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EFFECT OF LIGHT INTENSITY ON THE VARIABILITY OF FLUORESCENCE CHARACTERISTICS OF *Phaeocystis* sp. (PRYMNESIOPHYCEAE)

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Abstract. The influence of different light levels (20 and 130 μE m^{-2} s^{-1}) on the quantitative fluorescence characteristics of *Phaeocystis* sp. pigments was investigated by measuring the excitation spectra of *in vivo* fluorescence of chlorophyll *a* and accessory pigments. The fluorescence intensities corresponding to chlorophyll *a* and accessory pigments as well as the ratios of these intensities were analysed. Supporting measurements of *in vivo* absorption and microscopic cell counting were carried out. The dynamics of specific fluorescence, relative fluorescence efficiency, and pigment content in different growth stages were studied to provide the knowledge for data interpretation in fluorescence analysis of phytoplankton blooms. It was revealed that the ratios of fluorescence intensities of chlorophyll *a* induced through accessory pigments and directly through chlorophyll *a* (chlorophyll *c*/chlorophyll *a* and carotenoids/chlorophyll *a*) were stable during the growth of the selected culture. The ratios can be used as a parameter not influenced by the growth stage.

Key words: *Phaeocystis* sp., *in vivo* fluorescence, fluorometry, light adaptation.

INTRODUCTION

In vivo florometry has become an important part of different monitoring systems to estimate the abundance and dynamics of phytoplankton biomass. Mostly fixed wavelength fluorometers are used in continuous recording of fluorescence (Kahru et al., 1991; Guo & Dunstan, 1995). The measurements of excitation–emission spectra of fluorescence are more informative and allow determination of the major photosynthetic accessory pigments additionally to the total amount of chlorophyll *a*. The technique can roughly qualify the taxonomic composition of phytoplankton population (Cowles et al., 1993; Babichenko et al., in press).

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Although *in vivo* fluorescence measurements can be rapidly performed without any time-consuming treatments, accurate assessment of phytoplankton biomass is complicated because of the variability of the relationship between the chlorophyll *a* concentration and its *in vivo* fluorescence (Sosik & Mitchell, 1991; Althuis et al., 1994). The fluorescence of autotrophic algae depends on external factors and internal cellular adaptive responses to environmental conditions. The influence of light and nutrient availability on fluorescence characteristics has been documented for some laboratory cultures (e.g. Kiefer, 1973; Mitchell & Kiefer, 1988; Sosik & Mitchell, 1991; Geider et al., 1993; Johnsen & Sakshaug, 1993; Johnsen et al., 1997) and for natural phytoplankton communities (e.g. Neori et al., 1984; Kolber et al., 1990; Cowles et al., 1993; Guo & Dunstan, 1995). At the same time information on continuous dynamics of fluorescence characteristics of phytoplankton during the growth process is lacking in the literature. However, this knowledge is extremely important in any fluorescent analysis of phytoplankton blooms.

The present work was aimed to study the variability of fluorescence characteristics of photosynthetic pigments during the growth of *Phaeocystis* sp. by measuring excitation spectra of *in vivo* fluorescence of chlorophyll *a* and major accessory pigments at different light conditions. *Phaeocystis* was chosen for the experiment as one of the most widespread marine phytoplankters, regularly forming monospecific spring blooms in different areas of the world ocean (Reid et al., 1990; Lancelot et al., 1994). In addition the genus *Phaeocystis* is of great interest because it can possibly play a role in the regulation of climate by producing dimethylsulpho-propionate as one component of sulphur global cycle (Liss et al., 1994; Stefels et al., 1995). The results obtained in the laboratory experiment can serve to provide a correct interpretation of fluorescence data registered during *Phaeocystis* blooms.

MATERIALS AND METHODS

Culture growth conditions. The experiments were carried out on batch cultures of *Phaeocystis* sp. isolated from the North Sea (obtained from the algae culture collection of the University of Bergen, Norway). As the systematics of the genus *Phaeocystis* is still under discussion (Reid et al., 1990; Buma et al., 1991) we use in the text the extension "sp". Algae were cultivated in a 3-L glass flask using f/2 medium of Guillard & Ryther (1962) without the addition of silicate (Sigma Chemical Co, lot 76H2322) prepared on the basis of GF/F-filtered seawater with a salinity of 33 PSU. The cultures were grown at 4 °C and continuously illuminated with three cool-white fluorescent tubes (Philips TLD 36/33W). The cultures were cultivated at two different light conditions: at dimmed light (20 $\mu E\ m^{-2}\ s^{-1}$, D-culture) and at moderate light (130 $\mu E\ m^{-2}\ s^{-1}$, L-culture). The cultures were gently mixed periodically. *Phaeocystis* sp. during preculturing was in the colonial form, in the experiment only the flagellated stage was observed.

Spectral measurements. *In vivo* excitation spectra were recorded in the range of 400–660 nm at the fluorescence wavelength 680 nm with a spectrofluorimeter M32B (Laser Diagnostic Instruments Ltd., Estonia). Samples with very high culture densities were diluted with GF/F-filtered seawater before analysis.

In vivo and *in vitro* absorption spectral measurements in the spectral range 400–750 nm were carried out with a Hitachi spectrophotometer (model 1500). To record *in vivo* absorption spectra the filter-pad method was used (Yentsch, 1957, 1960). The volume to be filtered onto the Whatman GF/F filter was adjusted by means of fluorescence intensity measurements. The aim was to have approximately the same amount of pigments on the filter at any time of the analysis (Mitchell and Kiefer, 1988).

For *in vitro* absorption measurements, the cells were collected on GF/F filters. The pigments were extracted with 90% acetone at 4 °C for 24 h in the dark. Before spectral recording the extracts were cleared by filtration through GF/F filters. The concentrations of chlorophylls *a* (Chl*a*) and *c* (Chl*c*) were calculated using the equations of Jeffrey & Humphrey (1975) and the concentrations of total carotenoids (Car) with the equation of Parsons & Strickland (1963).

Microscoping counts. Phytoplankton cells were fixed with Lugol's solution. The cell densities in the cultures were counted in Bürker's chamber by a light microscope (Zeiss).

Calculations. The first measurements of the experiment (hour 0 in the figures) were carried out in a preculture before the inoculation and recalculated correspondingly to the dilution.

The cellular content of pigments in units, c (pg cell^{-1}), was calculated by dividing the concentration of the corresponding pigment, C (μg L^{-1}), by the number of cells, N (cells L^{-1}), in a water sample:

$$c = \frac{C}{N}.$$

The pigment-specific fluorescence intensity, $i(\lambda_{ex}, \lambda_{em})$, was expressed as the ratio of the *in vivo* fluorescence intensity, $I(\lambda_{ex}, \lambda_{em})$, to the concentration of the pigment:

$$i = \frac{I(\lambda_{ex}, \lambda_{em})}{C}.$$

The pigment-specific relative fluorescence efficiency, q, was calculated as the ratio of the *in vivo* fluorescence intensity and the *in vivo* absorption, $A(\lambda)$, normalized by the amount of pigment in a cell:

$$q = \frac{I(\lambda_{ex}, \lambda_{em})}{c \cdot A(\lambda)}.$$

RESULTS AND DISCUSSION

Pigmentation of two *Phaeocystis* sp. strains is well documented by Buma et al. (1991). The main light-harvesting pigments of *Phaeocystis* sp. are chlorophylls *a*, *c1*, *c2*, and *c3* and fucoxanthin. *Phaeocystis* sp. cells isolated from the North Sea contain only a small amount of diadinoxanthin and 19'hexanoyloxyfucoxanthin. Diadinoxanthin belongs to the group of photoprotective pigments involved in the rapid xanthophyll cycling (Demers et al., 1991; Moisan et al., 1998). Typical *in vivo* excitation spectra of fluorescence and *in vivo* and *in vitro* absorption spectra measured in the present experiment are shown in Figs. 1 and 2, respectively. *In vivo* absorption maximum at 484 nm is caused by fucoxanthin, diadinoxanthin and 19'hexanoyloxyfucoxanthin. The shoulder at 530 nm is due to the absorbing of light by fucoxanthin and 19'hexanoyloxyfucoxanthin (Johnsen et al., 1994). A mixture of chlorophylls *c* absorbs light in a range from 460 nm to 470 nm, while Chl*a* has an absorption from 430 nm to 440 nm (Rowan, 1989; Johnsen & Sakshaug, 1993). The absorption maxima of pigments extracted in organic solvent were blue shifted. The maxima of excitation spectra correspond to the direct excitation of Chl*a* (at 435 nm), and excitation of Chl*a* through the accessory pigment Chl*c1* + *c2* + *c3* at 460 nm, and fucoxanthin causes the shoulder at 480 nm to 550 nm. Diadinoxanthin is not involved in the process of light transfer to photosystem II (Johnsen & Sakshaug, 1996; Johnsen et al., 1997).

The growth of *Phaeocystis* sp. was characterized by a 3-day lag-phase for the L-culture and a 5-day lag-phase for the D-culture and a rapid logarithmic growth after that (Fig. 3a). A stationary phase of growth for the L-culture was reached at the day 9 and for the D-culture at the day 12. The dynamics of the pigment concentration and integral fluorescence was similar to that for cell numbers (not shown). At the same time the *c* values (Fig. 3b) and fluorescence intensity per cell (Fig. 3c) in the D-culture were 2–2.5 times higher compared with the L-culture. This is in correspondence with literature data (Gallagher et al., 1984;

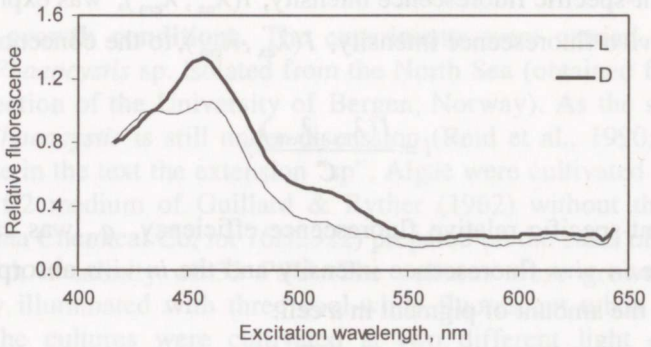

Fig. 1. Differences of *in vivo* fluorescence excitation spectra of *Phaeocystis* sp. cultures grown under different light conditions (day 9). Spectra are scaled to 1 at 435 nm of Chl*a* excitation. L-culture grown at 130 μE m^{-2} s^{-1}, D-culture grown at 20 μE m^{-2} s^{-1}.

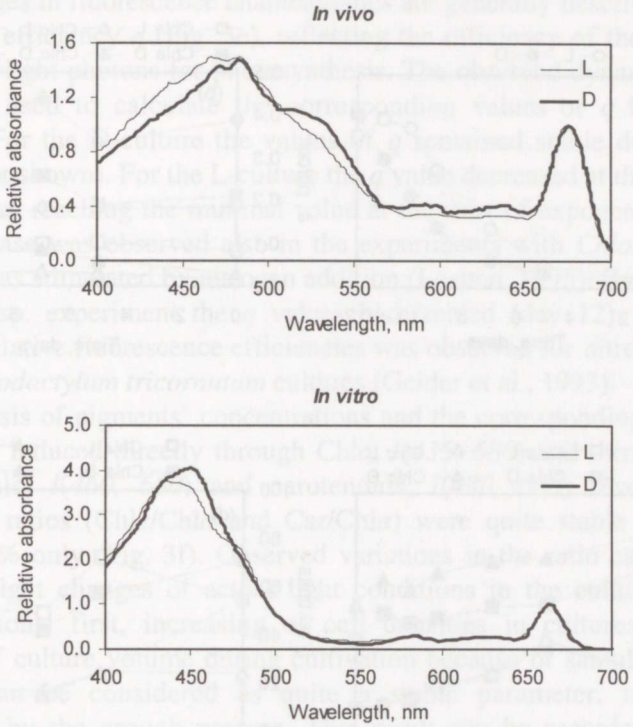

Fig. 2. Differences of absorption spectra of *Phaeocystis* sp. Spectra are scaled to 1 at red maximum of spectra. Abbreviations as in Fig. 1.

Kohata & Watanabe, 1988; Mitchell & Kiefer, 1988; Nelson & Prézelin, 1990; Johnsen & Sakshaug, 1993; Sciandra et al., 1997), where higher cellular pigment concentration and correspondingly higher fluorescence were observed for cells growing at lower illumination conditions. During algae cultivation the *c* values in the D-culture increased slightly at the beginning of exponential growth (day 7) and thereafter decreased slowly. In the L-culture the values of *c* were constant (Fig. 3b). The fluorescence of pigments per cell had a tendency to decrease through the experiment for both cultures (Fig. 3c), but in the D-culture the values started to diminish at the beginning of the experiment and for the L-culture after the start of intensive growth (day 5). A similar dynamics was observed in the cultivation experiment with *Chlorella* sp. by using nutrient additions (Leeben, 1995). Because of rather constant *c* values (Fig. 3b), the pigment-specific fluorescence *i* (Fig. 3d) showed a similar behaviour as fluorescence per cell (Fig. 3c). Such a decreasing of Chl*a*-specific fluorescence for the cultures at the start of intensive growth (at day 7) can be probably due to the high excitation energy transfer efficiency to reaction centres of photosystem II in a growing culture.

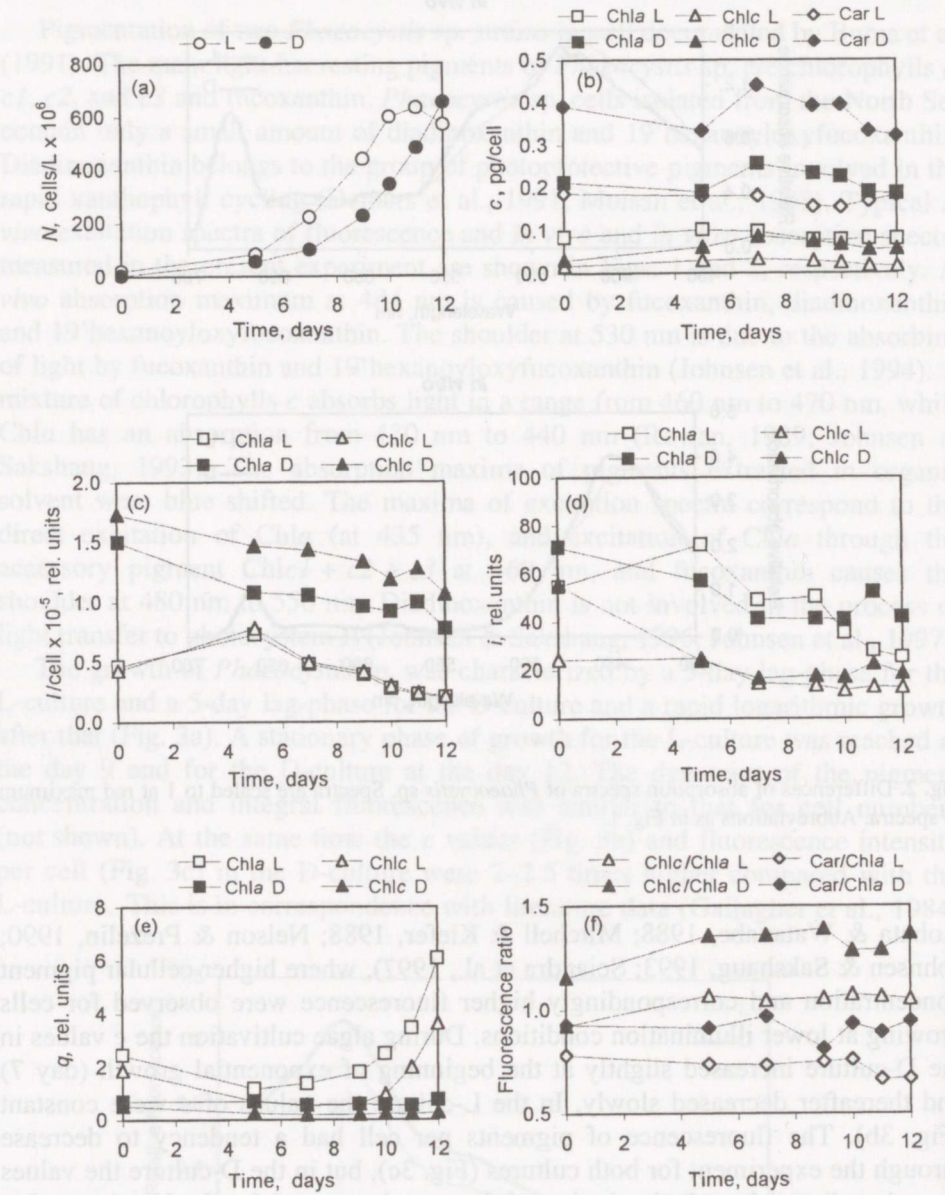

Fig. 3. Temporal development of *Phaeocystis* sp. cultures: (a) cell numbers, *N*; (b) cellular pigment content, *c*; (c) fluorescence per cell, *I*/cell; (d) pigment-specific fluorescence, *i*; (e) relative fluorescence efficiency, *q*; (f) ratio of fluorescence intensities excited through accessory pigments (Chl*c* at 460 nm, Car at 480 nm) and direct excitation of Chl*a* (at 435 nm). The values of *i* and *q* for Chl*c* are divided by 10. Abbreviations as in Fig. 1.

The changes in fluorescence characteristics are generally described by relative fluorescence efficiency q (Fig. 3e), reflecting the efficiency of the utilization of the absorbed light photons for photosynthesis. The observed dynamics of culture growth was used to calculate the corresponding values of q for the L- and D-cultures. For the D-culture the values of q remained stable during 3 weeks (last week not shown). For the L-culture the q value decreased at the beginning of the experiment reaching the minimal value at the start of exponential growth. A similar decrease was observed also in the experiments with *Chlorella* sp. when the growth was stimulated by nitrogen addition (Leeben, 1995). By the end of the *Phaeocystis* sp. experiment the q values had trebled (day 12). An analogous increase in relative fluorescence efficiencies was observed for nitrogen- and iron-starved *Phaeodactylum tricornutum* cultures (Geider et al., 1993).

The analysis of pigments' concentrations and the corresponding intensities of fluorescence, induced directly through Chl*a*, *I*(*435, 680*) and through accessory pigments Chl*c*, *I*(*460, 680*) and carotenoids, *I*(*480, 680*) revealed that the fluorescence ratios (Chl*c*/Chl*a* and Car/Chl*a*) were quite stable and varied in extent by 15% only (Fig. 3f). Observed variations in the ratio can be probably caused by slight changes of actual light conditions in the culture due to two opposite actions: first, increasing of cell densities in cultures and second, decreasing of culture volume during cultivation because of sampling. Therefore the ratios can be considered as quite a stable parameter, not influenced considerably by the growth process. This result can be regarded as the most important one for practical application of fluorescence excitation spectra for phytoplankton diagnostics.

CONCLUSION

Relationships between the fluorescence characteristics and concentrations of pigments at different light levels during cultivation of *Phaeocystis* sp. were investigated. Different dynamics of fluorescent characteristics of high- and low-light adapted phytoplankton cells complicates the interpretation of monitoring fluorescence data obtained in periods of phytoplankton blooms. Attempts to calibrate fluorescence data by the concentration of pigments without understanding the influence of different light conditions on phytoplankton will lead to errors in the quantitative estimation of pigments. By taking into account the results of the laboratory experiments it is possible to more correctly interpret fluorescent data for natural phytoplankton. The experiment with *Phaeocystis* sp. growth at different light conditions supported previously obtained results for *Chlorella* sp. cultivation at different nutrient additions: the ratio of fluorescence intensities of Chl*a* induced through accessory pigments and directly through Chl*a* (Chl*c*/Chl*a* and Car/Chl*a*) is not influenced much by the growth phase and defined by the pigment composition of cells (i.e. by taxonomic characteristics).

This makes it possible to diagnose the phytoplankton community at different stages of its development both quantitatively and qualitatively by using ratios of peak intensities in excitation spectra of fluorescence as fluorescent markers of the taxonomic group.

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VALGUSE INTENSIIVSUSE MÕJU *Phaeocystis* sp. (PRYMNESIOPHYCEAE) FLUORESTSENTSI KARAKTERISTIKUTE MUUTLIKKUSELE

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Laborieksperimendi käigus uuriti valguse erineva intensiivsuse (20 ja 130 µE m^{-2} s^{-1}) mõju mikrovetika *Phaeocystis* sp. pigmentide fluorestsentsi karakteristikutele. Selleks mõõdeti pigmentide fluorestsentsi ergastusspektreid *in vivo* ning klorofüll *a* ja lisapigmentide (klorofüll *c* ja karotinoidide) sisaldust. Analüüsiti klorofüll *a* ja lisapigmentide fluorestsentsi intensiivsust ning ka nende intensiivsussuhteid (klorofüll *c*:klorofüll *a* ja karotinoidid:klorofüll *a*). Lisaks registreeriti mikrovetika neeldumisspektrid *in vivo* ning loendati mikroskoobi abil rakkude hulk. Uuriti pigmendi fluorestsentsi, fluorestsentsi relatiivse efektiivsuse ja rakkude pigmendisisalduse dünaamikat kultuuride erinevates kasvufaasides. Kuigi *Phaeocystis* sp. fluorestsentsi karakteristikud ja pigmentide kontsentratsioon kultiveerimise jooksul varieerusid, muutusid pigmentide fluorestsentsi intensiivsussuhted kultuuri kasvufaasides vähe.