II. 光合成の環境適応機構

Photosynthetic responses of diatom species *Coscinodiscus centralis* Ehrenberg to strong light intensity at low temperature in North Water Polynya

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Abstract

Diatom species of *Coscinodiscus centralis* Ehrenberg was widely distributed in North Water Polynya in April and May 1998. This species often showed maximum cell densities near to the sea surfaces. Its photosynthetic rates did not show any photoinhibition up to 475 μmol photons m^{-2} s^{-1} and an index of onset of light saturation represented as I_k was 115 μmol photons m^{-2} s^{-1}. Responses of this species to the strong light intensity at low temperature should be supported both by an initial slope of α and by a maximum rates of P_s^b in photosynthetic rate - light intensity curve (P-I curve). Its α and P_s^b at 0°C were not similar to those estimated in the past studies in polar sea and were 3.35 X 10^{-2} (mgC mgChla^{-1} hour^{-1})/(μmol photons m^{-2} s^{-1}) and 3.84 mgC mg Chla^{-1} hour^{-1}, respectively. Its higher P_s^b was maintained against suppression of electron transport rates and carboxylation rates by Ribulose bisphosphate carboxylase/oxygenase (RuBisCO) at low temperatures. Relative electron transport rates (ETR) and carboxylation rates by RuBisCO decreased by 60 and 47% with temperature decrease form

10 to 0°C, respectively, although photochemical reactions of quantum yield of photosystem II decreased only by 12%.

Introduction

Photosynthetic organisms of algae in polar sea are commonly subjected to characteristic conditions of temperature and light. Water temperature is almost constant and near to freezing point of seawater. In contrast day light length remarkably changes in a year; no solar radiation occurs in winter although the length increase to all day long in summer. As a result Polar Regions can have a greater daily insolation even than tropical regions in midsummer (Kirk 1994). However many parts of the polar sea are covered by sea ice. Solar radiation is reduced to 2% or less of that reaching the ice surface due to absorption and reflection by ice and snow under seasonal and permanent sea ice (Horner & Schrader 1982, Sullivan et al. 1982, SooHoo et al. 1987). Low temperature and weak light intensity effects photosynthetic responses to light intensity represented by photosynthetic rate - light intensity curves (P-I curve). At low temperatures maximum rates per a unit amount of chlorophyll a (Chla) decreased as some photosynthetic reactions were suppressed (Davison 1991, Kirk 1994). The maximum rates also decreased under weak light intensity as contents of Chla increased in some algae (Yoder 1979, Kirk 1994). As accessory pigments increased together with Chla, the initial slope in P-I curve became much steep under weak light intensity (Geider 1987, Kirk 1994). These lower maximum rates and steep initial slope cause a saturation of photosynthesis under weak light intensity. Algal communities in polar sea inhabiting under weak light intensity

at low temperature showed saturation under weak light intensity (Suzuki et al. 1997).

In polynya, where little ice covers the sea surface in spring and summer, algae are often exhibited to great daily insolation directly. Algae in a Polynya would be exhibited to both strong light intensity and low temperature. Under strong light intensity at low temperature photosynthetic organisms tend to show photo-inhibitions and decrease its photosynthetic rates (Sonoike 1998). Photosynthetic organisms in the polynya need to get along with strong light intensity and low temperature to avoid a photoinhibition and maintain its photosynthesis. The responses to the strong light intensity at low temperatures could be one of the most important mechanisms for higher primary productions in polynya. In the present study we collected the cells of diatom species *C. centralis*, who distributed widely in North Water Polynya, and estimated the light responses of its photosynthesis under strong light intensity at low temperatures. With enough amounts of the cells, biochemical analysis was further estimated.

Materials and methods

Seawater samples were collected at stations described in Table 1 during the cruise of North Water Polynya in 1998. Two litters of the sample was filtrated (Wattman GF/F) and the algal cells on the filters were counted by microscopic observations and/or by eye. At stations B102 and B18, where the densities of large algal cells were high, the algal cells were collected by the vertical net tows from 50 m to the surface with a net of 200 μm mesh. The collected samples were left in

a thermos bottle (TIGER MWE-T350 Japan) in the dark at 0°C for 30 minutes. Large diatom cells were settled at first. Then the cells were picked up with a pipette and moved into filtrated surface sea water (FSW, here after) in another thermos bottle. The cells were settled and moved into FSW more two times in order to select the cells.

The photosynthetic rates of the cells were determined with the ^{14}C-tracer technique using a photosynthetron (Lewis and Smith, 1983) whose temperature was controlled by a water circulator (CTE42A Yamato-Komatsu Japan). Each incubation vials in the photosynthetron was set at each different distance from a halogen lamp and was illuminated from the bottom side. Different light intensities were simulated by the distances. The light intensity on the bottom surfaces of an each incubation vial was determined by a 2π quantum meter (LI-COR, LI-190SA). Using *Levenberg-Marquardt* method, the data we obtained on the photosynthetic rate under various light intensity below 475 μmol m^{-2} s^{-1} were fitted with the following equation ;

$$P^B = P_s^b \left(1-\exp\frac{-\alpha \cdot I}{P_s^b}\right) = P_s^b \left(1-\exp\frac{-I}{I_k}\right) \qquad (1)$$

where P^B is the actual photosynthetic rate (mgC $mgChla^{-1}$ $hour^{-1}$), P_s^b is the estimated maximum rate of the biomass-specific photosynthetic rate (mgC $mgChla^{-1}$ $hour^{-1}$), I is the light intensity (μmol photons m^{-2} s^{-1}), α is the initial slope of the P-I curve [(mgC $mgChla^{-1}$ $hour^{-1}$)/(μmol photons m^{-2} s^{-1})], and I_k ($= P_s^b / \alpha$) is the estimated light intensity at the onset of light saturation (μmol photons m^{-2} s^{-1}). The responses of

photosynthetic rates to changes in temperature were also determined at ca. 500 μmol photons m^{-2} s^{-1}, where the rate was saturated at 0°C.

Chlorophyll fluorescence of photosystem II was determined with PAM chlorophyll fluoromer system (TEACHING-PAM, WALZ, Germany) to assess photosynthetic properties (Schreiber et al. 1997). A grass chamber, in which temperature controlled water was circulated at a rate of ca. 9 litters min^{-1}, was fixed to the measuring head of PAM system and regulated the temperature of the samples in the head. Soon after changing temperature from 0°C to each estimation temperature, fluorescence were determined at 10 different light intensities with a data acquisition program of DA-TEACH (TEACHING-PAM, WALZ, Germany). Diatom samples were centrifuged at 500G for 10 minutes at 0°C and cells were collected as a pellet. In protein extraction buffers (100mM HEPES pH.8.0, 1.2mM EDTA, 25mM $NgCl_2$, 1.0mM DTT) the pellets were homogenized with an ultra sonic disrupter (U50, IWAKI, Japan) at 0°C and were centrifuged at 500G for 10 minutes. The supernatants were used as the crude extract of enzymes and carbon fixation rates with 1.0 mM Ribulose-1,5-bisphosphate were estimated as RuBisCO activities (Yokota 1991) after activations at 10°C for 3 minutes.

The pellets of the cells were frozen in liquid nitrogen and were stored at temperatures below -80°C for the observations of a scanning electron microscope (SEM) (Hayakawa et al. 1995). With this sample the variations of the gene of RuBisCO small sub-unit (*rbcS*) were determined. The frozen pellet was ground into powder with a mortar in liquid nitrogen. One gram of the powder was added to 10 ml of DNA extraction

buffer (50mM Tris-HCl pH.8.0, 20mM EDTA, 1% SDS, 1% Protainase K) and incubated at 50°C overnight. DNA was extracted with phenol : chloroform method. The molecules of *rbcS* in the samples were amplified by polymelase chain reactions (PCR) with Taq enzyme (Taq EX, Takara, Japan). Eighteen molecules of *rbcS* were cloned by TA-cloning method (The Original TA Cloning Kit with pCR 2.1, Invitrogen, U.S.) and determined each sequence (Dye Terminator Cycle Sequencing FS Core Kit, Perkin-Elmer, U.S.).

Chlorophyll a concentration was determined in a Turner Design fluorometer (Model10-100R) after extraction with N,N-dimethylformamide of each sample retained on a Whatman Gf/F filter (Suzuki and Ishimaru 1990).

Results

During the cruise on April and May 1998 single cells of *Coscinodiscus centralis* Ehrenberg and *C. asteromphalus* Ehrenberg were found (Dr. Lovejoy personal communications). We also found the large single cells, whose diameters were more than 200μm, at many stations and counted densities of its cells at 4 stations shown in table 1. Cell densities were less than 65 cells l^{-1} at station A49 and station A35 (Fig. 1). The densities at station A49 were ca. 60 cells l^{-1} at above 79m and decreased to 0 at 150m. At station A35 the densities were less than 32 cells l^{-1} at above 80m and increased to 53 cells l^{-1} at 150m, where all the cells were etiolated. Cell densities increased more than 150 cells l^{-1} at station A27 and B18. At station A27 densities were 109 cells l^{-1} at the surface and increased to 151 cells l^{-1} at 8m and decreased along with depth. No algal cells were found at 150m. At station B18 density was 111 cells l^{-1} at the surface and increased to 195 cells l^{-1} at

22 m and decreased to 10 cells l^{-1} at 100m.

At station B102 and B02, where we found good amounts of algal cells, the cells were collected from the depth between 50 and 0m. Large algal cells were settled in FSW 3 times and selected. After the selection it was hard to find algal cells except for large single cells of a centric diatom. By the SEM observation the large diatom was identified as *C. centralis*. Using the extracted DNA from the samples as a template, DNA segments for a small subunit of riblose bisphosphate carboxirase/oxygenase (*rbcS*) were amplified by Polimerase Chain Reaction (PCR). Eighteen molecules of DNA segments were cloned by a TA-cloning method. The base sequence of each molecule of the amplified 18 DNA segments was completely same (Table 2). The homology of the *rbcS* segments with those of *Thallassiosira nordenskioeldii* Cleve, *Detonula confervacea* (Cleve) Gran (Tomizaka 1998), *Skeletonema costatum* (Grev.) Cleve and *Chaetoceros* sp. (data were not shown) were 80, 83, 82 and 79, respectively, and were similarly different from these species.

Photosynthetic rates of the diatom samples were determined at various light intensity up to 475 μmol photons m^{-2} s^{-1} at 0°C with the ^{14}C-tracer technique (Fig. 2). The cells did not show any photo-inhibition in this light intensity range and reached to the maximum rates of 4.41 mgC mgChla^{-1} hour^{-1} at to 475 μmol photons m^{-2} s^{-1}. The relationship was approximated with saturation curves (Eq.(1)) without considering photo-inhibition. Estimated α was 3.35 X 10^{-2} (mgC mgChla^{-1} hour^{-1})/(μmol photons m^{-2} s^{-1}) and P_s^b was 3.84 mgC mg Chla^{-1} hour^{-1} and then I_k was 115 μmol photons m^{-2} s^{-1}.

The effects of temperature on maximum rates and initial

slopes were further estimated. In order to estimate effects on maximum rates, photosynthetic rates at 500 μmol photons m^{-2} s^{-1}, where the rats were almost saturated at 0°C, were determined at various temperatures up to 20°C and relative rates of photosynthesis to the rate at 0°C were estimated (Fig.3). Photosynthetic rates under the strong light intensity showed particular temperature dependency. Its maximum rate of 228% of the rate at 0°C was observed at 10°C. The rates decreased below and above there. At 5 °C the rates was almost same as those at 0 °C. The rate at 15 and 20°C were 96 and 91% of the rate at 0 °C.

To estimate effects on maximum rates, we further determined the rates of the CO_2 assimilation by RuBisCO, which is one of the key enzymes of maximum photosynthetic rates. Radio activities in assimilated ^{14}C by the crude extracts of diatom samples were determined. The activities showed a different temperature dependency from those of photosynthetic rates under the strong light intensity (Fig.4). The activity was 867 dpm min^{-1} at 0 °C and increased exponentially to 5170 dpm min^{-1} at 30 °C. In this temperature range the rate increased 89% by 10°C (Q10=1.89) on average. At 40°C maximum activities of 11500 dpm min^{-1} was determined. However the activity was unstable and decreased to 2430 dpm min^{-1} after incubations for 3 minutes at 40°C.

In order to estimate effects of low temperature on an initial slope in P-I curve, the responses of chlorophyll fluorescence to changes in light intensity were determined at various temperatures. Then quantum yields of photosystem II, which could indicate the light harvesting properties and could affected initial slopes of light response curves, were

estimated. Quantum yields generally decreased along with light intensity steeply under a weak intensity and gradually under a strong intensity (Fig. 5). Temperature affected the yields differently under a weak intensity from that did under a strong intensity. Under 60 μmol photons m^{-2} s^{-1} quantum yields showed similar values at between 0 and 15 °C. It decreased above at 20 °C and minimum value of 0.35 was determined at 25 °C. Under 1250 μmol photons m^{-2} s^{-1}, in contrast, quantum yields were effected by low temperatures. Minimum value of 0.1 was determined at 0 °C. The value increased to 0.21 at 10 °C and showed same values at temperatures between 10 and 20 °C. At 25 °C it decreased to 0.14. Quantum yields decreased with light intensity steeply at low temperatures and gradually at high temperatures. Under all the light intensity quantum yield showed higher values at intermediate temperatures of 10 and 15°C than those determined at high and low temperatures (Fig. 5).

Discussion

Single cells of diatom were found at many stations of North water polynya on April and May 1998. From the collected diatom cells, DNA segments for a part of *rbcS* gene were amplified and 18 molecules of them were cloned to determine their base sequences. Most of the photosynthetic organisms have *rbcS* different from the others. Thus some different *rbcS* should be amplified from the diatom sample contained some species. Completely same sequences of 18 segments indicated that most of the cells in the sample were belong to only one species and that contamination of the different photosynthetic organisms to the species could be less than 15% ($p<0.05$) under

assumption of same efficiencies of the primer for amplifying a *rbcS* segment in the sample (Table 2). By SEM observations we could not find diatom cells except for large centric one, which was identified as *Coscinodiscus centralis* Ehrenberg. At station A 27 and B18 maximum cell densities were found at 8 m and 22 m, respectively, and below there cell densities decreased along with depth. At around the depth for the maximum densities the cells could be growing and kept their densities. At station A49 and A35 the cell showed the broad peaks at around 50 m and 150 m, respectively, and some cells were etiolated. At deeper layers its settled cells could be exhibited to low light intensity and could not grow actively. The cells at 150m were all etiolated.

These algal growth and primary productions are mainly supported by their photosynthesis. Photosynthetic responses of algal populations were estimated at various sea areas and were varied corresponding to their inhabiting environments (Parsons et al. 1984). We also estimated the photosynthetic responses of the collected cells of *C. centralis*, who was expected to show characteristic responses to the environments in North Water polynya as they widely distributed there. At first we determined their photosynthetic rates under various light intensity at 0°C near to *in situ* temperature. Under weak light intensity the rates increased lineally along with light intensity followed by saturation. Up to 475 μmol photons m^{-2} s^{-1} they showed no clear photo-inhibition (Fig.2). Algal populations in polar sea often showed saturation at low light intensity and the index of onset light saturation of I_k was sometimes less than 5 μmol photons m^{-2} s^{-1}. I_k of 115 μmol photons m^{-2} s^{-1} in this study was quite higher than those estimated in

the past studies. Its photosynthesis would be respond to a direct light intensity in the North Water Polynya.

Light saturation are determined by initial slopes of α and maximum photosynthetic rates of P_s^b in the photosynthesis-light intensity (P-I) curve . High I_k should be resulted from low α and high P_s^b . There are several studies reporting the values of α for natural algal communities and the α varied corresponding to the environment widely . The α estimated in polar sea were generally high and sometimes were more than 0.2 (mgC mgChla^{-1} hour^{-1})/(μmol photons m^{-2} s^{-1}) (cf.) as they were often estimated with ice algal communities inhabiting under low light intensity at a bottom of sea ice. In this study estimated α was 3.35 X 10^{-2} (mgC mgChla^{-1} hour^{-1})/(μmol photons m^{-2} s^{-1}). Compared to ice algal communities the α of *C. centralis* was remarkably low.

There are also several studies reporting the values of P_s^b. Most of the natural algal populations showed P_s^b within the range between 0.1 and 6.0 mgC mgChla^{-1} hour^{-1}. Its value of P_s^b estimated in polar sea showed relatively low within the range and was sometimes less than 1.0 mgC mgChla^{-1} hour^{-1}. In this study P_s^b was estimated as 3.84 mgC mgChla^{-1} hour^{-1} and was higher than those estimated with the communities in polar sea. Photosynthetic responses of *C. centralis* were characterized by low α and high P_s^b. We can consider that *C. centralis* could be exhibited to a strong light intensity and responded to the intensity both by low α and by high P_s^b.

These photosynthetic responses were carried out at low temperatures in the polynya. At those low temperatures many reactions in organisms were suppressed . At low temperatures maximum photosynthetic rates were generally low and photo-

inhibitions occurred by an light intensity exceed to the low maximum rates . In stead of low temperatures *C. centralis* well corresponded to the strong light intensity. Thus at the low temperature we analyzed these photosynthetic responses. To estimate the effects of low temperatures on the initial slope of α, quantum yields of photosystem II (PSII, here after), which is one of the photochemical reactions determining the initial slope α, were determined by a chlorophyll fluorescence. Under 60 μmol photons m^{-2} s^{-1} quantum yields were almost constant at around 0.5 at temperatures between 0 and 20 °C. At 25°C it decreased to 0.35 (Fig. 5). Under weak light intensity photochemical reactions are not limited by following reactions and showed its properties . These results strongly suggested that the effects of temperatures on photochemical reactions of PSII could be small at temperatures between 0 and 20°C. Under 1250 μmol photons m^{-2} s^{-1}, temperature dependencies of quantum yields were different from those at 60 μmol photons m^{-2} s^{-1}. Quantum yields were almost constant at around 0.2 at temperatures between 10 and 20°C. However they decreased at low temperatures below 10 °C and were 0.10 at 0°C. Under strong light intensity part of the absorbed photons could not be used by the photosynthesis as it was limited by the following reactions of electron transports . Thus the decrease of yields at 0 °C would indicated the effects of low temperature on electron transport rates. We can estimate the properties of electron transport as a relative electron transport rate (ETR) at a certain light intensity as follows;

$$ETR = Q * R * a \qquad (2)$$

where Q is quantum yield and R is light intensity and a is the efficiency factor of photosynthesis of 0.4 . The ETR varied along with light intensity (Fig.6). The response of ETR to changes in light intensity at 0°C were similar to the light response curve of actual photosynthetic rates (Fig. 2) and saturated at around 500 μmol photons m^{-2} s^{-1}. Under low light intensity At 5°C ETR increased togather with that at 0°C but did not saturated up to 1000 μmol photons m^{-2} s^{-1}. At low light intensity ETR estimated at 10, 15 and 20°C also increased similarly to those at 0°C. However they did not stop increaseing up to ca. 250 at 1250 μmol photons m^{-2} s^{-1}. ETR at each temperatures determined at various light intensity below 1250 μmol photons m^{-2} s^{-1} were fitted with the following equation;

$$ETR = ETR^{*} \left(1-\exp\frac{-Q^{*}.I}{ETR^{*}}\right) \qquad (3)$$

where ETR is the relative electron transport rate (μmol photons m^{-2} s^{-1}), ETR^{*} is the expected maximum rate of the ETR (μmol photons m^{-2} s^{-1}), I is the light intensity (μmol photons m^{-2} s^{-1}), Q^{*} is the initial slope of the ETR-I curve, from which we can estimate an expected quantum yield at 0 μmol photons m^{-2} s^{-1} (Fig. 6). Expected maximum quantum yields and expected ETR at 0 μmol photons m^{-2} s^{-1}, which could be the maximum yields with no suppression by following reactions at temperatures between 0 and 25°C were shown in figure 7a and figure 7b, respectively. These results suggested much clearly the effects of low temperature on a photosynthesis. Maximum quantum yields of PSII changed little at temperatures between 0 and 15°C. They

showed a maximum value at 5°C and decreased only 7% from 5°C to 0°C (Q10=1.14) and 0.5% at temperatures from 5 to 15°C (Q10=0.95). Above 15°C maximum yields decrease steeply to 0.21 at 25°C (Q10=0.40). The photochemical reactions could be unstable at above 20 °C. Expected maximum ETR showed different temperature dependencies from maximum yields (Fig. 7b). Maximum ETR increased 2.5 times at temperatures between 0 and 10°C (Q10=2.5) and were maintained ca. 130 μmol photons m^{-2} s^{-1} with some fracturations at temperatures between 10 and 25°C. These results suggested that the properties of electron transport rates were suppressed at low temperatures below 10 °C.

We also estimated the effects of temperature on the rates of CO_2 assimilation by RuBisCO, which follows the reactions of light harvesting and electron transport and is one of the limiting reactions of maximum photosynthetic rates (Fig.4). The rates of *C. centralis* increased exponentially up to 30°C. Even at 40°C rates increased although the activities were not stable (Fig. 7). At low temperatures the rates decrease exponentially. At 0°C it was 52% of the rates at 10°C (Q10=1.89).

Each photosynthetic reaction of C. centralis showed different temperature dependencies from the others. At above 15°C its quantum yield inhibited by high temperatures. Maximum ETR did not increase at above 10 °C although it kept rates up to 25°C. Its carboxyration by RuBisCO was not inhibited up to 40°C. By low temperatures, in contrast, its quantum yields were suppressed little. Its maximum ETR and carboxyration by RuBisCO were suppressed at 0 °C. Its quantum yields at 0 °C were 88% of that at 10 °C, although its maximum ETR and carboxyration rates by RuBisCO at 0 °C decreased to 40 and 53% of those at

10 °C, respectively.

Temperature dependencies of photosynthetic rates at 500 μmol photons m^{-2} s^{-1} were different from those of ETR and of RuBisCO. The rate were suppressed at temperatures below and above 10°C. Its particular temperature dependencies suggested that maximum photosynthetic rates could not be limited by one simple reaction at all the temperature range but could be limited by several different reactions at each narrow temperature range. Possible limitations by a certain reactions decreased the rates with temperatures below 10 °C. But the rates were kept 43% of the rates at 10 °C even at 0 °C. These positive rates at 0°C indicated that no reactions in photosynthesis could be decreased to 0. At low temperatures near to 0 °C some enzymes lost their activities. Electron transport rates of a certain algae suppressed strongly at 0°C. Compare to these reactions, those of *C. centralis* could be well corresponded to the low temperatures in the polynya.

In spite of the correspondence these reactions could not avoid to decrease their activities at low temperatures and each reactions suppressed differently. These different suppressions would be important to consider the photosynthetic responses at low temperatures. Even at 0 °C *C. centralis* showed low α and high P_s^b and avoided photo-inhibitions up to 475 μmol photons m^{-2} s^{-1}. As suppressions of photochemical reactions by low temperature were not serious, *C. centralis* could easily decrease its properties of photochemical reactions and could control the values of α. In contrast the suppressions of carboxylation and electron transport rates were strong. To increase P_s^b *C. centralis* could need to increase their properties against suppression by low temperatures.

Considering the suppression at low temperatures the maintenance of P_s^b would be one of the most important mechanisms to keep high I_k and to avoid the photoinhibition in order to maintain higher primary productions under strong light intensity at low temperatures in North Water polynya.

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Table 1. Sampling date and station positions.

Station	Latitude (N)	Longitude (W)	Date
A49	76°20'	74°42'	23 Apr
A27	77°20'	73°47'	27 Apr
A35	76°57'	75°06'	01 May
B18	77°50'	73°08'	13 May
Sampling stations for determination of cell densities			
B102	76°36'	76°30'	07 May
B02	78°16'	74°51'	09 May
Sampling stations for determination of cell densities			

Table 2. Base sequence of amplified DNA segments from diatom samples at station B02 using two synthetic primers corresponding to positions 08-35 (5' TTACACAAGGTTGTTTCTVGTTCTTA CC 3') and 385-410 (5' AGTGTA CAAGCTAATC CAGAAGGTGG 3') in *rbcS* of *Thalasiossila nordenskioeldii* (Tomizawa 1998). Astarisks showed

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   1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
000 *******TTA CACAAGGTTG TTTCTVGTTC TTACCAGATT TAACTGACCA ACAAATTGAA
060 AAACAAGTTG CATATGCAAT TTCAAGAGGC TTGGCAATGA ATGTTGAATG GACGGATGAT
120 CCACACCCAC GTAACAGTTA CTGGGAATTA TGGGGTTTAC CATTATTTGA CATTAAAGAT
180 TCAGGTTCTG TAATGTATGA ACTTAACGAA GCTCGTAAAG CATGTCCAAA TGGCTACATT
240 CGTATGAATG CATTTGATGC TAGTTACGGG ACAGAAAGTT GCGTTATGTC TTTCATCGCA
300 AGCCGTCCAA GTAATGAACC AGGTTTCTAT TTAGATCGTA CAGAAGGACC AGGTCGTCAA
360 ATTATTTACT CTATTAAGAG TTATAGTGTA CAAGCTAATC CAGAAGGTGG **********
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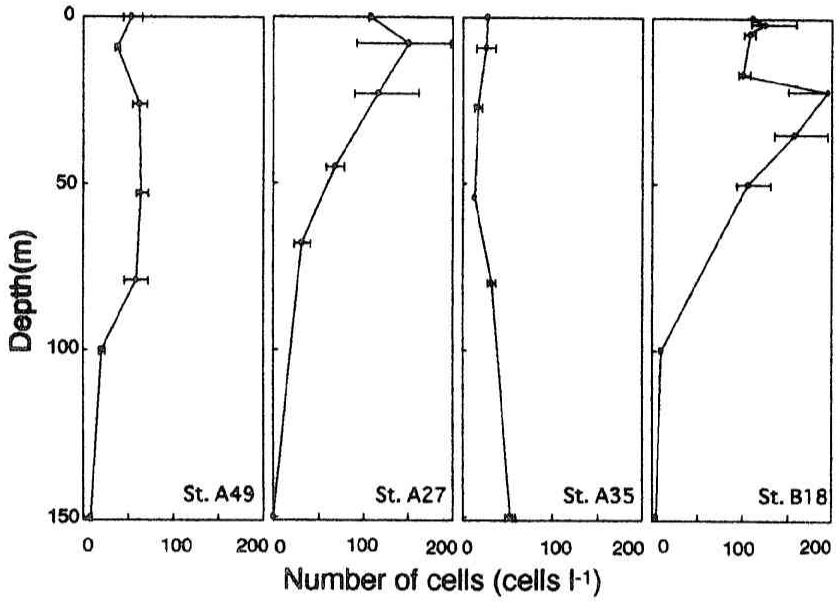

Figure 1。
Cell densities of *C。centralis*。Horizontal bars show ranges and closed circles show averages of 4 samples from each sampling depth。

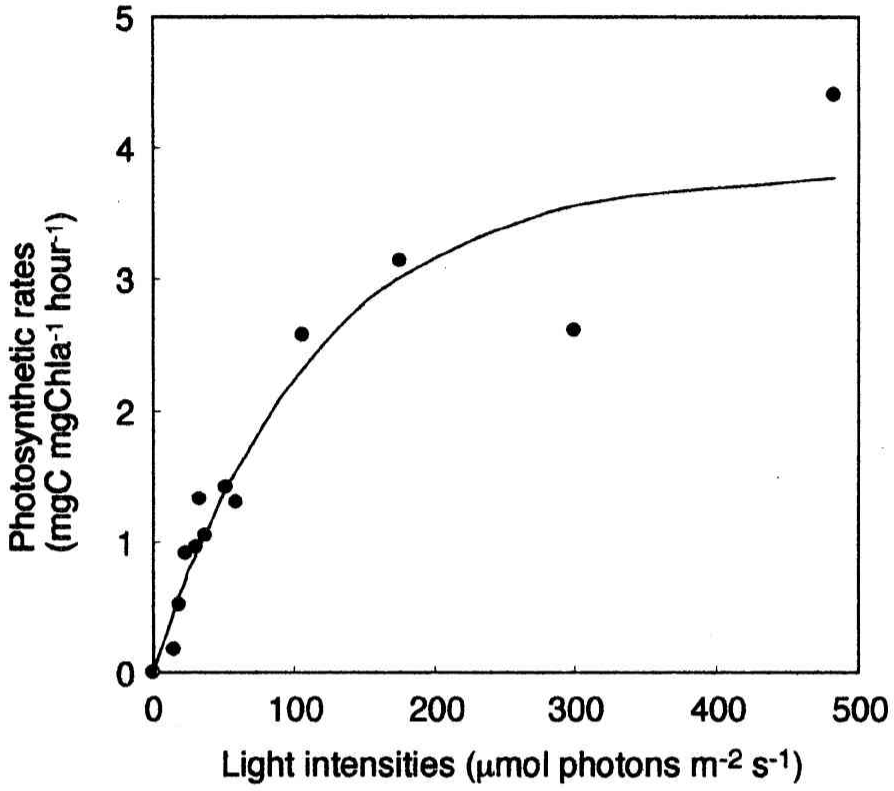

Figure 2.
Influence of light intensity on the photosynthetic rates of *C. centralis* determined by ^{14}C tracer technique at 0°C. Line was drawn using Eq. (1): $P^B = P_s^b [1-\exp(-\alpha \cdot I / P_s^b)]$; see text for further details.

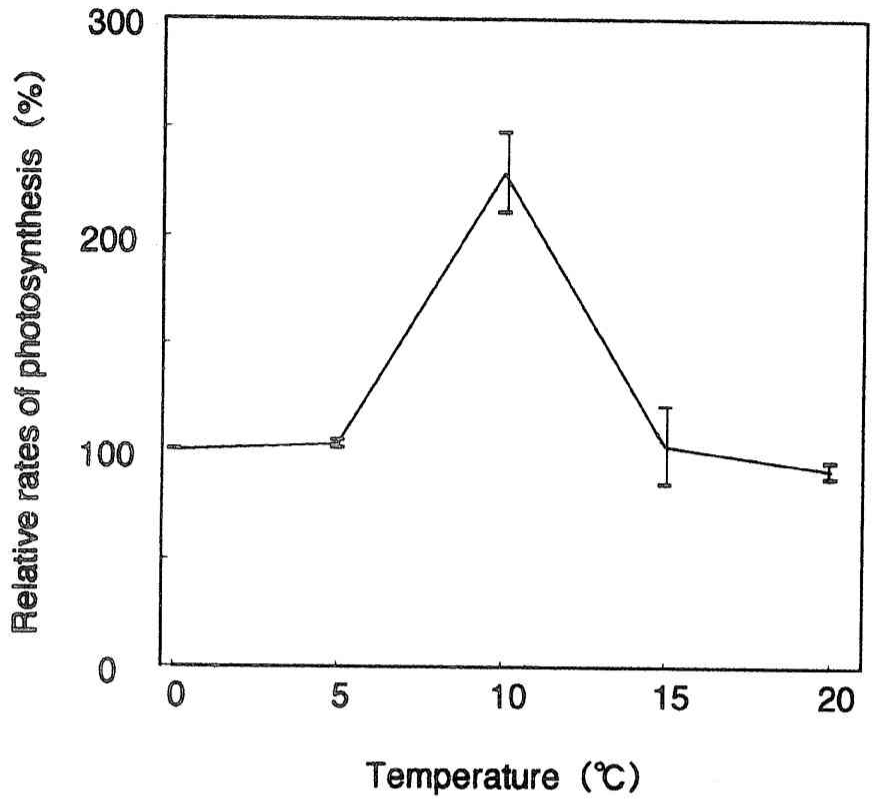

Figure 3.
Influence of temperature on the photosynthetic rate of *C. centralis* determined by ^{14}C tracer technique at 500 μmol photons m^{-2} s^{-1}. Vertical bars indicate range of the rates of 2 samples at each temperature.

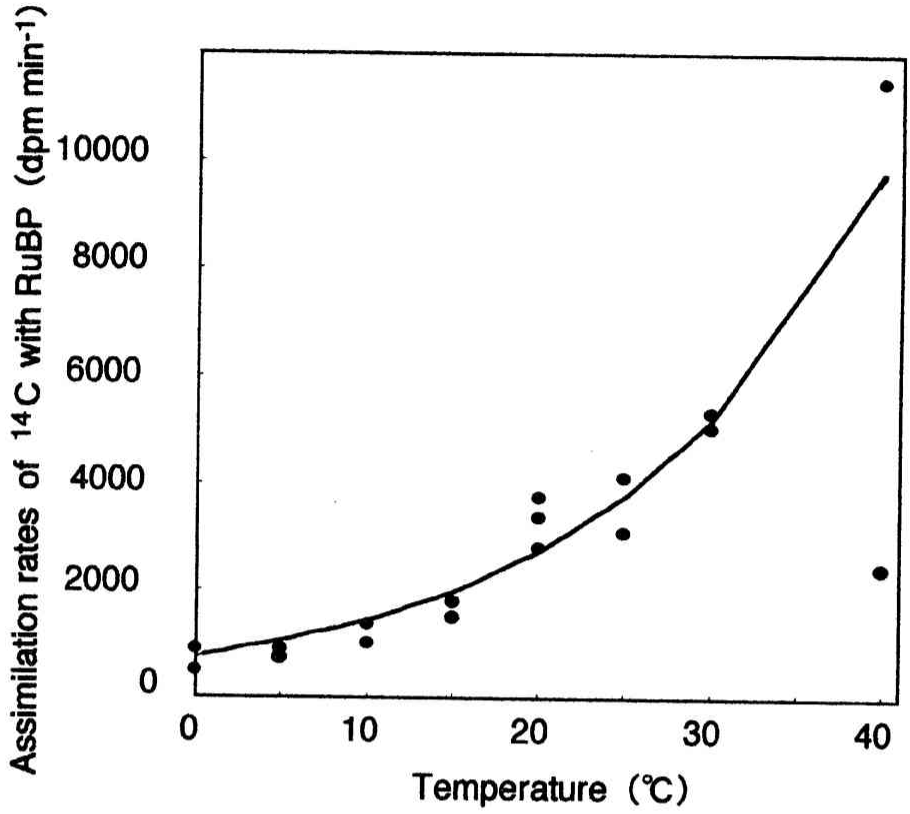

Figure 4.
Assimilation rates of ^{14}C by the crude extracts of *C. centralis* with 1.0 mM of Riblose bisphosphote (RuBP). Line was estimated by the least sequence method assuming the exponential increase of the rates at temperatures between 0 and 30℃.

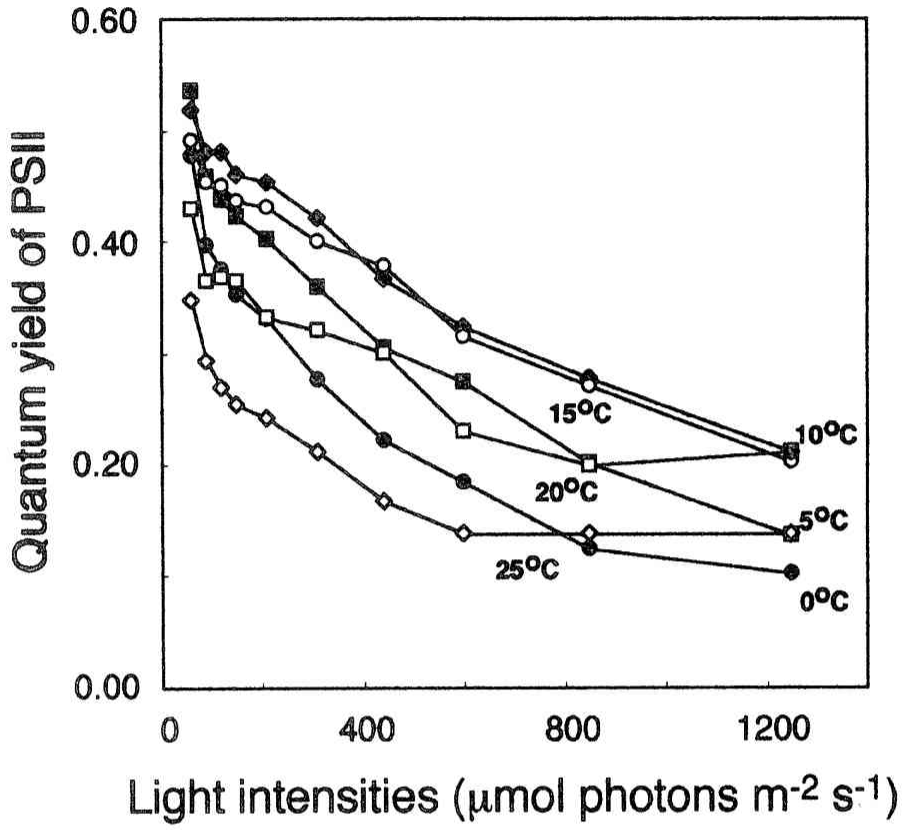

Figure 5.
Quantum yield of PSII determined by PAM method at temperatures between 0 and 25°C. Closed circles, closed squares, closed diamonds, open circles, open squares and open diamondes show the yields at 0,5,10,15,20 and 25 °C, respectively.

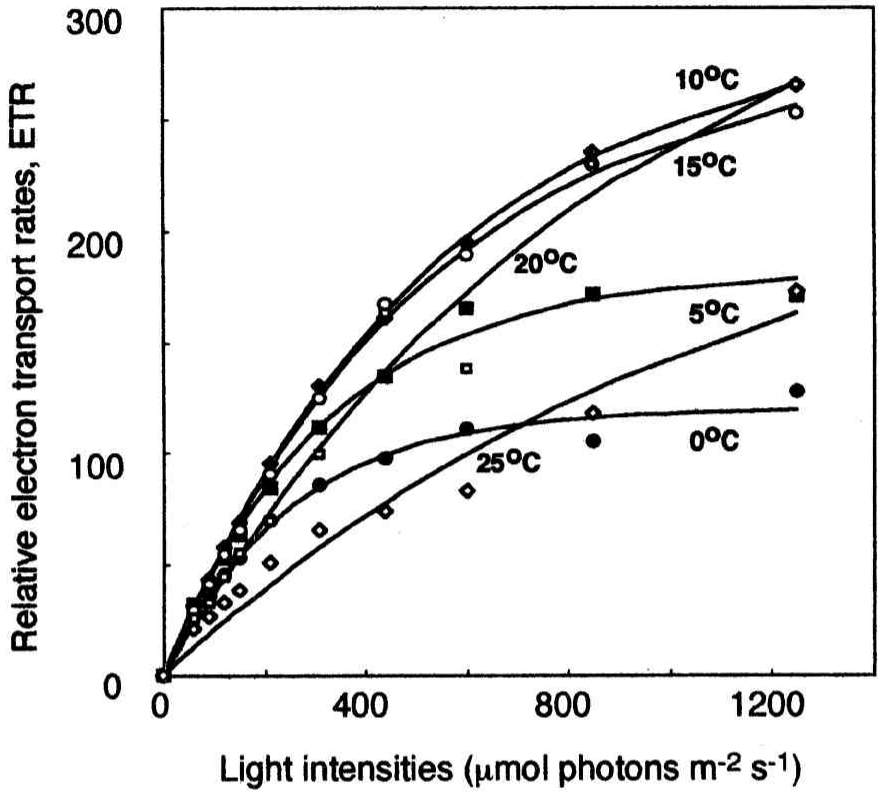

Figure 6.
Estimated relative electron transport rates (ETR) by Eq. (2): ETR = Q * R * a . Lines were drawn using Eq. (3): ETR= ETR^{*} (1-exp-Q^{*}.I/ ETR^{*}); see text for further details.

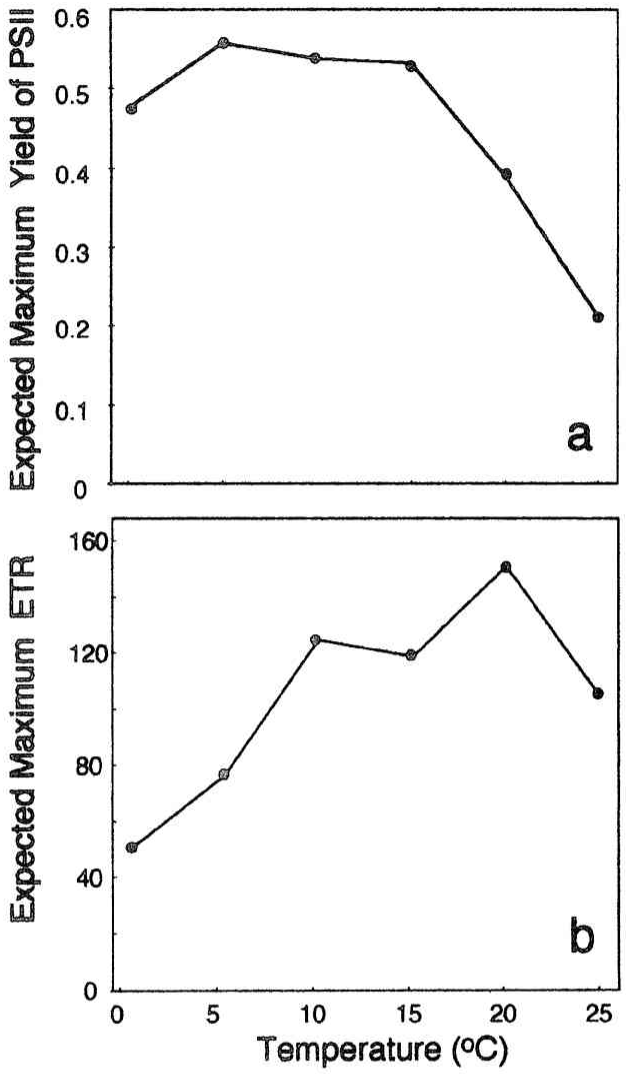

Figure 7.
Expected maximum yields of PSII (a) and ETR (b) estimated by Eq. (3) at temperatures between 0 and 25°C.