Spectral fluorometric characterization of Haptophyte dynamics using the FluoroProbe: an application in the eastern English Channel for monitoring *Phaeocystis globosa*

EMILIE HOULIEZ[1]*, FABRICE LIZON[1], MELILOTUS THYSSEN[2], LUIS FELIPE ARTIGAS[2] AND FRANÇOIS G. SCHMITT[1]

[1]UNIVERSITÉ LILLE NORD DE FRANCE, UNIVERSITÉ DES SCIENCES ET TECHNOLOGIES DE LILLE—LILLE 1, LABORATOIRE D'OCÉANOLOGIE ET DE GÉOSCIENCES—CNRS, UMR 8187, STATION MARINE DE WIMEREUX, 28 AVENUE FOCH, 62930 WIMEREUX, FRANCE AND [2]UNIVERSITÉ LILLE NORD DE FRANCE, UNIVERSITÉ DU LITTORAL CÔTE D'OPALE LABORATOIRE D'OCÉANOLOGIE ET DE GÉOSCIENCES—CNRS, UMR 8187, MAISON DE LA RECHERCHE EN ENVIRONNEMENT NATUREL, 32 AVENUE FOCH, 62930 WIMEREUX, FRANCE

*CORRESPONDING AUTHOR: houliez-emilie@voila.fr

Received July 7, 2011; accepted in principle September 30, 2011; accepted for publication October 12, 2011

Corresponding editor: Zoe Finkel

In this study, we examined the possibility of using the FluoroProbe for monitoring the dynamics of the Haptophyte *Phaeocystis globosa* in the coastal waters of the eastern English Channel. The FluoroProbe was recalibrated by recording a new fingerprint for *P. globosa* and the use of this new fingerprint was tested through a series of laboratory and *in situ* experiments. The annual dynamics of *P. globosa* estimated using the FluoroProbe and by flow cytometry were similar. A strong relationship was found between the FluoroProbe estimates of *P. globosa* biomass expressed in terms of chlorophyll *a* equivalent per litre (eq. $\mu g\,L^{-1}$) and flow cytometric cell counts ($r = 0.889$, $P < 0.001$, $n = 121$). The FluoroProbe can be used to detect the flagellated cells as well as the colonial cells of *P. globosa* but not to distinguish these two cell types in mixed assemblages. The use of the new fingerprint recorded for *P. globosa* improved the detection of *Isochrysis sp.* This suggests the possibility of using the FluoroProbe to monitor Haptophytes other than *P. globosa* by calibrating the device with species representative of the region of interest. However, it is important to note that the detection of *P. globosa* at the species level was possible in the eastern English Channel because it was the only Haptophyte species present with a biomass sufficient to be detected by the FluoroProbe. In areas where several Haptophyte species are simultaneously present, their discrimination will be impossible and in such situations the FluoroProbe can be used to monitor the dynamics of the combined Haptophyte group.

KEYWORDS: spectral fluorescence; *Phaeocystis globosa*; Haptophytes; algal blooms; phytoplankton quantification; chlorophyll *a*

doi:10.1093/plankt/fbr091, available online at www.plankt.oxfordjournals.org. Advance Access publication November 10, 2011

© The Author 2011. Published by Oxford University Press. All rights reserved. For permissions, please email: journals.permissions@oup.com

INTRODUCTION

Haptophyte microalgae are an important component of the world's oceanic phytoplankton, blooming seasonally in different ecosystems (Zapata *et al.*, 2004). Among Haptophytes, the genus *Phaeocystis* is one of the most widespread and can produce nearly monospecific blooms reaching a high carbon biomass (up to 10 mg C L^{-1}) in several coastal and oceanic waters (Schoemann *et al.*, 2005). In the eastern English Channel and southern Bight of the North Sea, *Phaeocystis globosa* is the dominant Haptophyte (Astoreca *et al.*, 2009; Lefebvre *et al.*, in press). This species forms massive blooms of mucilaginous colonies during spring (Cadée and Hegeman, 2002; Seuront *et al.*, 2006; Schapira *et al.*, 2008; Blauw *et al.*, 2010). One of the most visible manifestations of these blooms is the accumulation of foam on the seashore during their termination phase. Although different hypotheses have been proposed to explain the formation of these blooms (Lancelot *et al.*, 1987; Peperzak *et al.*, 1998; Meyer *et al.*, 2000) and the success of this species (Veldhuis and Wassmann, 2005), the environmental factors controlling *P. globosa* blooms remain poorly understood. This may be due in part to the complexity of its life cycle that makes monitoring difficult.

P. globosa has a polymorphic life cycle exhibiting phase alternation between different types of free-living cells (vegetative non-motile, vegetative flagellates and microzoospores) of 3–8 μm in diameter and mucilaginous colonies usually reaching millimetres in size (Peperzak *et al.*, 2000; Schoemann *et al.*, 2005; Rousseau *et al.*, 2007). The colonies of *P. globosa* are relatively easy to identify with a light microscope, but the flagellated cells are more difficult to recognize because of their small size and the difficulty of detecting their haptonema (a characteristic organelle of the class) under the light microscope. Moreover, the various fixatives used for preservation may damage the cells, rendering their enumeration somewhat imprecise (Antajan *et al.*, 2004).

Several alternative techniques to the light microscopy have been proposed to monitor the species of the genus *Phaeocystis*, such as electron microscopy (Puigserver *et al.*, 2003; Guiselin *et al.*, 2009), r-RNA targeted sandwich hybridization (Zhen *et al.*, 2008), ribosomal DNA analysis (Gaebler *et al.*, 2007) or pigment analysis by HPLC (Wright *et al.*, 1996). However, these methods have the limitations of being costly, laborious and destructive while providing limited coverage in space and time and rarely in real time (Millie *et al.*, 2002; Gregor and Marsalek, 2004; Gregor *et al.*, 2005; Richardson *et al.*, 2010). Moreover, these techniques, as well as the traditional techniques of cell counts by microscopy, require an experienced analyst and are costly in terms of man-hours (Beutler *et al.*, 2002).

Recently, flow cytometry has also been suggested as a method for monitoring species of the genus *Phaeocystis* (Rutten *et al.*, 2005; Veldhuis *et al.*, 2005; Guiselin, 2010). Although flow cytometry facilitates the monitoring of *Phaeocystis* species by considerably reducing the time of sample analysis and by enhancing the objectiveness of enumeration and the recognition of flagellated cells, some shortcomings persist. Colonial cells of *Phaeocystis* frequently reach several millimetres, size, while current flow cytometers are equipped with narrow nozzles (the more efficient are able to analyse cells with a maximum size of 1000 μm). Consequently, depending on the colonial cells and flow cytometer nozzle sizes, colonial cells of *Phaeocystis* are either counted as single entities or disrupted prior to entering the flow cytometer nozzle, making enumeration of this cell type somewhat imprecise and dependent on the flow cytometer type used (Veldhuis *et al.*, 2005; Guiselin, 2010). Moreover, even if flow cytometry allows *in situ* collection of data at relatively high frequency (typically several times an hour), this method remains costly, and subsequent data processing and interpretation are still needed and are time-consuming despite the introduction of several automated recognition techniques such as neural networks and automated statistical techniques (e.g. Balfoort *et al.*, 1992; Carr *et al.*, 1996; Caillault *et al.*, 2009; Malkassian *et al.*, 2011).

From this perspective there is a clear demand for tools and methods that can simplify phytoplankton quantification for monitoring purposes particularly since the assessment of changes in phytoplankton assemblages is a prerequisite for fully understanding primary production processes and for the assessment of water quality (Beutler *et al.*, 2002; Gregor *et al.*, 2005).

The use of spectral fluorescence would be a good alternative for identifying *P. globosa* if, in the region of interest, *P. globosa* is the only abundant Haptophyte. This method is based on selective excitation of the differing antenna and accessory pigments between taxonomic groups of algae with sequential light excitations using several light emitting diodes (for a review of this method see MacIntyre *et al.*, 2010). Several spectral fluorometers with varying excitation wavelength exist. These include the Mini-Tracka II (Chelsea Instruments, UK), the C6 platform for Cyclops-7-sensors (Turner Designs, USA), the Algae Online Monitor (Photon Systems Instruments, Czech Republic), the Multi-Exciter (JFE Alc Co., Ltd, Japan) or the Algae Online Analyser (AOA) and the FluoroProbe both from bbe-Moldaenke (Kiel, Germany) (Richardson *et al.*, 2010). Here we used the FluoroProbe described by Beutler *et al.* (Beutler *et al.*, 2002).

The first *in situ* application of the FluoroProbe was carried out by Leboulanger *et al.* (Leboulanger *et al.*, 2002). These authors monitored, after a reconfiguration of the device, the dynamics of the toxic cyanobacterium *Planktothrix rubescens* in Lake Bourget (France) and showed its utility for monitoring cyanobacteria. Later, Gregor and Marsalek (Gregor and Marsalek, 2004) validated the use of this probe for total chlorophyll *a* (chl *a*) determination in rivers and reservoirs by comparing their results with a standard spectrophotometry method. In a second study, Gregor *et al.* (Gregor *et al.*, 2005) tested the performance of the FluoroProbe in monitoring phytoplankton community composition in different eutrophic freshwater reservoirs in the Czech Republic and found a relatively good agreement between the FluoroProbe's determinations and cell counts by microscopy. Working in the Gulf of Mexico, See *et al.* (See *et al.*, 2005) reported mis-classification by the FluoroProbe of certain brown algae as either brown algae/green algae or brown algae/Cryptophyta mixtures; specifically it was the case where Haptophytes were classified as a brown algae/green algae mixture. Similar results of mis-classification of Haptophytes were also obtained by Richardson *et al.* (Richardson *et al.*, 2010) and MacIntyre *et al.* (MacIntyre *et al.*, 2010) working with the AOA a spectral fluorometer that functions on the same principle as the FluoroProbe.

The aim of this work was to test the possibility of using the FluoroProbe for monitoring the dynamics of the Haptophyte *P. globosa* in the coastal waters of the eastern English Channel. We tested the hypothesis that the FluoroProbe is not able to correctly discriminate Haptophytes and we tried to answer the following questions:

(1) Is the FluoroProbe able to discriminate the Haptophyte *P. globosa*? Is a reconfiguration of its fingerprints necessary?
(2) How does the use of a new fingerprint for *P. globosa* affect the discrimination of other phytoplankton groups?
(3) To what extent can the FluoroProbe discriminate the *P. globosa* signal in the presence of other groups of microalgae? Is the FluoroProbe able to discriminate the different life cycle stages of *P. globosa*?
(4) What are the effects of each algal group concentration within a phytoplankton assemblage on the FluoroProbe discriminations? How does the number of algal groups within a phytoplankton assemblage modify the quality of the FluoroProbe classifications?
(5) Are the results of the *P. globosa* discrimination by the FluoroProbe comparable to a cell counts method such as flow cytometry?

For the purpose of the present study, a new fingerprint was recorded to discriminate *P. globosa* and then validated by several laboratory and *in situ* experiments.

METHOD

Fluorescence measurements

Spectral fluorescence background

Measurements of spectral fluorometers are based on the principle of differentiation of algal populations by the spectral fluorescence approach. It is known that chlorophyll fluorescence is mainly emitted by chl *a* of photosystem II (PSII) antenna system, which consists of an evolutionarily conserved chl *a*-containing core and species-dependent peripheral antenna composed of differing accessory pigments (Rowan, 1989; Jeffrey *et al.*, 1997). In the "green" lineage, the peripheral antenna contains chl *a*, chl *b* and xanthophyll. In the "blue" lineage, phycobilisomes (principally composed of phycocyanin) function as peripheral antenna. The members of the "brown" lineage contain chl *a*, chl *c* and xanthophyll (often fucoxanthin or peridin). The peripheral antenna of the "red" lineage is composed of phycobilisomes, as in the "blue" lineage; however, phycoerythrin is the major pigment instead of phycocyanin and the peripheral antenna also contains chl *a* and chl *c* (Rowan, 1989; Jeffrey *et al.*, 1997). The spectral fluorescence approach is based on selective excitation of the differing antenna and accessory pigments between taxonomic groups of algae using light of varying wavelengths to obtain characteristic fluorescence excitation spectra (Yentsch and Yentsch, 1979; Millie *et al.*, 2002). Each of the four lineages is characterized by a specific excitation spectrum called a "fingerprint" resulting from the composition of their peripheral antenna (Beutler *et al.*, 2002). Using a mathematical technique such as Gaussian decomposition of spectra or linear unmixing, it is possible to determine the phytoplankton composition and chl *a* concentration associated with each algal group, within an unknown sample, by fitting the measured excitation spectra using a library of fingerprints that serve as a reference (MacIntyre *et al.*, 2010).

The FluoroProbe

The FluoroProbe (bbe-Moldaenke, Kiel, Germany) is a spectral fluorometer able to discriminate four spectral algal groups: brown algae (Heterokontophyta and Dinophyta), cyanobacteria (cyanobacteria with

phycocyanin), green algae (Chlorophyta) and Cryptophyta (Cryptophyta, Rhodophyta, cyanobacteria with phycoerythrin) in mixed assemblages. It uses five light emitting diodes (470, 525, 570, 590 and 610 nm) for sequential light excitation of accessory pigments and the relative fluorescence intensity of chl *a* is measured between 690 and 710 nm. The excitation spectrum obtained is compared by linear unmixing to a library of four fingerprints stored in the probe and the relative concentration of each algal group expressed in terms of the equivalent amount of chl *a* per litre (eq. $\mu g\ L^{-1}$) as well as the total chl *a* concentration are calculated. An additional diode (370 nm) is used for the excitation and subsequent subtraction of the fluorescence of dissolved organic matter ("yellow substances"). For a detailed description of the FluoroProbe, see Beutler *et al.* (Beutler *et al.*, 2002). In this study, all fluorescence measurements were made using the 25 mL cuvette of the FluoroProbe.

A new fingerprint for *Phaeocystis globosa*

As purchased, our FluoroProbe was provided with fingerprints for brown algae, cyanobacteria, green algae and Cryptophyta (original fingerprints, Fig. 1). To discriminate the Haptophyte *P. globosa*, we recorded a new fingerprint using natural coastal water dominated by this species (>90% determined by cell counts from flow cytometry and microscopic observations, data not shown). The probe was first immersed in 4 L of ultra-filtered (0.2 μm) coastal water to obtain a "natural blank" and then in 4 L of natural coastal water dominated by *P. globosa* (with a known chlorophyll concentration) to calibrate the new fingerprint (Fig. 1). The fingerprints obtained with cultures of *P. globosa* were similar to those obtained with natural coastal water. Consequently, only the recorded field signature was used for the subsequent detection of this species.

Laboratory experiments

To determine to what extent the FluoroProbe can discriminate the signal of *P. globosa* in the presence of other microalgae and how the use of this fingerprint potentially affects the discrimination of other groups, seventeen species belonging to different phytoplankton groups (Table I) were used to carry out a series of laboratory experiments. All cultures were grown under a 12 h light−dark cycle in white light Osram powerstart HQI-T 250W/D daylight (170 μmol photons $m^{-2}\ s^{-1}$) at 15°C. Cultures were regularly diluted with fresh medium to ensure they were nutrient replete.

Experiment 1

As indicated above, the phytoplankton composition of an unknown sample is determined by fitting the measured excitation spectra by a library of fingerprints that serve as

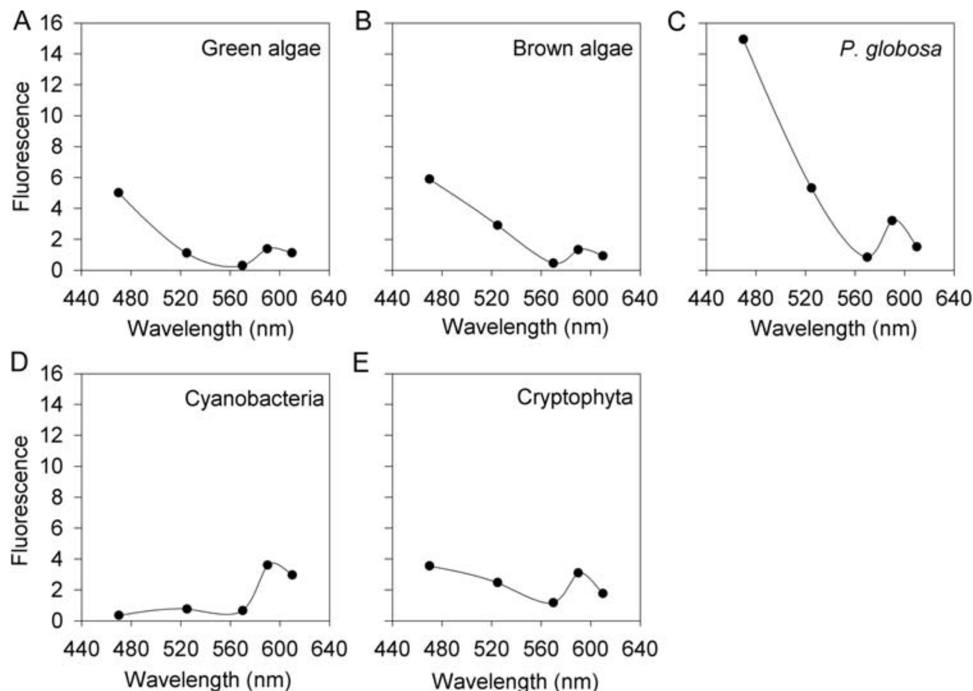

Fig. 1. Original fingerprints (**A**, **B**, **D** and **E**) and the new fingerprint of *Phaeocystis globosa* (**C**). Values are expressed in relative fluorescence for each excitation wavelength.

Table I: Division, species, strain code, culture medium and origin of the 18 phytoplankton cultures examined

Division	Species	Strain code	Culture medium	Origin
Haptophyta	*Phaeocystis globosa*	PLY 575	Keller's ESW	Plymouth Laboratory
	Phaeocystis globosa	PLY 699	Keller's ESW	Plymouth Laboratory
	Isochrysis sp.		f/2	LOG Wimereux
Chlorophyta	*Chlorella autotrofica*		f/2	LOG Wimereux
	Scenedesmus sp.		BG 11	Laboratory ECOBIO Rennes
	Chlamydomonas reginae	PLY 399	Erd Schreiber	Plymouth Laboratory
Cyanobacteria	*Microcystis aeruginosa*		BG 11	Laboratory ECOBIO Rennes
	Gloeothece sp.		f/2	LOG Wimereux
	Anabaena cylindrica		f/2	LOG Wimereux
Cryptophyta	*Rhodomonas marina*		f/2	LOG Wimereux
	Cryptomonas maculata	PLY 175	Erd Schreiber	Plymouth Laboratory
Rhodophyta	*Porphyridium cruentum*		f/2	LOG Wimereux
	Rhodella maculata	PLY 470	Erd Schreiber	Plymouth Laboratory
Bacillariophyta	*Thalassiosira oceanica*		f/2	LOG Wimereux
	Actinoptychus sp.		f/2	LOG Wimereux
	Coscinodiscus sp.		f/2	LOG Wimereux
	Schroederella sp.		f/2	LOG Wimereux
	Asterionellopsis glacialis	PLY 607	Erd Schreiber	Plymouth Laboratory

Culture medium: Keller's ESW (Keller *et al.*, 1987); f/2 (Guillard and Ryther, 1962; Guillard, 1975); BG 11 (Allen, 1968; Allen and Stanier, 1968; Rippka *et al.*, 1979); Erd Schreiber (Tompkins *et al.*, 1995)

a reference. The FluoroProbe determinations of algal groups are therefore strongly dependent on the fingerprints used and when a new fingerprint is employed it is essential to verify that it does not affect the discrimination of other groups. The potential error in the discrimination of the different groups caused by the use of the fingerprint of *P. globosa* was evaluated by comparing the discrimination of these groups using the original fingerprints (brown algae, cyanobacteria, green algae and Cryptophyta) with their discrimination using the fingerprint of *P. globosa*. These tests were done on pure cultures.

One of the disadvantages of the FluoroProbe is that it is only able to discriminate four phytoplankton groups so that the addition of a new fingerprint is only possible if one of the four default fingerprints is disabled. The potential effect on the discrimination of the different algal groups by the fingerprint of *P. globosa* was therefore evaluated by alternately replacing the four original fingerprints by the fingerprint of *P. globosa*. For example, the error in the prediction of cyanobacteria was evaluated by successively using three combinations of fingerprints (cyanobacteria + *P. globosa* + brown algae + Cryptophyta; cyanobacteria + green algae + *P. globosa* + Cryptophyta; cyanobacteria + green algae + brown algae + *P. globosa*). This operation was repeated for each phytoplankton group.

Experiment 2

The quality of the discrimination of the *P. globosa* signal in the presence of other microalgae was evaluated using different mixtures of algae with different proportions (the proportions are detailed in Figs 3 and 4). For this purpose, only three phytoplankton groups were considered because they are the dominant groups in the eastern English Channel. Three species were used: *Asterionellopsis glacialis* (Bacillariophyta), *Cryptomonas maculata* (Cryptophyta) and *P. globosa*. As *P. globosa* has a heteromorphic life cycle with colonial and flagellated cells, two cultures of *P. globosa* (one culture of flagellated cells and one of colonial cells) were used. Additional mixtures were made with algal groups for which the FluoroProbe was initially developed, i.e. a Bacillariophyta: *A. glacialis*, a Cryptophyta: *C. maculata* and a Chlorophyta: *Chlamydomonas reginae* to determine the quality of the discrimination of these groups and to compare the results with the discrimination of *P. globosa*. The subsamples of culture were diluted in ultra-filtered (0.2 μm) sea water to make the different mixtures. The contributions of each group in the different mixtures were determined using the FluoroProbe. Samples were filtered for chl *a* concentration measurements (see below) and these concentrations were used to calculate the expected proportions.

Field measurements

To validate the use of the new fingerprint for *in situ* monitoring of *P. globosa*, samples were taken in the coastal waters (50°45′57.42″N, 1°35′55.17″E) of the eastern English Channel (France). Sampling was carried out each week during 2009 and on several dates in the spring 2010. All samples were taken at high and low tide. They were placed into opaque containers and brought back to laboratory for determination of

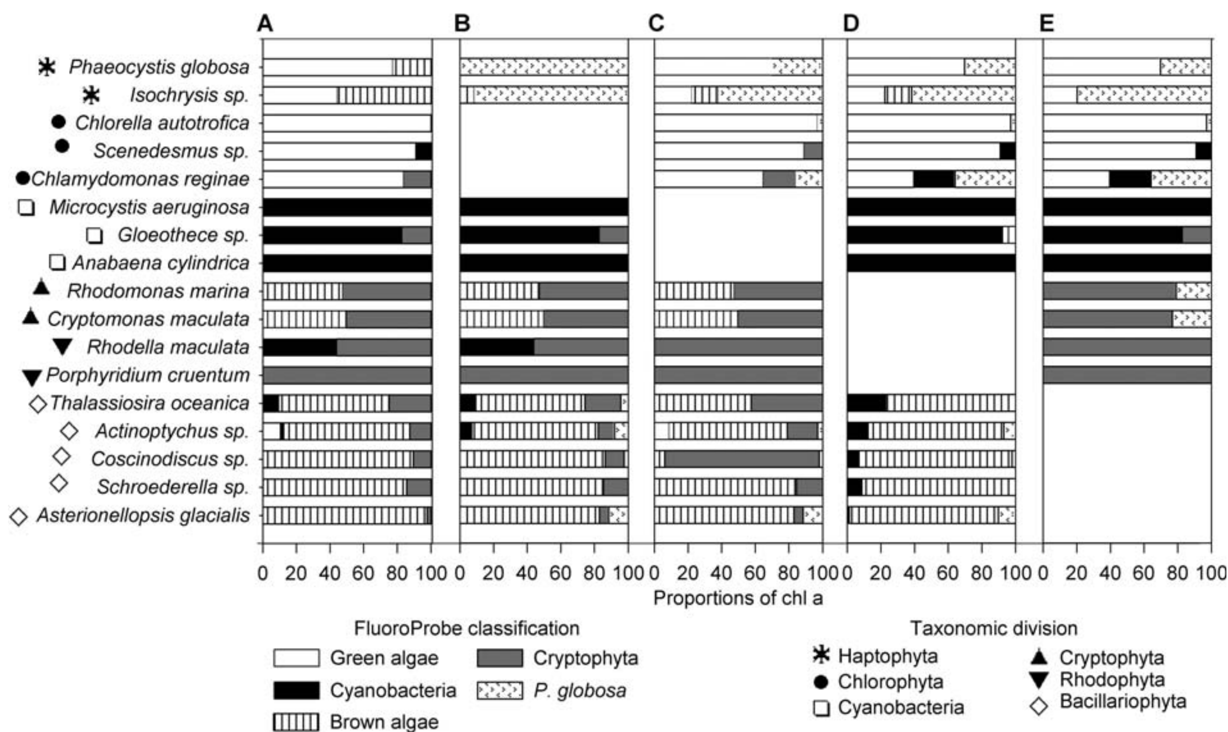

Fig. 2. FluoroProbe classification of 17 phytoplankton pure cultures using either the four original fingerprints or three original fingerprints + *Phaeocystis globosa*'s fingerprint. Original fingerprints (Cyanobacteria + brown algae + green algae + Cryptophyta) (**A**). Fingerprints of Cyanobacteria + brown algae + Cryptophyta + *Phaeocystis globosa* (**B**). Fingerprints of green algae + brown algae + Cryptophyta + *Phaeocystis globosa* (**C**). Fingerprints of Cynaobacteria + green algae + brown algae + *Phaeocystis globosa* (**D**). Fingerprints of Cynaobacteria + green algae + Cryptophyta + *Phaeocystis globosa* (**E**). Colours correspond to the FluoroProbe classification, whereas symbols situated in front of the species names correspond to the taxonomic division of species.

phytoplankton assemblage composition by the FluoroProbe and flow cytometry. Analyses with the FluoroProbe were always done within the 20 min following the sampling.

Chlorophyll *a* measurements

Chl *a* concentrations of pure culture and mixtures were determined by filtering known volumes of culture through Whatman 47-mm GF/F glass-fibre filters. The filters were stored at −80°C and subsequently extracted in 90% acetone. Chl *a* concentration was evaluated by fluorometry using a Turner Designs Model 10-AU fluorometer. The fluorescence was measured before and after acidification with HCl (Lorenzen, 1966; Aminot and Kérouel, 2004). The fluorometer was calibrated using known concentrations of commercially purified chl *a* (Sigma).

Flow cytometry

Samples were fixed for 15 min with glutaraldehyde 0.25% final concentration and stored at −80°C for later analysis. Thawed samples were analysed using a Cytosense Benchtop (CytoBuoy BV, Netherlands) equipped with a blue laser beam (488 nm, 50 mW). This instrument records the pulse shape of each particle passing through the laser beam at a speed of 2 m s^{-1}. For each particle, full pulse profile digitizing electronics enables morphological analysis. The pulse shape of the forward (FW) and sideward (SWS) scatter signals, the red (FLR, 668−734 nm), orange (FLO, 601−668 nm) and yellow (FLY, 536−601 nm) fluorescences were collected. Ten micrometre orange fluorescent polystyrene beads (Invitrogen Fluorosphere) were used as an external standard and analysed before and after each set of measurements to normalize scatter and fluorescence signals. Ultra-filtered (0.2 μm) sea water was used as sheath fluid and samples were run at 4.5 μL s^{-1}. Data were analysed using the Cytoclus software (CytoBuoy, bv). *P. globosa* was identified from the pulse shape and the profile using the levels of chl *a* fluorescence (FLR), the FW and the SWS according to Guiselin (Guiselin, 2010), Rutten *et al.* (Rutten *et al.*, 2005) and Veldhuis *et al.* (Veldhuis *et al.*, 2005). The Cytosense is able to analyse a wide range of cell sizes (1−800 μm and a few millimetres in length): flagellated as well as

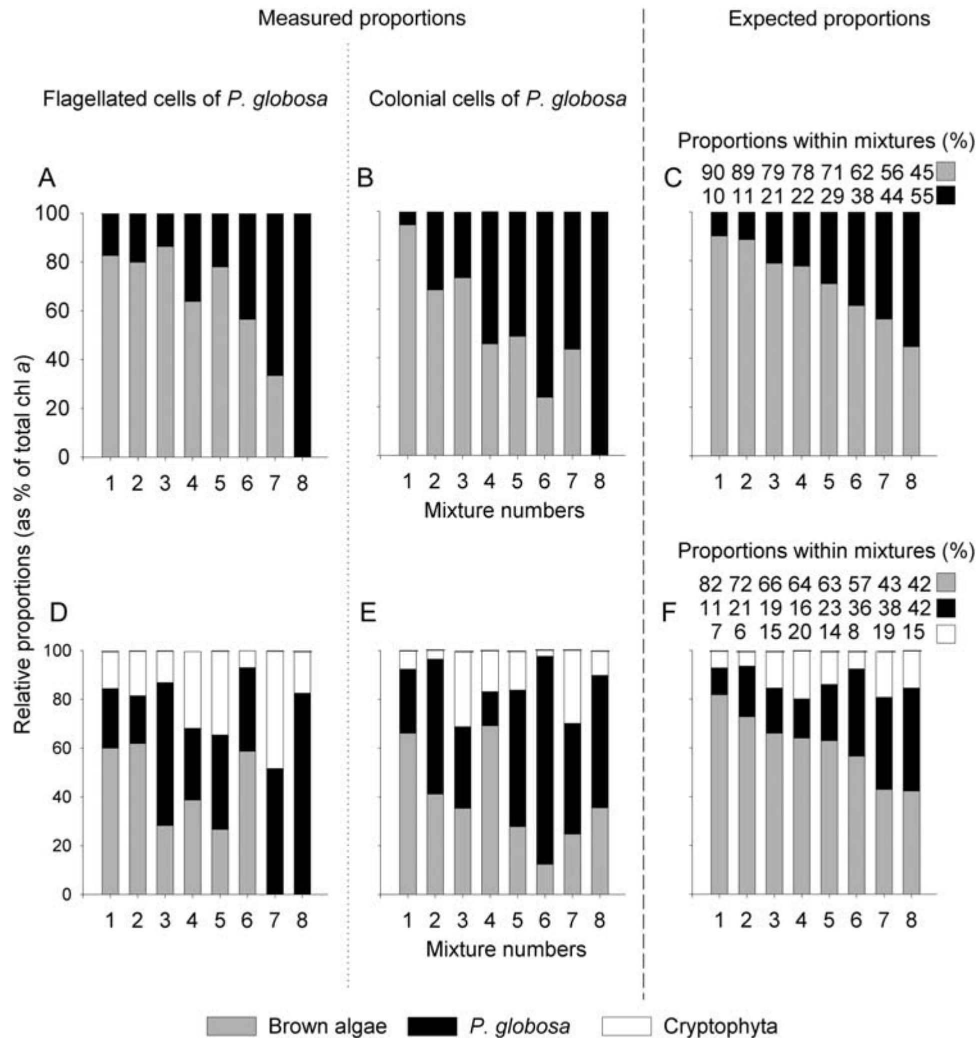

Fig. 3. Measured (**A**, **B**, **D** and **E**) and expected (**C** and **F**) relative proportions of *Phaeocystis globosa* in different mixtures with *Asterionellopsis glacialis* (A, B, C) or *Asterionellopsis glacialis* + *Cryptomonas maculata* (D, E, F). (A) and (D) correspond to mixtures with flagellated cells of *Phaeocystis globosa*, and (B) and (E) to mixtures with its colonial cells. *Phaeocystis globosa* is in black, *Asterionellopsis glacialis* is in grey and *Cryptomonas maculata* is in white. The bottom *x*-axis corresponds to the different mixture numbers. The top *x* numbers in (C) and (F) correspond to the proportions used to make the mixtures. The measured proportions were obtained using the FluoroProbe whereas the expected proportions were calculated from the chlorophyll *a* concentrations.

colonial cells of *P. globosa* were consequently both enumerated. Although the larger colonial cells (millimetre size) are theoretically too large for the flow cytometer nozzle, the gelatinous mucus of the colonies is fluid enough to pass through the orifice (Veldhuis *et al.*, 2005).

Statistical analysis

Fisher's exact test (one tail) was used to compare relative proportions of algal groups within mixtures. This test is advised when the Chi-square test assumptions are not respected; particularly when any expected frequency is <1 or when 20% of expected frequencies are ≤ 5 (Scherrer, 2007). It was run using the R-software (R Development Core Team, 2011). Absolute concentrations were compared using Student's *t*-test (Scherrer, 2007). Pearson's correlation analysis and simple linear regressions were performed to evaluate the relationships between the measured and the expected absolute chl *a* concentrations for *P. globosa*, *A. glacialis* (brown algae) and *C. maculata* (Cryptophyta); and between the FluoroProbe's results and cell abundances determined by flow cytometry (Scherrer, 2007). These statistical procedures were performed using the software SYSTAT 10.

RESULTS

Experiment 1: potential errors in the discrimination of algae groups induced by the fingerprint of *P. globosa*

The potential errors in the discrimination of different phytoplankton species induced by the fingerprint of *P. globosa* were evaluated by comparison of their discrimination using the original fingerprints with their discrimination using the fingerprint of *P. globosa* (Fig. 2). Only the discrimination of Chlorophyta and three species of Bacillariophyta (*Thalassiosira oceanica*, *Actynoptychus sp.* and *A. glacialis*) were significantly different using the fingerprint of *P. globosa* ($P < 0.01$, Fisher's exact test). The error in the discrimination of Bacillariophyta was lower than the Chlorophyta discrimination and affected the proportions of chl *a* that were already incorrectly classified by the FluoroProbe using the original fingerprints. With the original fingerprints, *P. globosa* ($n = 20$) was incorrectly classified as a mixture of brown algae ($23 \pm 0.2\%$) and green algae ($77 \pm 0.2\%$). Using the *P. globosa* fingerprint, all of the signal was attributed to *P. globosa* except when this fingerprint was used at the same time as the fingerprint of green algae: in this case $69 \pm 0.4\%$ of the signal was still mis-classified as green algae. Using the fingerprint of *P. globosa* improved the discrimination of other Haptophytes. Indeed, with the original fingerprints, the signal of *Isochrysis sp.* ($n = 12$) was incorrectly classified as $44 \pm 0.3\%$ of green algae and $56 \pm 0.3\%$ of brown algae, whereas with the fingerprint of *P. globosa* only $9 \pm 0.7\%$ of the signal was incorrectly classified as brown algae.

Experiment 2: ability of the FluoroProbe to discriminate the signal of *P. globosa* in mixed assemblages

Relative contributions

No significant statistical difference between the discrimination of the two life cycle stages of *P. globosa* in different assemblages was found ($P > 0.05$, Fisher's exact test; Fig. 3A versus B and D versus E). The results of the discrimination by groups using the fingerprint of *P. globosa* are in relatively good agreement with the expected relative proportions for mixtures of two phytoplankton groups. Indeed, even though visually there were some differences, the same trend was observed and no significant statistical difference was found between the observed and the expected relative proportions ($P > 0.05$, Fisher's exact test; Fig. 3A versus C and B versus C). Nevertheless, when *P. globosa* represents >55% of the mixture, as it was the case in mixtures 8, the FluoroProbe failed to discriminate Bacillariophyta and the totality of the signal was classified as *P. globosa* (Fig. 3A and B, mixtures 8). For mixtures of three phytoplankton groups, the discrimination of algal groups was more difficult with significant differences between the observed and the expected relative proportions ($P < 0.02$, Fisher's exact test; Fig. 3D versus F and E versus F). Indeed, the FluoroProbe underestimated brown algae (by 1.9–4.6 times), overestimated Cryptophyta (by 1.8–3.3 times) and in certain mixtures, Bacillariophyta were not detected (Fig. 3D, mixtures 7 and 8). In contrast, no significant difference was found between the observed and the expected relative proportions of *P. globosa* ($P > 0.05$, Fisher's exact test).

To determine if these results were specific to the use of the *P. globosa* fingerprint, mixtures were made with algal groups for which the FluoroProbe was initially developed, i.e. a Bacillariophyte: *A. glacialis*, a Cryptophyte: *C. maculata* and a Chlorophyte: *C. reginae* (Fig. 4). The same trends as with the fingerprint of *P. globosa* were observed: with mixtures of two algal groups, the results were actually in accordance with the expected relative proportions and no significant difference was found between the observed and expected proportions ($P > 0.05$, Fisher's exact test; Fig. 4A and B). With mixtures of three algal groups significant differences were found between the observed and the expected relative proportions ($P < 0.001$, Fisher's exact test). Cryptophyta were overestimated (by 1.7–2.6 times) and green algae were underestimated (by 1.6–16.1 times), whereas brown algae were not significantly different ($P > 0.05$, Fisher's exact test) (Fig. 4C and D). In certain mixtures green algae were not detected (Fig. 4C, mixtures 1, 3 and 6).

Absolute concentrations

Absolute concentrations of brown algae and Cryptophyta within mixtures were significantly different between the FluoroProbe assessments and the expected chl *a* concentrations, whereas no significant difference was found for *P. globosa* (Table II). Similarly to the relative proportions, no significant difference between the absolute concentrations of the two life cycle stages of *P. globosa* was found. The FluoroProbe-derived chl *a* concentrations relative to the expected concentrations of each algal group in mixtures are shown in Fig. 5. The disagreement between the expected and the measured chl *a* concentrations was not greater for *P. globosa* (using the new fingerprint) than for the algal groups for which the FluoroProbe was initially developed. Indeed, the coefficient of determination of the relationships between measured and expected concentrations for *P. globosa* was higher ($r^2 = 0.84$, $P < 0.001$) than the coefficient for other groups ($r^2 = 0.73$, $P < 0.001$ and $r^2 = 0.39$,

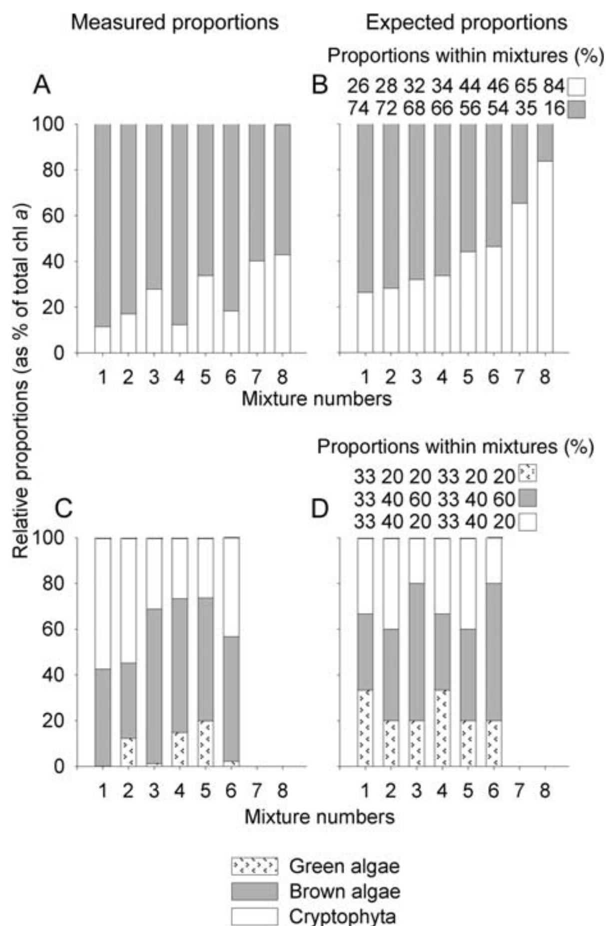

Fig. 4. Results of the FluoroProbe detection of *Asterionellopsis glacialis* (brown algae), *Cryptomonas maculata* (Cryptophyta) and *Chlamydomonas reginae* (green algae) in different mixtures with different proportions. The relative proportions measured with the FluoroProbe are represented in the left-side panel (**A** and **C**) and the expected relative proportions calculated from the chlorophyll *a* concentrations are in the right-side panel (**B** and **D**). Each line corresponds to different mixtures of cultures combined in different proportions: *Asterionellopsis glacialis* + *Cryptomonas maculata* (A and B) and *Cryptomonas maculata* + *Asterionellopsis glacialis* + *Chlamydomonas reginae* (C and D). The bottom *x*-axis corresponds to the different mixture numbers. The top *x* numbers in (B) and (D) correspond to proportions used to make mixtures.

$P = 0.01$ for *A. glacialis* and *C. maculata*, respectively), and the slope of the regression was closer to 1 for *P. globosa*. For each of these species, the slope of the regression was significantly different from 1 and indicated that the FluoroProbe underestimated chl *a* concentrations.

In situ use of the FluoroProbe to detect *P. globosa*

The fingerprint of *P. globosa* was field-tested using the FluoroProbe to monitor changes in phytoplankton community structure in the coastal waters of the eastern English Channel. The FluoroProbe's taxonomic classification using the original fingerprints or the fingerprint of *P. globosa* are shown in Fig. 6. With the original fingerprints, the classification was dominated by brown algae throughout the monitoring period with a minor contribution of Cryptophyta and cyanobacteria. The contribution of green algae was also low except during the spring bloom (from April to June) where their contribution was close to that of brown algae (Fig. 6A). Using the fingerprint of *P. globosa*, the classification showed a clear succession of phytoplankton communities during the bloom period (from February to August). The *P. globosa* bloom occurred from mid-April to mid-May and disappeared at the end of spring. This bloom was followed by and preceded by two blooms of brown algae (first bloom from February to mid-April; second bloom from May to August). Cryptophyta and cyanobacteria showed the same trends using the original fingerprints and their contributions stayed low (Fig. 6B).

Biomass values for *P. globosa* expressed in eq. µg chl $a\,L^{-1}$ were compared with cell counts from flow cytometry. The annual pattern of *P. globosa* variation was similar, as determined both from the FluoroProbe (Fig. 7A) and from flow cytometry (Fig. 7B). There was a strong linear relationship between the two methods ($y = 7.03 \times 10^{-4}\ x$, $r = 0.889$, $P < 0.001$, $n = 121$; Fig. 7C).

DISCUSSION

The necessity of using new fingerprints

Using the original fingerprints, *P. globosa* was classified by the FluoroProbe as a brown algae/green algae mixture. Indeed, the field test on the annual changes in phytoplankton community structure showed that using the original fingerprints, the classification was dominated by brown algae except during April–May where the contribution of green algae was close to brown algae. In contrast cytometric analyses did not report the presence of green algae but the presence of the Haptophyte *P. globosa*.

Similar observations of mis-classification of Haptophytes were reported by MacIntyre *et al.* (MacIntyre *et al.*, 2010), Richardson *et al.* (Richardson *et al.*, 2010) and See *et al.* (See *et al.*, 2005) who compared predictions of algal group composition by the FluoroProbe or the AOA to those derived from taxonomic classifications using CHEMTAX (Mackey *et al.*, 1996) based on high-performance liquid chromatography (HPLC) pigment concentrations. Working in

Table II: Student's t*-test comparing of absolute chlorophyll* a *concentrations of green algae, brown algae, Cryptophyta, flagellated cells and colonial cells of* P. globosa *in different mixtures*

Mixtures	Type of comparison	Group	*P*-value
Flagellated cells of *P. globosa* + *A. glacialis*	Observed versus Expected	Brown algae	0.013
		P. globosa	**0.342**
Colonial cells of P. globosa + A. glacialis	Observed versus Expected	Brown algae	0.006
		P. globosa	**0.387**
C. maculata + *A. glacialis*	Observed versus Expected	Cryptophyta	<0.001
		Brown algae	**0.186**
Flagellated cells of P. globosa + A. glacialis + C. maculata	Observed versus Expected	*P. globosa*	**0.185**
		Cryptophyta	**0.442**
		Brown algae	0.003
Colonial cells of *P. globosa* + *A. glacialis* + *C. maculata*	Observed versus Expected	*P. globosa*	**0.361**
		Cryptophyta	0.001
		Brown algae	0.007
P. globosa + *A. glacialis*	Flagellated cells versus Colonial cells	*P. globosa*	**0.187**
		Brown algae	**0.945**
P. globosa + *A. glacialis* + *C. maculata*	Flagellated cells versus Colonial cells	*P. globosa*	**0.143**
		Brown algae	**0.574**
		Cryptophyta	**0.485**

Significant *P*-values are indicated in bold.

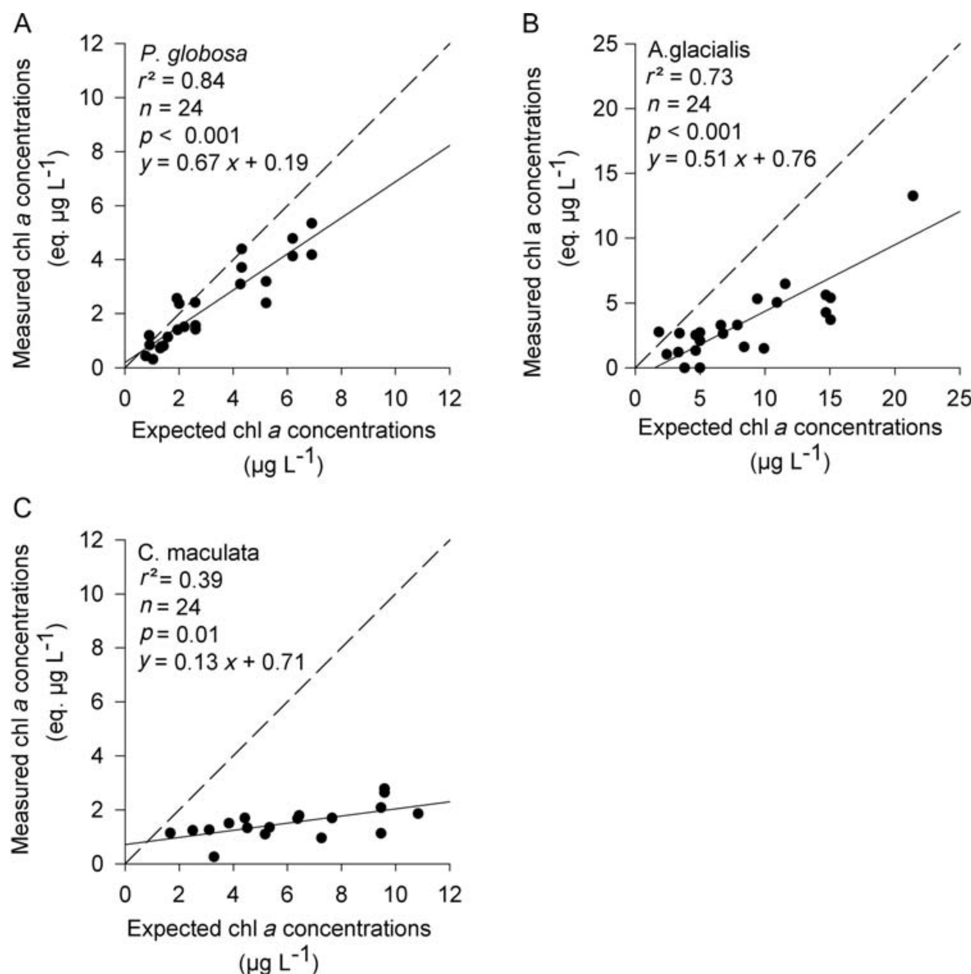

Fig. 5. Relationships between the measured and the expected chlorophyll *a* concentrations for *Phaeocystis globosa* (**A**), *Asterionellopsis glacialis* (brown algae) (**B**) and *Cryptomonas maculata* (Cryptophyta) (**C**). The measured proportions were obtained using the FluoroProbe, whereas the expected proportions were calculated from the chlorophyll *a* concentrations. The solid line is the regression line. The dotted line indicates the 1:1 ratio. Some points are superimposed.

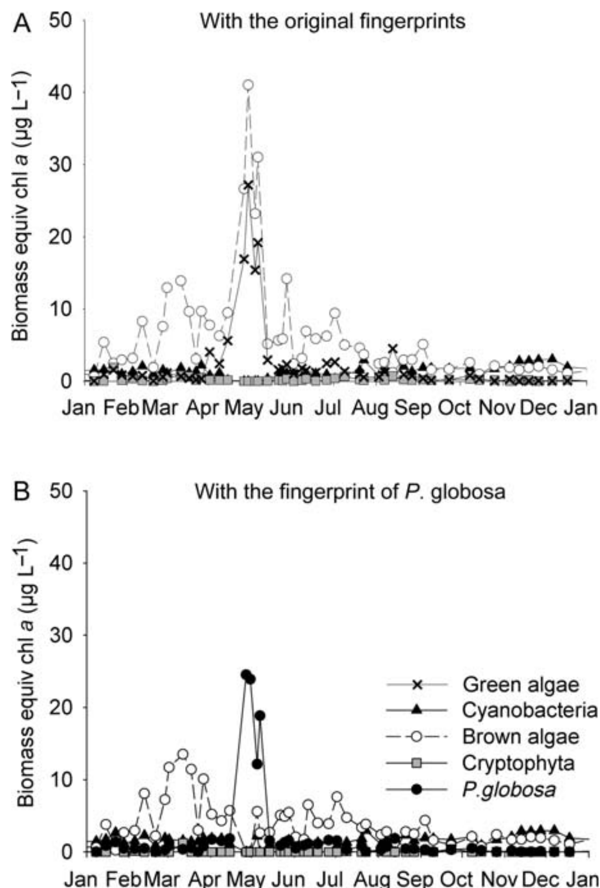

Fig. 6. Temporal variations in the biomass of four phytoplankton groups measured with the FluoroProbe at high tide in 2009. Results were obtained using either the four original fingerprints (cyanobacteria + green algae + brown algae + Cryptophyta) (**A**) or the fingerprints for cyanobacteria + brown algae + *Phaeocystis globosa* + Cryptophyta (**B**). The relative amount of each phytoplankton group is expressed in terms of the equivalent amount of chlorophyll *a* per litre (eq. µg L^{-1}).

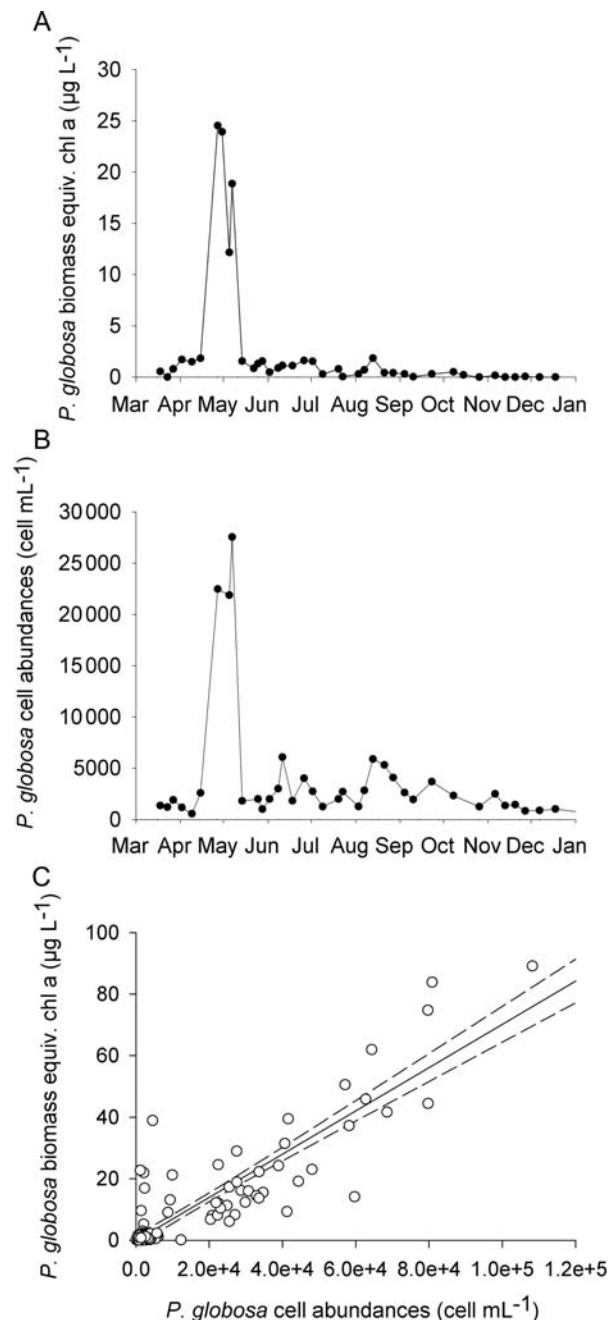

Fig. 7. Temporal dynamics of *Phaeocystis globosa* at high tide in 2009. Biomass of *Phaeocystis globosa* measured with the FluoroProbe in equivalent amount of chl a per liter (eq. µg L^{-1}) (**A**). Cell abundances of *Phaeocystis globosa* determined by flow cytometry (cell mL^{-1}) (**B**). Relationship between the FluoroProbe results and cell abundances determined by flow cytometry ($y = 7.03 \times 10^{-4}\ x$, $r = 0.889$, $P < 0.001$, $n = 121$) (**C**). In (C), the solid line is the regression line and confidence intervals (95%) for the regression line are indicated by dashed curves. This relationship was obtained by pooling data from samples taken at high and low tide in 2009 and 2010.

the Gulf of Mexico, MacIntyre *et al.* (MacIntyre *et al.*, 2010) and See *et al.* (See *et al.*, 2005) obtained similar results: the fluorescence-based classification showed a dominance of green algae (>60%) with a minor contribution of brown algae, while the pigment-based classification showed a dominance of diatoms and dinoflagellates and detected the presence of 19′-hexanoyloxyfucoxanthin: a characteristic pigment of Haptophytes (Jeffrey *et al.*, 1997). In these studies, the apparent dominance of green algae may therefore be due in part to the presence of Haptophytes especially since culture experiments, conducted by Richardson *et al.* (Richardson *et al.*, 2010) and See *et al.* (See *et al.*, 2005) showed, respectively, the misclassification of *Isochrysis galbana* and *Emiliana huxleyi*. *I. galbana* was classified as a mixture of brown algae (60 ± 1.7%) and green algae (40 ± 2%) and *E. huxleyi* was classified as 75% of green algae and 25% of brown algae.

These authors concluded that the reasons underlying the mis-classification of Haptophytes are unclear and proposed two hypotheses. The first is that a single group of brown algae (generally diatoms) is used as a calibration species' for fingerprints, whereas in natural samples, brown algae could be really a combination of diatoms, dinoflagellates and Haptophytes so that variations in the shape of the calibration versus non-calibration species spectral signatures result in misidentifications. The second is that there are pigment similarities between green algae and Haptophytes. Although they failed to identify a unique reason for this mis-classification, their observations suggest that it is most likely related to the shape of the fingerprint of Haptophytes.

The record of a new fingerprint for the Haptophyte *P. globosa* allowed us to observe that the shape of this fingerprint differs effectively from the fingerprint of brown algae (Fig. 1). The goal of our study was not to identify the reasons why the shape of the *P. globosa* fingerprint is different from other brown algae but to study the possibility of using these differences as an advantage for discriminating this species from other phytoplankton groups. Our results demonstrated that it is possible to use this new fingerprint to monitor the dynamics of *P. globosa* without a major effect on the identification of other phytoplankton groups.

Efficiency of the *P. globosa* fingerprint in laboratory experiments

Laboratory experiments showed relatively good agreement between the observed and the expected relative proportions of *P. globosa* in different mixtures and the use of the *P. globosa* fingerprint did not strongly affect the classification of other groups because the same results were obtained using either the original fingerprints or the *P. globosa* fingerprint. Only the discrimination of green algae and brown algae species could be affected by the use of the *P. globosa* fingerprint.

The major interference was observed between the fingerprints of green algae and *P. globosa* so that it is not possible to use these two fingerprints at the same time. The reasons underlying this interference are not clear but could be related to the shape of the *P. globosa* fingerprint that shows a certain similarity with that of green algae. This group is rarely encountered in ecosystems where *P. globosa* is present. The fingerprint of *P. globosa* can therefore be used to replace the fingerprint of this group.

The detection of brown algae is affected when *P. globosa* represents >55% of the total biomass. In this case, the FluoroProbe is unable to detect their presence and the whole signal is classified as *P. globosa*. In the field, a similar situation of strong dominance by *P. globosa* can be observed during the spring blooms; however, during these periods diatom biomass is always low when *P. globosa* represents >55% of the total biomass and these periods are short. For example, during the years 2007, 2008 and 2009, these periods represented a mean duration of ±1 month with a mean diatom biomass of 122 ± 78 μC L^{-1}, whereas the mean *P. globosa* biomass was 377 ± 251 μC L^{-1} (Grattepanche *et al.*, 2011).

The tests with the two life cycle stages of *P. globosa* (flagellated and colonial cells) did not show any significant difference between the discrimination of these two life cycle stages in different assemblages. Consequently, the FluoroProbe can be used to detect the flagellated cells as well as the colonial cells of *P. globosa*. Although the FluoroProbe is not able to differentiate these two life cycles, it has at least the advantage of not underestimating the part played by the flagellated cells in the dynamics of *P. globosa* in comparison with traditional methods of cell counts with a microscope. Moreover, this probe is very easy to use. It can rapidly generate data on the spatio-temporal dynamics of phytoplankton groups and does not require any particular specialized training from the user.

Efficiency of the *P. globosa* fingerprint in an *in situ* study

The *in situ* use of the *P. globosa* fingerprint to monitor the changes in phytoplankton community structure in the coastal waters of the eastern English Channel showed a clear succession of phytoplankton blooms during the spring with the *P. globosa* bloom that occurred in April–May and preceded and followed two blooms of brown algae. The annual dynamics of *P. globosa* determined by the FluoroProbe and from flow cytometry were similar and a strong relationship was found between the values of *P. globosa* expressed in eq. μg chl *a* L^{-1} and cell abundances from flow cytometry. These observations are very consistent with the spring phytoplankton successions reported by Grattepanche *et al.* (Grattepanche *et al.*, 2011) during the same year (2009) next to our study site and are in agreement with the previously reported phytoplankton successions during the spring blooms in the eastern English Channel (Breton *et al.*, 2000; Seuront *et al.*, 2006) and the North Sea (Gieskes and Kraay, 1975; Cadée and Hegeman, 1986; Rousseau *et al.*, 2000, 2002; Tungaraza *et al.*, 2003; Stelfox-Widdicombe *et al.*, 2004; Muylaert *et al.*, 2006). Although the use of the *P. globosa* fingerprint can lead to an underestimation of diatoms during the spring

P. globosa bloom, when this species represents >55% of the total biomass (cf. laboratory experiments), the estimations of brown algae are much closer to the previously reported phytoplankton successions (Breton *et al.*, 2000; Seuront *et al.*, 2006) and to the microscopic observations of Grattepanche *et al.* (Grattepanche *et al.*, 2011) using this fingerprint than using the original fingerprints.

Absolute chl *a* concentrations and potential limits of the method

Errors in absolute concentration assessments could be for various reasons. The FluoroProbe underestimations of chl *a* concentrations, observed in our study, were probably caused by the fact that the FluoroProbe is calibrated according to HPLC analysis, whereas expected chl *a* concentrations were evaluated by fluorometry. It is known that HPLC usually provides lower chl *a* concentrations than spectrophotometric or fluorometric methods. This is due to allomers and other chlorophyll derivates that are detected as chl *a* in spectrophotometric and fluorometric methods, while they are separated chromatographically by HPLC (Meyns *et al.*, 1994; Jeffrey *et al.*, 1997). Our results agree with results of Gregor *et al.* (Gregor *et al.*, 2005) and Gregor and Marsalek (Gregor and Marsalek, 2004) that reported the FluoroProbe underestimations of chl *a* concentrations in comparison with a spectrophotometric method. The calibration is probably not the unique error factor in absolute concentration determination. Variations in the ratio of fluorescence to chl *a* (F^{chl}) were probably also involved.

According to MacIntyre *et al.* (MacIntyre *et al.*, 2010 and references therein), F^{chl} varies between species, with light exposure, nutrient availability and cell size. The classification algorithm used by the FluoroProbe for assessing chl *a* concentration does not integrate these variations and uses an invariant F^{chl} for each algal group. Consequently, natural variations in F^{chl} result in mis-classifications by the FluoroProbe. Moreover, the FluoroProbe has an open measuring chamber; it is, therefore, susceptible to additional variation in F^{chl} due to bright actinic light when it is used *in situ*. To limit this, it is advised to always use the FluoroProbe equipped with its black plastic case or to use the Flow-Through unit (bbe Moldaenke, Kiel, Germany).

To summarize, although the FluoroProbe did a relatively good job at monitoring the dynamics of *P. globosa*, as it is the case with all spectral fluorometers, determination of absolute contributions of phytoplankton groups can be subject to some bias (MacIntyre *et al.*, 2010). These biases are not related to the use of the *P. globosa* fingerprint but are related to the spectral fluorescence technique and the capacities of the FluoroProbe *per se*. Until a new classification algorithm integrating F^{chl} variations is proposed, it is recommended to complement measurements conducted with the FluoroProbe with less frequent discrete sample collections for microscopic or flow cytometric analyses. However, even if the FluoroProbe does not completely replace the traditional methods of cell counts, it has the advantage of considerably reducing the number of samples to analyse by using these methods. The FluoroProbe remains a very good tool for monitoring the *P. globosa* dynamics because it is able to detect the flagellated cells as well as the colonial cells of *P. globosa*, but also because, its high frequency measurements will never be equalled by the more accurate but more expensive and laborious traditional cell counts methods.

If we have to answer to the question "Which is the more appropriate method for monitoring *P. globosa* dynamics?" we shall answer that this depends on the kind of information required. The FluoroProbe is an excellent tool for projects requiring both a low-cost analysis and an easy to use method for obtaining real-time information at high spatio-temporal resolution about *P. globosa* dynamics (without differentiation between flagellated and colonial cells of *P. globosa*) together with the knowledge of the relative contribution of the major phytoplankton groups. For those looking for information about *P. globosa* dynamics at high resolution with a precise knowledge of absolute contributions of the major phytoplankton groups, the FluoroProbe coupled with less frequent sample collections for cell counting is a good combination. In such a situation, the interest of the FluoroProbe is that it provides higher spatio-temporal resolution and reduces the number of samples for cell counting. Such a combination reduces the time and cost of sample analysis in comparison with a monitoring program that would be carried out (as far as possible) at the same resolution but that would be only based on cell counting methods (flow cytometry or microscopic observations). Finally, if the objectives are to obtain precise counts of colonial and flagellated cells of *P. globosa* with detailed information about the species composition of the phytoplankton community, traditional microscopy coupled with an enumeration of flagellated cells of *P. globosa* by flow cytometry or electron microscopy is probably the only solution. However, in this case, standard routine measurements with high spatio-temporal resolution are very likely excluded mainly because of the costs and time needed to carry out such analyses.

CONCLUSIONS

Overall results of this study enable us to envisage the use of the FluoroProbe for long-term monitoring of the population dynamics of *P. globosa* with a higher temporal resolution than classical cell count methods. Indeed, good agreement was found between the FluoroProbe's results and cell counts by flow cytometry. The advantages of the FluoroProbe are its acquisition frequency (2 s) that permits a large amount of data to be obtained in very short time and the fact that all measurements are performed on-line without any delay between the measurement and the final results. This offers the possibility of collecting information on the dynamics of *P. globosa* at rates comparable with physico-chemical data that may improve our knowledge on the environmental factors controlling these blooms and may open new lines of research. Finally, the improvement of the detection of *Isochrysis sp.* using the *P. globosa* fingerprint suggests that the FluoroProbe may be used to monitor the dynamics of other Haptophytes in other ecosystems by calibrating the device with species representative of the region of interest. It is, nevertheless, important to bear in mind that when using the FluoroProbe, the monitoring of Haptophytes at the species level is only possible in areas where a single Haptophyte species is encountered. In areas where several species are simultaneously present, the FluoroProbe will be unable to distinguish them and, in this case, it can be used for monitoring the dynamics of the combined Haptophyte group.

ACKNOWLEDGEMENTS

The authors are grateful to Elsa Breton for providing cultures and her advice on culture maintenance and to Mélodie Penel and Jessica Chicheportiche for chlorophyll *a* analyses. They thank Yvan Lagadeuc and Stéphane Fraisse for *Microcystis aeruginosa* and *Scenedesmus sp.* cultures. Sébastien Lefebvre and Tammi L. Richardson are acknowledged for their critical reading and constructive comments on a previous draft of the manuscript. Finally, we thank the associate editor and two anonymous reviewers for their remarks.

FUNDING

This study forms part of the PhD thesis of E. Houliez. This work was financially supported by a grant from the French "Ministère de l'Enseignement Supérieur et de la Recherche" and the DYMAPHY INTERREG IVA "2 Mers Seas Zeeën" project co-funded by the European Union (ERDF funds).

REFERENCES

Allen, M. M. (1968) Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.*, **4**, 1–4.

Allen, M. M. and Stanier, R. Y. (1968) Growth and division of some unicellular blue-green algae. *J. Gen. Microbiol.*, **51**, 199–202.

Aminot, A. and Kérouel, R. (2004) *Hydrologie des écosystèmes marins. Paramètres et analyses*, Editions de l'Ifremer, Brest. pp. 335.

Antajan, E., Chrétiennot-Dinet, M. J., Leblanc, C. *et al.* (2004) 19′-Hexanoyloxyfucoxanthin may not be the appropriate pigment to trace occurrence and fate of *Phaeocystis*: the case of *P. globosa* in Belgian coastal waters. *J. Sea Res.*, **52**, 165–177.

Astoreca, R., Rousseau, V., Ruddick, K. *et al.* (2009) Development and application of an algorithm for detecting *Phaeocystis globosa* blooms in the Case 2 Southern North Sea waters. *J. Plankton Res.*, **31**, 287–300.

Balfoort, H. W., Snoek, J., Smiths, J. R. M. *et al.* (1992) Automatic identification of algae: neural network analysis of flow cytometric data. *J. Plankton Res.*, **14**, 575–589.

Beutler, M., Wiltshire, K. H., Meyer, B. *et al.* (2002) A fluorometric method for the differentiation of algal populations *in vivo* and *in situ*. *Photosynthes. Res.*, **72**, 39–53.

Blauw, A. N., Los, F. J., Huisman, J. *et al.* (2010) Nuisance foam events and *Phaeocystis globosa* blooms in Dutch coastal waters analyzed with fuzzy logic. *J. Mar. Syst.*, **83**, 115–126.

Breton, E., Brunet, C., Sautour, B. *et al.* (2000) Annual variations of phytoplankton biomass in the Eastern English Channel: comparison by pigment signatures and microscopic counts. *J. Plankton Res.*, **22**, 1423–1440.

Cadée, G. C. and Hegeman, J. (1986) Seasonal and annual variations in *Phaeocystis pouchetii* (Haptophyceae) in the westernmost inlet of the Wadden Sea during 1973 to 1985 period. *Neth. J. Sea Res.*, **20**, 29–36.

Cadée, G. C. and Hegeman, J. (2002) Phytoplankton in the Marsdiep at the end of the 20th century; 30 years monitoring biomass, primary production, and *Phaeocystis* blooms. *J. Sea Res.*, **48**, 97–110.

Caillault, E., Hébert, P. A. and Wacquet, G. (2009) Dissimilarity-based classification of multidimensional signals by conjoint elastic matching: Application to phytoplanktonic species recognition. *Commun. Comput. Inf. Sci.*, **43**, 153–164.

Carr, M. R., Tarran, G. A. and Burkill, P. H. (1996) Discrimination of marine phytoplankton species through the statistical analysis of their flow cytometric signatures. *J. Plankton Res.*, **18**, 1225–1238.

Gaebler, S., Hayes, P. K. and Medlin, L. K. (2007) Method used to reveal genetic diversity in the colony-forming prymnesiophytes *Phaeocystis antarctica*, *P. globosa* and *P. pouchetti*-preliminary results. *Biogeochemistry*, **83**, 19–27.

Gieskes, W. W. C. and Kraay, G. W. (1975) The phytoplankton spring bloom in Dutch coastal waters of the North Sea. *Neth. J. Sea Res.*, **9**, 166–196.

Grattepanche, J. D., Breton, E., Brylinski, J. M. *et al.* (2011) Succession of primary producers and micrograzers in a coastal ecosystem dominated by *Phaeocystis globosa* blooms. *J. Plankton Res.*, **33**, 37–50.

Gregor, J., Geris, R., Marsalek, B. *et al.* (2005) *In situ* quantification of phytoplankton in reservoirs using a submersible spectrofluorometer. *Hydrobiologia*, **548**, 141–151.

Gregor, J. and Marsalek, B. (2004) Freshwater phytoplankton quantification by chlorophyll a: a comparative study of *in vitro*, *in vivo* and *in situ* methods. *Water Res.*, **38**, 517–522.

Guillard, R. R. (1975) Culture of phytoplankton for feeding marine invertebrates. In Smith, W. L. and Chanley, M. H. (eds), *Culture of Marine Invertebrate Animals*. Plenum Press, New York, pp. 1–17.

Guillard, R. R. and Ryther, J. H. (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. *Can. J. Microbiol.*, **8**, 229–239.

Guiselin, N. (2010) Caractérisation des événements phytoplanctoniques en zone côtière: tests de techniques alternatives et développement d'indicateurs de qualité des masses d'eau. PhD Thesis. Université du Littoral Côte d'Opale, France.

Guiselin, N., Courcot, L., Artigas, L. F. *et al*. (2009) An optimised protocol to prepare *Phaeocystis globosa* morphotypes for scanning electron microscopy observation. *J. Microbiol. Methods*, **77**, 119–123.

Jeffrey, S. W., Mantoura, R. S. and Wright, S. W. (eds) (1997) *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*. United Nations Educational, Scientific and Cultural Organization, Paris. 661 pp.

Keller, M. D., Selvin, R. C., Claus, W. *et al*. (1987) Media for the culture of oceanic ultraphytoplankton. *J. Phycol.*, **23**, 633–638.

Lancelot, C., Billen, G., Sournia, A. *et al*. (1987) *Phaeocystis* blooms and nutrient enrichment in the continental coastal zones of the North Sea. *Ambio*, 16, 38–46.

Leboulanger, C., Dorigo, U., Jacquet, S. *et al*. (2002) Application of a submersible spectrofluorometer for rapid monitoring of freshwater cyanobacterial blooms: a case study. *Aquat. Microb. Ecol.*, **30**, 83–89.

Lefebvre, A., Guiselin, N., Barbet, F. and Artigas, LF. (2011) Long-term hydrological and phytoplankton monitoring (1992–2007) of three potentially eutrophic systems in the eastern English Channel and the Southern Bight of the North Sea. *ICES J. Mar. Sci*, **68**, 2029–2043.

Lorenzen, C. J. (1966) A method for continuous measurement of *in vivo* chlorophyll concentration. *Deep-Sea Res. I.*, **13**, 223–247.

MacIntyre, H. L., Lawrenz, E. and Richardson, T. L. (2010) Taxonomic discrimination of phytoplankton by spectral fluorescence. In Suggett, D. J. *et al*. (ed), *Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications*, pp. 129–169.

Mackey, M. D., Mackey, D. J., Higgins, H. W. *et al*. (1996) CHEMTAX - A program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. *Mar. Ecol. Prog. Ser.*, **144**, 265–283.

Malkassian, A., Nerini, D., Van Dijk, M. A. *et al*. (2011) Functional analysis and classification of phytoplankton based on data from an automated flow cytometer. *Cytom. Part A*, **79A**, 263–275.

Meyer, A. A., Tackx, M. and Daro, N. (2000) Xanthophyll cycling in *Phaeocystis globosa* and *Thalassiosira sp.*: a possible mechanism for species succession. *J. Sea Res.*, **43**, 373–384.

Meyns, S., Illi, R. and Ribi, B. (1994) Comparisons of chlorophyll-a analysis by HPLC and spectrophotometry: where do the differences come from? *Arch. Hydrobiol.*, **132**, 129–139.

Millie, D. F., Schofield, O. M. E., Kirkpatrick, G. J. *et al*. (2002) Using absorbance and fluorescence spectra to discriminate microalgae. *Eur. J. Phycol.*, **37**, 313–322.

Muylaert, K., Gonzales, R., Franck, M. *et al*. (2006) Spatial variation in phytoplankton dynamics in the Belgian coastal zone of the North Sea studied by microscopy, HPLC-CHEMTAX and underway fluorescence recordings. *J. Sea Res.*, **55**, 253–265.

Peperzak, L., Colijn, F., Gieskes, W. W. C. *et al*. (1998) Development of the diatom-*Phaeocystis* spring bloom in the Dutch coastal zone of the North Sea: the silicon depletion versus the daily irradiance threshold hypothesis. *J. Plankton Res.*, **20**, 517–537.

Peperzak, L., Colijn, F., Vrieling, E. G. *et al*. (2000) Observations of flagellates in colonies of *Phaeocystis globosa* (Prymnesiophyceae): a hypothesis for their position in the life cycle. *J. Plankton Res.*, **22**, 2181–2203.

Puigserver, M., Chrétiennot-Dinet, M. J. and Nezan, E. (2003) Some Prymnesiaceae (Haptophyta, Prymnesiophyceae) from the Mediterranean Sea, with the description of two new species: *Chrysochromulina lanceolata* sp. nov. and *C. pseudolanceolata* sp. nov. *J. Phycol.*, **39**, 762–774.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*, R foundation for statistical computing, Vienna, Austria.

Richardson, T. L., Lawrenz, E., Pinckney, J. L. *et al*. (2010) Spectral fluorometric characterization of phytoplankton community composition using the Algae Online Analyser. *Water Res.*, **44**, 2461–2472.

Rippka, R., Deruelles, J. and Waterbury, J. B. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.*, **111**, 1–61.

Rousseau, V., Becquevort, S., Parent, J. Y. *et al*. (2000) Trophic efficiency of the planktonic food web in a coastal ecosystem dominated by *Phaeocystis* colonies. *J. Sea Res.*, **43**, 357–372.

Rousseau, V., Chrétiennot-Dinet, M. J., Jacobsen, A. *et al*. (2007) The life cycle of *Phaeocystis*: state of knowledge and presumptive role in ecology. *Biogeochemistry*, **83**, 29–47.

Rousseau, V., Leynaert, A., Daoud, N. *et al*. (2002) Diatoms succession, silicification and availability in Belgian coastal waters (southern North Sea). *Mar. Ecol. Prog. Ser.*, **236**, 61–73.

Rowan, K. S. (1989) *Photosynthetic Pigments of Algae*, Cambridge Univ Press, Cambridge, 334 pp.

Rutten, T. P. A., Sandee, B. and Hofman, A. R. T. (2005) Phytoplankton monitoring by high performance flow cytometry: a successful approach? *Cytom. Part A*, **64**, 16–26.

Schapira, M., Vincent, D., Gentilhomme, V. *et al*. (2008) Temporal patterns of phytoplankton assemblages, size spectra and diversity during the wane of a *Phaeocystis globosa* spring bloom in hydrologically contrasted coastal waters. *J. Mar. Biol. Assoc. U.K.*, **88**, 649–662.

Scherrer, B. (2007) *Biostatistique*, Volume 1, 2e édition, Gaëtan Morin éditeur. 816 pp.

Schoemann, V., Becquevort, S., Stefels, J. *et al*. (2005) *Phaeocystis* blooms in the global ocean and their controlling mechanisms: a review. *J. Sea Res.*, 53, 43–66.

See, J. H., Campbell, L., Richardson, T. L. *et al*. (2005) Combining new technologies for determination of phytoplankton community structure in the northern Gulf of Mexico. *J. Phycol.*, **41**, 305–310.

Seuront, L., Vincent, D. and Mitchell, J. G. (2006) Biologically induced modification of seawater viscosity in the Eastern English Channel during a *Phaeocystis globosa* spring bloom. *J. Mar. Syst.*, **61**, 118–133.

Stelfox-Widdicombe, C. E., Archer, S. D., Burkill, P. H. *et al*. (2004) Microzooplankton grazing in *Phaeocystis* and diatom-dominated waters in the southern North Sea in spring. *J. Sea Res.*, **51**, 37–51.

Tompkins, J., Deville, M. M., Day, J. G. *et al*. (1995) *Culture Collection of Algae and Protozoa. Catalog of Strains*. Ambleside, UK, 204 pp.

Tungaraza, C., Rousseau, V., Brion, N. *et al*. (2003) Contrasting nitrogen uptake by diatom and *Phaeocystis*-dominated phytoplankton assemblages in the North Sea. *J. Exp. Mar. Biol. Ecol.*, **292**, 19–41.

Veldhuis, M. J. W., Brussaard, C. P. D. and Noordeloos, A. A. M. (2005) Living in a *Phaeocystis* colony: A way to be a successful algal species. *Harmful Algae*, **4**, 841–858.

Veldhuis, M. J. W. and Wassmann, P. (2005) Bloom dynamics and biological control of a high biomass HAB species in European coastal waters: A *Phaeocystis* case study. *Harmful Algae*, **4**, 805–809.

Wright, S. W., Thomas, D. P., Marchant, H. J. *et al*. (1996) Analysis of phytoplankton of the Australian sector of the Southern Ocean: Comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorisation program. *Mar. Ecol. Prog. Ser.*, **144**, 285–298.

Yentsch, C. S. and Yentsch, C. M. (1979) Fluorescence spectral signatures: the characterization of phytoplankton populations by the use of excitation and emission spectra. *J. Mar. Res.*, **37**, 471–483.

Zapata, M., Jeffrey, S. W., Wright, S. W. *et al.* (2004) Photosynthetic pigments in 37 species (65 strains) of Haptophyta: implications for oceanography and chemotaxonomy. *Mar. Ecol. Prog. Ser.*, **270**, 83–102.

Zhen, Y., Mi, T. and Yu, Z. (2008) Detection of *Phaeocystis globosa* using sandwich hybridization integrated with nuclease protection assay (NPA-SH). *J. Environ. Sci.*, **20**, 1481–1486.