Response of spring diatoms to CO$_2$ availability in the western North Pacific

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Ocean acidification

Atmospheric CO$_2$ increases

Release of anthropogenic CO$_2$

Increase in air temperature

Global warming

Decrease in seawater pH: “Ocean acidification”

Increase in dissolved CO$_2$ in seawater

Riebesell (2004)
In the western subarctic Pacific

- Highest biological drawdown of partial pressure of CO$_2$ ($p$CO$_2$) in surface waters among the world’s oceans (Takahashi et al., 2002).
- The high $p$CO$_2$ drawdown is attributable to massive spring diatom bloom (Midorikawa et al., 2003; Ayers and Lozier, 2012).

Little is known about response of the spring diatoms to CO$_2$ availability in seawater.
On deck CO₂ bottle incubation experiment

- Sampling date: May 8, 2011
- Site: Stn PH3 (41°N, 144°E)
- Sampling depth: 10 m
- Incubation period: ca. 3 days

Initial conditions at 10 m:
- Temperature: 5.0 °C
- Salinity: 33.1
- Chl a: 0.71 µg L⁻¹
- Nitrate: 14.0 µM
- Phosphate: 0.95 µM
- Silicate: 11.8 µM
- pCO₂: 342.8 µatm

R/V Tansei Maru (JAMSTEC/ Univ. Tokyo)
- Bubbling of CO\(_2\) gases
- Flow rate: 50 mL min\(^{-1}\)
- Temperature: 5 ºC
- PAR: 50% light level

**Incubation conditions**

**Air + CO\(_2\)**
- Air filters
- Bubbling of CO\(_2\) gases
- Flow rate: 50 mL min\(^{-1}\)
- Temperature: 5 ºC
- PAR: 50% light level

**CO\(_2\) levels:**
- 180 µatm CO\(_2\)
- 350 µatm CO\(_2\) (ambient)
- 750 µatm CO\(_2\)
- 1,000 µatm CO\(_2\)

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**Clean Niskin bottle sampling**

Pre-filtration with 197 µm teflon mesh

12 L polycarbonate bottles (4 CO\(_2\) conditions × triplicate = 12 bottles)
Changes over time in chlorophyll (Chl) a concentration determined with high-performance liquid chromatography (HPLC)

The net growth of phytoplankton assemblages was suppressed by an increase in CO₂ level.
Changes in algal community composition

Contributions of each algal taxa to Chl a biomass on **Day 0** as estimated with the program CHEMTAX (Mackey et al., 1996; Latasa, 2007)

![Pie chart showing the contributions of different algal taxa to Chl a biomass on Day 0.](chart)

- **Cryptophytes** (38%)
- **Diatoms** (35%)
- **Haptophytes**
- **Pelagophytes**
- **Chlorophytes**
- **Prasinophytes**

The decreases in Chl a level at higher CO$_2$ levels after incubation were probably due to declines in diatom abundance.

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On **Day 3**

net growth rates of each taxonomic pigment marker

- **Alloxanthin** (d$^{-1}$)
- **Fucoxanthin** (d$^{-1}$)

* Tukey’s HSD, $p < 0.05$, $n = 3$
RubisCO
(Ribulose-1,5-bisphosphate carboxylase/oxygenase)

• In algae, CO$_2$ is fixed in the Calvin Benson cycle catalyzed by the enzyme RubisCO.

• The large subunit of RubisCO is encoded by $rbcL$ gene, which can be regulated by environmental factors (John et al., 2007).

• Diatom abundance and photosynthetically active diatoms can be inferred from $rbcL$ DNA or cDNA fragments (Endo et al., 2014; 2016)

$$y = 0.678x + 2.41$$
$$r^2 = 0.602$$

John et al. (2007)
Copy numbers of diatom-specific $rbcL$ gene as determined by quantitative PCR (qPCR)

Diatom-specific PCR primers: John et al. (2007)

A significant correlation was found between fucoxanthin and diatom-specific $rbcL$ gene levels → **Copy number of diatom-specific $rbcL$ gene can become an indicator for diatom biomass.**
Relative contributions (%) of each diatom family to the \textit{rbcL} DNA or cDNA libraries from the initial and each CO$_2$ treatment on day 3 as estimated with next-generation sequencing (Ion Torrent) technology.

"Bacillariaceae" contains some pennate diatoms such as \textit{Pseudo-nitzschia}, \textit{Nitzschia}, and \textit{Cylindrotheca} genera.
Percent differences in *rbcL* contribution (%) between the control (350 $\mu$atm $p$CO$_2$) and other $p$CO$_2$ treatments in the diatom DNA or cDNA libraries.

### rbcL DNA library (abundance)

| Family                  | 180 $\mu$atm | 750 $\mu$atm | 1000 $\mu$atm |
|-------------------------|--------------|--------------|---------------|
| Chaetocerotaceae        |              |              |               |
| Coscinodiscaceae        |              |              |               |
| Cymatosiraceae          |              |              |               |
| Rhizosoleniaceae        |              |              |               |
| Stephanodiscaceae       |              |              |               |
| Thalassiosiraceae       |              |              |               |
| Achananthaceae          |              |              |               |
| Bacillariaceae          |              |              |               |
| Naviculaceae            |              |              |               |
| Fragilariaaceae         |              |              |               |
| Unidentified diatoms    |              |              |               |
| Other eukaryotes        |              |              |               |

### rbcL cDNA library (photosynthetic activity)

| Family                  | 180 $\mu$atm | 750 $\mu$atm | 1000 $\mu$atm |
|-------------------------|--------------|--------------|---------------|
| Chaetocerotaceae        |              |              |               |
| Coscinodiscaceae        |              |              |               |
| Cymatosiraceae          |              |              |               |
| Rhizosoleniaceae        |              |              |               |
| Stephanodiscaceae       |              |              |               |
| Thalassiosiraceae       |              |              |               |
| Achananthaceae          |              |              |               |
| Bacillariaceae          |              |              |               |
| Naviculaceae            |              |              |               |
| Fragilariaaceae         |              |              |               |
| Unidentified diatoms    |              |              |               |
| Other eukaryotes        |              |              |               |

The vulnerable diatom families suggested: Bacillariaceae, Naviculaceae, Fragilariaaceae

The predominant diatom family suggested: Chaetocerotaceae
Changes over time in the photosynthetic competence ($F_v/F_m$) of phytoplankton among CO$_2$ treatments during incubation as determined by FIRe fluorometry.

The results indicate the photosystem II activity of the phytoplankton assemblages was little affected by CO$_2$ availability.

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Summary

We investigated the impact of different CO$_2$ levels on spring diatoms in Oyashio waters of the western North Pacific.

- Net growth rates of fucoxanthin, a diatom marker, decreased at higher CO$_2$ levels during incubation.
- Diatom-specific rbcL DNA copies can also become an indicator of diatom biomass in the study area.
- Diatom-specific rbcL DNA and cDNA analyses revealed that Chaetocerataceae, Thalassiosiraceae and Fragilariaceae might be vulnerable in the high CO$_2$ world expected in the near future, whereas Bacillariaceae could be a strong group against CO$_2$ changes.
Thank you!