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Impacts of elevated CO₂ on phytoplankton community composition and organic carbon dynamics in nutrient-depleted Okhotsk Sea surface waters

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Abstract

Impacts of the increasing CO₂ in seawater (i.e. ocean acidification) on phytoplankton physiology may have various and potentially adverse effects on phytoplankton dynamics and the carbon cycle. We conducted a CO₂ manipulation experiment in the Sea of Okhotsk in summer 2006 to investigate the response of the phytoplankton assemblage and dynamics of organic carbon. During the 14-day incubation of nutrient-depleted surface water with a natural phytoplankton assemblage under 150, 280, 480, and 590 μatm pCO₂, the relative abundance of fucoxanthin-containing phytoplankton such as diatoms and prymnesiophytes decreased with increasing pCO₂. The amount of DOC accumulation also decreased with increasing pCO₂, while differences in POC accumulation between the treatments were small and did not show a clear trend with the pCO₂. Change in the phytoplankton community composition under different pCO₂ conditions will alter the organic carbon dynamics as found in the present experiment. Compared to results in the literature from nutrient-replete conditions indicating a potential enhancement of phytoplankton production with elevated pCO₂, the present results indicated a different physiological response of phytoplankton under nutrient-depleted conditions. These results indicate that the continuing increase in atmospheric CO₂ can significantly affect the structure of marine ecosystems and carbon cycle in nutrient-depleted subpolar surface waters.

1 Introduction

Increasing anthropogenic CO₂ in the atmosphere through mainly the burning of fossil fuels is penetrating rapidly into the surface ocean (Sabine et al., 2004), and lowering the pH in surface seawater as a result of the acidifying effects of CO₂ in water (Caldeira and Wickett, 2003). Surface ocean pH has already decreased 0.1 units since the industrial revolution, and has been projected to decrease an additional 0.1–0.35 units within this century (IPCC, 2007). Although ocean acidification will potentially have

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significant direct impacts on a wide range of marine organisms (Raven et al., 2005; Kleypas et al., 2006), the responses of individual organisms, populations, communities, and ecosystems are largely unknown (Vézina and Hoegh-Guldberg, 2008; Doney et al., 2009). Considerable research efforts have mainly focused on predicting the impact of ocean acidification on calcifying marine organisms (Shirayama and Thornton, 2005; Gazeau et al., 2007; Hoegh-Guldberg et al., 2007). However, studies on planktonic primary producers in relation to ocean acidification are still limited despite the established importance of these organisms in marine ecosystems and biogeochemical cycles (Riebesell, 2004; Rost et al., 2008). Therefore, manipulative experiments are required for assessing the impacts of acidification on phytoplankton, and for integrating the impacts into biogeochemical modeling approach (Orr et al., 2009).

Several past studies using natural phytoplankton assemblages have predicted that change in CO₂ concentration could alter the phytoplankton species composition. For example, Tortell et al. (2002) showed that an increment of CO₂ changed the taxonomic composition of the phytoplankton assemblage in the Equatorial Pacific, in which 150 ppm favor the prymnesiophyte *Phaeocystis* sp. and 750 ppm favor the diatoms. Kim et al. (2006) showed that increasing CO₂ stimulated the growth of the diatom *Skeletonema costatum* in coastal waters of southern Korea. Tortell et al. (2008) also reported that elevated CO₂ enhanced primary production and relative abundance of chain-forming *Chaetoceros* in the Southern Ocean. On the other hand, in the Bering Sea, diatoms were replaced by autotrophic nanoflagellates under a high CO₂ condition (Hare et al., 2007). Recently, Riebesell et al. (2007) demonstrated that increased CO₂ concentration enhanced the efficiency of the biological carbon pump during a diatom bloom in a coastal mesocosm experiment. These studies indicate that CO₂ increase can alter not only the phytoplankton species composition but also the organic carbon dynamics in the sea. However, it should be noted that all these results were obtained from artificial algal blooms induced under macro- and/or micro-nutrient replete conditions. At present, macro- and/or micronutrients are depleted in most surface areas of the oceans. Physiological responses of phytoplankton can be expected to differ

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between under nutrient-depleted and replete conditions. Additionally, in such nutrient-depleted waters, regenerated production via the microbial loop may become dominant (Azam et al., 1983). Further studies are clearly essential to address the effects of CO₂ on phytoplankton community composition and carbon dynamics in nutrient-depleted waters.

We thus conducted a CO₂ manipulated bottle incubation experiment using the natural plankton community collected from nutrient-depleted surface water in the Sea of Okhotsk in summer 2006, as a part of the framework of the Plankton Ecosystem Response to CO₂ Manipulation Study (PERCOM). Care was taken to ensure that no nutrient enrichment of the incubation bottles occurred and we measured changes over time in phytoplankton community structure and organic carbon accumulation, and discuss the effects of CO₂ on these parameters.

2 Materials and methods

2.1 Sampling and experimental setup

The study was carried out aboard the R/V *Professor Khromov* in summer of 2006. Surface seawater sampling for a CO₂ manipulation experiment was conducted at a station (49.5° N, 148.25° E; 1050 m depth) in the Sea of Okhotsk on 25 August 2006 (Fig. 1). The water was collected from 10 m depth with acid-cleaned Niskin-X bottles on a Kevlar wire. A total of 160 L of seawater was poured into four 50 L polypropylene carboys (40 L seawater for each of the given CO₂ concentrations) through silicon tubing with a 243 μm mesh Teflon net to remove any large plankton. Subsamples were taken from the each carboy and poured into three acid-cleaned 9 L polycarbonate bottles (12 bottles in total) for incubation. Similarly, initial samples also were collected from the each carboy. All of the sampling was carried out using trace metal clean techniques to avoid any effects of trace elements other than CO₂ on phytoplankton growth.

Incubation was conducted on deck in a temperature-controlled water-circulating tank

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for 14 days under in situ temperature of 13.5°C and 50% surface irradiance. To control CO₂ concentrations in the incubation bottles, air mixtures containing 180, 380, 750, and 1000 ppm CO₂, purchased from a commercial gas supply company (Nissan-Tanaka Co., Japan), were bubbled into triplicate incubation bottles. The flow rate of the gas was set at 100 mL min⁻¹ for the first 24 h, and thereafter maintained at 50 mL min⁻¹. Subsamples were collected from each incubation bottle on days 1, 3, 5, 9, and 14 at 10:00 a.m. during the course of the experiment.

2.2 Sample treatments and analyses

Subsamples for total dissolved inorganic carbon (DIC) and total alkalinity (TA) were collected in 120 mL glass vials, poisoned with HgCl₂. Concentrations in DIC and TA were measured by coulometric titration (Johnson et al., 1985) and single point titration (Culberson et al., 1970), respectively. CO₂ and pH were calculated by using a set of CO₂ constants as “K1, K2 from Mehrbach et al., (1973) and refitted by Dickson and Millero (1987)”, and on pH scale as “total hydrogen scale” under in situ temperature of 13.5°C and salinity of 32.52. In addition, certified reference material distributed by A. G. Dickson (Scripps Institution of Oceanography) was used.

Subsamples for nutrients were collected in 10 mL plastic tubes and stored at -20°C until analysis on land. Nitrate plus nitrite (NO₃+NO₂), phosphate (PO₄), and silicate (SiO₂) concentrations were determined with a segmented continuous flow analyzer (QuAAtro, Bran+Luebbe). Ammonium (NH₄) was analyzed fluorometrically (Holmes et al., 1999).

Size-fractionated chlorophyll-*a* (chl-*a*) samples were collected sequentially on 10 μm pore size 47 mm polycarbonate filter without vacuum and then 25 mm GF/F filter under gentle vacuum (<0.01 MPa), and the filters extracted immediately with *N,N*-dimethylformamide (DMF) at -20°C in the dark over 24 h (Suzuki and Ishimaru, 1990). Chlorophyll-*a* concentrations were measured onboard with a fluorometer (Model 10-AU, Turner Designs) by the non-acidification method of Welschmeyer (1994). For phytoplankton pigment analysis, subsamples from each of the triplicate incubation bot-

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5 tles were pooled together and filtered onto GF/F filters, which were stored at -85°C until analysis on land. The pigments extracted in DMF were measured with a high-performance liquid chromatography (CLASS-VP system, Shimadzu) according to the method detailed in Suzuki et al. (2005).

10 Particulate organic carbon (POC) were collected on pre-combusted (450°C , 4 h) 25 mm GF/F filter under a gentle vacuum at $<0.01\text{ MPa}$ and the filter samples were stored at -20°C until analysis on land. Before POC analysis, the filter samples were freeze-dried, and then exposed to HCl fumes overnight in a desiccator to remove inorganic carbon from the samples. Carbon contents on the filter were measured with a CN elemental analyzer (NA-1500, Fisons). Subsamples for dissolved organic carbon (DOC) were filtered through pre-combusted (450°C , 4 h) 47 mm GF/F filters and collected in precombusted (550°C for 4 h) glass ampoules, which were stored at -20°C until analysis on land. Dissolved organic carbon was measured by a high-temperature combustion method (TOC-V, Shimadzu). We measured at least one consensus reference material for DOC (CRM; #05-04), distributed by D. A. Hansell's laboratory (University of Miami), with the samples during every analysis run. Our measurements of $44.5 \pm 0.7\ \mu\text{mol DOC L}^{-1}$ (mean \pm SD, $n=9$) for the CRMs were within the consensus values of $44\text{--}45\ \mu\text{mol L}^{-1}$.

20 For enumeration of ultraphytoplankton and heterotrophic bacteria, subsamples were preserved with paraformaldehyde (PFA; 0.2% in final concentration) and frozen at -85°C until analysis on land. The samples were analyzed with a flow cytometry (XL ADC system, Beckman Coulter) outlined in Suzuki et al. (2005). Microscopic examination was conducted on formaldehyde- (1% in final concentration) and glutaraldehyde-preserved (1% in final concentration) samples. Cells of coccolithophores were examined on PFA-preserved (0.4% in final concentration) samples using a scanning electron microscope (SEM; JMS-840A, JEOL) according to the protocol detailed in Hattori et al. (2004).

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3 Results and discussion

We could create significant gradients in $p\text{CO}_2$ and pH among the four treatments by bubbling the four different air- CO_2 mixtures into the incubation seawater medium (Fig. 2). Mean seawater $p\text{CO}_2$ and pH in 180, 380, 750, and 1000 ppm CO_2 treatments during the experiment were 145–148, 275–276, 479–489, and 583–597 μatm and 8.40, 8.18, 7.97, and 7.89, respectively. The CO_2 systems were manipulated significantly by comparison with the initial value of $p\text{CO}_2$ of 195–200 μatm and pH of 8.30. The seawater $p\text{CO}_2$ did not fully equilibrate with the bubbling air CO_2 concentrations. This may be attributable to the insufficient flow rates of the air bubbling. Our results clearly shows that direct measurements of at least two parameters in seawater CO_2 system are necessary to confirm whether the CO_2 condition in the experimental system equilibrates with the bubbling gas. Hereafter we will refer 150, 280, 480, and 590 μatm for the 180, 380, 750, and 1000 ppm CO_2 treatments, respectively.

Macronutrients were depleted within the surface mixed layer and the phytoplankton biomass in terms of chl-*a* concentration was low (Table 1). Nitrogenous nutrients were completely depleted, and PO_4 and SiO_2 concentrations were 0.25 and 1.06 $\mu\text{mol L}^{-1}$, respectively, before incubation. Initial chl-*a* biomass was comprised of 0.30 $\mu\text{g L}^{-1}$ of small-sized (<10 μm) algal cells and 0.01 $\mu\text{g L}^{-1}$ of large-sized (>10 μm) ones. Phytoplankton cells larger than 5 μm in size were rarely observed using light microscopy. Diatoms were scarcely observed in that size range. Some dinoflagellates such as, *Prorocentrum*, *Gonyaulax*, and *Ceratium* were identified, but their abundances were low. The ultraplankton community (ca. <5 μm in size), revealed by flow cytometry, was comprised of *Synechococcus*, eukaryotic ultraphytoplankton, and heterotrophic bacteria. Initial abundances of the cells were 9.0×10^3 , 1.8×10^4 , and 3.8×10^5 cells mL^{-1} , respectively.

During the 14-day incubation experiment, chl-*a* concentrations fluctuated between 0.1 and 0.4 $\mu\text{g L}^{-1}$, but the variation pattern was similar in all treatments (Fig. 3). Chlorophyll-*a* was mainly derived from small-sized cells throughout the experiment.

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Liu et al. (2009) reported that the growth rate of the in situ phytoplankton assemblage at the site where the present study was carried out was estimated to be 0.38 d^{-1} , representing the mean doubling time of the cells was approximately 2 days. If we set the endpoint as the end of the experiment (day 14), net chl-*a* production ranged from -0.04 to -0.16 between the treatments (Fig. 4a). No significant difference in the net chl-*a* production was observed between the treatments. Change in the concentrations of NH_4 , NO_3+NO_2 , and PO_4 were small (Table 1). Silicate was consumed to 53% of the initial concentration; the consumption in $150 \mu\text{atm}$ was significantly larger than other treatments (Fig. 4b; Tukey's test, $p < 0.05$).

Although apparent changes in chl-*a* biomass were small under the nutrient depleted conditions, fucoxanthin/chl-*a* ratios showed variations between the treatments (Fig. 4c). At the end of the experiment, a decreasing trend was observed in the fucoxanthin/chl-*a* ratio (g g^{-1}) from 0.30 in $150 \mu\text{atm}$ to 0.16 in $590 \mu\text{atm}$. These fucoxanthin/chl-*a* ratios were higher than the initial value of 0.12. Since the pigment samples were pooled from the triplicate bottles in each treatment, the statistical significance for these pigments values could not be evaluated. It is known that fucoxanthin is contained in diatoms, prymnesiophytes, chrysophytes, and raphidophytes (Jeffrey et al., 1997). In this study, 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin, which are chrysophyte and cryptophyte markers, respectively (Jeffrey et al., 1997), were very low throughout the experiment. In addition, raphidophytes were not detected using light microscopy. These results indicate that diatoms might sensitively respond to changes in CO_2 concentration and under high CO_2 environmental conditions their growth relative to other taxonomic groups in the assemblage will be reduced. They are supported by the higher efficiency of carbon fixation by ribulose-1,5-bisphosphate carboxylase-oxygenase (Ru-bisco) in diatoms than other algal groups (Tortell, 2000). The results of higher diatom contribution in the assemblage in the lowest $p\text{CO}_2$ were also consistent with SiO_2 consumption in each treatment (Fig. 4b). A similar result could not be obtained from our microscopic examination because of the lack of significant data on diatoms identified in the $>5 \mu\text{m}$ fraction. Alternatively, variation of cellular fucoxanthin content in diatoms

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may explain the difference in fucoxanthin/chl-*a* ratios between the treatments. Increasing fucoxanthin/chl-*a* ratios were reported with increasing nitrogen limitation (Hou et al., 2007). Light intensity also influences the cellular fucoxanthin content but does not alter the fucoxanthin/chl-*a* ratio (Goericke and Welshmeyer, 1992). Since nutrient and light conditions were assumed to be equal between the treatments, the change in cellular fucoxanthin content is unlikely to be the cause of the difference in fucoxanthin/chl-*a* ratios at the end of the experiment.

For the effect of CO₂ increase on coccolithophores, no coccolithophores were detected either at the initial or at the end of the experiment with SEM. Cell abundance of heterotrophic bacteria was stable at around 4×10^5 cells mL⁻¹ throughout the incubation period, and no significant difference in the net change in bacterial abundance was observed between the treatments (Fig. 4d). *Synechococcus* and autotrophic ultra-eukaryotes showed decreasing trends from 9×10^3 to around 2×10^3 cells mL⁻¹ and from 1.8×10^4 to $0.4\text{--}1.0 \times 10^4$ cells mL⁻¹, respectively, during the experiment. No significant differences in the net change in the abundances of *Synechococcus* (Fig. 4e) and ultra-eukaryotes (Fig. 4f) were also observed between the treatments.

Effects of $p\text{CO}_2$ change on carbon dynamics appeared more in DOC than in POC. Net accumulation of POC ranged from $1.2 \pm 1.4 \mu\text{mol L}^{-1}$ in 280 μatm to $-1.7 \pm 0.5 \mu\text{mol L}^{-1}$ in 590 μatm (Fig. 4g). Net POC accumulation in 280 μatm was significantly higher than that in 480 μatm and 590 μatm (Tukey's test, $p < 0.05$). On the other hand, net DOC accumulations decreased from $4.9 \pm 2.8 \mu\text{mol L}^{-1}$ to $0.49 \pm 0.50 \mu\text{mol L}^{-1}$ with increasing $p\text{CO}_2$ (Fig. 4h). Net DOC accumulation in 150 μatm was significantly higher than that in 480 μatm and 590 μatm (Tukey's test, $p < 0.05$). Difference in net DOC accumulation of $4.4 \mu\text{mol L}^{-1}$ between 150 μatm and 590 μatm was larger than that of POC of $2.9 \mu\text{mol L}^{-1}$ between 280 μatm and 590 μatm . The clear trend of decreasing net DOC accumulation with increasing $p\text{CO}_2$ can be explained by changes in production and/or decomposition processes. If bacterial DOC decomposition was not changed between the treatments, DOC production was elevated in the 150 μatm condition. If DOC production did not change between the treat-

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ments, DOC decomposition may have been enhanced in the 480 μatm and 590 μatm condition. In the present experiment, temporally stable and indistinguishable dynamics in cell abundance of heterotrophic bacteria between the treatments (Fig. 4d) partly support the former case, although we have no data on rate processes such as bacterial production. Furthermore, DOC production can change between the treatments, because the community structure may have changed between the treatments. Similar decreasing trend of fucoxanthin/chl-*a* ratio and ΔDOC with increasing $p\text{CO}_2$ (Fig. 4c and 4h) support this. Pigments analysis suggested that relative abundance of diatoms were enhanced in the 150 μatm condition (Fig. 4c). The amount of exudation of photosynthetic products as DOC is highly species-specific (Nagata, 2000). Thus net DOC production can vary under different phytoplankton species compositions. This is likely to be the most reasonable explanation for our results. On the other hand, bacterial DOC decomposition is an enzymatically-driven reaction, so the process would be sensitive to the change in pH. Bacterial cell-associated and free dissolved enzymes to hydrolyze DOC (e.g. α - and β -glucosidase) play a major role in DOC decomposition (Arnosti, 2002). However, there is no studies on pH dependency of the degradative reactions with these enzymes. We need to further clarify the cause of the differences in DOC dynamics under different $p\text{CO}_2$ conditions.

The present results suggest that the future direction of nutrient depleted surface ocean biogeochemistry in the subpolar region is less diatom dominance and less DOC production in the high CO_2 world. Diatoms are responsible for the biological carbon pump even in the nutrient depleted low productive season in the subarctic regions (Honda et al., 1997, 2002; Takahashi et al., 2000). Therefore decrease in diatoms production would have negative impact on the marine carbon uptake and thus play a positive feedback on global warming and ocean acidification. The trends observed in the present study lead to the opposite conclusion to various recent studies carried out under nutrient-replete conditions (Riebesell et al., 2007; Tortell et al., 2008). In these previous studies, the growth of diatoms and organic carbon production accelerated under high CO_2 conditions, and it was concluded that ocean acidification may

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act as a negative feedback on global environmental issues associated with increasing atmospheric CO₂. Our results indicate that the response of phytoplankton to pCO₂ increase may be altered by their physiological status. Under nutrient limited conditions, reaction of a given species to the pCO₂ increase may reflect the intracellular biochemical reactions directly (e.g. characteristic of Rubisco as shown in Tortell, 2000). Carbon concentrating mechanisms, including the role of intra- and extracellular carbonic anhydrases, also are key processes for regulating a given species' reaction to pCO₂ increase.

For DOC dynamics, on the other hand, previous studies demonstrated that DOC production may be enhanced under high CO₂. Engel et al. (2004) and Riebesell et al. (2007) revealed that the formation of transparent exopolymer particles (TEP), which are made of the aggregation of dissolved carbohydrates, was accelerated under high CO₂ condition. High CO₂ potentially enhances photosynthetic carbon assimilation through lowering energetic costs associated with carbon acquisition, as found for natural assemblages (Hein and Sand-Jensen, 1997; Tortell et al., 2008) and isolated single strains (Riebesell et al., 1993; Rost et al., 2003; Hutchins et al., 2007). Some parts of the synthesized organic carbon fail to be incorporated into proteins and lipids, and are exuded to outside of the cell as DOC. When the community structure does not change or single isolated species are used in a experiment, DOC production may increase with increasing pCO₂. However, in the case that the phytoplankton species composition changed in the experimental system, intraspecies difference in DOC production under different pCO₂ conditions may be masked by the interspecies difference in DOC production as concluded for the present experiment. Decrease in DOC production can have significant impacts on biogeochemical cycles of carbon and nutrients through the change in bacterial activity (Thingstad et al., 2008). Decrease in labile DOC production may lower the competitive advantage of bacteria against phytoplankton for inorganic nitrogen and phosphorus, and thus potentially have a positive effect on phytoplankton nutrient acquisition especially in nutrient-depleted environments. Decrease in semi-labile and refractory DOC production may decrease carbon fixation as

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DOC which is transported into the ocean interior. Given that decrease in dissolved carbohydrate production will lower the ambient TEP concentration, which enhances the particulate aggregation, it may down-regulate the sinking POC flux. On the other hand, direct effect of pH reduction is also reported to reduce the TEP-driven particle aggregation and sedimentation processes (Mari, 2008). Therefore the assessment of the impacts of change in DOC and TEP production is not straightforward, but potentially have a significant impact on marine ecosystem and carbon cycle.

Thus, the phytoplankton community structure and organic carbon dynamics are fully coupled, so we need a better understanding of the impact of ocean acidification on these critical components of marine biogeochemistry. Previous experimental results have recently been incorporated into a simulation model to assess the impacts of ocean acidification on the biological carbon pump (Hofmann and Schellnhuber, 2009). The present study suggests that responses of planktonic primary producer to $p\text{CO}_2$ increase is different between nutrient-replete and deplete condition. Further research is required based on in situ CO_2 manipulation experiments using a nutrient limited (including iron as a micro-nutrient) plankton ecosystem and results incorporated into global models.

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Table 1. Initial and final conditions of nutrients and chlorophyll-*a* (chl-*a*) concentrations (mean ±1 standard deviation) pooled for all treatments.

	NH ₄ (μmol L ⁻¹)	NO ₃ +NO ₂ (μmol L ⁻¹)	PO ₄ (μmol L ⁻¹)	SiO ₂ (μmol L ⁻¹)	Small chl- <i>a</i> (μg L ⁻¹)	Large chl- <i>a</i> (μg L ⁻¹)
Initial	0.09±0.02	0.05±0.02	0.25±0.01	1.06±0.05	0.30±0.01	0.01±0.00
Final	0.10±0.06	0.02±0.01	0.22±0.01	0.68±0.12	0.21±0.07	0.01±0.00

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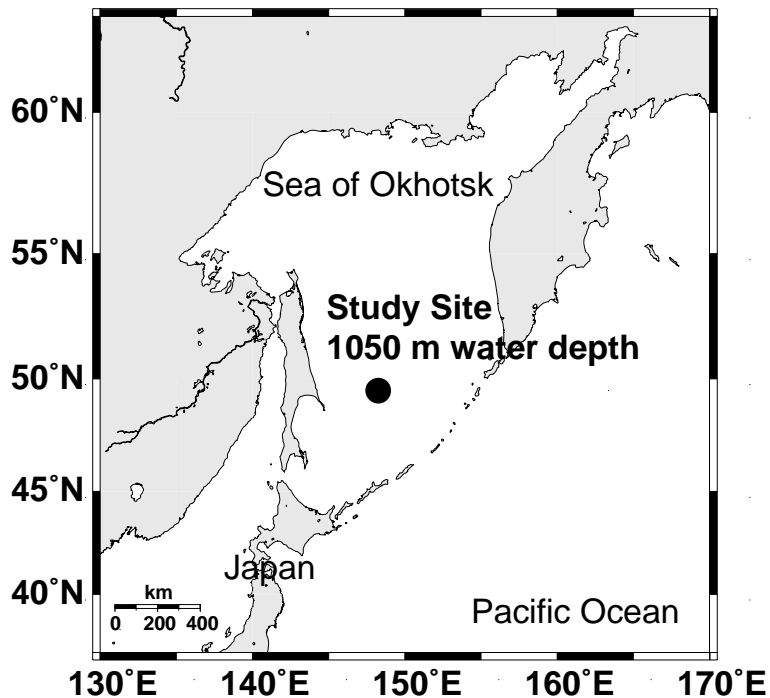


Fig. 1. Location of a sampling station for the CO₂ manipulation experiment in the Sea of Okhotsk.

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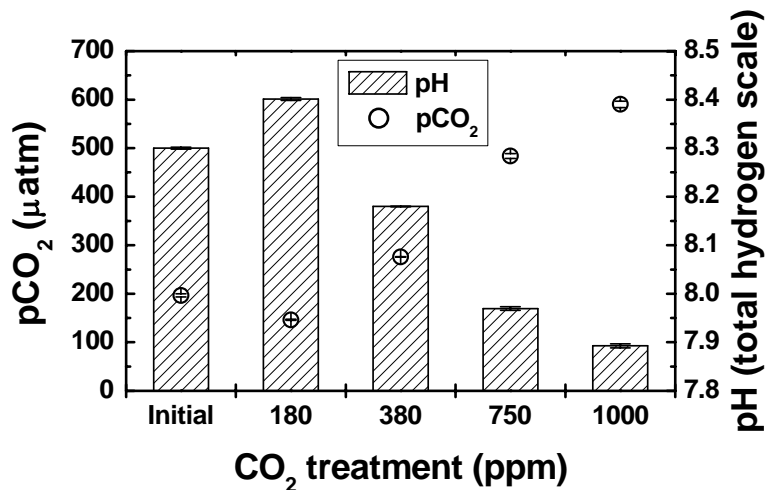


Fig. 2. Seawater pCO₂ and pH (mean with range) at the initial and during the course of the incubation experiment in the four different CO₂ treatments.

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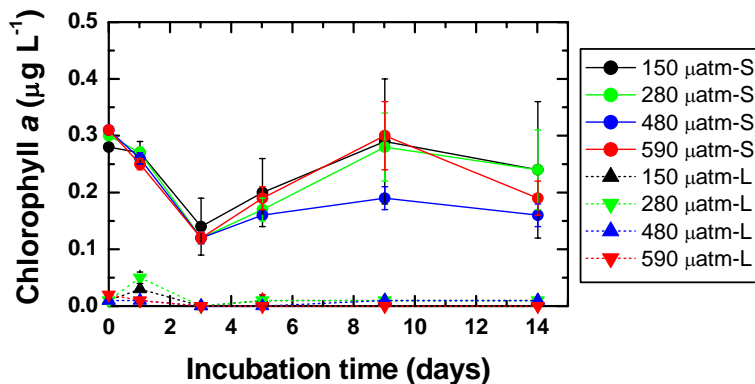


Fig. 3. Time course of chlorophyll-*a* concentrations (mean ±1 standard deviation of triplicate bottles) in small- (S; <10 µm) and large-sized cells (L; >10 µm) for the four treatments during the experiment.

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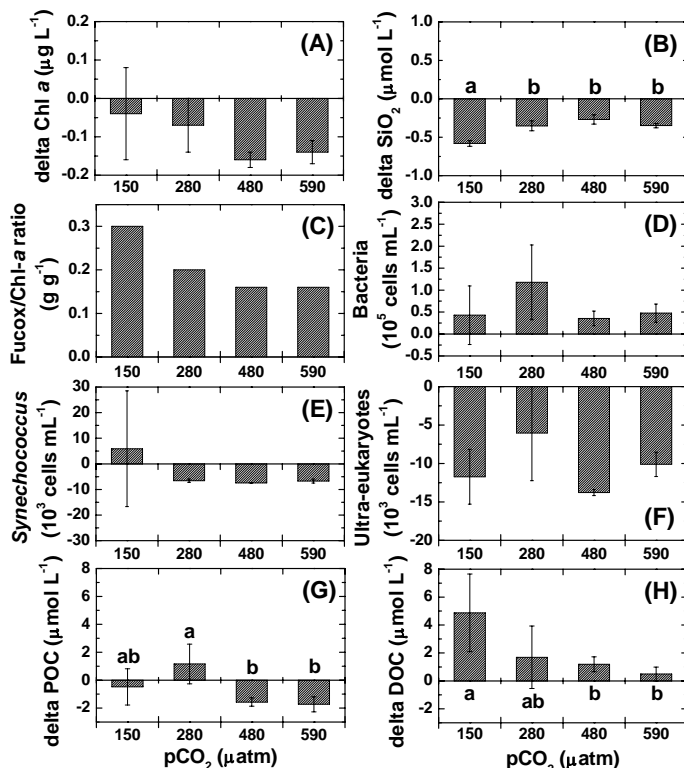


Fig. 4. Differences between the initial and end of the experiment values (delta; the end minus the initial value) in **(A)** chlorophyll-*a* (chl-*a*), **(B)** silicate (SiO₂), **(D)** heterotrophic bacteria, **(E)** *Synechococcus*, **(F)** autotrophic ultra-eukaryotes, **(G)** particulate organic carbon (POC), and **(H)** dissolved organic carbon (DOC), and **(C)** fucoxanthin (Fucox) to chl-*a* ratios at the end of the 14-day incubation experiment. Pooled samples of triplicate bottles were analyzed for Fucox/chl-*a* ratios. Different letters above bars denote significant difference between the treatments (Tukey's test, $p < 0.05$).

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