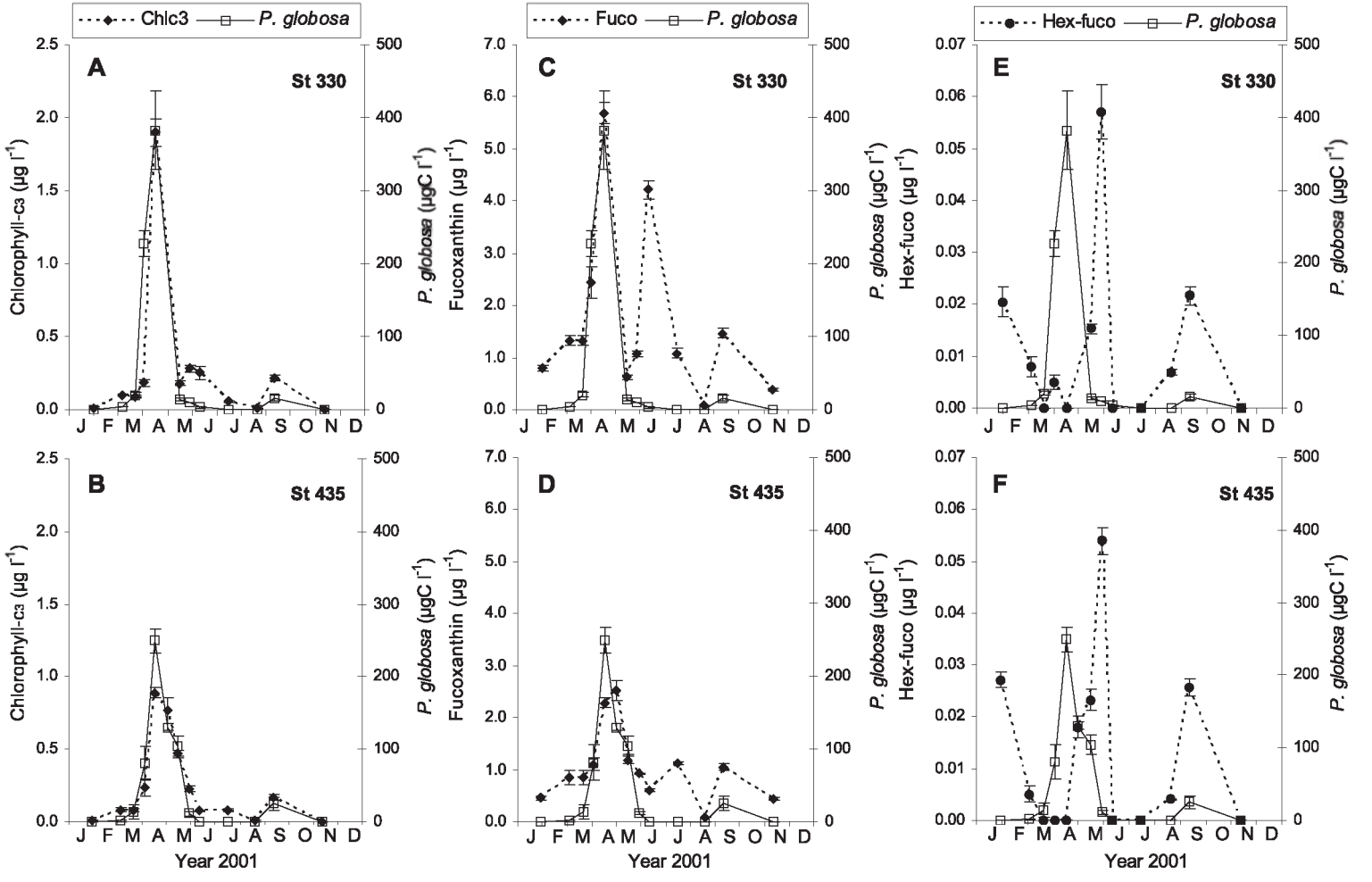

Fig. 5. Seasonal evolution of *P. globosa* cell carbon biomass (µgC l^{-1}): (A and B) chlorophyll-c_3, (C and D) fucoxanthin, and (E and F) 19′-hexanoyloxyfucoxanthin (µg l^{-1}) at stations 330 and 435 of the Belgian monitoring grid, in 2001.

Table 2
Correlations (Spearman rank) between *Phaeocystis globosa* carbon biomass and haptophyte specific pigments

		Chl-c_3	Fuco	Hex-fuco
Phaeocystis globosa	r_s	0.862	0.724	0.074
	n	25	25	25
	p	< 0.001	< 0.001	> 0.05

terised by the presence of chlorophyll-c_3 and fucoxanthin, the latter pigment being surprisingly dominant. The fucoxanthin to chlorophyll-a ratio (Table 1) is comparable to values reported for diatoms (Mackey et al., 1996; Llewellyn and Gibb, 2000). As the phytoplankton spring bloom community of Belgian coastal waters mainly consists of diatoms and colonies of *P. globosa* (Lancelot et al., 1998; Rousseau et al., 2002) we conclude that fucoxanthin has no value as a biomarker of *P. globosa* occurrence in field samples. Chlorophyll-c_3 on the other hand has been reported as a ubiquitous pigment of *Phaeocystis* species (Claustre et al., 1990; Vaulot et al., 1994; Breton et al., 2000; Cotonnec et al., 2001) but it is also found in many other haptophytes (Jeffrey and Wright, 1994) some diatoms (Stauber and Jeffrey, 1988; Llewellyn and Gibb, 2000) and one chrysophyte (Vesk and Jeffrey, 1987). Microscopic observations of our samples revealed the co-occurence with *P. globosa* of three diatom species known to contain chlorophyll-c_3. These are *Thalassionema nitzschioides*, *Rhizosolenia setigera* and *Asteroplanus karianus*. Their total biomass represented less than 3% of *P. globosa* biomass during the bloom. Hence most of the chlorophyll-c_3 found in our samples could be attributed to *P. globosa*, as also suggested by the significant correlation between chlorophyll-c_3 concentration and *P. globosa* biomass. We admit, however, that very few diatom species among those reported in our samples have been examined for their pigment composition. Further analysis of pigment composition and variability of major diatom species (e.g. *G. delicatula*, *R. imbricata* and *Pseudo-nitzschia delicatissima*) blooming during the *Phaeocystis* bloom is needed before chlorophyll-c_3 can safely be used as an indicator of *P. globosa* in field samples.

One major result of the pigment analysis of the *P. globosa* strain is the absence of the two fucoxanthin derivatives, hex-fuco and but-fuco. The existing literature on the pigment composition of cultured strains of *Phaeocystis* shows a wide inter-specific as well as intra-specific variation of these two pigments. Hex-fuco is a dominant carotenoid in *P. antartica* with but-fuco being present at trace levels (Buma et al., 1991; Vaulot et al., 1994). Both pigments are also characteristic of *P. pouchetii* (Wright and Jeffrey, 1987; Jeffrey and Wright, 1994; Llewellyn and Gibb, 2000) and were used as indicators of *P. pouchetii* in field samples (Wright and Jeffrey, 1987). However Gieskes and Kraay (1986) reported that a strain of *P. pouchetii* isolated from the southern part of the North Sea did not contain hex-fuco (see comment in Bjørnland et al., 1988). Likewise Jeffrey and Wright (1994) reported that this pigment was absent in one of the six strains of *P. pouchetii* they analysed. Vaulot et al. (1994) identified both pigments in North European strains including *P. globosa*, but at a much lower concentration than in tropical or Mediterranean strains. The fact that none of the acyl-fucoxanthin pigments were identified in our culture can thus be considered a distinctive feature of *P. globosa* in Belgian coastal waters. This is confirmed by field observations showing no correlation between hex-fuco concentrations and *P. globosa* biomass. The relatively low concentrations of hex-fuco measured in May at the very end of the *P. globosa* spring bloom (Fig. 4) may have resulted from nutrient limitation as suggested by Buma et al. (1991). These authors indeed observed a relative increase of hex-fuco during the stationary stage of a *Phaeocystis* culture isolated from the Southern Bight of the North Sea, whereas this pigment was not detected during exponential growth. Recently, variations in light and iron conditions have also been proved to result in fluctuations of hex-fuco content in *Phaeocystis* sp. of several orders of magnitude (Van Leeuwe and Stefels, 1998; Schlüter et al., 2000). Records of hex-fuco in our field samples may also point to the presence of another hex-fuco-containing haptophyte species, such as *I. rotunda*. Indeed this species was accidentally isolated with *Phaeocystis* colonies from the April samples. The strain of *I. rotunda* that we isolated contained, in addition to its major carotenoids (fucoxanthin and hex-fuco), chlorophyll-c_3 and trace levels of but-fuco. The latter was not clearly identifiable in field samples, probably because of its low concentration. We assume then that the *I. rotunda* abundance was negligible compared to that of *Phaeocystis*. As

the two species cannot be easily distinguished with optical microscopy (inverted or epifluorescence), a confusion between free-living flagellated cells of *P. globosa* and *I. rotunda* was possible (Fig. 2C and D and Fig. 2E and F). This species has been previously mentioned in Belgian coastal waters (M'harzi et al., 1998) in samples collected in February around sand banks of the Belgian coast. It was first isolated by Reynolds from surface waters near Bear Island (southwest of Spitsbergen), and has been found in many places: in the region of the Shetland Islands, the northwest of Ireland, the Plymouth area, the Mid-Channel (Reynolds, 1974), in the Friday Harbor area, Washington (Green and Pienaar, 1977), in the offshore waters of the East Australian current (Hallegraeff, 1983), in the Kiel Bight and Kiel Fjord (Jochem, 1990) and in the Inner Oslofjord (Backe-Hansen and Throndsen, 2002). It is then recorded in places where *Phaeocystis* is also present and it is probable that the two species co-occur but that *I. rotunda* is usually overlooked during *Phaeocystis* surveys.

5. Conclusion

This study confirms that there is a far greater range of pigment compositions across haptophyte species than originally reported. Recently the 4 haptophyte pigment types suggested by Jeffrey and Wright (1994) have been expended to eight (Zapata et al., 2004). Pigment distribution in *P. globosa* differs from that of other *Phaeocystis* species by the absence of hex-fuco and but-fuco. This result precludes the use of hex-fuco as an indicator of *P. globosa* in phytoplankton samples (Breton et al., 2000; Meyer, 2002) and also as a biomarker of the ecological fate of *Phaeocystis* in the pelagic and the sediments. Especially, it challenges conclusions of *Phaeocystis* resistance to copepod grazing in Belgian coastal waters and adjacent areas based on the absence of hex-fuco in the copepod diet (Breton et al., 1999; Gasparini et al., 2000). Moreover, the pigment suite of *P. globosa* is shared by some chlorophyll-c_3 containing diatoms of the Belgian coastal waters, which makes the interpretation of pigments in field samples difficult. *I. rotunda* which was identified in our samples thanks to electronic microscopy also contains chlorophyll-c_3 as a major pigment with fucoxanthin and the two acyl-fucoxanthins. These pigments are also characteristic of a marine chrysophyte and three bloom-forming dinoflagellates (Vesk and Jeffrey, 1987; Bjørnland and Liaaen-Jensen, 1989). We thus conclude that the pigment signature of *P. globosa* is not specific enough for its safe identification in natural mixed assemblages. More generally, we recommend that HPLC pigment analysis of field samples should always be coupled with microscopic studies of phytoplankton taxa from representative samples, and combined with pigment signature analysis of the major taxa present.

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