Published in the Proceedings of the SPIE International Symposium on "Optics and Photonics: Remote sensing of the coastal oceanic environment" held in San Diego, USA, 31st July-1st August 2005

Optical Properties of algal blooms in an eutrophicated coastal area and its relevance to Remote Sensing.

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ABSTRACT

The Southern Bight of the North Sea is characterised by a large influence of river inputs, which results in eutrophication of the area. High concentrations of plankton biomass and suspended matter have been reported for this area, in relation with blooms of different species and resuspension of bottom sediments. In spring the haptophyte *Phaeocystis globosa* blooms throughout the area reaching up to 30 mg Chlorophyll m^{-3} or more nearshore. This event is followed in June by red tides of the dinoflagellate *Noctiluca scintillans*. These blooms are concurrent with different species of diatoms. The strong optical signature of these blooms is clear to human observers making them potentially detectable in satellite imagery. As a first step in this direction, sampling has been carried out in the area, during *Phaeocystis* and *Noctiluca* blooms in 2003 and 2004. Phytoplankton pigments and inherent optical properties (particle, detrital and phytoplankton absorption) have been measured spectrophotometrically, and *in situ* using an ac-9 for total absorption and particle scattering. Field data were compared with optical properties of pure species obtained in laboratory. In parallel, water-leaving reflectance has been also measured. In this paper we characterise the optical signatures of diatoms, *Phaeocystis* and *Noctiluca* and their contribution to total absorption. The impact on water-leaving reflectance spectra is evaluated; in order to assess the conditions in which remote sensing can provide information for monitoring the timing, extent and magnitude of blooms in this coastal area.

Keywords: HAB, *Phaeocystis*, *Noctiluca*, diatoms, optical properties, water-leaving reflectance.

1. INTRODUCTION

The Southern Bight of the North Sea is a highly eutrophicated area as a consequence of nutrient input from several large river discharges [1,2,3]. This high nutrient input corresponds to anthropogenic nitrate excess, and results in an enhancement of the phytoplankton blooms and a shift in phytoplankton species dominance; from diatom towards flagellate-dominated community. In the Southern Bight of the North Sea, blooms of undesirable species such as the large colony-forming *Phaeocystis globosa* in spring[3] are followed by red tides of the dinoflagellate *Noctiluca scintillans* in June (Dr. Elsa Breton, personal communication). Both *Phaeocystis* and *Noctiluca* co-occur with diatoms at various proportions. These blooms although not toxic are reported as Harmful Algal Blooms (HAB) and have a negative impact on the environment in spring, especially on the water quality and tourism, due for example to *Phaeocystis* foam accumulation on the beaches [3,4].

These organisms reach high cellular densities resulting in a strong discoloration of the water clear to human observers. *Phaeocystis* blooms are associated with high concentrations of pigments, turning the water into a brownish colour whilst the accumulation of *Noctiluca* cells at surface imparts the water a reddish colour (Figure 1).

As a consequence, it is expected that both *Phaeocystis* and *Noctiluca* can be detected with satellite imagery. Remote sensing signals have been criticised for providing only bulk composite signals for a given water mass where the signatures for different phytoplankton species are difficult to discriminate[5]. Moreover, in nearshore, estuarine and inland waters, suspended sediments and dissolved organic compounds make the optical properties even more complex. Recent approaches have proved that partial discrimination of species based on absorption characteristics is possible[6,7], at least

for in-water optical measurements. Actually, fourth-derivative analyses have been used to efficiently discriminate between toxic dinoflagellates and other phytoplankton species based on a specific pigment signature[7].

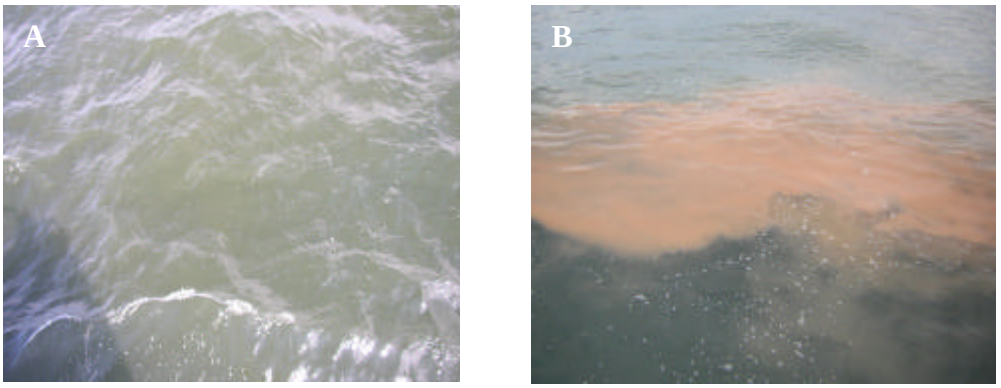

Figure 1: Photographs of A) *Phaeocystis globosa* (May 2004, courtesy of Management Unit of the North Sea Mathematical Models) and B) *Noctiluca scintillans* (29/6/2005) blooms in the Southern Bight of the North Sea.

In this paper the optical signatures of these two bloom-forming species as well as diatoms are investigated. Measurements in the field and on pure cultures are used in order to better understand the causes of variation. Using remote sensing reflectance data, the possibility of detecting these signatures from space is explored. Discrimination of the different taxonomic groups on the basis of their absorption spectra is a challenge for the development of satellite imagery. This is a first approach to improve the knowledge of the optical characteristics of two important HAB species in the area and to provide information on the geographical extent and magnitude of blooms.

2. METHODS

2.1. Field samples

Sampling was conducted in Belgian and adjacent coastal waters during 3 campaigns in April-May, July 2004 and July 2005 aboard RV Belgica. Sampling stations are reported in Figure 2.
At all stations a 20 L seawater sample was taken at the surface for: phytoplankton absorption, High Performance Liquid Chromatography (HPLC) chlorophyll *a* and pigment composition, phytoplankton community composition and ac-9 measurements. Filtration was performed immediately after sampling and ac-9 measurements were made just after filtration.

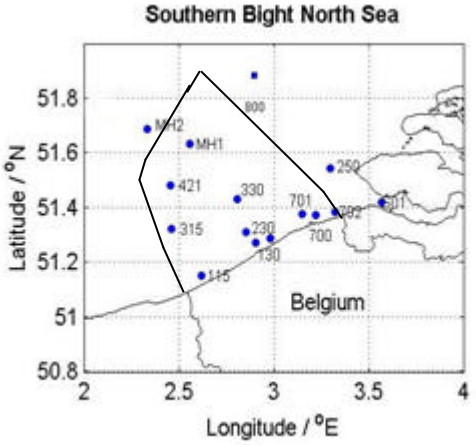

Figure 2: Sampling stations in the Belgian Coastal Zone and adjacent water in the Southern Bight of the North Sea.

2.2. Cultures

Phaeocystis globosa is a prymnesiophyte whose life cycle includes an alternance between free-living cells and mucilaginous colonies of different sizes[8] and *Noctiluca scintillans* is an heterotrophic dinoflagellate which is commonly found during June and July in the area forming patches of orange-red colour, especially near the coast during sunny, calm sea conditions. The optical signatures of these main blooming species were determined on pure cultures and compared with those of two common diatoms blooming in the area, the early spring *Skeletonema costatum* and *Guinardia delicatula* concurrent of *Phaeocystis* in spring[9].

Pure strains of *Phaeocystis globosa, Skeletonema costatum* and *Guinardia delicatula* were isolated from the central Belgian Coastal Zone. They were maintained in a culture room at 8°C under a 10h:14h light:dark cycle at a light intensity of 100 µmol quanta m^{-2} s^{-1}. Growth medium consists of 0.2 µm filtered seawater enriched with F20 medium modified[10].

Noctiluca scintillans cells were isolated from the field during the July 2004 cruise at different sampling stations. They were concentrated and maintained at seawater temperature. Sampling for light absorption and HPLC was carried out.

2.3. Chlorophyll *a*, pigment and phytoplankton analyses.

Between 0.05 and 1 L of seawater were filtered onto 25 mm glass fiber filters (Whatman GF/F) for HPLC chlorophyll *a* determination. The filters were kept in liquid nitrogen on board and then stored at –80°C in the laboratory until analysis. HPLC determination was conducted following[11] modified[12]. Chlorophyll *a* was detected by absorption at 436 nm and identified based on comparison of retention time and spectra with standards.

Phytoplankton samples were preserved with 1% of a Lugol:Glutaraldehyde solution, stored in the dark at 4°C. Phytoplankton was analysed with an inverted microscope (Leitz Fluovert).

2.4. Phytoplankton light absorption

Seawater was filtered onto a 25 mm glass fiber filter (Whatman GF/F), the volume varied depending on sample particle load from 0.05 to 1 L. Filters were kept in liquid nitrogen on board and then stored at –80°C in the laboratory until analysis. The absorbance spectra of particles $OD_P(l)$ and non-algal particles $OD_{NAP}(l)$ retained on the filter was determined following the Transmittance-Reflectance method[13]. Transmittance and reflectance were measured between 300 and 800 nm with a Uvikon 930 dual beam spectrophotometer equipped with a 6 cm-integrating sphere. Pathlength amplification was corrected using an algorithm[14] which has been validated for several phytoplankton species and detrital particles. To obtain the absorbance spectrum of non-algal particles retained in the filter $OD_{NAP}(l)$, the filter was bleached with a solution of sodium hypochlorite (0.13% active chlorine)[15]. Absorbance values at each wavelength were converted into absorption coefficients as:

$$a_{P/NAP}(l) = 2.303 * OD_{P/NAP}(l) / X \quad (1)$$

where X is the ratio of filtered volume to the filter clearance area.

No correction to absorption for scattering in the near infrared was performed since the T-R method has been proved to correct for scattering and because there is also evidence that some mineral particles absorb in the near-infrared region[16]. The $a_{NAP}(l)$ spectra was corrected by fitting an exponential function to the data between 340 and 750 nm.

The phytoplankton absorption coefficient $a_{ph}(l)$ was obtained from

$$a_{ph}(l) = a_P(l) - a_{NAP}(l) \quad (2)$$

The spectral fourth derivative was computed for each species spectrum to resolve the position of the absorption maxima attributable to photosynthetic pigments[7].

2.5. ac-9 measurements

At all stations total non-water absorption a(λ) and beam attenuation c(λ) coefficients were measured with an ac-9 profiler (WetLabs, Inc). The spectral resolution consists of the following 9 bands: 412, 440, 488, 510, 555, 630, 650, 676 and 715 nm.

Water was pumped from a 20L bottle sample filled with surface seawater, into the ac-9 that was placed vertically in a thermoregulated bath with circulating seawater. Data were recorded during 2 minutes, and then the median was taken over 0.5-minute noise-free data. Temperature and salinity corrections were performed on the raw data[17]. Absorption was corrected also for scattering by subtracting a(715) from a(λ)[18].

The particle scattering coefficient $b_p(\lambda)$ is obtained from

$$b_p(\lambda) = c(\lambda) - a(\lambda) \quad (3)$$

A pure water calibration was performed daily using freshly produced Milli-Q water, to check for deviations from the annual WetLabs calibration[19].

2.6. Reflectance

Water-leaving radiance reflectance, r_w, is calculated from simultaneous above-water measurements of downwelling irradiance, E_d^{0+}, upwelling radiance, L_{sea}^{0+}, and sky radiance, L_{sky}^{0+}, using three TriOS-RAMSES hyperspectral spectroradiometers:

$$r_w = \frac{p\left(L_{sea}^{0+} - r_{sky} L_{sky}^{0+}\right)}{E_d^{0+}} \quad (4)$$

where r_{sky} is the reflection coefficient for the wave-roughened air-water interface. This corresponds to "Method 1" of the NASA protocols[20]. The instruments are mounted on a steel frame with zenith angles of the sea- and sky-viewing radiance sensors of 40°. The frame is fixed to the prow of the ship, facing forwards to minimise ship shadow and reflection. The ship is manoeuvred on station to point the radiance sensors at a relative azimuth angle of 135° away from the sun. More information on this system and on data processing can be found in [21].

2.7. *Noctiluca* red tide sampling

On 29 June 2005 a big patch or front of *Noctiluca scintillans* approximately 10m wide and 100m long was encountered by RV Belgica (Figure 3). By crossing the patch, water-leaving reflectance measurements were made just before, in and after crossing the *Noctiluca* patch. Also ac9 measurements were done and samples taken for phytoplankton absorption and HPLC pigment determination.

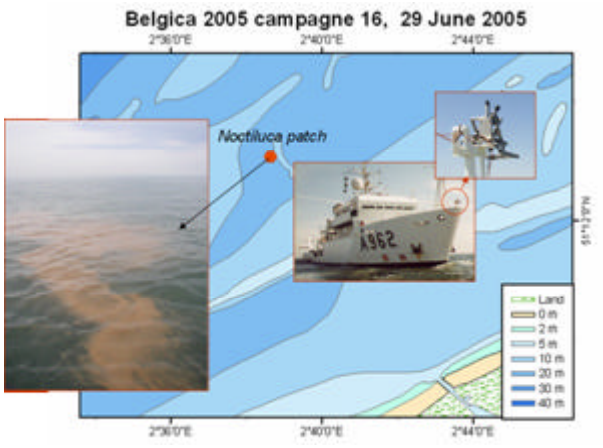

Figure 3: Position of the *Noctiluca* patch encountered by RV Belgica on 29 June 2005 and measured by a combination of 3 TriOS-RAMSES radiance sensors and ac9 absorption and scattering meter.

3. RESULTS

3.1. Optical properties of natural communities.

3.1.1. Seasonal variation.

Average phytoplankton absorption spectra of April-May and June cruises (Figure 4) show clear differences in shape, indicating a different species composition. The blue maximumin July is shifted towards 400 nm probably indicating phaeopigment contribution, while in April-May is at 440 nm. Also the blue/red band ratio is 1.3 in April-May and 2.8 in July. The HPLC pigment results show a predominance of chl $c_{(1+2)}$, chl c_3 and fucoxanthin during April-May and fucoxanthin and peridinin in July (Figure 5A). This will suggest a community dominated by diatoms and prymnesiophytes in April-May and diatoms and dinoflagellates in July. In agreement, phytoplankton counts data confirms that the dominant species are *Phaeocystis* and the diatom *Guinardia delicatula* in April-May (Figure 5B) and *Noctiluca* and *Guinardia delicatula* in July (data not shown). Note that *Noctiluca* is a non-pigmented dinoflagellate that is only possible to identify by microscope count.

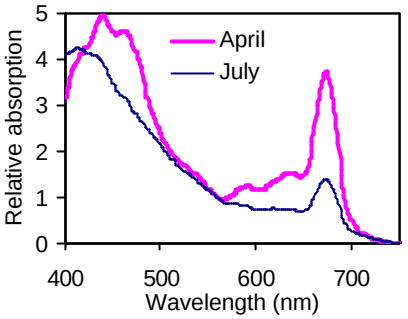

Figure 4: Mean phytoplankton absorption spectra normalised to 560 nm, for April-May and July cruises.

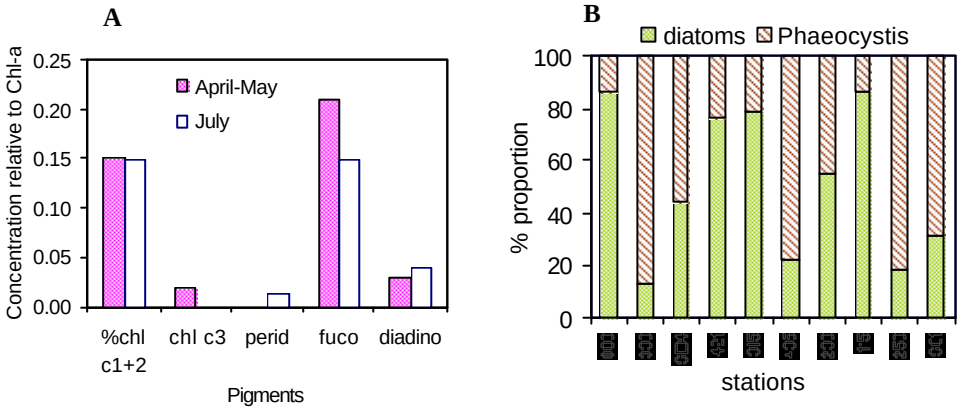

Figure 5: A) HPLC pigment composition of representative samples from April-May and July cruises, chl $c_{(1+2)}$: chlorophyll $c_{(1+2)}$, chl c_3: chlorophyll c_3, perid: peridinin, fuco: fucoxanthin, diadino: diadinoxanthin and B) Relative proportion of *Phaeocystis* and diatom cell density at representative stations sampled during the April-May cruise.

3.1.2. Species-dominated characteristics.

While clear differences are observed between absorption spectra measured in April-May and July (Figure 4), discrimination of the different taxonomic groups on basis of their absorption spectra is much less evident when comparing spectra of samples with different dominant groups (Figure 6A). However, absorption differences exist in the blue region, especially at 410 and 480 nm, allowing a distinction of *Noctiluca* from Phaeocystis and the diatoms. Between 500 and 600 nm, measured spectra are indeed very similar even if the dominating group is changing.

Scattering data obtained with ac9 (Figure 6B) show that during April-May the *Phaeocystis*-dominated assemblage has much higher scattering than the diatom-dominated assemblage. This is probably related to the range of sizes of *Phaeocystis* including small flagellates (3µm) up to big colonies (2mm) compared with the range of diatoms (11 to 84 µm). Phytoplankton scattering is however lower than that measured for the Noctiluca-dominated assemblage of July.

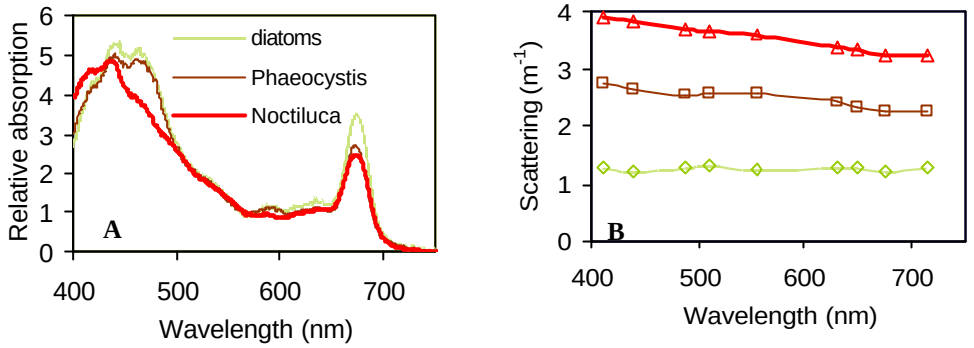

Figure 6: Measured A) Relative phytoplankton absorption and B) Total scattering for different groups dominating the field samples.

3.1.3. *Noctiluca* optical characteristics.

Results of optical properties measured at a *Noctiluca* red tide event the 29th June 2005 (Figure 7), show that absorption is dominated by a maximum at 488 nm and a secondary peak at 630 nm. These maxima correspond to absorption by carotenoids and chlorophyll c respectively[22] and are indicative of the kind of food that *Noctiluca* is eating. The shape of this spectrum is different from the one found for a field sample with many species (Figure 6A), suggesting that pure *Noctiluca* absorption features are probably masked by absorption from other species in that figure. The scattering values are high compared to those found in a mixed field sample. The lowest values are found in the blue part of the spectrum and the highest in the red part. It is interesting to note that the highest light absorption is found in the blue and the highest scattering in the red part of the spectrum.

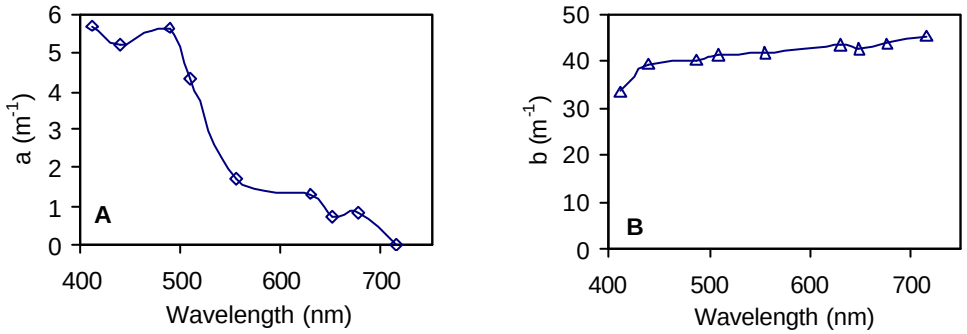

Figure 7: ac9 A) absorption and B) scattering measurements of a *Noctiluca* red tide.

3.2. Experiments in cultured species

Measurements were made on pure cultures of diatoms, *Phaeocystis* and *Noctiluca* in order to study their spectral signature and to better understand the observed variability of absorption spectra in the field samples.

3.2.1. *Phaeocystis* and diatoms.

The absorption spectra of the species are shown in Figure 8. The shape of the absorption spectrum of *Phaeocystis* differs from that of diatoms, especially at 403, 460, 480 and 585 nm, wavelengths that corresponds to absorption of phaeophytin-a, chl c_3, fucoxanthin or diadinoxanthin and chl c_3 respectively[22]. Even if *Phaeocystis* and diatoms both contain Fucoxanthin[22], the proportions are different en each species. This suggests that by using ratios between these wavelengths, these two groups could be discriminated. It is interesting to note that both diatoms show enhanced absorption around 410 nm probably as a result of phaeopigment presence.

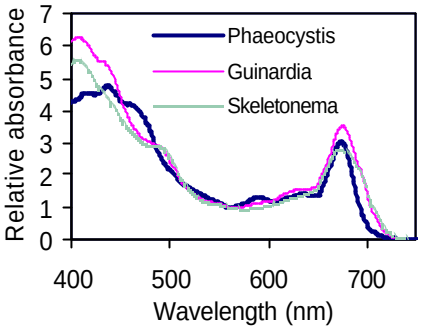

Figure 8: Absorption spectra of *Phaeocystis*, *Guinardia* and *Skeletonema* pure cultures.

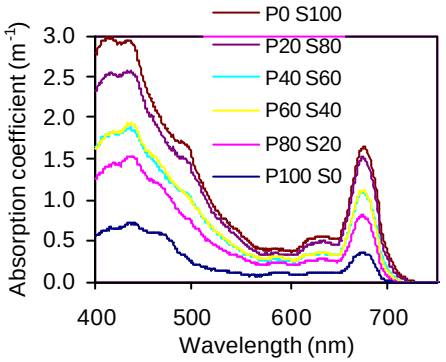

Figure 9: Absorption spectra of varying proportions of *Phaeocystis* (P) and *Skeletonema* (S) pure cultures. The proportions are given.

In order to investigate the absorption spectra of a mixed species assemblage, as found in the field, and the variation of their shape, *Phaeocystis* and *Skeletonema* were mixed in varying proportions (Figure 9). The results show that the shape of the spectra changes from one pure species to the other. If the pure spectra from both species are compared they have different maxima in absorption, but between the mixed spectra, it is difficult to establish whether they are representative of one or the other species. As a result, the spectra of the mixed species contain optical signatures from both species, making them difficult to discriminate.

3.2.2. Noctiluca

As *Noctiluca* is a part of the assemblage during summer we investigated the absorption characteristics of this dinoflagellate, in particular whether it could be discriminated from spectra from other species. Results from an incubation experiment show that the shape of the absorption spectrum of *Noctiluca* changes between stations, probably related to their gut content and to the kind of food they are eating since they lack of own pigmentation (Figure 10). At coastal stations (700, S01) the shape resemble the one from non-algae particles but with a chlorophyll *a* peak at 676 nm. At offshore stations (800, 435) there is a notable peak at 473 nm and another at 510 nm. Further HPLC analyses of *Noctiluca* samples will confirm this hypothesis.

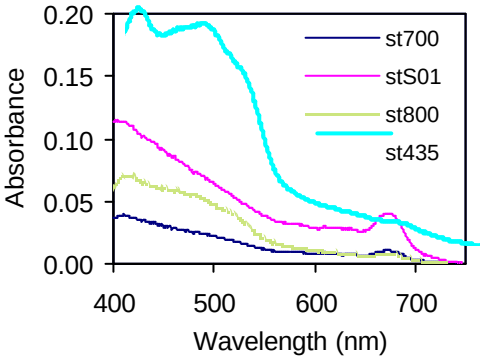

Figure 10: *Noctiluca* absorbance spectra at different stations.

3.2.3. Discrimination of species spectra

Analysis of the fourth derivative of the absorption spectra of *Phaeocystis*, diatoms and *Noctiluca* confirms these peaks and show that there are differences between the three groups at certain wavelengths, notably in the blue-green part of the spectrum, where for example *Phaeocystis* show chl-c3 related peak and diatoms show a fucoxanthin or diadinoxanthin related peak (Figure 11). This technique has proved to efficiently discriminate between some pigment-specific dinoflagellates in mixed samples[7], and it appears to be useful tool to discriminate between diatoms and *Phaeocystis* around 500 nm and between *Noctiluca* and the others species between 450-600 nm.

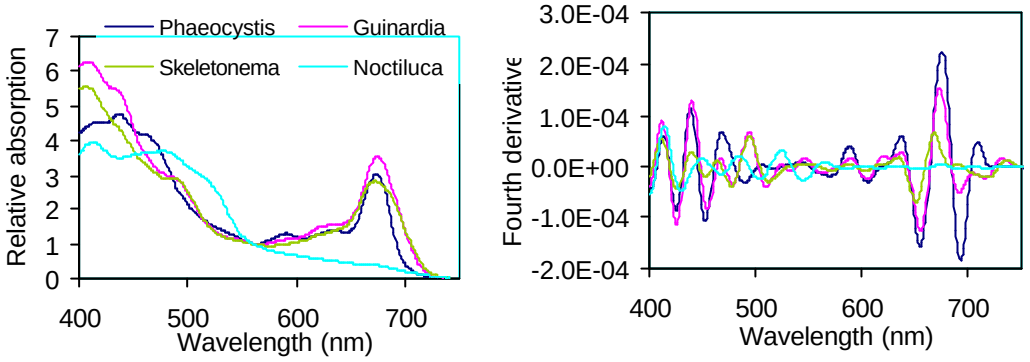

Figure 11: Absorption spectra of *Phaeocystis*, *Guinardia*, *Skeletonema* and *Noctiluca* pure cultures.

3.3. Impact on reflectance and remote sensing algorithms

3.3.1. Seaborne reflectance measurements across a *Noctiluca* bloom

The magnitude of reflectance recorded for the spectral range 580-800 nm is very high and probably corresponds to an optically "saturated" situation where additional scattering would not give any further increase reflectance[23] (Figure 12). Such high values of reflectance are not well represented by any existing model of reflectance as function of inherent optical properties. The abrupt change in reflectance between the spectral range 400-520 nm and 580-700 nm is consistent with the orange-red colour seen by human observers. The browner colour observed at other times for water with very high non-algae particle loads or certain phytoplankton blooms would correspond to a smoother increase in reflectance from blue through green to red wavelengths.

In terms of the inherent optical properties, the reflectance spectrum observed for the *Noctiluca* front corresponds to very high scattering throughout the visible spectrum and very high absorption for the range 400-520 nm with relatively lower absorption from 580-800 nm despite the fact that pure water absorption for wavelengths above 700 nm is generally considered as high under other circumstances (Figure 12). Although this measurement system has not been designed or adequately tested for the range 350-400 nm it is interesting to note the reduction of reflectance for these shorter wavelengths which presumably corresponds to even higher absorption in this range. The nature of the absorbing compounds for that spectral range is unknown.

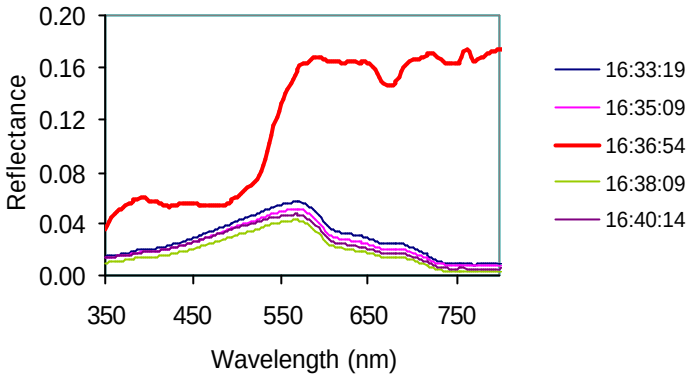

Figure 12: Water-leaving reflectance measurements before (at UTC time 16:33:19 and 16:35:09), in (at UTC time 16:36:54, thick line), and after (at UTC time 16:38:09 and 16:40:14) the *Noctiluca* patch.

3.3.2. Relevance to remote sensing algorithms

Clearly the feature observed in Figure 12 (thick line) is an extreme case. It is interesting to consider how this would be processed by remote sensing algorithms. Because of the extremely high reflectance in the range 600-830 nm, the water is optically saturated and the red chlorophyll *a* absorption feature generally seen as a reflectance minimum near 670 nm is *less* pronounced than for phytoplankton blooms with what is conventionally considered as high chlorophyll *a* concentration e.g. 30-100 mg/m^3. It is likely that no conventional chlorophyll *a* retrieval algorithm could perform adequately under such circumstances except perhaps the wavelength-adaptive "CRAT" algorithm[24], which uses no assumptions about the functional form of dependence of reflectance on inherent optical properties. In this case CRAT would return a chlorophyll a concentration of about 280 mg/m^3 for this reflectance spectrum (see Figure 3[24]). Even the meaning of chlorophyll *a* "concentration" is potentially misleading for such a case of a very near-surface bloom. Indeed the striking visual appearance of this bloom is probably related to the fact that during calm sunny weather this species floats to the surface giving very high accumulations in the top few centimetres of the water column. A browner appearance would presumably be observed if the organism descends a few tens of centimetres in the water column, giving sufficient distance for the high absorption of pure water to reduce upwelling red light. This corresponds to the different appearance of the species collected in bucket onboard ship and floating at different depths.

Red tide index type algorithms based on detecting a peak reflectance near 710 nm are also not well-adapted to such a reflectance spectrum because of the high reflectances measured for the range 650-710 nm. Such algorithms are generally designed for much lower reflectances where the 710 nm reflectance peak contrasts more strongly with reflectance at other wavelengths.

Although not designed for such a purpose it is interesting to note that certain algorithms for quantification of total suspended matter (TSM) concentration such as those based on reflectance at a single red or near infrared wavelength[25] will detect effectively the enormous change in reflectance and attribute this to an abrupt change in backscattering and hence TSM. While probably not able to estimate accurately TSM because of optical saturation and not able to distinguish between algae and non-algae particles, such algorithms will detect a TSM front in remote sensing imagery in accordance with the reality.

Obviously the spatial scale of this feature (10m by a few 100m) is too small for it to be resolved in large area ocean colour sensors such as SeaWiFS, MODIS and MERIS, although higher spatial resolution airborne imagery can resolve such features. In the case of under-resolved blooms or wider area but less extreme blooms it is likely that the absorption properties of this species will influence significantly the reflectance spectrum in much the same way as non-algae particles and/or phytoplankton particles do, depending on the absorption spectrum relevant to the moment (cf. Figure 10) and on the concentration of absorbing components. It remains a challenge for the future to determine whether this species can be distinguished from remotely sensed reflectance spectra for such less extreme conditions.

4. CONCLUSIONS

In the present study we have shown that the discrimination of species based on absorption data is not always possible due to the pigmentation signatures of them, which sometimes are very similar, as the case of *Phaeocystis* and diatoms. We have also shown that certain species like the dinoflagellate *Noctiluca* have a distinctive absorption spectrum that is not caused by own pigmentation, but could help when discriminating from other species. This has an obvious impact on the reflectance spectra, since the colour of the ocean is modelled by processes involving pigmentation and scattering.

The optical measurements performed in the *Noctiluca* red tide event are interesting and have raised several questions regarding where do the characteristic red colour of this bloom come from. As a first step in this study we have found particular absorption and scattering results, which can be an explanation to this question. What is not clear yet is whether this event could be detected with remote sensing in cases where apart from blooming species there are other particles absorbing light. There is still much research undergoing this interesting event and there are many questions left open for future work.

ACKNOWLEDGMENTS

This study was funded by the STEREO programme of the Belgian Federal Science Policy Office in the framework of the BELCOLOUR project. The captain and crew of the Research Vessel Belgica are thanked for their assistance with seaborne measurements. Special acknowledgments to Dr. Elsa Breton for isolation of *Noctiluca scintillans* and for helpful assistance and comments.

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