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Carbon cycle coupling of photosynthesis and calcification in the coccolithophore, *Emiliania huxleyi*

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Abstract:

Carbon dynamics of *E. huxleyi* is of great concern in terms of global carbon cycles. We monitored cell concentration, nutrients, C_T, pH and carbon production together with stable organic and inorganic carbon isotopes for 30 days within a 1 m^3 axenic culture tank. Our results, repetitively, indicated that during the exponential growth phase and most of the stationary growth phase, *E. huxleyi* always serves as a net CO_2 sink; and only during late stationary growth phase, CO_2 was released to the atmosphere. During the whole period, *E. huxleyi* acted as a sink of CO_2. From the changes in $\Delta\delta^{13}C$ values and the concentrations of carbonate species, it was concluded that HCO_3^- as well as CO_2 were acceptable carbon sources for organic growth when CO_2 availability in sea water or internally liberated during calcification was limited.

KEYWORDS: coccolithophore, *Emiliania huxleyi*, calcification, carbon cycle, stable carbon isotope

1.Introduction

Coccolithophore blooms are widespread in the ocean. Their roles in oceanic and global carbon cycling are of potentially great importance, because of not only their capacity for organic matter production by photosynthesis but also their unique ability to synthesize external plates of calcite, called coccoliths. Recently *E. huxleyi* blooms have been observed extensively in the North Atlantic in satellite images (Brown and Yoder 1994; Batch et al. 1991; Holligan et al. 1983, 1993) and in field observations (Brown and Yoder 1994; Batch et al. 1991; Holligan et al. 1983, 1993; Fernandez et al. 1993; Robertson et al. 1991), and it has been concluded that dense blooms of *E. huxleyi* become sources of CO_2 to the atmosphere. Because CO_2 is liberated through the calcification as follows.

$$Ca^{2+}+2HCO3^{-}\rightarrow CaCO_3+CO_2+H_2O \quad (1)$$

However, detection of *E. huxleyi* populations from satellite images (Holligan et al. 1983) or field observations has only been possible during late stages of bloom development and their impact on the carbon cycle during bloom onset and growth has been unknown. Here laboratory experiments were conducted in a 1 m^3 axenic culture tank (Watanabe et al. 1988, 1991, Kim et al. 2004). The use of the large tank enabled frequent and long term observation, including isotope

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analyses which require large volume of sample water. We discuss whether *E. huxleyi* is source of CO_2 or sink of CO_2 by comparing organic and inorganic carbon production. When 1 mol of inorganic production is made, 0.6 mol of CO_2 is increased. Thus, when the ratio of calcium carbonate production rate and organic production rate is smaller than 1.66, *E. huxleyi* is sink of CO_2. We present data describing the carbon cycle coupling of photosynthesis and calcification

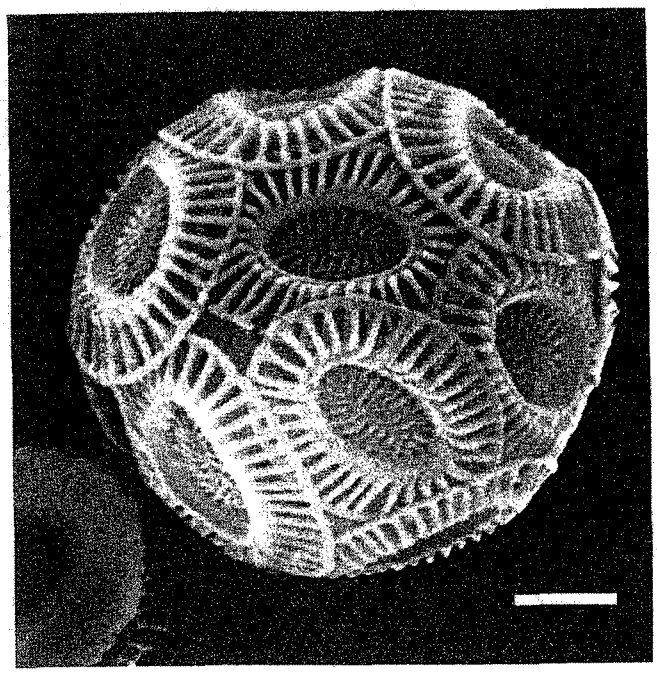

Fig.1 *E. huxleyi* (Paasche 2002)

in a complete *E. huxleyi* bloom cycle based on the variation in organic and inorganic stable isotopes which imply changes of carbon sources during the life cycle of *E. huxleyi* .

2.Materials and Methods

Experiment Design-.E. huxleyi (Lohm) Hay et Mohler (Prymnesiophyceae: CCMP 374 at the Bigelow Laboratory for Ocean Science) was precultured axenically using f/50 medium (which means 50times dilution of f medium) in Erlenmyer flask(2L) at 18° C, ca 150 μE m^{-2} s^{-1} under a 12:12 LD photoperiod for ten days. The NIES tank, a large axenic culture tank (Watanabe et al. 1988, 1991) (1 m i.d. ´ 2 m; working volume ~ 1 m3) , was used to grow E. huxleyi under axenic conditions by using f/50 medium and precultured E. huxleyi cells in Erlenmyer were inoculated axenically into the NIES tank to give an initial cell concentration of 1.03 × 103 cells ml^{-1} at the beginning of the experiment. Illumination was given from a 5-kw xenon lamp operated at 530μE m^{-2} s^{-1} (average at the surface) with a 12:12 L/D regime (L: 0600 ~ 1800 hours). Temperature was kept at 18° C. Sterilized air (200 ml min^{-1}) was introduced

gently from the bottom of the tank to maintain a fully mixed condition. On day 17, f/20 medium was added in order to simulate upwelling of nutrient in the open sea. Same experiments were repeated several times.

Sampling and analyses- At 1300 hours on every day 2 liters of water were sampled. Cells were counted with a Coulter TA-II counter. For particulate C measurement, sample water was filtered through precombusted (400 °C for 4 h) Whatman GF/F glass-fiber filter. The filters were rinsed with 0.5 M ammonium formate and stored at -20 °C until analysis. After the filters had been dried in a forced-air oven at 80 °C for 48 h, particulate C on the filters was measured with a CHN analyzer (MT-3, Yanaco, Japan). Also organic carbon was measured after the acidification of the sample in order to remove $CaCO_3$. Sample water was filtered (Pall Ultipore N_{66} filter, 0.45 μm pore size, rinsed with HNO_3) and rinsed with distilled water. The filter was placed in a tuftainer vial (7-m*l* volume) with 1 m*l* HNO_3 and heat-decomposed in a high-pressure digestion bomb (140 °C, 4 h). The resulting solution was diluted to 25 m*l* by distilled water and used to measure calcium by flame atomic absorption spectrum (Shimadzu AA-640-12, C_2H_2-Air). The results were converted to cell content of calcium and cell content of inorganic carbon. For the measurements of pH and C_T, seawater was sampled by gravity from a sampling line at the side of the tank in which cavitation was avoided. Sample water in 25-m*l* syringe was filtered through a 0.45 μm Whatman cellulose nitrate filter attached to another 25-m*l* syringe without exposing to air. C_T was determined by using a coulometer (Dickson et al. 1992; Johnson et al. 1987) (UIC Inc. model 5012 CO_2 coulometer and model 5130 acidification module). Precision of < 0.5 % was obtained. pH was measured by HM-60V (Toa Denpa). The precision and accuracy were both 0.1.

Isotopic analysis- Organic and Inorganic stable carbon isotope ratio (Particulate Organic Carbon and Particulate Inorganic Carbon) were measured. Data were calculated according to $\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{ref} - 1] \times 1000$ with respect to PDB standard. Sample water (300 m*l* ~ 1000 m*l*) was filtered through precombusted (450°C for 4 h) Whatman GF/F glass-fiber filters. The filters were rinsed with distilled water to remove dissolved inorganic carbon and then stored at -20°C until analysis. Samples for measurement of $\delta^{13}C$ (POC) were acidified by 1 N HCl in order to remove PIC on filter. Conversion of the remaining organic carbon into CO_2 and subsequent extraction of evolved CO_2 were done by the double tube combusion method and the cryogenic purification method, respectively (Minagawa et al. 1984). Samples for the measurements of $\delta^{13}C$ (PIC) were reacted by 100 % phosphoric acid at 60°C in a vacuum system without removing POC on the filter, and evolved CO_2 was extracted by the method described above. The purified CO_2 gas was analyzed with a Finnigan Mat 252/B isotope ration mass spectrometer. Based on the analysis of standards (pure antipyrine and $CaCO_3$), the method is accurate and precise to better than ±0.03 % . We estimated the daily calcium carbonate and photosynthetic organic production rates on the basis of changes in the measured pH and C_T values (Smith 1973; Suzuki et al. 1995). (Fig. 2a). CO_2 was calculated by C_T and pH.

3.Results

Whole Experiments- The results obtained were repetitive. Biological and chemical conditions are explained below.

Nutrients and cell growth- Initial nutrient concentrations were 35.6 μM (NO_3-N) and 1.75 μM (PO_4-P).The growth rate was $\mu = 0.87\ d^{-1}$ during the exponential growth phase between day 1 and day 8, and on day 10 the cells reached the stationary phase with a maximum cell concentration of 9.6×10^5 cells·m*l* $^{-1}$ (Fig. 2a). After addition of

nutrient on day 17, the cells showed growth again ($\mu = 0.18\ d^{-1}$) with a maximum cell concentarion of 7.6×10^5 cells·ml^{-1} on day 23. The cell concentrations then gradually decreased and reached 4.4×10^5 cells·ml^{-1} on day 30.

pH and Ca - Initial values of C_T and pH were 1908 μM and 8.12, respectively (Fig. 2c). During the exponential and stationary growth phase, C_T continued to decrease until day 25 (C_T was 981.9 μM and pH was 9.086). Only after day 25 did the cells stop growth (Fig. 2a) and an increase in C_T and decrease in pH were observed (Fig. 2c) (C_T was 1001.1 μM and pH was 8.99 on day 30), which indicates the release of CO_2 to the seawater after day 25. We estimated the daily calcium carbonate and photosynthetic organic production rates on the basis of changes in the measured pH and C_T values (Smith 1973; Suzuki et al. 1995). (Fig. 2a). CO_2 was calculated by C_T and pH. During the exponential growth phase the organic production rate showed a peak value of 123 μM-C·day^{-1} on day 7 and calcium carbonate production rate showed a peak value of 40 μM-C·day^{-1} on day 9. During stationary phase organic and calcium carbonate production rates were almost kept constant. After nutrient enrichment, organic production increased again, but after day 26 organic production became negative. Calcium carbonate production rate was 3 ~ 4 μM-C·day^{-1} after nutrient enrichment.

Carbonate- The ratio of calcium carbonate production rate to organic carbon production rate required to maintain no change of pCO_2 in seawater has been calculated to be 1.66 (Ware et al. 1992). When the ratio is smaller than this, *E. huxleyi* serves as a sink of CO_2 (Suzuki et al. 1995). The ratio shown in Fig. 3b was smaller than this threshold value during exponential and stationary growth phases, except for the late stage of stationary growth phase (day 15 and 16) and after day 25. It is clearly shown in Fig. 3b that *E. huxleyi* serves as a sink of CO_2 during the exponential growth phase and most of the stationary growth phase in which the ratio is smaller than the threshold value of 1.66 and carbon dioxide liberated during the calcification was used for photosynthetic organic production. pCO_2 did not in fact increase (Fig. 2d). Changes of pCO_2 in seawater were determined mainly by the balance of photosynthesis and calcification.

Isotopes- Ratios of stable carbon isotopes for particulate organic carbon (δ^{13}C(POC)) and particulate inorganic carbon (δ^{13}C(PIC)) on day 4 were -23.8 ‰ and 0.1 ‰, respectively (Fig.4a, 4b). δ^{13}C(POC) was in the range reported for C_3 photosynthesis, in which CO_2 was the substrate required (Berry 1989). δ^{13}C(PIC) depends on the isotopic composition of the inorganic carbon dissolved in seawater and δ^{13}C(PIC) is almost same as the value of δ^{13}C for dissolved inorganic carbon in seawater (~-0.08 ‰ on day 4).

4.Discussion

Activity of E. huxleyi at the end of the experiment - After day 25 organic carbon production was negative (Fig. 3a) and uptake of nitrogen stopped (Fig. 2b), which indicates the cessation of photosynthetic carbon production. Phosphate concentration in the medium had been zero after day 18 and *E. huxleyi* cells had been under extreme P-starvation for more than 12 days. However, cell concentrations showed only a relatively gradual decrease and cellular contents of organic carbon and inorganic carbon were still increasing after day 25 (Fig. 2a). An increase of C_T and a decrease of pH were observed, but decomposition of organic carbon by bacteria was not possible, since this culture was axenic. Therefore CO_2 was released from calcification and respiration, and *E. huxleyi* cells were still alive. Where did energy come from? It has been observed that *Chrysochromulina hirta* Manton (Prymnesiophyceae) was able to take up aggregated food particles using a haptonema, which is a thread-like organelle characteristically present in members of the class Prymnesiophycea (Kawachi et al. 1991). The capture of food and its transport by the

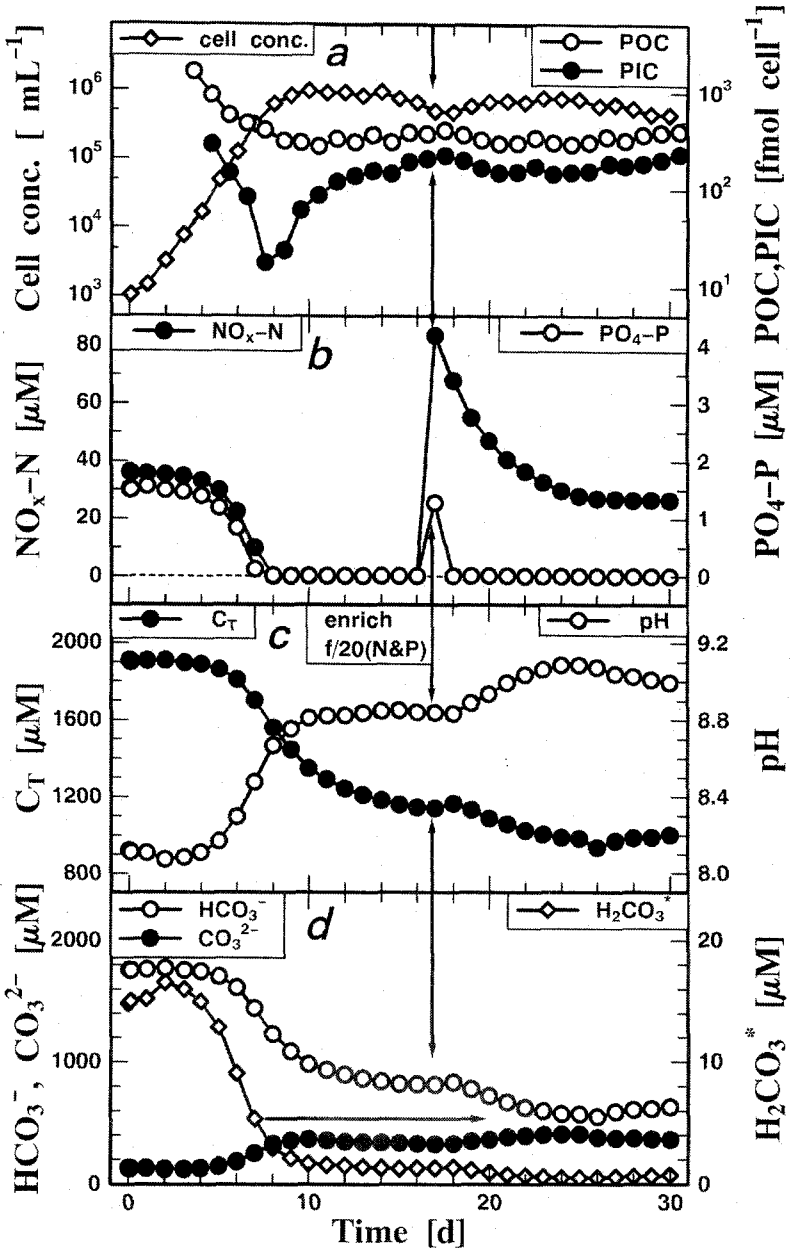

Fig. 2 (***a***.) Growth of *E. huxleyi* (***b***.) Dissolved NO_3-N and PO_4-P concentrations. (***c***.) total dissolved inorganic carbon (C_T) and pH. (***d***.) Concentrations of carbonate species based on equilibrium. Arrow shows the timing of nutrient enrichment.

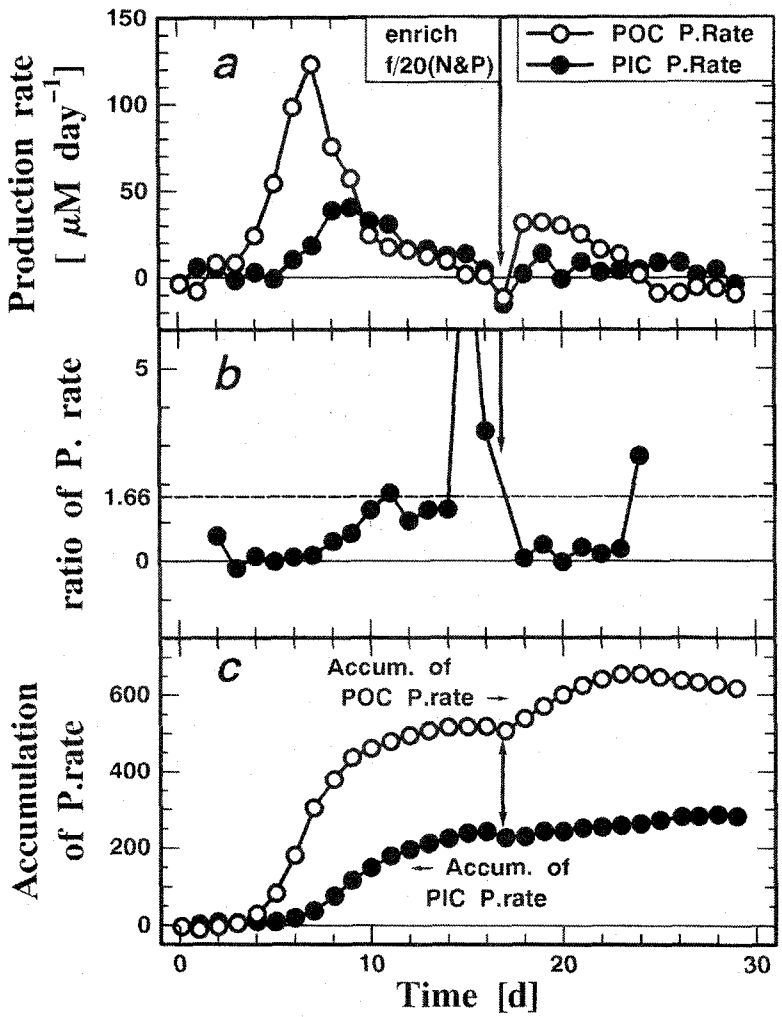

Fig. 3 (*a.*) Photosynthetic organic production rate and calcium carbonate production rate estimated on basis of changes in the measured values of C_T and pH. (*b.*) Ratio of calcium carbonate production rate and organic production rate. (*c.*) Cumulative values of calcium carbonate production and photosynthetic organic production. Arrow shows the timing of nutrient enrichment.

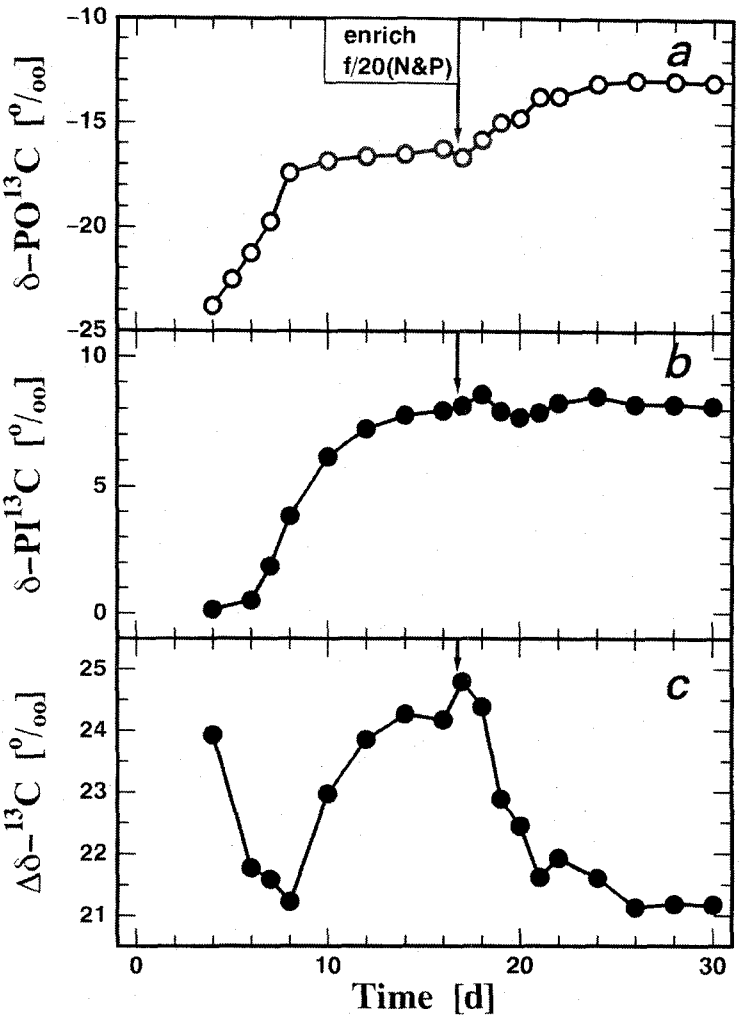

Fig. 4 (*a.*) δ^{13}C of particulate organic carbon (δ^{13}CPOC)) and particulate inorganic carbon (δ^{13}C(PIC)). (*b.*) $\Delta\delta^{13}$C = δ^{13}C(PIC) – δ^{13}C(POC). Arrow shows the timing of nutrient enrichment.

haptonema was observed only when the cells were swimming with the haptonema fully extended forward and the two flagella beating backwards alongside the body of the cell. Little is known about the life history stage of *E. huxleyi*. It has a non-motile, plated phase ("C" cells), which reproduces vegetatively via simple binary fission (Balch et al. 1993). Scaled or "S" cells are motile with a pair of flagella and appear to be formed when "C" cells become senescent. After day 25 these "S" cells might be formed when "C" cells become senescent and could have been adapted more to heterotrophic activities. It was reported that *E. huxleyi* excreted much of the organic substrate and was also able to utilize dissolved organic substrate for growth (Flynn 1990; Van Bleijswijk et al. 1994). Phosphate-stressed cells might have utilized organic substrates available in the medium.

Implications from the isotopic analysis-The carbon isotope ratio of phytoplankton could be explained on the basis of environmental differences in temperature (Falkowski 1991; Hinga et al. 1994), light intensity (Thompson and Calvert 1995), pH (Hinga et al. 1994) and carbon availability (Hinga et al. 1994). Since temperature and light intensity were kept constant in this experiment, carbon availability was the major factor to determine the carbon isotope ratio in this experiment.

δ^{13}C(POC) increased linearly from -23.8 ‰ to -17.4 ‰ and the concentration of H_2CO_3 decreased from 15.0 μM to 2.9 μM in exponential growth phase (Fig. 4a). δ^{13}C(POC) showed small increase from -17.4 ‰ to -16.2 ‰ in stationary growth phase (between day 8 and day 16), when the concentration of H_2CO_3 was less than 1 μM (Fig. 1d). δ^{13}C(PIC) started to increase (from 0.1 ‰ to 1.9 ‰) on day 7 when dissolved phosphate depleted completely in the medium (Fig. 2b) and enhancement of calcification due to phosphate depletion was observed (Paasche and Brubak 1994). During the period between day 7 and day 16 δ^{13}C (PIC) continued to increase from 1.9 ‰ to 8.0 ‰. After nutrient enrichment on day 17, δ^{13}C(POC) immediately showed an increase (Fig. 4a), but after 3 days lag (day 20) δ^{13}C(PIC) showed an increase when phosphate was completely depleted while nitrogen was available (Fig. 2b). Here it was clearly shown that production of calcium carbonate was enhanced under phosphorus limitation.

The delta del, $\Delta\delta^{13}C = \delta^{13}C(PIC) - \delta^{13}C(POC)$, expresses the overall fractionation factor and is numerically similar with epsilon, which considers the isotopic discrimination between CO_2 aq and the organic carbon (Fig. 4c). $\Delta\delta^{13}$C increased between day 8 and day 17, and the concentration of H_2CO_3 ($H_2O + CO_2$) continued to decrease. From these observations it was concluded that CO_2 was the carbon source utilized during this period and CO_2 liberated during the calcification was used for photosynthetic organic production and δ^{13}C(PIC) and δ^{13}C(POC) were interconnected with each other. On the other hand, when the POC production rate exceeded that of PIC, *E. huxleyi* needed an other carbon source than liberated CO_2. Especially after day 17 (nutrient enrichment) organic production increased (Fig. 3a), while the calcification was negligibly small due to phosphate enrichment. During this period the concentration of HCO_3^- decreased from 835.0 μM (day 18) to 554.7 μM (day 26); however the availability of CO_2 was very low due to increase of pH from 8.833 (day 18) to 9.072 (day 26) (the concentration of H_2CO_3 was less than 1 μM). From these observations it was concluded that HCO^- as well as CO_2 were acceptable carbon sources for organic growth when CO_2 availability was limited. This conclusion was supported by the decreases in $\Delta\delta^{13}$C during the period of high POC production (day 4 to day 8 and day 17 to day 26), because a smaller carbon isotope discrimination has been reported when HCO_3^- was used as carbon source (Falkowski 1991)..

Cumulative values (Fig. 3c) of calcium carbonate production and photosynthetic carbon production (in which production rate were integrated over one day in Fig. 3a) became almost identical with δ^{13}C(PIC) and δ^{13}C(POC) in Fig. 4a and 4b, suggesting that the isotope ratio of particulate carbon was reflecting a decrease in C_T (Thompson and Calvert). This suggests that values of δ^{13}C(PIC) and δ^{13}C(POC) express the total production of calcium carbonate

production and photosynthetic carbon production, and therefore derivatives of $\delta^{13}C$(PIC) and $\delta^{13}C$(POC) changes express the production rates of calcium carbonate and photosynthetic carbon, respectively. Thus, true production rates of organic and inorganic carbon could be obtained from $\delta^{13}C$(POC) and $\delta^{13}C$(PIC), even when sedimentation of cells and detachment of coccoliths occur. These analyses are important when the results would be applied into field observations. The possibility of accurate estimation of these production rates is particularly important in the field, where the estimation of these production rates was extremely difficult due to the effects of sedimentation and detachment of coccoliths.

Sink or Source- Most of the experimental period *E. huxleyi* acted as a sink of CO_2. These laboratory results can explain why the release of CO_2 to the atmosphere was observed in the field survey of *E. huxleyi* bloom in the North Atlantic Ocean. The field survey of the bloom was possible only in a late stage of development, when the color of the ocean turned milky white due to coccoliths and satellite images could recognize distinct patches of *E. huxleyi* bloom. Typically the *E. huxleyi* bloom lasted approximately 3 weeks (Holliban et al. 1993), and therefore the laboratory experiment covered more than the entire bloom period. The integrated net carbon budget over the experimental period (Fig. 2C, 3C) indicated that the bloom of *E. huxleyi* acted as a sink of CO_2, even if the release of CO_2 was observed at the late stage of stationary phase. The experiment used f/50 medium and initial nutrients concentrations were 35.6 μM (NO3-N) and 1.75 μM (PO4-P) which were almost 10 times higher than nutrients concentrations observed in the surface layer of the ocean. However, along the US coast of the North Atlantic Sea E.huxleyi blooms have been observed in the area of Gyre where high nutrients were upwelling from the bottom. In this experiment such condition with high nutrients concentrations in the surface layer were simulated in a large axenic tank. In a Gyre water mass is isolated from ambient sea water with limited dispersion and the condition similar as a batch culture could be created. Therefore it is reasonable to conclude that the experimental results obtained in this analysis simulate reasonably the nutrient condition occuring in the area of Gyre, even if f/50 medium was used.

Future work-Our system is axenic, thus, we could not know the effect of bacteria on the carbon cycle. This should be analyzed furthermore.

5.Conclusion

E. huxleyi act as sink of CO_2 during the most of the life cycle. Isotopic analyses implied the ability to use HCO_3^- when CO_2 is depleted.

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