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

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Ocean acidification

Atmospheric CO₂ increases

Release of anthropogenic CO₂

Increase in air temperature

Global warming

Increase in dissolved CO₂ in seawater

Decrease in seawater pH: “Ocean acidification”

Riebesell (2004)
In the western subarctic Pacific

- Highest biological drawdown of partial pressure of CO$_2$ ($\rho$CO$_2$) in surface waters among the world’s oceans (Takahashi et al., 2002).
- The high $\rho$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
- ρCO₂: 342.8 µatm

R/V Tansei Maru (JAMSTEC/ Univ. Tokyo)
• Bubbling of CO₂ gases
• Flow rate: 50 mL min⁻¹
• Temperature: 5 ºC
• PAR: 50% light level

Clean Niskin bottle sampling
Pre-filtration with 197 µm teflon mesh
12 L polycarbonate bottles
(4 CO₂ conditions × triplicate = 12 bottles)

Incubation conditions

Air + CO₂

空气过滤器

Bubbling of CO₂ gases
Flow rate: 50 mL min⁻¹
Temperature: 5 ºC
PAR: 50% light level

CO₂ levels:
• 180 µatm CO₂
• 350 µatm CO₂ (ambient)
• 750 µatm CO₂
• 1,000 µatm CO₂
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)

The decreases in Chl a level at higher CO₂ levels after incubation were probably due to declines in diatom abundance.

On Day 3
net growth rates of each taxonomic pigment marker

* 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 \textit{rbcL} gene as determined by quantitative PCR (qPCR)

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

\[
y = 6.29 \times 10^8 x - 1.76 \times 10^7
\]
\[r^2 = 0.910, \quad n = 25\]

A significant correlation was found between fucoxanthin and diatom-specific \textit{rbcL} gene levels → \textbf{Copy number of diatom-specific \textit{rbcL} gene can become an indicator for diatom biomass.}
Relative contributions (%) of each diatom family to the *rbcL* DNA or cDNA libraries from the initial and each CO₂ treatment on day 3 as estimated with next-generation sequencing (Ion Torrent) technology.

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

\textbf{\textit{rbcL DNA library (abundance)}}

\begin{itemize}
  \item Chaetoceraceae
  \item Coscinodiscaceae
  \item Cymatosiraceae
  \item Rhizosoleniaceae
  \item Stephanodiscaceae
  \item Thalassiosiraceae
  \item Achnanthaceae
  \item Bacillariaceae
  \item Naviculaceae
  \item Fragilariaceae
  \item Unidentified diatoms
  \item Other eukaryotes
\end{itemize}

\textbf{\textit{rbcL cDNA library (photosynthetic activity)}}

\begin{itemize}
  \item Chaetoceraceae
  \item Coscinodiscaceae
  \item Cymatosiraceae
  \item Rhizosoleniaceae
  \item Stephanodiscaceae
  \item Thalassiosiraceae
  \item Achnanthaceae
  \item Bacillariaceae
  \item Naviculaceae
  \item Fragilariaceae
  \item Unidentified diatoms
  \item Other eukaryotes
\end{itemize}

Difference in contribution (%)

\textbf{The vulnerable diatom families suggested}

\textbf{The predominant diatom family suggested}
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!