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RESEARCH ARTICLE

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A methodological approach to investigate steady state fucoxanthin chlorophyll *a*/*c* binding protein mRNA levels in Wadden Sea sediments

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Abstract A method was established to investigate the steady state levels of mRNAs from genes encoding fucoxanthin chlorophyll *a*/*c* binding proteins (Fcp) of diatoms in situ. During the study, which was performed with Wadden Sea sediments from the German North Sea shore near Dangast, oxygenic photosynthesis was carried out mainly by pennate diatoms. Field samples were taken after tidal exposure from dawn up to late afternoon at 2-hourly intervals, and frozen in liquid nitrogen. In the laboratory, total RNA was isolated by isopycnic ultracentrifugation in caesium chloride gradients. Yields of approximately 10–300 µg RNA per gram wet sediment were obtained. Defined amounts of total RNA were blotted onto nylon membranes and hybridised with probes against the *fcp2* and 18S rDNA genes of *Cyclotella cryptica*. To estimate the steady state amount of *fcp* mRNAs, *fcp* signal intensities were normalized to the signal intensities obtained from hybridisation to an 18S rDNA gene probe. In the two time-course studies performed to demonstrate the applicability of the method, the steady state levels of *fcp* mRNA increased up to 12-fold with the onset of light, reaching a maximum 6–8 h after sunrise before they decreased again. Possible reasons for this time-course are discussed.

Keywords Diatoms · Field studies · Gene expression · Light-harvesting complexes

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Introduction

Photosynthetic eukaryotes contain light-harvesting complexes (LHCs) that absorb light energy and transfer it to the reaction centres of photosystem I and photosystem II. While the LHCs of higher plants and green algae are constituted by chlorophyll *a*/*b* (chl *a*/*b*) binding proteins (Cabs) [9, 15], LHCs of chromophytic algae are composed of fucoxanthin chlorophyll *a*/*c* binding proteins (Fcps) [5, 7, 10, 17]. Fcps are phylogenetically related to Cabs and share with them several highly conserved amino acids that function in pigment binding or are responsible for the three-dimensional structure [5]. They are nuclear-encoded by multigene families, the *fcp* genes [6, 11]. The transcription of most *fcp* genes is light-induced and follows a diurnal pattern. Thus, steady state *fcp* mRNA levels of the centric diatoms *Cyclotella cryptica* and *Thalassiosira weissflogii* are highest approximately 6–8 h after the beginning of an illumination period and decrease later [18, 22]. Additionally, transcript levels and the expression of *fcp* genes depend on the photo fluence rate applied and decrease with increased photo fluence rate [2, 22].

To date, all expression studies on *fcp* genes have been carried out exclusively in the laboratory with cultured material under defined conditions. However, under field conditions gene expression might be influenced by cellular and external factors simultaneously. For our experiments, we chose the Wadden Sea sediments of Dangast, which are dominated by diatoms as a phototrophic algal group [14]. Oxygen evolution and carbon fixation are high in these sediments, although the tides cause extreme changes in moisture, salinity, temperature and irradiance [4, 25]. All these varying environmental parameters might influence the expression of *fcp* genes; thus, to understand this ecosystem it is essential to study all these effects in combination, i.e. in situ. To this end, laboratory methods have to be modified and adjusted for use on natural sediments. In a previous publication [13], we described the application of immunochemical

techniques to the investigation of diatomaceous Fcp protein levels in field samples. The aim of the present work was to develop and apply a method for the detection and quantification of *fcp* mRNAs in these sediments.

Materials and methods

Field sampling

Wadden Sea field samples were withdrawn from muddy intertidal sediments located on the North Sea shore near the harbour of Dangast (Germany) on 15 May and 26 September 2001. Sunrise was at 04:26 and 06:17 local time, sunset at 20:17 and 18:14, respectively. High tides were at 06:43 and 18:58 on 15 May and at 07:51 and 20:48 on 26 September, low tides at 00:20 and 12:25 on 15 May and at 01:23 and 14:01 on 26 September. The samples were collected after the water had drained off between 06:00 and 16:00 on 15 May and between 08:00 and 19:00 on 26 September, mostly at 2-hourly intervals from three sampling sites in parallel, which were approximately 20 m from the high tide border line. For sampling, the uppermost 4–8 mm of the brown diatom layer was scraped off with a spatula and homogenized. Aliquots of 5 g were taken, wrapped in aluminium foil and immediately plunged into liquid nitrogen. The samples were then transferred to the laboratory where they were stored at −70°C.

Isolation of RNA

Total RNA from Wadden Sea sediments was isolated according to [28] with modifications. The cells within the 5 g aliquots of sediment samples were broken in liquid nitrogen using a mortar and pestle. Guanidine thiocyanate buffer [10 ml; 4 M guanidine thiocyanate, 100 mM Tris HCl (pH 7.5), 0.5% (w/v) sodium lauryl sarcosinate] and 100 µl β-mercaptoethanol were added to prevent RNA degradation. The DNA was sheared with a syringe and particles in the suspensions were pelleted by centrifugation (Beckman TJ-6 centrifuge; 4,960 *g* for 10 min at 20°C). Total RNA was isolated by ultracentrifugation in CsCl density gradients (Beckman L8-M ultracentrifuge, rotor SW40; 182,000 *g* for 24 h at 20°C). The RNA pellets were washed with 70% ethanol and dissolved in 400 µl TE buffer [10 mM Tris-HCl (pH 7.6), 1 mM EDTA] containing 0.1% (w/v) SDS. The RNA was precipitated with ethanol at −20°C overnight, pelleted by centrifugation (Heraeus Biofuge Fresco; Hanau, Germany; 13,000 rpm for 10 min at 4°C), washed with 70% ethanol, and finally dried in a vacuum dryer (Vacuum Concentrator; Bachofer, Reutlingen, Germany). The pellets were dissolved in 400 µl diethylpyrocarbonate (DEPC)-treated distilled H_2O (0.1% v/v DEPC) and the concentration of RNA solutions was measured at 260 nm in a Hitachi U-3000 spectrophotometer. The OD_{260}/OD_{280} ratios were measured in parallel. The RNA fractions of the three samples taken in parallel at each time point were combined and finally stored at −70°C.

Cultures and growth conditions of reference organisms

Four diatoms, two cryptophytes, two rhodophytes, two haptophytes, and one green alga were used to check the specificity of the gene probes. The organisms, sources, growth media and references are compiled in Table 1. The organisms were cultivated either without aeration or gassed with sterile air at 18–22°C. Cultures were grown in Erlenmeyer flasks containing 100–2,000 ml culture medium or in glass tubes (45 cm length, 4 cm diameter, 400 ml culture volume) at an irradiance of 4–5 W m^{-2} and a light:dark regime of 16:8 h. The cells were harvested by centrifugation and kept frozen at −20°C until genomic DNA was isolated.

Isolation of DNA

Total DNA of algal cultures was isolated according to [10] with some modifications. The pelleted algal cells were resuspended in 10 ml lysis buffer [50 mM Tris-HCl (pH 8.0), 100 mM EDTA, 1% (w/v) SDS, 10 mM dithiothreitol] and incubated for 30 min at 37°C. Protein was removed by repeated extraction with phenol, phenol/

Table 1 Sources and growth media of the reference organisms used to check thespecificity of the gene probes

Organism	Source, culture collection, strain #	Growth medium
Cyclotella cryptica (Bacillariophyceae)	SAG[a], strain 1070-1 [29]	Cyclotella medium [32]
Skeletonema costatum (Bacillariophyceae)	CCMP[b], strain 1332	f/2 [12]
Phaeodactylum tricornutum (Bacillariophyceae)	SAG, strain1090-1a [29]	M5 [29]
Navicula sp. (Bacillariophyceae)	Isolated from Wadden Sea sediment, Dangast, Germany	M5
Dunaliella sp. (Chlorophyceae)	G. Gerdes (Terramare, Wilhelmshaven, Germany)	ASN III [27]
Rhodella violacea (Rhodophyceae)	SAG, strain 30.97	ASNIII
Galdieria sulphuraria (Rhodophyceae)	Culture collection of the University of Marburg, Germany, probably descendant of SAG, strain 107.79 [19]	Allen's medium [1]
Emiliana huxleyi (Haptophyceae)	SAG, strain B33.90 [29]	M5
Isochrysis sp. (Haptophyceae)	SAG, strain B927-2 [29]	M6 [29]
Rhodomonas sp. (Cryptophyceae)	U. Sommer (Institut für Meereskunde, University of Kiel, Germany) [3]	ASN III
Cryptomonas sp., strain S2 (Cryptophyceae)	Isolated from a freshwater pond near Marburg, Germany [26]	Moor-Chu [30]

[a]Algal culture collection (Sammlung von Algenkulturen) at the University of Göttingen
[b]Provasoli-Guillard National Center for Culture of Marine Phytoplankton, West Boothbay Harbor, Me

chloroform (1:1) and chloroform. CsCl (1 g/ml aqueous solution) and ethidium bromide (500 µl of a 10 mg/ml stock solution) were added to the aqueous phases and DNA was isolated by density gradient ultracentrifugation in a Beckman L8-M Ultracentrifuge (rotor 70.1Ti; 186,000 ×*g* for 48 h at 20°C). The DNA band was harvested with a syringe. After removal of the ethidium bromide with CsCl-saturated isobutanol, the DNA solutions were dialysed against TE buffer and precipitated with ethanol at −20°C overnight. The DNA was pelleted by centrifugation for 10 min at 4°C and 13,000 rpm (Heraeus Biofuge Fresco), washed with 70% ethanol, dried in a vacuum dryer and finally dissolved in 400 µl TE buffer. After measuring the concentrations at 260 nm in a Hitachi U-3000 spectrophotometer, the DNA fractions were stored at −20°C.

Agarose gel electrophoresis

The quality of the DNA fractions was checked by running 5 µg aliquots on gels of 1% (w/v) agarose in TAE (40 mM Tris-HCl, 0.115% acetic acid, 10 mM EDTA, pH 8.0) for 90 min at 60 V. The quality of the RNA fractions was checked by running 5–10 µg aliquots on denaturing formaldehyde gels according to [28].

RNA and DNA dot blots

For RNA dot blotting experiments, 10 µg RNA of each of the sampled and pooled RNA fractions was dissolved in 400 µl formaldehyde buffer (DEPC-treated H_2O:20×SSC (3 M sodium chloride:300 mM sodium citrate pH 7.0):formaldehyde in a ratio of 5:3:2), heated to 68°C for 10 min, immediately chilled on ice and spotted onto dry nylon membranes (Biodyne Plus Nylon Membrane; Pall Gelman Laboratory, Ann Arbor, Mich.) using a Minifold I Dot-Blot System (Schleicher & Schuell, Dassel, Germany). The RNA was fixed by baking the membranes at 80°C for 2 h. RNA dot blots were prepared in triplicate for each of the sampled and pooled RNA fractions and for each gene probe. For DNA dot blotting experiments, 100 ng algal DNA aliquots were dissolved in 400 µl TE buffer, heated to 95°C for 10 min, immediately chilled on ice and processed as described for the RNA dot blots.

Synthesis of gene probes

The probes were amplified and labelled with a PCR DIG Probe Synthesis Kit (Boehringer Mannheim, Germany) according to the manufacturer's instructions. For probing total RNA from sediment samples for *fcp* mRNA, a 428 bp PCR product encoding the mature Fcp2 protein was amplified using the *fcp2* cDNA clone from the centric diatom *C. cryptica* as template [7] (EMBL/GenBank accession number AJ000545) and the primers 5′-CTCGGATTCTTCGATCC-3′ and 5′-AGTCCAAGAATACCCATCAT-3′. As an 18S rDNA probe, a 750 bp fragment of the 18S rDNA gene of *C. cryptica* was amplified from chromosomal DNA by PCR. The primer sequences were 5′-GGTGGTGCATGGCCG-3′ and 5′-ATCCTTCTGCAGGTTCACC-3′ [22]. PCR was performed with 30 cycles of a 45 s denaturing step at 95°C, followed by a 60 s annealing step at 49°C in the case of *fcp2* and 50°C for the 18S rRNA fragment, and a 60 s elongation step at 72°C. The probes were heated to 95°C for 10 min and dissolved in DIG Easy Hyb hybridisation solution (Boehringer Mannheim) prior to use.

Hybridisation experiments

Hybridisation experiments were performed according to *The System User's Guide for Filter Hybridisation* (Boehringer Mannheim). As heterologeous gene probes were used, after overnight hybridisation at 40°C, two washing steps of moderate stringency at 30°C with 2×SSC followed by two washing steps of higher stringency at 50°C with 0.5×SSC were carried out. For hybridisation of RNA dot blots, all solutions were treated with DEPC and autoclaved prior to use. For signal detection, the anti-Digoxigenin-alkaline phosphatase-conjugate and CSPD (DIG Luminescent Detection Kit, Boehringer Mannheim) were used according to the producer's recommendations. X-ray films (Lumi Film, Boehringer Mannheim) were exposed for periods of 1–6 h (18S rDNA gene probe) or up to 24 h (*fcp2* gene probe).

RNA dot blot quantification

The X-ray films were recorded and stored as digitised files using a Herolab Easy CCD Camera (type 429 h), a Herolab RH-3 illumination chamber and a personal computer equipped with the E.A.S.Y. Analysis System software (Herolab, Wiesloch, Germany). The signal intensity of the dots was calculated using the E.A.S.Y. Image Plus software [22]. As three dots were quantified in parallel for each sampling time and each gene probe, the mean (± standard deviation) was calculated.

Light measurements and statistical analyses

Photon fluence rates were measured with an Almemo 2290-2 measuring device (Ahlborn Mess- und Regeltechnik, Holzkirchen, Germany) equipped with a DK-PHAR2 Underwater Quantum Sensor (Deka Sensor Technology, Teltow, Germany). Measured values are given in µmol photons m^{-2} s^{-1}. Statistical analyses (*t*-test) were performed with the Sigma Plot 3.0 software package (Jandel Scientific Software, Erkrath, Germany).

Results

Sampling site

The measured photon fluence rates and air temperatures are shown in Fig. 1. On 15 May, the photon fluence rates increased from approximately 20 µmol photons m^{-2} s^{-1} in the early morning up to values of to 1,800 µmol photons m^{-2} s^{-1} measured around noon. On 26 September, the photon fluence rates did not exceed 800 µmol photons m^{-2} s^{-1}. In parallel, the temperatures rose by up to 9°C on both days. Light microscopy examination of sediment samples revealed that pennate diatoms, i.e. species of the genera *Navicula* and *Nitzschia*, were the dominating phototrophic algae inhabiting the sediments. Cyanobacteria, green algae, euglenoids and other chromophytic algae could not be seen. The dominance of diatoms predestined these sediments for investigating the steady state levels of *fcp* mRNAs in the field.

Yields, purity and quality of total RNA

Sediment samples were taken at 2-hourly intervals and subjected to RNA isolation. We combined several samples taken at different spots for each timepoint thus avoiding errors caused by possible local variations in the algal flora. The RNA yields ranged from approximately 10–300 µg RNA/g wet sediment, while the $OD_{260/280}$ ratios of RNA fractions ranged from 1.6 to 2. Northern

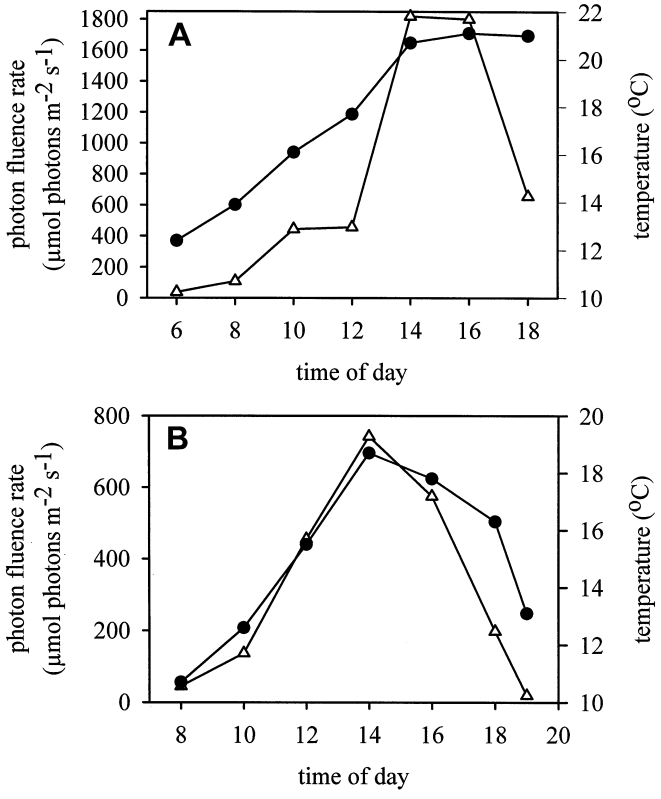

Fig. 1 Temperature curves (*filled circles*) and photon fluence rate curves (*triangles*) measured on **A** 15 May 2001 and **B** 26 September 2001

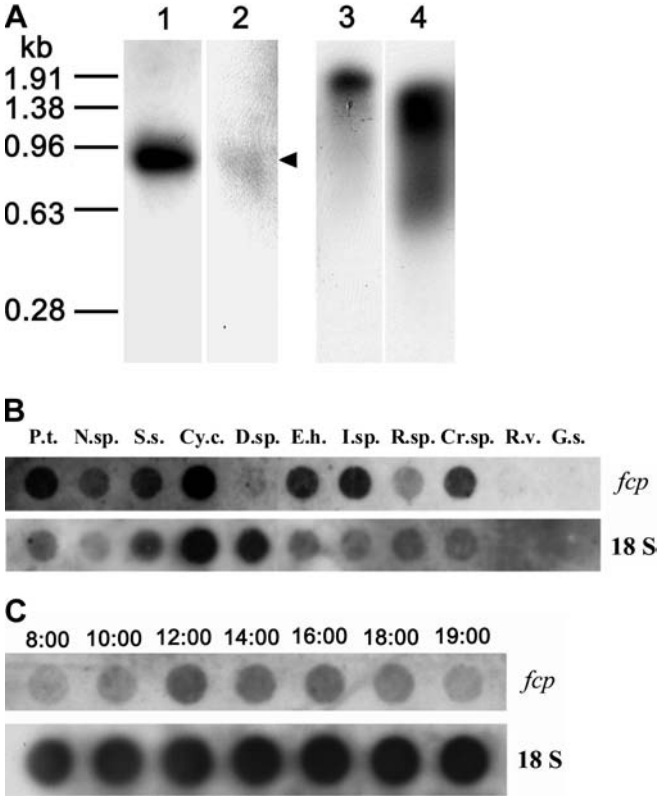

Fig. 2 **A** Northern blot analysis of RNA from Wadden Sea sediment (*lanes 2* and *4*) and from *Cyclotella cryptica* (*lanes 1* and *3*) separated by denaturing agarose gel electrophoresis and blotted onto nylon membranes. Lanes: *1*, *2 fcp2* gene probe, *3*, *4* 18S rDNA gene probe. The *fcp* signal was rather low in the case of RNA isolated from Wadden Sea sediments (*arrow* in *lane 2*). Some degradation was observed for the 18S rRNA from Wadden Sea sediment samples (*lane 4*), as the signal did not result in a distinct but rather a broad band. RNA size markers are indicated on the left. **B** Dot blots demonstrating the applicability of the gene probes. DNA of algal cultures (100 ng) was blotted onto nylon membrane and hybridised with the *fcp2* (upper panel) and 18S rDNA (lower panel) gene probes of *Cyclotella cryptica*. Target DNAs: *P.t. Phaeodactylum tricornutum*, *N.sp. Navicula* sp., *S.s. Skeletonema subsalsa*, *Cy.c. Cyclotella cryptica*, *D.sp. Dunaliella* sp., *E.h. Emiliana huxleyi*, *I.sp. Isochrysis* sp., *R.sp. Rhodomonas* sp., *Cr.sp. Cryptomonas* sp., strain S2, *R.v. Rhodella violacea*, *G.s. Galdieria sulphuraria*. **C** Representative dot blots of 10 µg RNA isolated from Wadden Sea sediment on 26 September 2001 hybridised with either the *fcp* gene probe (upper panel) or the 18S rDNA gene probe (lower panel). The values above the dots show the local time

blotting experiments revealed that RNAs of distinct sizes hybridised with the *fcp2* and 18S rDNA gene probes. We observed faint hybridisation signals for *fcp2* but strong signals for 18S rDNA (Fig. 2A, lanes 2 and 4). The hybridizing RNAs from sediment samples were similar in size to those which could be seen when total RNA of *C. cryptica* was run on denaturing formaldehyde agarose gels, northern blotted and hybridised with the two gene probes (Fig. 2A, lanes 1 and 3). Some degradation was observed for RNA isolated from Wadden Sea sediment when the 18S rDNA gene probe was used. Repeated extraction of the same sediment samples did not result in additional RNA recovery, thus demonstrating that the extractable RNA was recovered with a single extraction step. Prior to use of the RNA fractions in dot blotting experiments, the applicability of the two gene probes was confirmed.

Applicability of the gene probes

DNA from several algae (see Table 1) was dot blotted onto nylon membranes and hybridised with the probes. As shown in Fig. 2B (upper row), the *fcp2* gene probe gave rise to a strong hybridisation signal with DNA of *C. cryptica* and *Phaeodactylum tricornutum*. Positive signals were also found for the diatoms *Navicula* sp. and *Skeletonema subsalsa*, the haptophytes *Emiliana huxleyi* and *Isochrysis* sp., and the two cryptophytes *Rhodomonas* sp. and *Cryptomonas* sp., strain S2. No signals were detected for the rhodophytes *Rhodella violacea* and *Galdieria sulphuraria* and for the green alga *Dunaliella* sp. This shows that our probe is specific for mRNAs of Chl *c*-containing algae, but did not hybridise with *cab* mRNAs and mRNAs for red algal LHCs. The 18S rDNA gene probe (Fig. 2B, lower row) gave rise to hybridisation signals with DNAs of *C. cryptica*, *S. subsalsa*, *P. tricornutum* and *Dunaliella* sp. The signals for *Navicula* sp. and the other algae were weaker. As both gene probes gave rise to hybridisation signals with diatom samples and since other algae were hardly found in the collected Wadden Sea sediments, the probes were

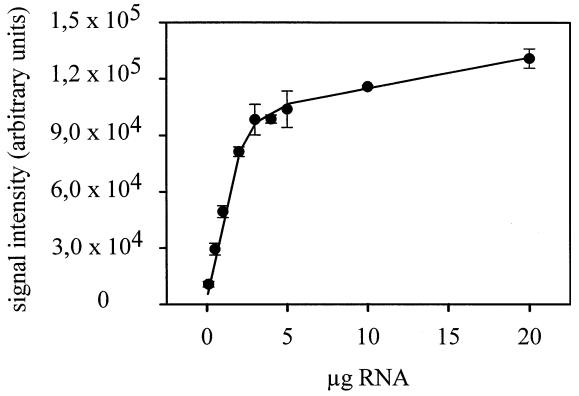

Fig. 3 Correlation between RNA amount applied and signal intensity. RNA (0.2–20 µg) was loaded and hybridised with the *fcp* gene probe. The data are the means of three measurements

judged suitable for subsequent hybridisation experiments with the RNA fractions isolated from these samples.

Determination of optimal signal range

It was to be expected that the signal intensity would saturate with increasing RNA loading and/or exposure time. To determine the range in which the signal intensity is well correlated with RNA amount at a given exposure time, increasing amounts of RNA (0.2–20 µg) were hybridised with the *fcp* probe. The result is shown in Fig. 3. Up to 90,000 counts there is a linear correlation between RNA load and signal intensity. Beyond this point, the slope of the curve is significantly decreased, although we did not reach true saturation with the RNA amount and exposure time applied. Hence it should be noted that only samples yielding less than 90,000 counts are compared with each other.

Steady state levels of *fcp* mRNA

Aliquots of 10 µg RNA of the samples taken at 2-hourly intervals on the two sampling days were dot blotted and hybridised with the probes. Representative results of the RNA dot blot time series of 26 September are shown in Fig. 2C. The RNA fractions that hybridised to the *fcp2* gene probe differed in their signal intensities over the time of sampling (Fig. 2C, upper row), while the 18S rDNA gene probe of *C. cryptica* gave rise to signals of almost equal intensities, irrespective of the time during the day (Fig. 2C, lower row). As quantification of steady state mRNA levels of *fcp* genes from Wadden Sea diatoms by RNA dot blots needed verification of equal loading, the hybridisation signals of the 18S rDNA gene probe was used as an internal standard to estimate the relative *fcp* transcript levels. The results of the two sampling days are compiled in Fig. 4A, B.

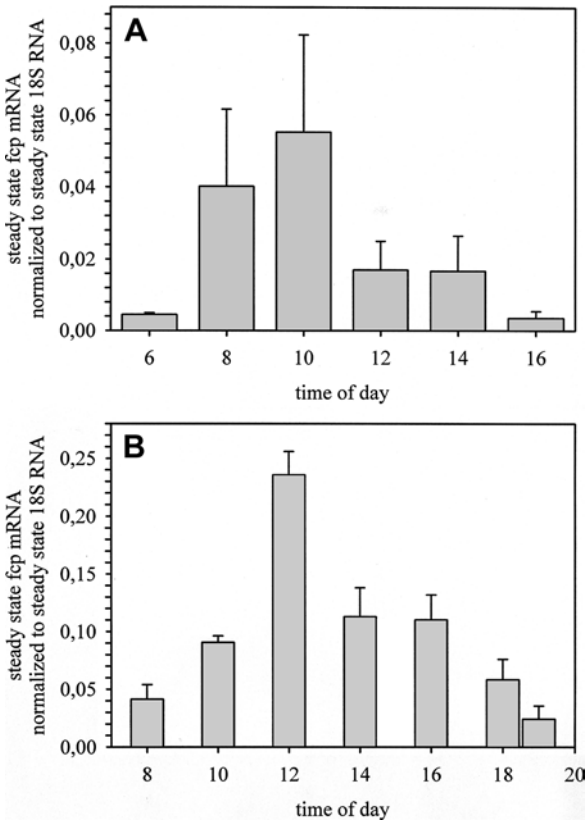

Fig. 4A, B Bar graphs showing the steady state levels of Wadden Sea sediment *fcp* mRNA estimated by normalising the *fcp* signal intensities to the corresponding 18S rDNA signal intensities. **A** Steady state levels of *fcp* mRNA on 15 May 2001. **B** Steady state levels of *fcp* mRNA on 26 September 2001. *Bars* Mean ± standard deviation

We observed an increase of the relative steady state *fcp* mRNA levels from the first sampling towards noon on both days. The maximum concentrations of steady state *fcp* mRNA on the sampling days were found to occur at 10:00 and 12:00, respectively. On 15 May, the relative steady state *fcp* mRNA level was approximately 12-fold higher at 10:00 than at 06:00, whereas on 26 September the value calculated for 12:00 exceeded that calculated for 08:00 by only 5- to 6-fold. The differences were statistically significant ($P = 0.05$ on 15 May, and $P = 0.006$ on 26 September). In the afternoon the steady state *fcp* mRNA levels dropped on both days and reached values similar to those measured at 06:00 or 08:00, respectively.

Discussion

Molecular studies with field samples have two main advantages: (1) organisms can be studied under the influence of all naturally occurring parameters, giving a better idea of what happens in an ecosystem than laboratory experiments; (2) if the DNA probes used are specific enough, the response of single taxa to external parameters (which may or may not be manipulated by

the investigator) can be studied without the need to isolate and cultivate the organisms in question. In recent years several laboratories have focussed on the detection of rRNAs and investigated gene expression in the field [16, 21, 24]. To our knowledge, the current study is the first that presents a method for monitoring steady state levels of distinct mRNA populations in a natural Wadden Sea sediment. Sampling can be carried out in the field with simple and inexpensive equipment. The isolation of RNA is based on routine protocols and its quality can be checked easily by agarose gel electrophoresis. Some degradation was observed for RNA isolated from Wadden Sea sediment when the 18S rDNA gene probe was used. It thus might be possible that some *fcp* mRNA degradation had also occurred, as the *fcp* mRNA signal was very faint (see Fig. 2A). However, this should have no major effects when the RNA is used subsequently in RNA dot blotting experiments.

We tested our gene probes with a variety of algae. The 18S rDNA gene probe resulted in strong or moderate hybridisation signals for diatoms and fainter signals for the other algae. Using the *fcp2* gene of *C. cryptica* as a probe, strong and moderate hybridisation signals were found with DNA of diatoms, while only moderate signals were registered with some of the other algae tested. This result was expected, as a heterologeous gene probe was used and several of the algae are known to contain membrane intrinsic light harvesting proteins, i.e. Cabs and Fcps [8, 11]. Cabs and Fcps contain several highly conserved domains and amino acids; thus positive hybridisation signals should occur. Further studies will gain much from the use of homologeous probes deduced from diatoms that have been isolated from the investigated sediments. Homologeous probes will allow tracking of at least those diatoms from which the probes were deduced. Although we used heterologeous gene probes, both were shown to be well suited for our purpose. As the transcripts of *fcp* and 18S rDNA genes from the same sampled material were detected in our dot blotting experiments, the steady state *fcp* mRNA levels could be determined by normalising the *fcp* signal intensities to the corresponding 18S rDNA signal intensity, assuming that 18S rRNA levels are constant and that the evenness of the measured 18S rRNA signal intensities are not due to signal saturation. This approach has also been used in laboratory experiments performed on unialgal cultures, higher plants and bacteria [16, 20, 22]. Normalisation to the 18S rRNA signal might be critical for those organisms that show different degrees in hybridising to the *fcp* and 18S rDNA probes of *C. cryptica*. However, we assume that the species composition of the sediments remains constant during the sampling period and therefore this factor can be neglected. For future experiments, the method might easily be transferred to sediments inhabited by a more diversified alga population, provided that the probes to be used are highly specific for the genes and/or organisms of interest.

On 15 May and 26 September, maximum *fcp* mRNA levels were detected at 10:00 and 12:00, respectively. The concentration increased 5- to 6-fold in the September sample and approximately 12-fold in the May sample. At later times of day, we measured decreasing values. This pattern may be caused by a diurnal oscillation of *fcp* transcripts. Leblanc and coworkers [18] investigated the photoregulation of *fcp* gene expression in the centric planktonic diatom *T. weissflogii*. They found a 5- to 6-fold increase in response to white light irradiation, with a peak occurring approximately 6–8 h after the onset of light. For *C. cryptica*, a diurnal expression pattern could be shown for *fcp* genes belonging to the *fcp1/fcp2/fcp3/fcp5* gene cluster. Maximum steady state *fcp* mRNA levels occurred approximately 5 h after the onset of light [22]. This data corresponds to our findings of a peak 6–8 h after sunrise.

However, diurnal oscillations of *fcp* gene expression have only been reported for centric, planktonic diatoms and it is not yet known whether the diatom taxa in our sediment samples show the same feature. Some organisms, such as the chrysophyte *Gyraudiopsis stellifer*, show no *fcp* transcript oscillations [23]. Therefore the decrease in *fcp* transcript level might also be related to the increasing photon fluence rates. On 15 May, photon fluence rate values above 400 μmol photons m^{-2} s^{-1} were measured after 10:00, while on 26 September this value was reached around noon. As shown in laboratory experiments, gene expression of *fcp* and *cab* genes is lowered under high light conditions compared to low light conditions [2, 31]. Further experiments are needed to show which factor is responsible for the *fcp* mRNA decrease in our case. Additionally, one has to bear in mind that the measured changes in the steady state levels of *fcp* mRNA may be due to alterations in transcription rates as well as to mRNA turnover and degradation rates. Currently, we cannot distinguish between these possibilities. We also have to emphasise that our data only show the overall changes in transcript levels. We cannot exclude the possibility that certain diatom species, which might be present in minor amounts, show a different type of *fcp* expression pattern.

Diatomaceous *fcp* genes might also be regulated by other parameters. Many parameters change in the course of a day in Wadden Sea sediments; the most important physical parameters besides incident light are moisture, salinity and temperature. It is known that temperature has a great impact on photosynthesis [4]. Our knowledge on the adaptation of the photosynthetic apparatus on the level of gene expression to varying environmental parameters in the field is still limited. Additional investigations are needed to understand the regulation of gene expression in the field and the method provided here is a helpful and promising starting device to achieve this.

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