Harmful Algae 75 (2018) 27–34

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Circadian and irradiance effects on expression of antenna protein genes and pigment contents in dinoflagellate *Prorocentrum donghaiense* (Dinophycae)

Xinguo Shi[a,b], Ling Li[a], Senjie Lin[a,c,*]

[a] State Key Laboratory of Marine Environmental Science and College of Ocean and Earth Sciences, Xiamen University, Xiamen, 361012, China
[b] College of Biological Science and Engineering, Fuzhou University, Fuzhou, 350108, China
[c] Department of Marine Sciences, University of Connecticut, Groton, CT 06340, United States

ARTICLE INFO

Article history:
Received 20 September 2017
Received in revised form 5 April 2018
Accepted 5 April 2018
Available online 16 April 2018

Keywords:
PCP
acpPC
Gene expression
Pigment contents
Prorocentrum donghaiense

ABSTRACT

PCP and acpPC are the two major antennae proteins that bind pigments in peridinin-containing dinoflagellates. The relationship between antennae proteins and cellular pigments at molecular level is still poorly understood. Here we identified and characterized the two antennae protein genes in dinoflagellate *Prorocentrum donghaiense* under different light conditions. The mature PCP protein was 32 kDa, while acpPC was a polyprotein each of 19 kDa. Both genes showed higher expression under low light than under high light, suggesting their possible role in a low light adaptation mechanism. The two genes showed differential diel expression rhythm, with PCP being more highly expressed in the dark than in the light period and acpPC the other way around. HPLC analysis of cellular pigments indicated a diel change of chlorophyll *c2*, but invariability of other pigments. A stable peridinin: chlorophyll *a* pigment ratio was detected under different light intensities and over the diel cycle, although the diadinoxanthin: chlorophyll *a* ratio increased significantly with light intensity. The results suggest that 1) PCP and acpPC genes are functionally distinct, 2) PCP and acpPC can function under low light as an adaptive mechanism in *P. donghaiense*, 3). the ratios of diadinoxanthin:chlorophyll *a* and peridinin: chlorophyll *a* can potentially be used as an indicator of algal photophysiological status and a pigment signature respectively under different light conditions in *P. donghaiense*.

1. Introduction

Dinoflagellates and other phytoplankton live in a variable light environment and during photoacclimation may modulate their pigment abundance and distribution. Cellular concentrations of photosynthetic pigments are expected to correlate with the expression of individual chlorophyll-binding proteins (Iglesias-Prieto and Trench, 1997). Changes in pigments under variable light intensities experienced by dinoflagellates and other phytoplankton are usually associated with antenna remodeling (Peers et al., 2009). Each phytoplankton species has a specific distribution of pigment in its pigment-protein complexes and the distribution is rearranged during photoacclimation (Iglesias-Prieto and Trench, 1997). For example, high light can induce loss of photosynthetic pigments and lead to photobleaching and photoinhibition, as documented in *Symbiodinium* (Takahashi et al., 2008), a genus of dinoflagellates mostly endosymbiotic with corals and other marine invertebrates. The loss of photosynthetic pigments through the loss of antenna protein, particularly acpPC is believed to be responsible for coral bleaching (Takahashi et al., 2008). Thus, understanding the responses of the two antennae proteins to the variable ambient light environment will provide valuable information regarding the functional significance of changes in cellular pigment concentration (Iglesias-Prieto and Trench, 1997).

PCP (peridinin- chlorophyll *a*-binding protein) and acpPC (chlorophyll *a*-chlorophyll *c2*-peridinin-protein) are two major light harvesting components in peridinin-containing (i.e. "typical") dinoflagellates (Michael and Miller, 1998; Takahashi et al., 2008), which form two antennas with associated pigments in the photosynthesis system (Iglesias-Prieto and Trench, 1997; Takahashi et al., 2008). PCP is a water-soluble protein, which only occurs in light harvesting complex in dinoflagellates, usually located in

* Corresponding author at: Department of Marine Sciences, University of Connecticut, Groton, CT 06340, USA.
E-mail address: senjie.lin@uconn.edu (S. Lin).

https://doi.org/10.1016/j.hal.2018.04.002
1568-9883/

the thylakoid membrane (Nassoury et al., 2001) to bind its luminal side and can exist in a short (of 15 kDa weight) or a long form (of 30–35 kDa weight) (Reichman and Vize, 2014). The long form is believed to have evolved from the short form via gene duplication (Hofmann et al., 1996). In *Glenodinium* sp., PCP synthesis is light regulated at the transcriptional level (Roman et al., 1988). In *Lingulodinium polyedrum*, PCP was reported to be regulated at the translational level by circadian signals (Nassoury et al., 2001). The photosynthetic capacity in *L. polyedrum* is maximal in the middle of the day and minimal in the middle of the night (Hollnagel et al., 2002), while the protein abundance is higher in the period from late of the day to the end of the night (Nassoury et al., 2001). In *Prorocentrum donghaiense*, this protein has also been reported to decrease under nitrogen limitation (Zhang et al., 2015). In contrast to PCP's water-soluble nature, acpPC usually is embedded in thylakoid membranes in dinoflagellates with functions similar to chlorophyll *a*/*b*-binding proteins in higher plants (Hiller et al., 1995). The gene usually encodes a polyprotein that can form as many as 10 different tandem repeated mature polypeptides, with ~19 kDa each (Hiller et al., 1995). In *Symbiodinium* spp., it was reported that acpPC was regulated by heat stress at the translational level (Takahashi et al., 2008) and the majority of the photosynthetic pigments were associated with this protein (Iglesias-Prieto and Trench, 1997). In *Amphidinium carterae*, acpPC gene expression was shown to be regulated by light intensity (Michael and Miller, 1998). So far, the relationship between photosynthetic pigments and these antenna proteins in dinoflagellates has been understudied.

P. donghaiense is a typical red tide dinoflagellate species, forming large scale blooms in East China Sea every year (Lu et al., 2005). Previous studies showed that the high turbidity and low light condition were conducive to *P. donghaiense* bloom formation (Sun et al., 2008). Pigments in this species have been used as a biological indicator of cell physiological status (Hou et al., 2007). It is well recognized that the dynamic process of the red tide event formed by *P. donghaiense* can be monitored by satellite based on the specific cellular pigment in this species (Lei et al., 2011). In this study, in order to further understand the functional significance of changes in cellular pigment concentration at the molecular level, the relationship between cellular pigments and gene expression of pigment-binding protein genes at different circadian times and under different light intensities was investigated. HPLC analysis was used to measure cellular contents of pigments over a diel cycle and under light intensities. PCP and acpPC genes were isolated, and their expression profiles in the diel cycle and under different growth light intensities were characterized.

2. Method

2.1. Algal culture and sample collection for gene isolation

P. donghaiense culture was grown in 1-L seawater medium (without silicate) and was treated with a cocktail of antibiotics including ampicillin (200 mg/L), kanamycin (100 mg/L) and streptomycin (100 mg/L) to minimize bacterial presence (Lin et al., 2015). The culture was kept at 20 ± 1 °C under a 14:10 h light dark cycle (LD) at a photon flux of 100 μmol photons m^{-2} s^{-1}. Cell concentration was monitored using a Sedgwick-Rafter counting chamber under a microscope. Exponential phase cells (~10^7 cells per sample) were harvested by centrifugation at 3000×*g* under 20 °C for 10 min. For RNA isolation, the cell pellet was resuspended in 1 mL TRIzol Reagent (Invitrogen, Carlsbad, CA), mixed thoroughly by vortex and stored at −80 °C for subsequent RNA extraction.

2.2. Diel sample collection for pigment and gene expression analyses

A master culture was first synchronized as previously reported (Shi et al., 2013). The synchronized culture was then transferred into 7.5-L L1 medium in triplicate. The culture condition was the same as described above. When the cultures entered the early exponential phase (3 days after transfer), a sample was taken from each of the triplicate cultures every 2 h for a 24 h period. At each time point, 25 mL and 300 mL samples were harvested for pigments analysis and RNA extraction, respectively. Pigment samples were filtered onto Whatman GF/F membrane of 25 mm diameter with 0.7-μm pore size under a gentle vacuum (<150 mmHg). The membrane was folded and wrapped with aluminum foil and frozen in liquid nitrogen. The frozen sample was stored at −80 °C for subsequent pigment extraction. The RNA samples were collected using centrifugation. The cell pellets were suspended in 1 mL TRIzol Reagent, mixed thoroughly, and stored at −80 °C until RNA extraction.

2.3. Light manipulation to study responses of PCP expression, pigment content, and photochemical efficiency in P. donghaiense

The synchronized culture was transferred into 1-L L1 medium in 9 bottles under 100 μmol photons m^{-2} s^{-1} to be set up in triplicate for each of the three light conditions used in the experiment. When the culture entered into exponential phase, each set of triplicate cultures was transferred to a different light density, 20 μmol photons m^{-2} s^{-1}, 100 μmol photons m^{-2} s^{-1} and 600 μmol photons m^{-2} s^{-1}, respectively. All the experiment was conducted under a 14:10 h LD light cycle. The light density experiment was started at middle light phase. Samples were collected in 0 h, 24 h and 48 h after transfer to respective light densities. Cell concentration was monitored as described above every day. Growth rate was calculated by [ln(N2)–ln(N1)]/(t2–t1), where N2 and N1 are cell concentrations at time t2 and t1, respectively. After 24 and 48 h treatment, 25 mL and 300 mL samples were harvested at the same time point of the day for pigments analysis and RNA extraction as described above, respectively in each time point. The maximum quantum efficiency of PSII photochemistry Fv/Fm = (Fm–Fo)/Fm was measured using FIRe fluorometer system (Satlantic, Halifax, NS, Canada) as described previously (Cui et al., 2017).

2.4. Pigment analysis

The frozen filter was treated with freeze drier under −80 °C for 12 h. The dried filter was soaked in 2 mL N, N-dimethylformamide and extracted in a freezer (−20 °C) for 2 h (Furuya et al., 1998). The extractions were then filtered using Whatman GF/F filters of 13 mm diameter with 0.7 um pore size (Swinnex Filter Holder) to remove cell debris and then mixed with the same volume of ammonium acetate solution (1 M). An aliquot of the extracted mixture was partially injected into an Agilent HPLC system equipped with a 3.5 μm Eclipse XDB C8 column (100 × 4.6 mm; Agilent Technologies). The HPLC equipment was consisted of a Shimadzu LC-20A pump with a low-pressure gradient unit FCV-20AL, an on-line degasser DGU-3A, and a photodiode array UV–vis detector SPD-M20AV with wavelength resolution in 1.2 nm size. The mobile phase were A (methanol: 1 M ammonium acetate, 80:20) and B (methanol). The LC gradients were (min, solvent A%, solvent B%): (0, 100, 0), (16, 45, 55), (27, 0, 100), (32, 0, 100), (40, 100, 0) and the flow rate was maintained at 1 mL min^{-1}. The specific peak of each pigment was identified based on their retention time and absorption spectrogram compared with those of pure standards purchased from Danish Hydraulic Institute (DHI) Water and

Environment, Hørsholm, Denmark. Quantitation was titrated using this standard according to its manufacturer's instrument.

2.5. RNA extraction and cDNA synthesis

Total RNA was extracted using TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) coupled with QiagenRNeasy Mini kit (Qiagen) following previously reported protocol (Lin et al., 2010). RQ1 DNase (Promega) was used to eliminate potential genomic DNA contamination according to the manufacturer's protocol and further purified using QiagenRNeasy Mini kit. RNA concentrations were estimated using NanoDrop ND-2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the qualities of the total RNA were evaluated using the absorbance ratios of 260/280 nm and 260/230 nm.

First-strand cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI) and 100 ng oligo-(dT)16 primers with 300 ng total RNA as input for each sample. In order to isolate 3′-complete cDNA of the gene, GeneRacer oligo-dT (Invitrogen, Carlsbad, CA, USA) was used to construct cDNA library (named Racer3-cDNA).

2.6. Gene isolation and sequence analysis

Gene specific primers (Table 1) were designed based on annotated partial cDNA sequences of RNA-Seq dataset (Shi et al., 2017). PCR with the synthesized cDNA as the template and the primers designed here was carried out to amplify PCP coding genes. The amplicons were purified and sequenced. To obtain full-length cDNAs of PCP gene, specific primers were designed from the sequenced fragment to pair with Racer3 and DinoSL to isolate 3′-end and 5′-end of gene using Racer3-cDNA library as templates, respectively (the NCBI accession number is MG523423). The amplicons were purified and sequenced, and the sequences from both ends were assembled to generate full-length gene sequences. The sequences of PCP and acpPC were analyzed by BLAST against GenBank nr database. Online software ORF Finder (Rombel et al., 2002) was used to predict protein-coding region of the genes.

2.7. Analyses of PCP and acpPC gene expression using reverse transcription quantitative PCR (RT-qPCR)

The expression levels of the PCP and acpPC in diel and light manipulated samples were determined using RT-qPCR. The full cDNA fragment of PCP was amplified, cloned and then PCR-amplified again to yield standards (Hou et al., 2010). The amplicon was purified and quantified using NanoDrop, and then serially diluted by 10-fold to obtain a gradient of 10^2–10^7gene copies per 5 μL. Each reaction was carried out in a total volume of 12 μL containing 250 nM of each primer, 5 μL cDNA or DNA, and 6 μL 2×SYBR Green Super mix. qPCR was performed in 96-well plates on a CFX96 Real-time PCR System (BioRad, USA) with iQTM SYBR® Green Supermix (BioRad, USA). For both the 10-fold serial dilution standard and the experimental cDNA, qPCR was run in triplicate (for technical replicates) for each of the biological triplicate. GAPDH was included in the qPCR as a reference gene for normalization of target gene expression levels (Shi et al., 2015). The transcript abundances of the target genes and the reference gene were analyzed using CFX software (Bio-Rad, Hercules, CA, USA).

2.8. Prediction of structural features of PCP

Based on the deduced amino acid sequence of PCP, the signal sequence in the N-terminal region was characterized using SignalP program (website: www.cbs.dtu.dk/services/SignalP/). The potential trans-membrane regions were identified using web software Tmpred (http://www.ch.embnet.org/software/TMPRED_form.html). Based on this software, the tansmembrane segment of a peptide can be obtained by their hydrophobicity profile prediction (Hofmann and Stoffel, 1993). In order to compare the common characteristics of PCP in dinoflagellate, the N-terminal of other reported PCP sequences (L13613 and CAA90654) were included in this analysis.

3. Results

3.1. Cell growth and photosynthetic efficiency

Under normal light intensity (100 μmol photons m^{-2} s^{-1}), the averaged cell growth rate in the 48 h was 0.65 d^{-1}. Under high light (600 μmol photons m^{-2} s^{-1}) and low light (20 μmol photons m^{-2} s^{-1}) conditions, the growth rate decreased to 0.065 d^{-1} and 0.32 d^{-1}, respectively (Fig. 1A). Under low light condition, photosynthetic efficiency increased significantly (one-tailed *t*-test, $p < 0.01$, n = 3; Fig. 1B). In contrast, the photosynthetic efficiency significantly decreased under high light condition (one-tailed *t*-test, $p < 0.01$, n = 3; Fig. 1B).

3.2. Pigment profile of P. donghaiense and its response to diel and irradiance variations

By comparing with pigment standards, chlorophyll *c2* (Chl *c2*), peridinin, diadinoxanthin, *Chl a* and β-carotene were identified in *P. donghaiense* cells (Fig. S1). Cellular Chl *c2* pigment content showed a clear diel change pattern in the 24-h sampling period, higher in light phase and lower in dark phase. This pigment stayed largely unchanged in the first five sampling time points of dark phase, then ramped up sharply to reach a pick level in 6 h (2 h after light switch-on). Thereafter, this pigment started to decrease slowly and to reach the minimum at the time point before 4 h of light/dark transition. Overall, the cellular content of Chl *c2* showed a 2.4-fold variation throughout the whole diel sampling period

Table 1
Primers used in this study.

Primer name	Sequences (5′–3′)	Application
Dino-SL	TCCGTAGCCATTTTGGCTCAAG	Dinoflagellate mRNA 5′ end cDNA synthesis and PCR (forward)
Pd_acpCP_qF	CAGGCCTGAAGTTCTCGGAC	*P. donghaiense* acpPC qPCR (forward)
Pd_acpCP_qR	TCAGGATCCGAGGACGTGATC	*P. donghaiense* acpPC qPCR (reverse)
Pd_PCP_qF	TCCGACATCTTCATCAAGCCTC	*P. donghaiense* PCP qPCR (forward)
Pd_PCP_qR	ATGCTGCCGATGGCCTTGTGGTG	*P. donghaiense* PCP qPCR (reverse)
Pd_acpCP_F	TGCGCGCCTTCGAGAGCGAGA	*P. donghaiense* acpPCPCR (forward)
Pd_acpCP_R	ATGTTGCGACGTCGCCGTCGGA	*P. donghaiense* acpPCPCR (reverse)
Pd_PCP_F	ACGTGGTGAAGAAGAACCAGGTG	*P. donghaiense* PCP PCR (forward)
Pd_PCP_R	GTCGATGCTGCCGATGGCCTTG	*P. donghaiense* PCP PCR (reverse)
Pdong-gapdh-QF	GTGTTCCTYACCGACGAGAAGATC	*P. donghaiense* GAPDH qPCR (forward)
Pdong-gapdh-QR3	CGCARTTCATGTCAGTCTTGTAGG	*P. donghaiense* GAPDH qPCR (reverse)

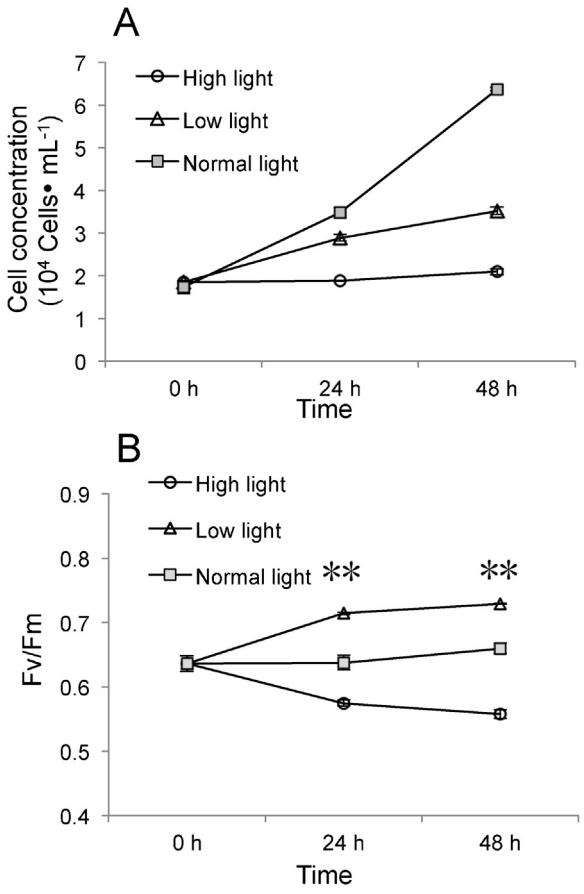

Fig. 1. Cell concentration (A) and photochemical efficiency (Fv/Fm Ratio) (B) of *Prorocentrum donghaiense* grown under different light intensities. Triangle, low light (20 μmol photons m^{-2} s^{-1}); circle, high light (600 μmol photons m^{-2} s^{-1}); square, normal light (100 μmol photons m^{-2} s^{-1}). Error bars depict standard deviations from triplicate cultures.

(Fig. 2). Other cellular photosynthetic pigments fluctuated only slightly over the circadian cycle. The variation of peridinin, Chl *a*, diadinoxanthin and β-carotene showed 1.19, 1.44, 1.39 and 1.44-fold change over the sampling period, respectively (Fig. 2).

Since diadinoxanthin and Chl *c2* are bound by acpPC protein specifically, cellular contents of these two pigments were selected for further analysis to detect the difference between night period and day period of the diel cycle. For darkness, data from 4 sampling time points at 0:00, 2:00, 4:00 and 6:00 were averaged; for light period, data from 6 sampling time points at 10:00, 12:00, 14:00, 16:00, 18:00 and 20:00 were averaged; at each time point, data were obtained from triplicate cultures. The two phase-transition points were removed for this analysis. Compared to night period, both of the average cellular contents of diadinoxanthin and Chl *c2* were higher in day period, although the day-night difference was statistically significant only for diadinoxanthin (one-tailed *t*-test, $p < 0.05$; Fig. S2).

Under the high light condition, all the five classes of cellular pigments showed a low level in both 24 h and 48 h exposure (Fig. S3A–B). For the low light cultures, all the five classes of cellular pigments decreased in the first 24 h. After that, three classes of pigments, peridinin, Chl *a* and Chl *c2*, showed higher cellular content in the low light treated cultures in 48 h (Fig. S3B).

The ratios of other pigments to *Chl a* were calculated for each sample. Most of the pigment ratios, such as Peridinin: Chl *a* (Fig. 3), were stable both over the 24 h diel cycle and under differential light intensities. The only exception was diadinoxanthin: Chl *a*, which increased significantly when light intensity increased from low to normal and to high levels (one-tailed *t*-test, $p < 0.05$) (Fig. 4).

3.3. Gene sequence and inferred protein structures

The original gene fragment information of PCP and acpPC was obtained from the RNA-Seq dataset of *P. donghaiense* (Shi et al., 2017). Based on the information, RACE was conducted in this study in an attempt to isolate the full-length cDNA sequences. This was successful for PCP, which turned out to be a 1421 bp. ORF Finder indicated that the PCP cDNA encoded a 365-aa precursor protein, with a predicted molecular weight of about 37 kDa. BLAST against NCBI nr database analysis for the PCP sequence returned *Symbiodinium* sp. chloroplast soluble peridinin- Chl *a* binding protein precursor as the top hit, with 69% identity. Signal peptide and trans-membrane region analysis indicated that the most likely cleavage site of the precursor protein was between residues Ala-53 and Asp-54, which would produce a mature PCP protein with 312-aa in length (Fig. S4). Thus, the molecular weight of mature PCP is predicted to be 32 kDa.

Based on RNA-Seq dataset, full-length cDNA sequence of this gene contains at least 4 tandem repeats. Our attempt to isolate full-length cDNA of acpPC failed due to low PCR efficiency for long fragment and tandem repeat organization of this gene. We obtained a 1440 bp cDNA fragment by PCR (the NCBI accession number is MG523422). BLAST analysis indicated that this fragment contained a complete coding region of a ~19 kDa polypeptide flanked by partial sequences of two other tandem repeat polypeptides. Based on 3′-RACE sequencing result of this gene, the predicted molecular mass of the last polypeptide was also about 19 kDa.

3.4. Dynamics of PCP and acpPC expression over the diel cycle and under different irradiances

The expression level of the PCP gene as normalized to the reference gene GAPDH showed a clear diel rhythm in the 24-h sampling period (Fig. 5A). The transcript abundance stayed largely unchanged in the first three sampling time points (from late light period to early dark period). Then the expression level decreased steadily throughout the dark period, and reached the minimum when cells had entered the light phase for 2 h. The transcript abundance increased in the next 8 sampling time points to reach a maximum late in the light period. The expression of PCP showed a 2.40-fold variation throughout the whole sampling period. In the diel sample set, the averaged transcript abundance of PCP in light phase (average of 12 samples from 4 time points sampling) was significantly higher than that in the dark phase (average of 18 samples from 6 time points sampling) (one-tailed *t*-test, $p < 0.05$, n = 18; Fig. S4). In differential light intensity cultures, PCP transcript abundance exhibited significant up-regulation under low light condition (one-tailed *t*-test, $p < 0.01$; Fig. 6A). On average there was about 1.58-fold increase when compared to the normal light condition. The transcript abundance was detected basically in the same level between the high light and the normal light conditions (one-tailed *t*-test, $p > 0.05$; Fig. 6A).

In contrast to the diel trend of PCP expression, acpPC expression was low at the beginning of the dark phase and showed an abrupt increase at the dark/light transition, thereafter the expression level stayed high and relatively stable, although fluctuations occurred throughout the circadian cycle (Fig. 5B**).** As a result, the averaged transcript abundance of acpPC was significantly lower in dark phase than in the light phase (one- tailed *t*-test, N = 18; $p < 0.05$; Fig. S4). In differential light intensity cultures, the normalized acpPC transcript abundance was significantly higher (by about 1.26-fold) when the cultures were grown at low light condition

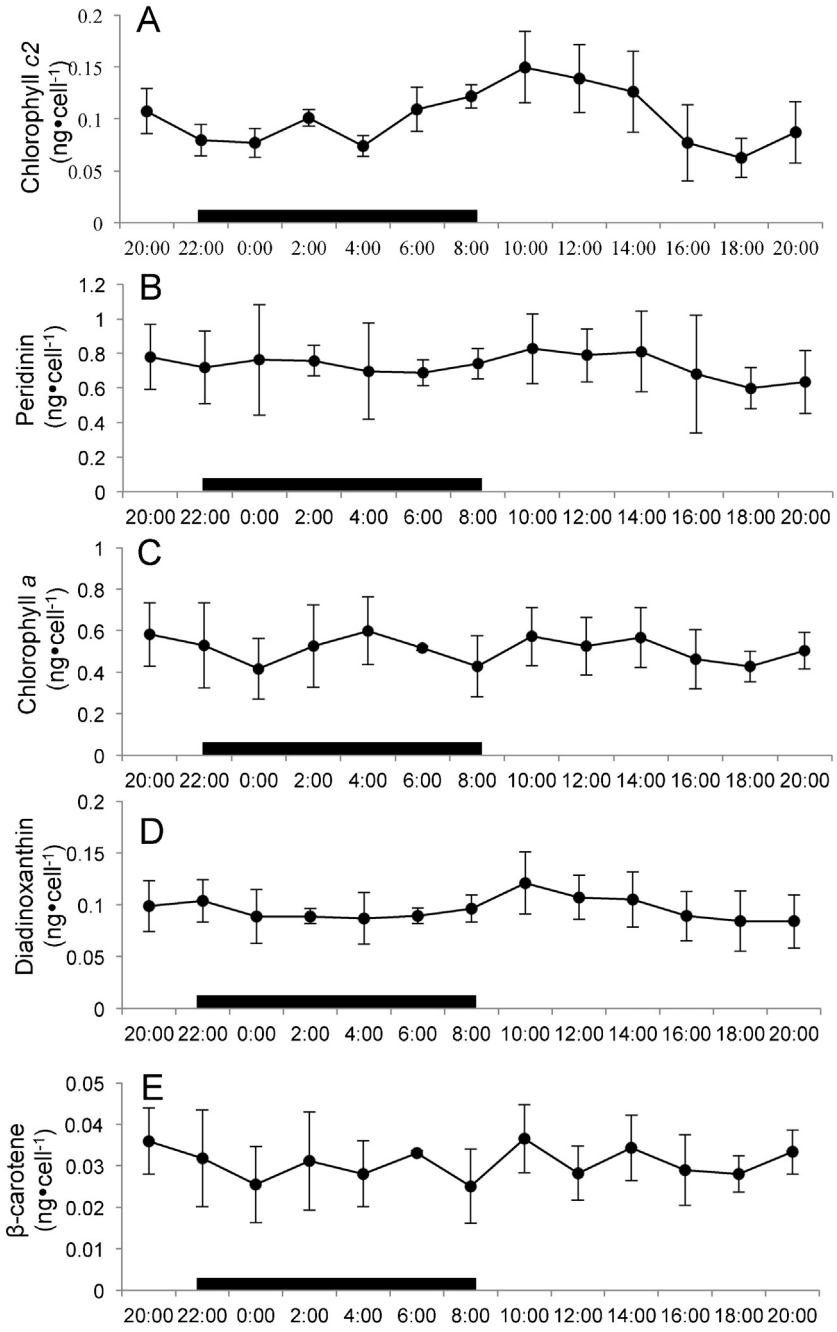

Fig. 2. Cellular pigment dynamics over a 24-h sampling period in the 14:10 light:dark cycle under normal light condition (100 μmol photons m^{-2} s^{-1}). Black bar indicates dark period whereas other time points are in light period. A, Chl *c2*; B, peridinin; C, diadinoxanthin; D, Chl *a*; E, β-carotene.

than at normal light condition (one-tailed *t*-test, $p < 0.05$; Fig. 5B). There was no significant difference in acpPC expression level between the normal and high light conditions (one-tailed t-test, $p > 0.05$; Fig. 6B).

4. Discussion

It is known that the main classes of pigments in dinoflagellates (peridinin, Chl *a* and Chl *c*) are bound by two antennae proteins, PCP and acpPC (Miller et al., 2005). PCP and acpPC complex together represent the light-harvesting components of dinoflagellate cells. Despite the importance, how the pigments and their associated proteins respond to variable light condition at the molecular level is still poorly understood. The present study represents one of the first efforts to simultaneously document pigment profiles and the expression patterns of pigment binding protein genes under light variation, and will provide baseline information for future research.

4.1. The variation of pigment ratio

Pigment ratios of algal species, especially the diagnostic pigment ratios, are used as a tool to identify and quantify the contribution of phytoplankton groups in marine and freshwater system (Descy et al., 2009). The ratios can be changed, for instance by nutrient limitation and light inhibition (Higgins et al., 2011). In this study, diadinoxanthin: Chl *a* was detected to increase significantly under high light. This apparent increase of

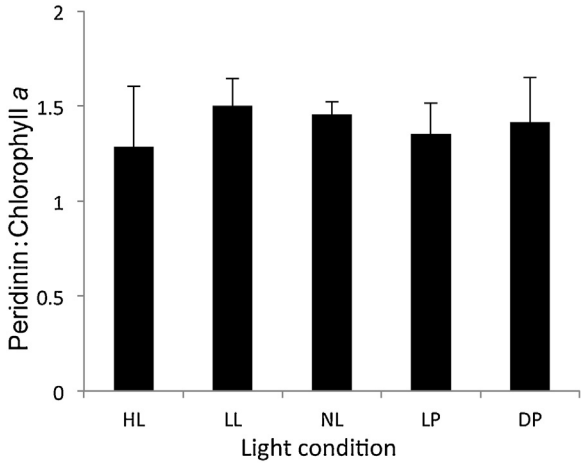

Fig. 3. The peridinin: Chl *a* ratio under different light intensities and light/dark phases. HL, high light; LL, low light; NL, normal light; LP, light phase; DP, dark phase. For light intensity samples, shown are means (bar height) and standard deviations (error bars) from triplicate cultures sampled at both 24 h and 48 h treatment combined. For light/dark phase samples, shown are means (bar height) and standard deviations (error bars) from triplicate cultures sampled at all the light period time points (10:00, 12:00, 14:00, 16:00, 18:00 and 20:00) and dark period time points (0:00, 2:00, 4:00 and 6:00), respectively.

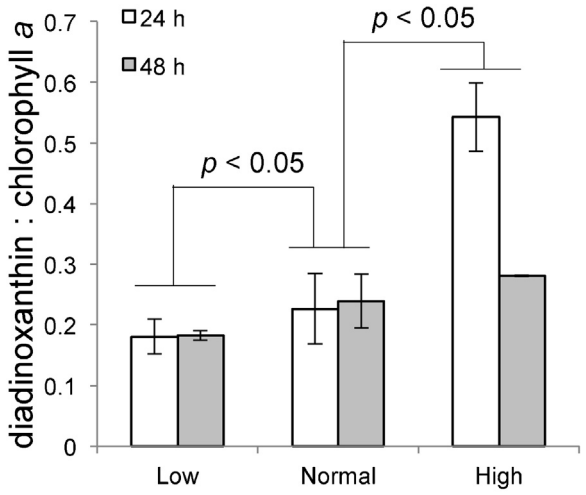

Fig. 4. The diadinoxanthin: Chl *a* ratio under different light intensities. Error bars depict standard deviations from triplicate cultures.

diadinoxanthin: Chl *a* under high light may have resulted from the fact that the basic function of diadinoxanthin is photo-protection, i.e. to protect against photo-inhibition under high light (Lavaud et al., 2002). It seems that increasing concentration of diadinoxanthin per unit Chl *a* is a common strategy for algal cells to cope with high light stress (Iglesias-Prieto and Trench, 1997). Similar results also have been reported in *Prorocentrum minimum*, *Alexandrium excavatum*, *Thalassiosira pseudonana* and other phytoplankton (Demers et al., 1991; Higgins et al., 2011; Jovine et al., 1995). Thus, the ratio of diadinoxanthin to Chl *a* could potentially be used as an indicator of algal photophysiological status at least in some dinoflagellates.

Peridinin: Chl *a* has been used as a diagnostic pigment ratio to investigate relative abundance of dinoflagellates in a phytoplankton (Schlüter et al., 2000). This analysis can be performed using programs such as CHEMTAX (Mackey et al., 1996), which requires measurements of total amount of each major pigment in the community and diagnostic pigment ratios of the lineage. In this study, we found the ratio of peridinin: Chl *a* was relatively stable in both diel samples over 24 h period and under different light intensities, ranging 1.3–1.5. In *Heterocapsa* sp. (Latasa and Berdalet, 1994) and *Prorocentrum micans* (Schlüter et al., 2000), similarly stable peridinin: Chl *a* ratios (0.8 and 0.4, respectively) also have been identified under different cultivation conditions. The stability of this ratio validates its suitability as a proxy for estimating abundance of dinoflagellates.

4.2. Expression profile of antennae protein genes and cellular content of pigments

It is well recognized that dinoflagellates generally show limited transcriptional responses to many environmental stimuli (Morey et al., 2011; Moustafa et al., 2010; Lin, 2011 for review). In this study, however, antenna gene expression was detected to have significant variations under different light intensity and in diel samples. This result put the antenna genes with some others as the minority of genes in dinoflagellates that show transcriptional regulation. PCP gene showed higher expression level under low light than under normal light condition in *P. donghaiense*. It seems that enhanced PCP gene expression is common strategy for dinoflagellates coping with low light. In *A. carterae*, PCP transcript level was promoted by low light condition by 86-fold relative that in high light condition (Michael and Miller, 1998). Such stimulated PCP expression also has been reported in *L. polyedrum* (Roman et al., 1988), *Heterocapsa pygmaea* (Triplett et al., 1993) and *Prorocentrum minimum* (Jovine et al., 1995). It is believed that such an expression profile is related to the basic function of PCP, feeding light energy primarily into photosystem II (Wong et al., 1979). Based on all these studies on different dinoflagellate species, a general conclusion may be made that the expression of PCP in dinoflagellates is stimulated by low light intensity.

The expression level of acpPC gene also was enhanced by low light in the present study. This gene expression profile is consistent with that previously reported in *A. carterae* and *Symbiodinium* spp. (Michael and Miller, 1998; Iglesias-Prieto and Trench, 1997). Because PCP and acpPC together represent main light-harvesting components of peridinin containing dinoflagellates (Prézelin, 1987), it has been suggested that the increased cellular contents of PCP and acpPC complexes are associated with increased number and size of the photosynthetic unit (Iglesias-Prieto and Trench, 1997, 1994). The observed photosynthetic unit increase should lead to PCP and acpPC increase. This hypothesis is consistent with the observation in this study that main cellular pigments increased after 48 h treatment under low light. This consistent observation suggests similar and possibly coordinated regulatory mechanisms for elevated pigment and binding protein synthesis under low light condition. This phenomenon may be because a low irradiance makes it necessary for cells to expand its light harvesting capacity.

It is intriguing that PCP and acpPC showed somewhat converse diel expression dynamics, which suggests differential circadian rhythm response and probably some kind of mechanistic differentiation between the two genes. Functioning in association with the unique carotenoid peridinin, PCPs are unrelated to all other proteins, and it harvests light through peridinin and transfers the energy to Chl *a* (Hofmann et al., 1996). In contrast, acpPCs are functionally similar to the Chl *a/b* proteins of higher plants and green algae and it binds Chl *a*, Chl *c2*, peridinin and diadinoxanthin (Miller et al., 2005). The increase of Chl *c2* during the dark phase seems consistent with increasing expression of acpPC, suggesting concurrent synthesis and close functional association. Previous report indicated that acpPC function through the mediation of phytochrome in green plants (Terzaghi and Cashmore, 1995). Yet no phytochrome has been detected or implicated in

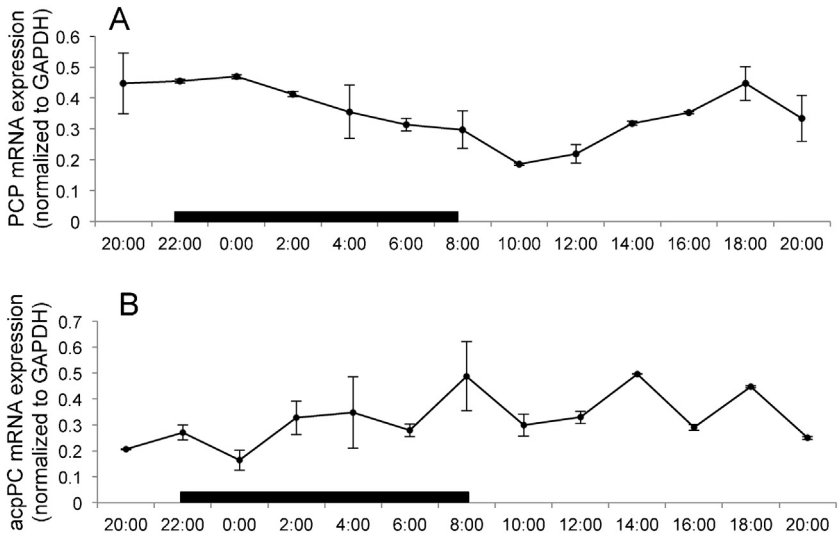

Fig. 5. GAPDH normalized diel expression profile of *P. donghaiense* antenna gene. Black bar indicates dark period whereas other time points are light period. A, PCP gene transcription dynamics over 24-h sampling period. B, acpPC gene transcription dynamics over 24-h sampling period. Error bars depict standard deviations of triplicate cultures.

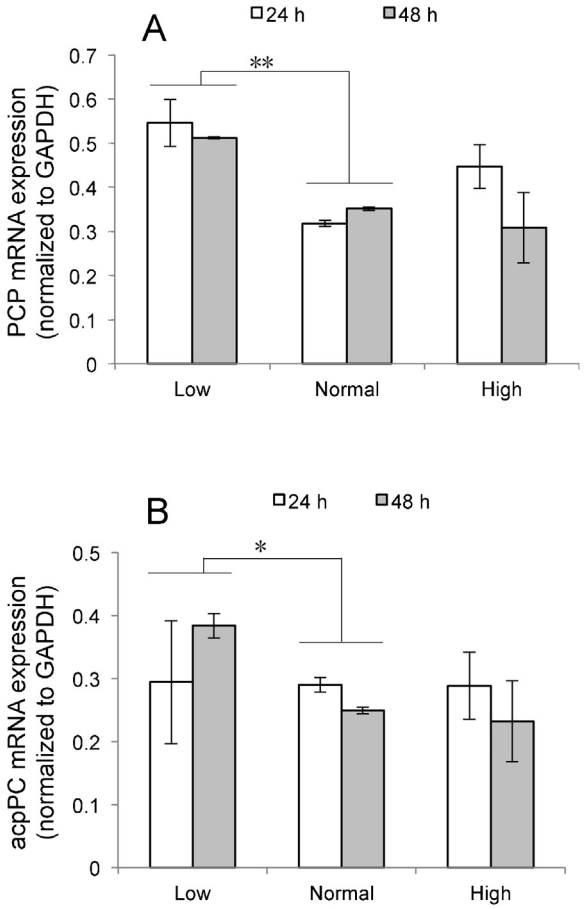

Fig. 6. GAPDH normalized expression profile of *P. donghaiense* antenna gene under different light intensities. A, PCP gene expression profile under different light intensities. B, acpPC gene expression profile under different light intensities. * indicate $p < 0.05$. ** indicate $p < 0.01$. Error bars depict standard deviations of triplicate cultures at the 2 sampling times (24 h and 48 h).

dinoflagellates. Currently, it is largely unclear how the two antennae protein genes are regulated molecularly, but recent findings of epigenetic elements provide some interesting perspective. In *A. carterae*, the expression of these two genes was accompanied by demethylation of CpG and CpNpG motifs in or near coding region of the two genes (Michael and Miller, 1998). DNA methylation produces epigenetic signal to lock genes in the "off" position, and thus to block gene activity (Phillips, 2008). CpG motif exists in both PCP and acpPC, in contrast to CpNpG motifs, which has been observed for acpPC loci only. Thus PCP and acpPC have differential methylatable loci in *A. carterae*. Methylation coupled demethylation is a common epigenetic mechanism to regulate gene expression, with methylation repressing and demethylation promoting gene expression respectively. In *A. carterae*, CpNpG demethylation is light-induced (Michael and Miller, 1998), and the expression level of acpPC would increase under light period due to demethylation event. In this study, the elevated expression level of acpPC at the dark-light transition in *P. donghaiense* is consistent with this scheme. Whether the light-induced CpNpG demethylation is one of the reasons that result in different diel expression profiles of PCP and acpPC needs to be investigated in the future.

Acknowledgements

The project was supported by National Key R&D Program of China grant 2016YFA0601202, the National Natural Science Foundation of China grants # 41606121, 31661143029, 41330959 and China Postdoctoral Science Foundation Grant 2016M602070.[SS]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.hal.2018.04.002.

References

Cui, Y., Zhang, H., Lin, S., 2017. Enhancement of non-photochemical quenching as an adaptive strategy under phosphorus deprivation in the dinoflagellate Karlodinium veneficum. Front. Microbiol. 8, 404.

Demers, S., Roy, S., Gagnon, R., Vignault, C., 1991. Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of Alexandrium excavatum (Dinophyceae) and Thalassiosira pseudonana (Bacillariophyceae): a photoprotection mechanism. Mar. Ecol. Prog. Ser. 185–193.

Descy, J.-P., Sarmento, H., Higgins, H.W., 2009. Variability of phytoplankton pigment ratios across aquatic environments. Eur. J. Phycol. 44 (3), 319–330.

Furuya, K., Hayashi, M., Yabushita, Y., 1998. HPLC determination of phytoplankton pigments using N, N-dimethylformamide. J. Oceanogr. 54 (2), 199–203.

Higgins, H.W., Wright, S.W., Schluter, L., 2011. Quantitative interpretation of chemotaxonomic pigment data. In: Suzanne, Roy. (Ed.), Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography. Cambridge University Press, pp. 257–313.
Hiller, R.G., Wrench, P.M., Sharples, F.P., 1995. The light-harvesting chlorophyll a-c-binding protein of dinoflagellates: a putative polyprotein. FEBS Lett. 363 (1–2), 175–178.
Hofmann, E., Wrench, P.M., Sharples, F.P., Hiller, R.G., Welte, W., Diederichs, K., 1996. Structural basis of light harvesting by carotenoids: peridinin-chlorophyll-protein from Amphidinium carterae. Science 272 (5269), 1788–1791.
Hollnagel, H.C., Pinto, E., Morse, D., Colepicolo, P., 2002. The oscillation of photosynthetic capacity in Lingulodinium polyedrum is not related to differences in RuBisCo, peridinin or chlorophyll a amounts. Biol. Rhythm Res. 33 (4), 443–458.
Hou, J.J., Huang, B.Q., Cao, Z.R., Chen, J.X., Hong, H.S., 2007. Effects of nutrient limitation on pigments in Thalassiosira weissflogii and Prorocentrum donghaiense. J. Integr. Plant Biol. 49 (5), 686–697.
Hou, Y., Zhang, H., Miranda, L., Lin, S., 2010. Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: microalgal pcna as the model gene. PLoS One 5 (3), e9545.
Iglesias-Prieto, R., Trench, R.K., 1994. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Mar. Ecol. Prog. Ser. Oldendorf 113 (1), 163–175.
Iglesias-Prieto, R., Trench, R., 1997. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll–protein complexes to different photon-flux densities. Mar. Biol. 130 (1), 23–33.
Jovine, R.V., Johnsen, G., Prézelin, B.B., 1995. Isolation of membrane bound light-harvesting-complexes from the dinoflagellates Heterocapsa pygmaea and Prorocentrum minimum. Photosynth. Res. 44 (1), 127–138.
Latasa, M., Berdalet, E., 1994. Effect of nitrogen or phosphorus starvation on pigment composition of cultured Heterocapsa sp. J. Plankton Res. 16 (1), 83–94.
Lavaud, J., Rousseau, B., Van Gorkom, H.J., Etienne, A.-L., 2002. Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom Phaeodactylum tricornutum. Plant Physiol. 129 (3), 1398–1406.
Lei, H., Pan, D., Bai, Y., Chen, X., Zhou, Y., Zhu, Q., 2011. HAB detection based on absorption and backscattering properties of phytoplankton, SPIE Remote Sensing. Int. Soc. Opt. Photon. pp. 81751F–81751F–81758.
Lin, S., Zhang, H., Zhuang, Y., Tran, B., Gill, J., 2010. Spliced leader-based metatranscriptomic analyses lead to recognition of hidden genomic features in dinoflagellates. Proc. Natl. Acad. Sci. U. S. A. 107 (46), 20033–20038.
Lin, S., Cheng, S., Song, B., Zhong, X., Lin, X., Li, W., Li, L., Zhang, Y., Zhang, H., Ji, Z., 2015. The Symbiodinium kawagutii genome illuminates dinoflagellate gene expression and coral symbiosis. Science 350 (6261), 691–694.
Lin, S., 2011. Genomic understanding of dinoflagellates. Res. Microbiol. 162, 551–569.
Lu, D., Goebel, J., Qi, Y., Zou, J., Han, X., Gao, Y., Li, Y., 2005. Morphological and genetic study of Prorocentrum donghaiense Lu from the East China Sea, and comparison with some related Prorocentrum species. Harmful Algae 4 (3), 493–505.
Mackey, M., Mackey, D., Higgins, H., Wright, S., 1996. CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 265–283.
Michael, R., Miller, D.J., 1998. Light-regulated transcription of genes encoding peridinin chlorophyll a proteins and the major intrinsic light-harvesting complex proteins in the dinoflagellate Amphidinium carterae Hulburt (Dinophycae) changes in cytosine methylation accompany photoadaptation. Plant Physiol. 117 (1), 189–196.
Miller, D.J., Catmull, J., Puskeiler, R., Tweedale, H., Sharples, F.P., Hiller, R.G., 2005. Reconstitution of the peridinin–chlorophyll a protein (PCP): evidence for functional flexibility in chlorophyll binding. Photosynth. Res. 86 (1), 229–240.
Morey, J.S., Monroe, E.A., Kinney, A.L., Beal, M., Johnson, J.G., Hitchcock, G.L., Van Dolah, F.M., 2011. Transcriptomic response of the red tide dinoflagellate, *Karenia brevis*, to nitrogen and phosphorus depletion and addition. BMC Genomics 12, 346.
Moustafa, A., Evans, A.N., Kulis, D.M., Hackett, J.D., Erdner, D.L., Anderson, D.M., et al., 2010. Transcriptome profiling of a toxic dinoflagellate reveals a gene-rich protist and a potential impact on gene expression due to bacterial presence. PLoS One 5, e9688.
Nassoury, N., Fritz, L., Morse, D., 2001. Circadian changes in ribulose-1, 5-bisphosphate carboxylase/oxygenase distribution inside individual chloroplasts can account for the rhythm in dinoflagellate carbon fixation. Plant Cell 13 (4), 923–934.
Peers, G., Truong, T.B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A.R., Hippler, M., Niyogi, K.K., 2009. An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. Nature 462 (7272), 518–521.
Phillips, T., 2008. The role of methylation in gene expression. Nat. Educ. 1 (1), 116.
Prézelin, B.B., 1987. Photosynthetic physiology of dinoflagellates. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Scientific, Oxford, pp. 174–223.
Reichman, J.R., Vize, P.D., 2014. Separate introns gained within short and long soluble peridinin-chlorophyll a-protein genes during radiation of Symbiodinium (Dinophyceae) clade a and B lineages. PLoS One 9 (10), e110608.
Roman, S.J., Govind, N.S., Triplett, E.L., Prézelin, B.B., 1988. Light regulation of peridinin-chlorophyll a-protein (PCP) complexes in the Dinoflagellate, Glenodinium sp. use of anti-Pcp antibodies to detect pcp gene products in cells grown in different light conditions. Plant Physiol. 88 (3), 594–599.
Rombel, I.T., Sykes, K.F., Rayner, S., Johnston, S.A., 2002. ORF-FINDER: a vector for high-throughput gene identification. Gene 282 (1), 33–41.
Schlüter, L., Møhlenberg, F., Havskum, H., Larsen, S., 2000. The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. Mar. Ecol. Prog. Ser. 192, 49–63.
Shi, X., Zhang, H., Lin, S., 2013. Tandem repeats, high copy number and remarkable diel expression rhythm of form II RuBisCO in Prorocentrum donghaiense (Dinophyceae). PLoS One 8 (8), e71232.
Shi, X., Li, L., Guo, C., Lin, X., Li, M., Lin, S., 2015. Rhodopsin gene expression is regulated by the light dark cycle, light spectrum and light intensity in the dinoflagellate Prorocentrum. Front. Microbiol. 6, 555.
Shi, X., Lin, X., Li, L., Li, M., Palenik, B., Lin, S., 2017. Transcriptomic and microRNAomic profiling reveals multi-faceted mechanisms to cope with phosphate stress in a dinoflagellate. ISME J. 11 (10), 2209–2218.
Sun, B., Wang, X., Li, Y., Wang, C., Wang, A., Liang, S., Zhang, C., 2008. Effects of irradiance on blooms of the dinoflagellate Prorocentrum donghaiense Lu in the coastal area in East China Sea. Huan Jing Ke Xue 29 (2), 362–367.
Takahashi, S., Whitney, S., Itoh, S., Maruyama, T., Badger, M., 2008. Heat stress causes inhibition of the de novo synthesis of antenna proteins and photobleaching in cultured Symbiodinium. Proc. Natl. Acad. Sci. 105 (11), 4203–4208.
Terzaghi, W.B., Cashmore, A.R., 1995. Light-regulated transcription. Annu. Rev. Plant Biol. 46 (1), 445–474.
Triplett, E.L., Jovine, R.V., Govind, N., Roman, S., Chang, S., Prezelin, B., 1993. Characterization of two full-length cDNA sequences encoding for apoproteins of peridinin-chlorophyll a-protein (PCP) complexes. Mol. Mar. Biol. Biotechnol. 2, 246–254.
Wong, D., Prézelin, B.B., Sweeney, B.M., 1979. Chlorophyll a fluorescence of Gonyaulax polyedra grown on a light-dark cycle and after transfer to constant light. Photochem. Photobiol. 30 (3), 405–411.
Zhang, Y., Zhang, S., He, Z., Lin, L., Wang, D., 2015. Proteomic analysis provides new insights into the adaptive response of a dinoflagellate Prorocentrum donghaiense to changing ambient nitrogen. Plant Cell Environ. 38 (10), 2128–2142.