Characterization of diurnal photosynthetic rhythms in the marine diatom *Skeletonema costatum* grown in synchronous culture under ambient and elevated CO$_2$

**Xiongwen Chen**$^{A,B}$ and **Kunshan Gao**$^{B,C}$

$^A$Department of Biology, Hubei Normal University, Huangshi 435002, Hubei, China. Corresponding author; email: xiongwenchen@eyou.com

$^B$Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei, China.

$^C$Marine Biology Institute, Shantou University, Shantou 515063, Guangdong, China.

**Abstract.** Photosynthetic performance was examined in *Skeletonema costatum* (Greville) Cleve. under 12:12-h light:dark (LD) cycle at ambient CO$_2$ (350 µL L$^{-1}$) and elevated CO$_2$ (1000 µL L$^{-1}$). At ambient CO$_2$, the cellular chlorophyll a content, the light-saturated photosynthetic rate ($P_m$), the initial slope of the light saturation curves ($\alpha$), the photochemical efficiency of PSII ($F_v/F_m$), the apparent carboxylating efficiency (ACE) and the photosynthetic affinity for CO$_2$$\left[1/K_m(CO_2)\right]$ all showed rhythmical changes with different amplitudes during the light period. The $P_m$ had similar changing pattern in the light period with the ACE and $1/K_m(CO_2)$ rather than with the $\alpha$ and $F_v/F_m$, indicating that rhythmical changes of photosynthetic capacity may be mainly controlled by the activity of C-reduction associated with CO$_2$ uptake during the light period. The CO$_2$ enrichment reduced the ACE and the affinity to CO$_2$, and increased the $\alpha$, cellular chlorophyll a content and $P_m$ based on cell number. By contrast, the changing patterns of all photosynthetic parameters examined here during the light period had almost the same for cells grown at ambient CO$_2$ and elevated CO$_2$, suggesting that the photosynthetic rhythms of *S. costatum* are not affected by CO$_2$ enrichment.

**Keywords:** apparent carboxylating efficiency, efficiency of the light reaction, photochemical efficiency of PSII, photosynthetic capacity, photosynthetic CO$_2$ affinity.

**Introduction**

Human activities and fossil-fuel burning have led to considerable anthropogenic emissions of carbon dioxide (CO$_2$). It has been predicted that the atmospheric CO$_2$ concentration in the end of this century will be 2–3 times that of the present, which may trigger global warming (Wigley and Raper 1992). It is important to assess the physiological and ecological impact of CO$_2$ enrichment on photosynthesis and growth of aquatic plants (Bowes 1993; Beardall and Giordano 2002). Impacts of CO$_2$ enrichment on the photosynthetic characteristics have been widely studied in algae. The light-saturated rate of photosynthesis is not affected by CO$_2$ enrichment in many marine macroalgae (Beer and Koch 1996; Beardall et al. 1998) and in some marine microalgae (Muñoz and Merrett 1989; Raven 1997), but most marine embryophytes (i.e. seagrasses) benefit from atmospheric CO$_2$ enrichment (Beer and Koch 1996; Beardall et al. 1998). The photosynthetic affinity for CO$_2$ and carbonic anhydrase activity in many microalgae declines with CO$_2$ enrichment (Bowes 1993; Matsuda et al. 2002; Colman et al. 2002). The photochemical efficiency of PSII increases with the increasing CO$_2$ in *Chlamydomonas reinhardtii* (Spalding et al. 1984).

Marine diatoms, a group of unicellular photosynthetic eukaryotes, are responsible for approximately 40% of marine primary productivity. Considerable research has been carried out on effect of CO$_2$ enrichment on the growth and photosynthesis of marine diatoms (Colman and Rotatore 1995; Raven and Falkowski 1999; Lane and Morel et al. 2000; Burkhardt et al. 2001; Morel et al. 2002). *Skeletonema costatum* is a unicellular marine diatom that is widely distributed in coastal waters all over the world and constitutes a major component of natural assemblages of most marine phytoplankton. The effects of CO$_2$ enrichment on diel variation of their growth and photosynthesis have rarely been studied. In this paper, response of diel variation

Abbreviations used: ACE, apparent carboxylating efficiency; CCM, inorganic carbon concentrating mechanisms; DIC, dissolved inorganic carbon; LD, light:dark; $P_m$, light-saturated photosynthetic rate.
of photosynthesis during the cell cycle of *S. costatum* to CO₂ enrichment is investigated, which is beneficial to improving our capacity to estimate its gross daily productivity under elevated CO₂.

Diurnal periodicity in photosynthesis has been widely documented to existence in many algae (Senger and Bishop 1967; Sournia 1974; Winter and Brandt 1986; Gacrzebak et al. 2001). The photosynthetic capacity of synchronously grown cells of algae is strongly modulated during the cell cycle, the observed results show the presence of various mechanisms of controlling the photosynthetic capacity during the cell cycle. For example, the C-reduction system has been shown to be the rate-limited step in synchronized cultures of *Euglena gracilis* (Walther and Edmunds 1973). Mishkind et al. (1979) showed that the rhythmical variations in photosynthetic activity during the cell cycle of *Ulva* may be modulated by the reoxidation of the plastoquinone pool by the donor for PSI. In addition, the phosphorylation of thylakoid membrane proteins in green algae *Scenedesmus obliquus* (Heil and Senger 1986) and *Chlamydomonas reinhardtii* (Marcus et al. 1986) was considered to be correlated with variations of their photosynthetic activity. The experiments presented here also examine the possible mechanism of controlling photosynthetic capacity during the cell cycle of *S. costatum*.

**Materials and Methods**

*Skeletonema costatum* (Greville) Cleve. (strain 2042) was obtained from the Institute of Oceanography, the Chinese Academy of Sciences, Cultures were grown at 20°C and 200 μmol m⁻² s⁻¹ with a 12 h light/12 h dark cycle (12:12 LD cycle) in filtered seawater, which was enriched with 3.0 mM KNO₃, 0.1 mM Na₂HPO₄, 70 μM NaSiO₃, 1.0 μM FeSO₄ and 25 μM EDTA·Na. Cultures were aerated with air containing approximately 350 or 1000 μL L⁻¹ CO₂. The medium pH ranged from 8.1 to 8.4 and from 7.7 to 7.9 at 350 and 1000 μL L⁻¹ CO₂, respectively. Cell density was controlled within a range of 0.5 × 10⁶–3 × 10⁸ cells L⁻¹ by dilution every day at the beginning and the end of illumination. Cells at the mid-exponential phase were harvested for the experiments.

Photosynthetic oxygen evolution was measured with a Clark-type oxygen electrode (YSI 5300, YSI incorporated, Yellow Springs, OH). The apparent carboxylating efficiency (ACE) of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) was estimated from the initial slope of the DIC-dependent photosynthetic oxygen evolution curves.

Photochemical efficiency of PSII (Fv/Fm) was measured by using a Plant Efficiency Analyzer (PEA, Hansatech instruments Ltd, Pentney, UK). Cells were sampled at defining time intervals, and after acclimation to dark for 10 min tested for Fv/Fm.

Chlorophyll a concentration was determined by the spectro-photometric method according to Jeffrey and Humphrey (1975). Cells were counted microscopically by using a haemocytometer.

**Results**

When *S. costatum* cells were grown in the 12 h light/12 h dark cycles, the cell numbers of the alga grown at the two CO₂ levels in the bulk increased steadily throughout the light period and they were 1.6 and 2.1 times higher after the 12 h light period for the alga grown at 350 and 1000 μL L⁻¹ CO₂, respectively (Fig. 1A). The chlorophyll a concentrations in the bulk of the two CO₂ culture both remained almost constant during the first and the last 2 h of the light period, but they increased 4.4- and 5.4-fold during the middle 8 h of the light period for the alga grown at 350 and 1000 μL L⁻¹ CO₂, respectively (Fig. 1B). The chlorophyll a contents of the two CO₂-grown cells were nearly constant during the first 4 h light period, increased steadily and reached a maximum at 10 h of light period (Fig. 1C). The contents of cellular chlorophyll a were higher for the alga grown at 1000 μL L⁻¹ CO₂ than that at 350 μL L⁻¹ CO₂ (Fig. 1C).

Changes of the light-saturated photosynthetic rate, Pm, for the alga grown at the two CO₂ levels during the light period are shown in Fig. 2. The Pm based on chlorophyll a content showed clear rhythmical change during the light period, exhibiting a maximal rate at 6 h in the light phase and a minimum at the end of light phase, and there was little difference between Pm of alga grown at low and high CO₂ (Fig. 2A). By contrast, Pm based on cell number showed a maximal rate at 8 to 10 h in the light period and a minimum at the beginning of the light period and the rate was higher at high CO₂ than at low CO₂ (Fig. 2B).

Variations of the initial slope of the light saturation curve, α, of the alga grown at the two CO₂ levels during the light period were shown in Fig. 3. The values of α reached a maximum and a minimum at the beginning and at the end of the light period, respectively, and the α on average during the light period was lower at the low CO₂ than at the high CO₂ (Fig. 3). The photochemical efficiency of PSII, Fv/Fm, of the alga decreased slightly over the light period, and was slightly higher in the high-CO₂-grown cells than that in the low-CO₂-grown cells (Fig. 4).
The photosynthetic CO₂ affinities, \(1/K_m(\text{CO}_2)\), of the alga were highest at 6 h of the light period and the CO₂ affinity was markedly higher in cells grown at the low CO₂ than that at the high CO₂ (Fig. 5). Figure 6 showed that the apparent carboxylating efficiencies (ACE) of the alga rhythmically changed and reached a maximum at 3 h of the light period, and the ACE was markedly higher at the low CO₂ than at the high CO₂.

Discussion

Prézelin et al. (1977) reported that the photosynthetic pigment (chlorophyll a and \(c_2\)) contents in marine dinoflagellates Glenodinium sp. and Ceratium furca were constant over circadian cycles, but Jacquet et al. (2001) showed that the chlorophyll contents for three marine Prochlorococcus spp. and five marine picoeukaryotes increased throughout the light period and decreased during the dark period. Our results here showed that the chlorophyll a contents of \(S. \text{ costatum}\) remained almost constant during the first 4 h and increased during the subsequent 6 h culture and then fell in the last 2 h during the light period. The data in Fig. 1 showed that the rate of cell division was relatively constant during the light period (Fig. 1A), but the rate of chlorophyll a synthesis fluctuated (Fig. 1B) and the rate was, on average, higher than that of cell division during the light period. Thus, the variation of the chlorophyll a content during the light period largely resulted from the fluctuating ratio of the rate of the chlorophyll a synthesis v. the cell division in \(S. \text{ costatum}\). The variation of the chlorophyll a content resulted in a difference in the pattern of \(P_m\) between that based on chlorophyll a content and that based on cell number during the light period.

Rhythmic change of photosynthetic capacity, \(P_m\), during the cell cycle has been documented for several

Fig. 1. Changes of (A) cell number and (B) chlorophyll a concentration in the bulk and (C) chlorophyll a content of cell for Skeletonema costatum cells grown at 200 µmol m⁻² s⁻¹ and 350 (■) or 1000 (●) µL L⁻¹ CO₂ during the light period. Values are mean ± s.e. of three separate experiments.

Fig. 2. Changes of light-saturated photosynthetic rate, \(P_m\), of Skeletonema costatum cultured at 200 µmol m⁻² s⁻¹ and 350 (■) or 1000 (●) µL L⁻¹ CO₂ during the light period. (A) based on cellular chlorophyll a content, (B) based on cell number. Values are mean ± s.e. of three separate experiments.
groups of algae and mixed populations of phytoplankton (MacCaull and Platt 1977; Prézelin et al. 1977; Mishkind et al. 1979; Kaftan et al. 1999), the time at which \( P_m \) is maximal varies but is most often in the morning or midday. Our data showed that the \( P_m \) of \( S. \) costatum varied rhythmically during the light period; the maximal \( P_m \) peaked in the midday when expressed on chlorophyll a but in the afternoon when expressed on the basis of cell number. The differences may be due to the diel variation of chlorophyll a content.

The factors that control photosynthetic activity during the cell cycle have not been identified. Some studies indicate that PSII activity and phosphorylation of thylakoid protein may play a role in controlling the change of the photosynthetic activity (Marcus et al. 1986; Allen 1992; Kaftan et al. 1999), and that the diel pattern of photosynthetic activity is directly correlated with the initial slope of the light-saturation curve (Harding et al. 1987; Erga and Skjoldal 1990), whereas other studies point out that C-reduction may be the rate-limiting factor (Walther and Edmunds 1973; Myers and Graham 1975). In the present study, the changing pattern of the \( P_m \) of \( S. \) costatum during
the light period was proportional to that of apparent carboxylating efficiency and that of photosynthetic CO₂ affinity, but was not correlated with that of the initial slope of the light saturation curve (α), which is indicative of the efficiency of the light reaction, and that of the photosynthetic chemistry efficiency of PSII ($F_v/F_m$). These results indicated that the changing pattern of the photosynthetic capacity during the light period might be controlled by the activity of C-reduction associated with C-uptake in S. costatum in dark reaction. *Skeletonema costatum* has been reported to have the capacity to actively take up both CO₂ and HCO₃⁻ (Korb et al. 1997). Our experiments showed that the alga mainly use free CO₂ from the medium (unpublished data), suggesting that the daily changes in the photosynthetic CO₂ affinity may be attributed to the diurnal alterations of photosynthetic capacity during the light period.

The results presented in this paper showed that the $P_m$ based on chlorophyll $a$ was not significantly different between ambient CO₂ and enriched CO₂, whereas the $P_m$ based on cell number increased with CO₂ enrichment (because of the increased chlorophyll $a$ content per cell under enriched CO₂). The result indicated that the estimation on effect of CO₂ enrichment on photosynthetic capacity was related to the normalized units, and implied that *S. costatum* benefited from CO₂ enrichment. The α and $F_v/F_m$ increased with increasing CO₂, indicating that the efficiency of light-harvesting and energy conversion in photosynthesis were increased. Down-regulation of Rubisco and inorganic carbon concentrating mechanisms (CCM) activities has been reported in many species (Wong 1979; Vu et al. 1983; Bowes 1993; Colman 2002; Matsuda et al. 2002). The present study indicated that the apparent carboxylating efficiency and the photosynthetic affinity for CO₂ markedly decreased under elevated CO₂ in *S. costatum*, suggesting that the similar down-regulation of Rubisco and CCM activities may also exist in this alga.

The effects of elevated CO₂ on photosynthetic rhythms during the cell cycle have been studied. Nara et al. (1989) reported that the changing patterns of the photosynthetic activity and the CA activity and the affinity of cell for CO₂ of *Chlorella ellipsoidea* C-27 were almost the same for cells synchronized at high (700 µM) and low (11 µM) levels of CO₂. Our data showed that the $P_m$ based on cell number, the chlorophyll $a$ content, the photosynthetic chemistry of PSII and the efficiency of the light reaction all increased to various degrees with elevated CO₂ and that the apparent carboxylating efficiency and the photosynthetic affinity for CO₂ markedly decreased under high CO₂ concentration in *S. costatum*. However, the changing patterns of all these photosynthetic parameters during the light period were almost the same for *S. costatum* cells grown at high and low levels of CO₂, suggesting that the photosynthetic rhythms was insensitive to CO₂ enrichment.

---

**References**


