Response of spring diatoms to CO_2 availability in the western North Pacific

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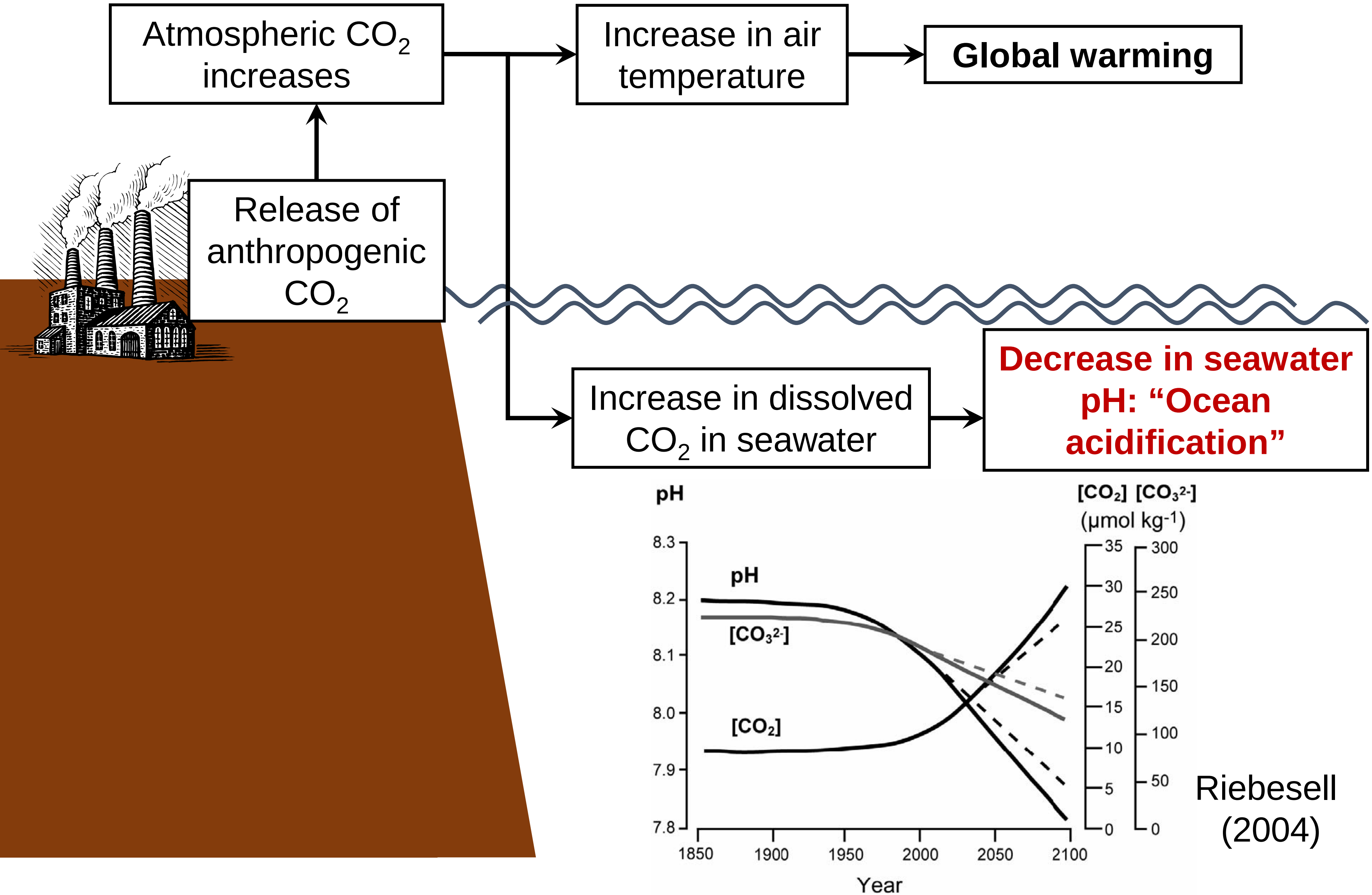
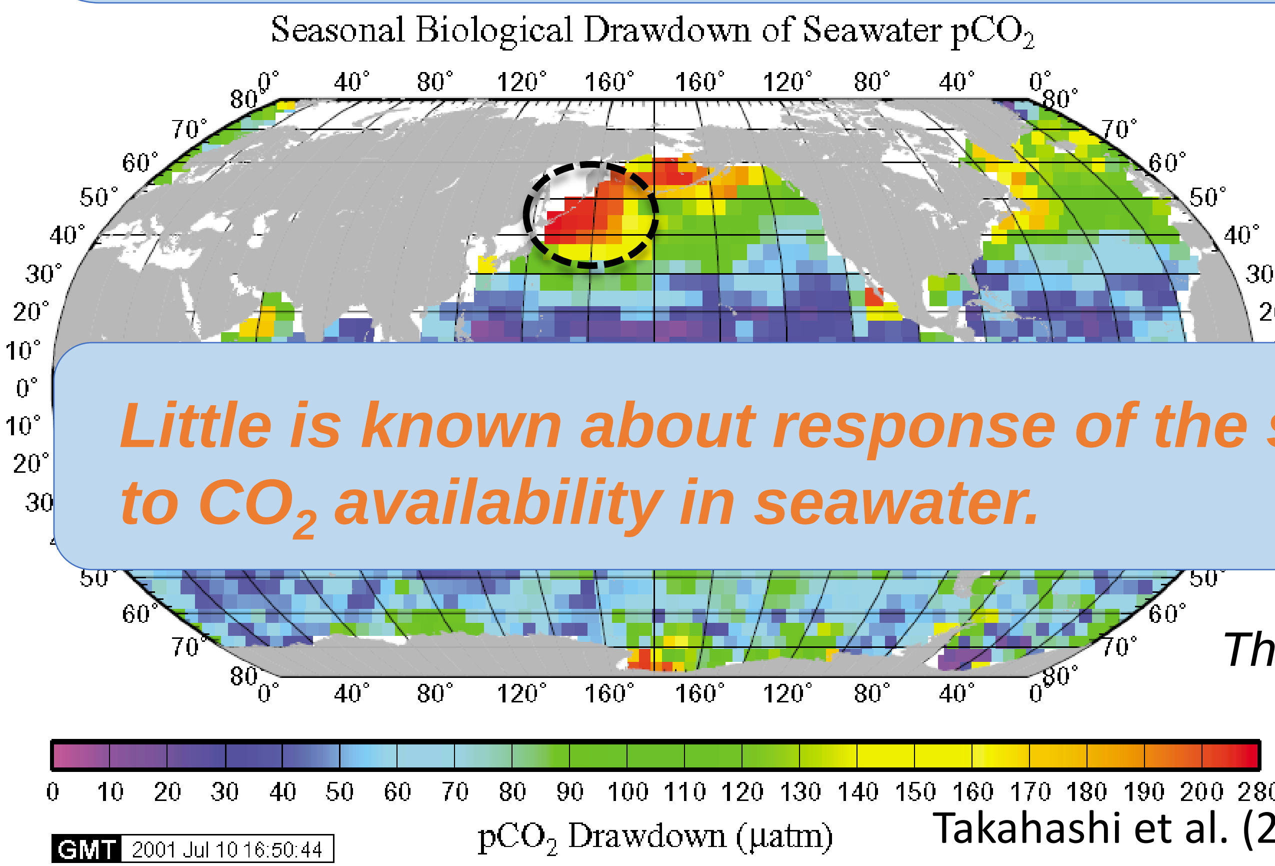
Ocean acidification

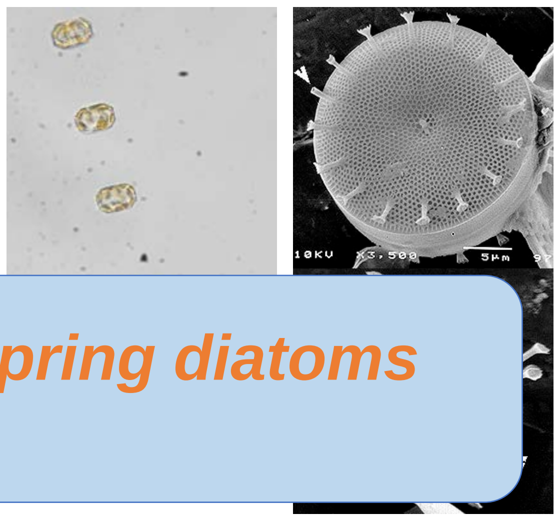


In the western subarctic Pacific

- Highest biological drawdown of partial pressure of CO_2 (pCO_2) in surface waters among the world's oceans (Takahashi et al., 2002).
- The high pCO_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.

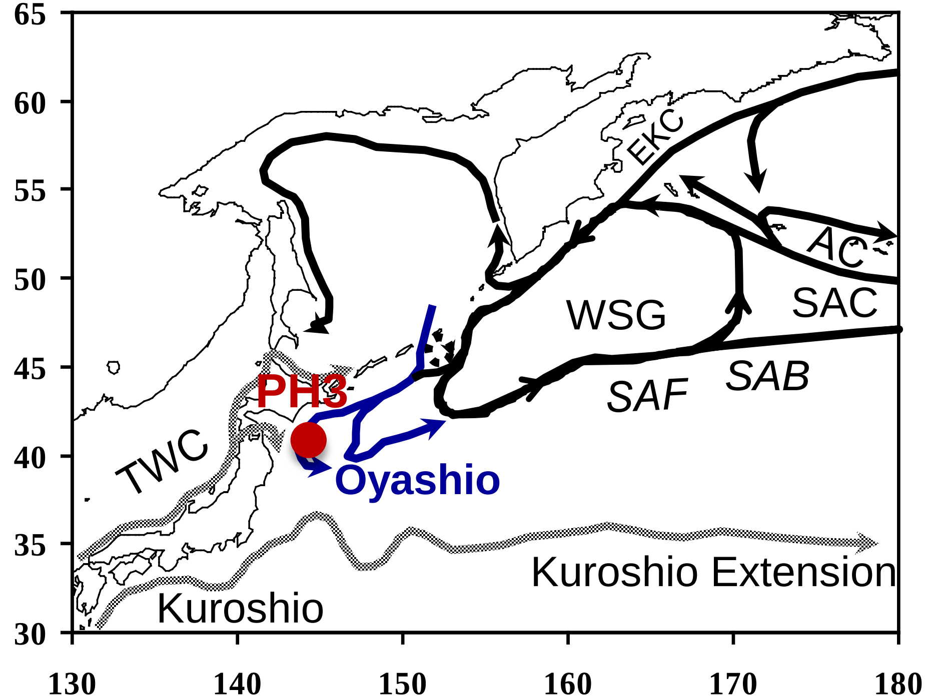


Thalassiosira nordenskioeldii

Takahashi et al. (2002)

On deck CO_2 bottle incubation experiment

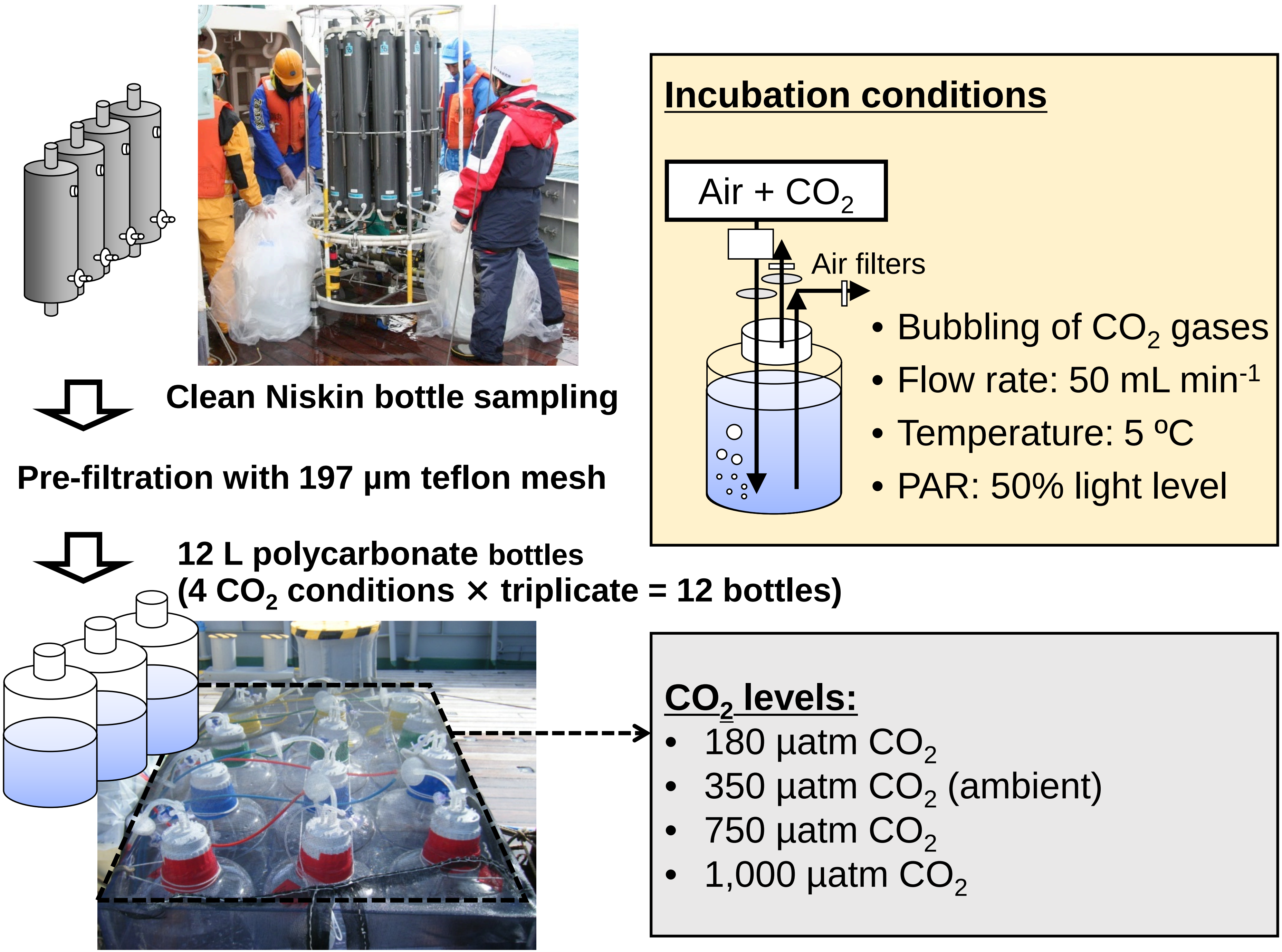
- Sampling date: May 8, 2011
- Site: Stn PH3 (41°N, 144°E)
- Sampling depth: 10 m
- Incubation period: ca. 3 days



R/V *Tansei Maru* (JAMSTEC/ Univ. Tokyo)

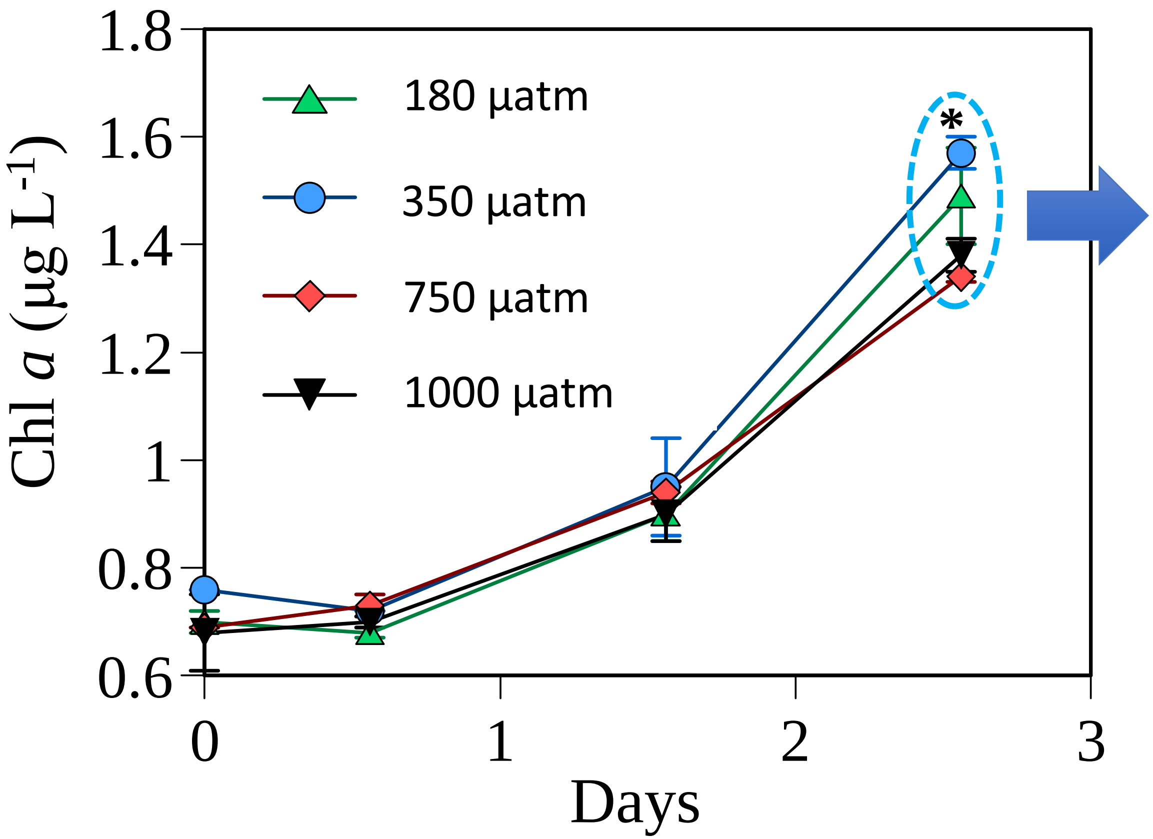
Initial conditions at 10 m:

- 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
- pCO_2: 342.8 µatm



Clean Niskin bottle sampling
Pre-filtration with 197 µm teflon mesh
12 L polycarbonate bottles
(4 CO_2 conditions × triplicate = 12 bottles)
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

Changes over time in chlorophyll (Chl) *a* concentration determined with high-performance liquid chromatography (HPLC)



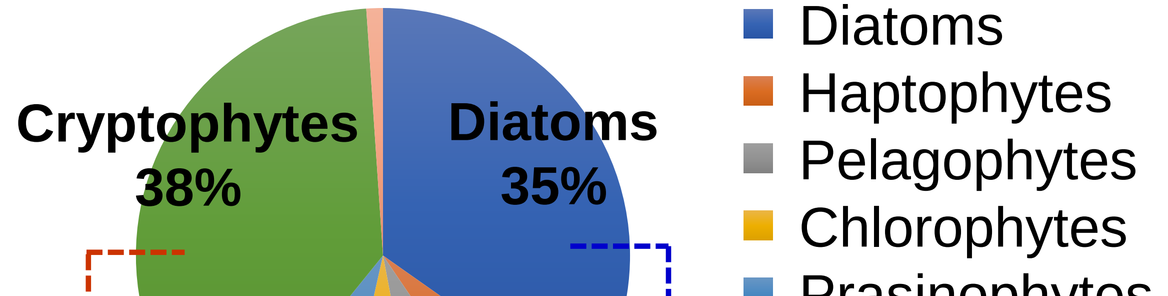
Chl *a* level
at 350 μatm pCO_2
>
Chl *a* level
at 750 and 1000 μatm
pCO_2

(Tukey's honest significant test (HSD), $p < 0.05$, $n = 3$)

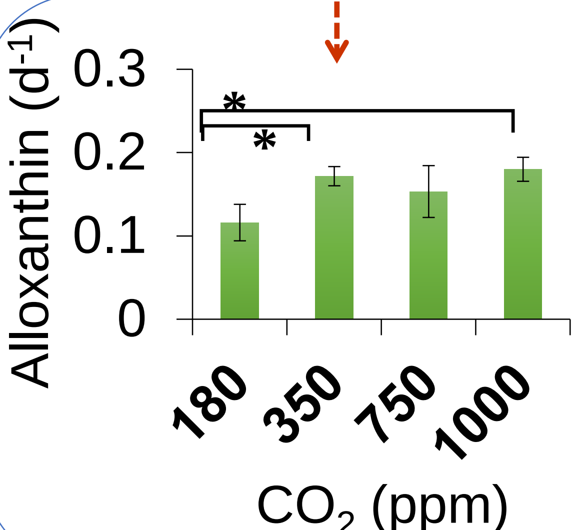
The net growth of phytoplankton assemblages was suppressed by an increase in CO_2 level.

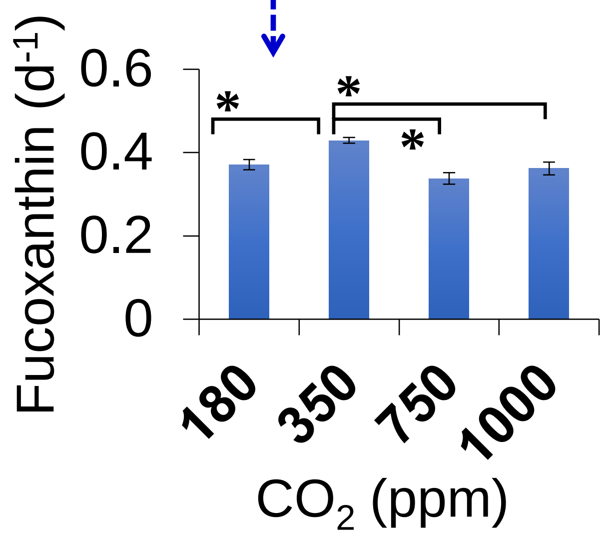
Changes in algal community composition

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 CO_2 levels after incubation were probably due to declines in diatom abundance.





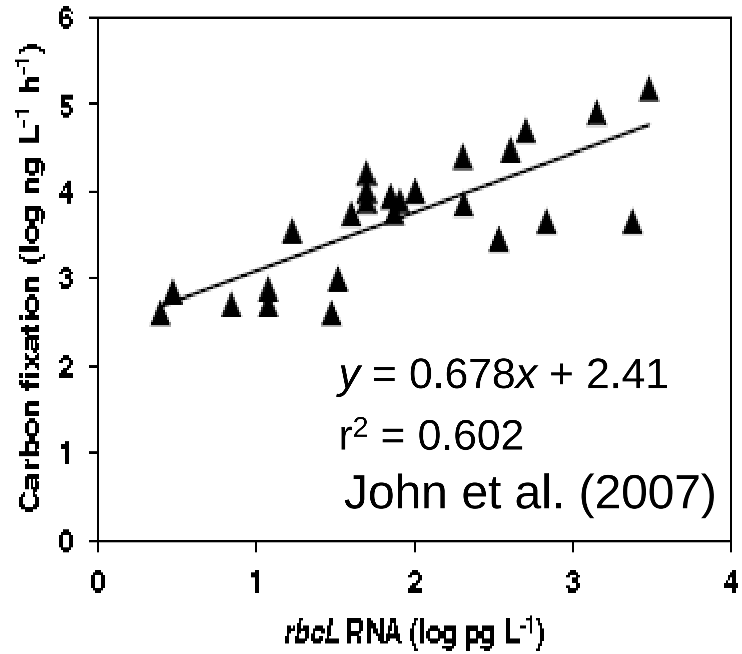
On **Day 3**

net growth rates of each taxonomic pigment marker

* Tukey's HSD, $p < 0.05$, $n = 3$

RubisCO
(Ribulose-1,5-bisphosphate carboxylase/oxygenase)

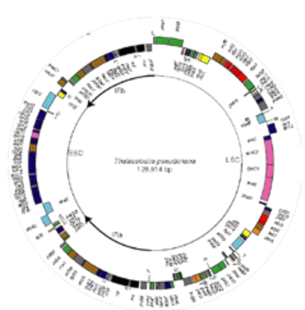
- In algae, CO_2 is fixed in the Calvin Benson cycle catalyzed by the enzyme RubisCO.
- The large subunit of RubisCO is encoded by *rbcL* gene, which can be regulated by environmental factors (John et al., 2007).
- Diatom abundance and photosynthetically active diatoms can be inferred from *rbcL* DNA or cDNA fragments (Endo et al., 2014; 2016)

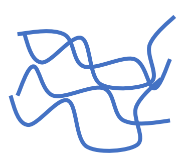


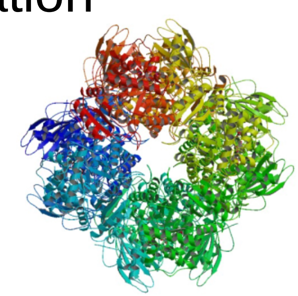
Transcription

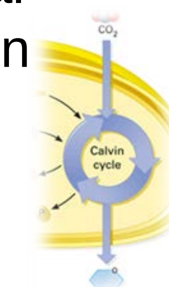
Translation

Functional expression









Plastid genome (DNA)

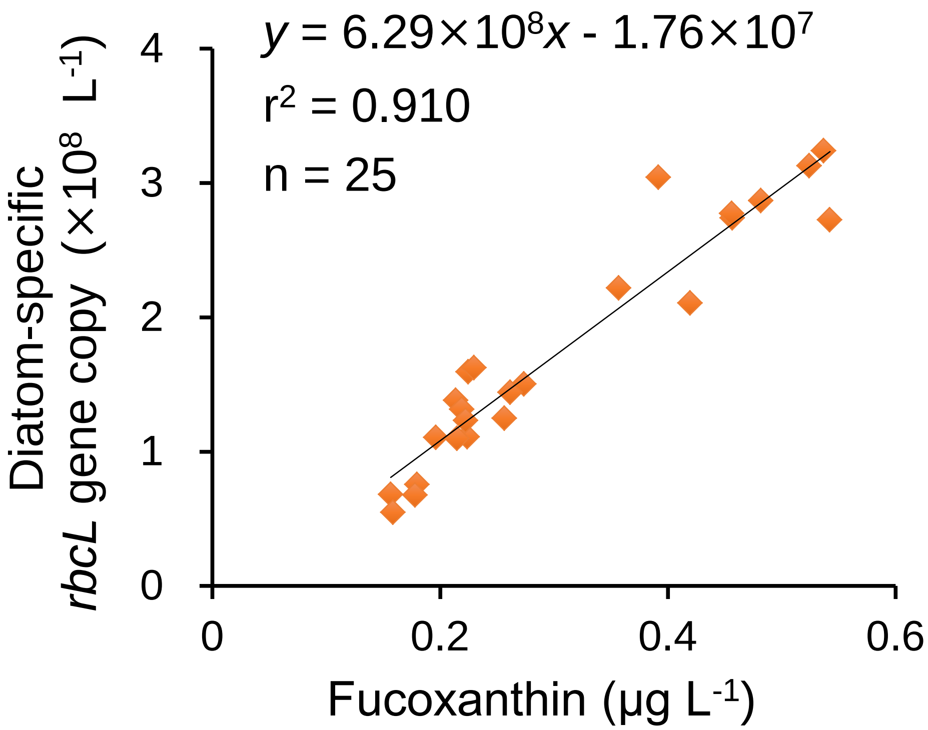
rbcL mRNA (cDNA)

RubisCO

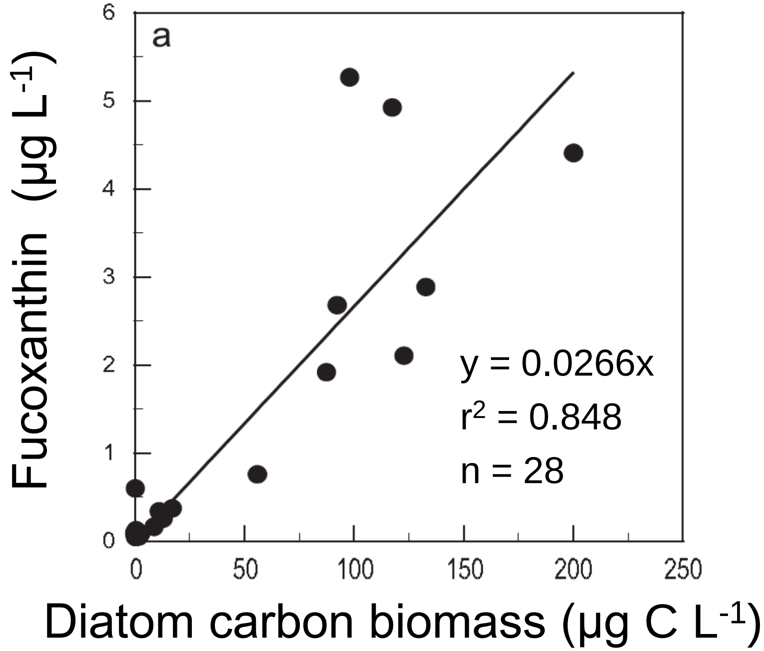
CO_2 fixation

Copy numbers of diatom-specific *rbcL* gene as determined by quantitative PCR (qPCR)

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

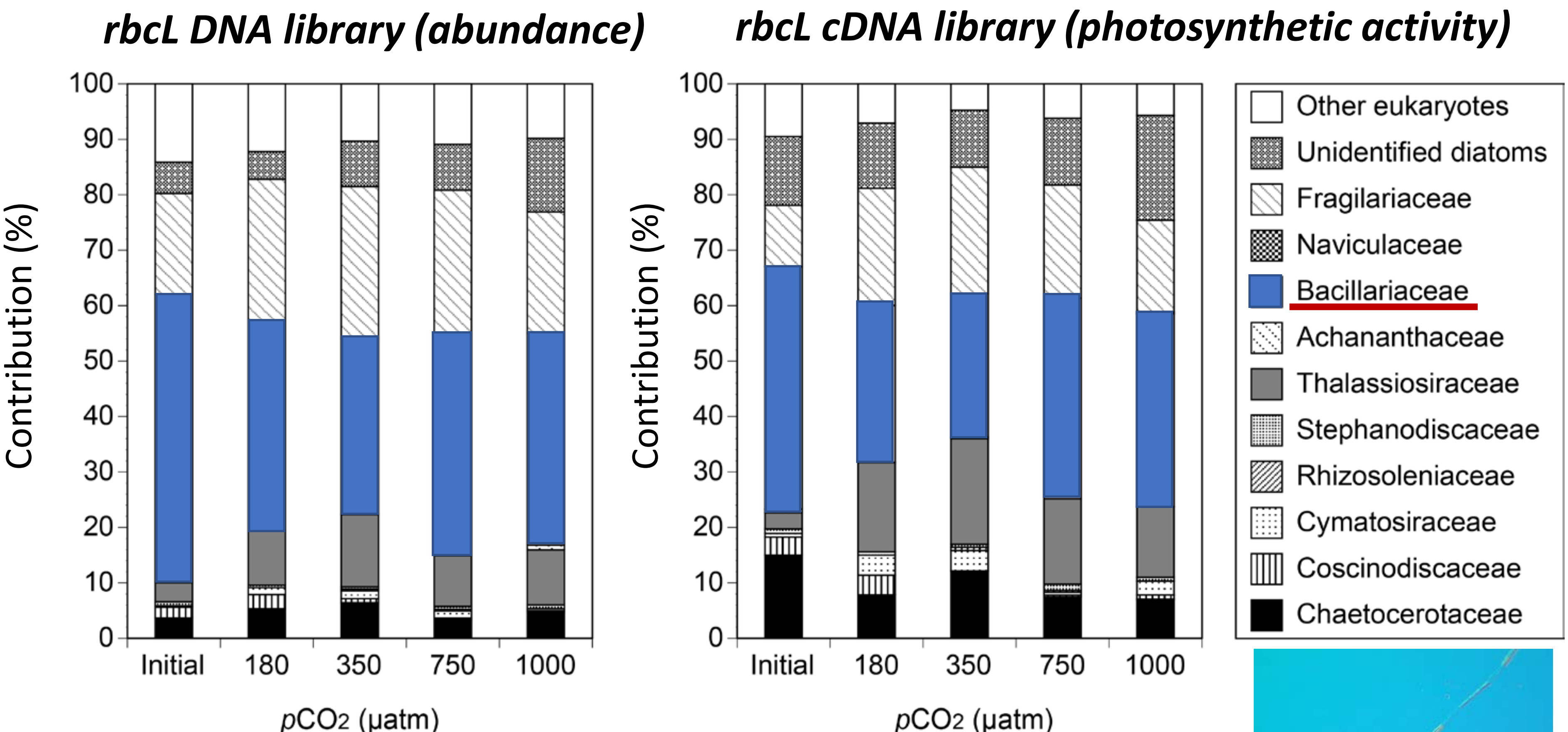


Spring Oyashio diatom bloom (Suzuki et al., 2011)



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 *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

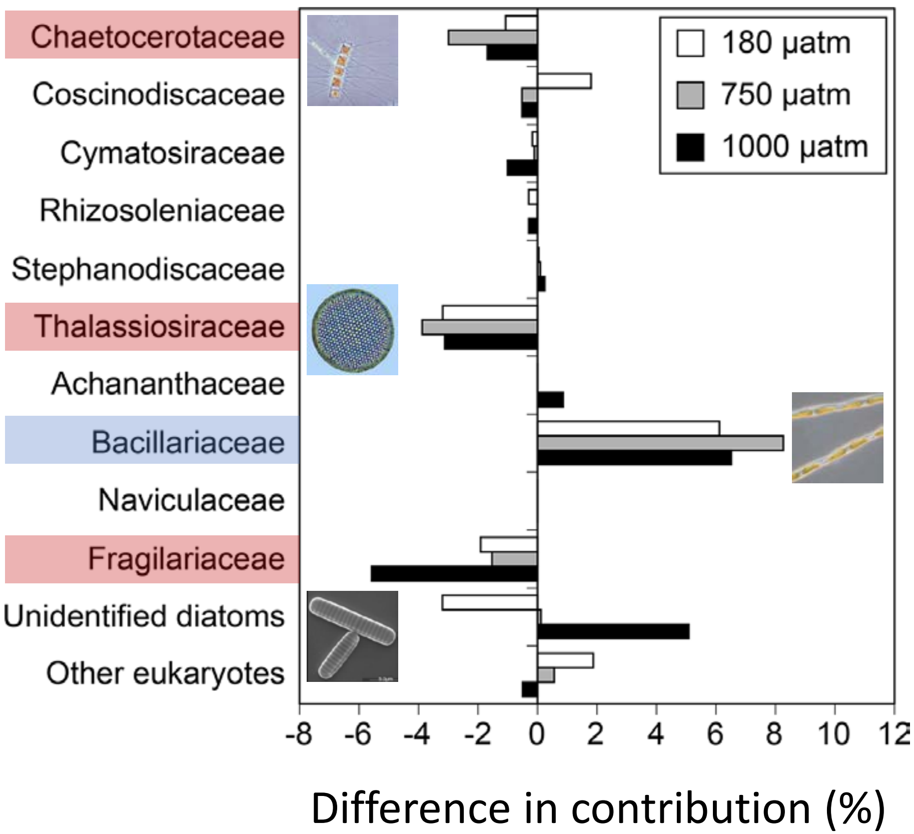


"Bacillariaceae" contains some pennate diatoms such as *Pseudo-nitzschia*, *Nitzschia*, and *Cylindrotheca genera*.

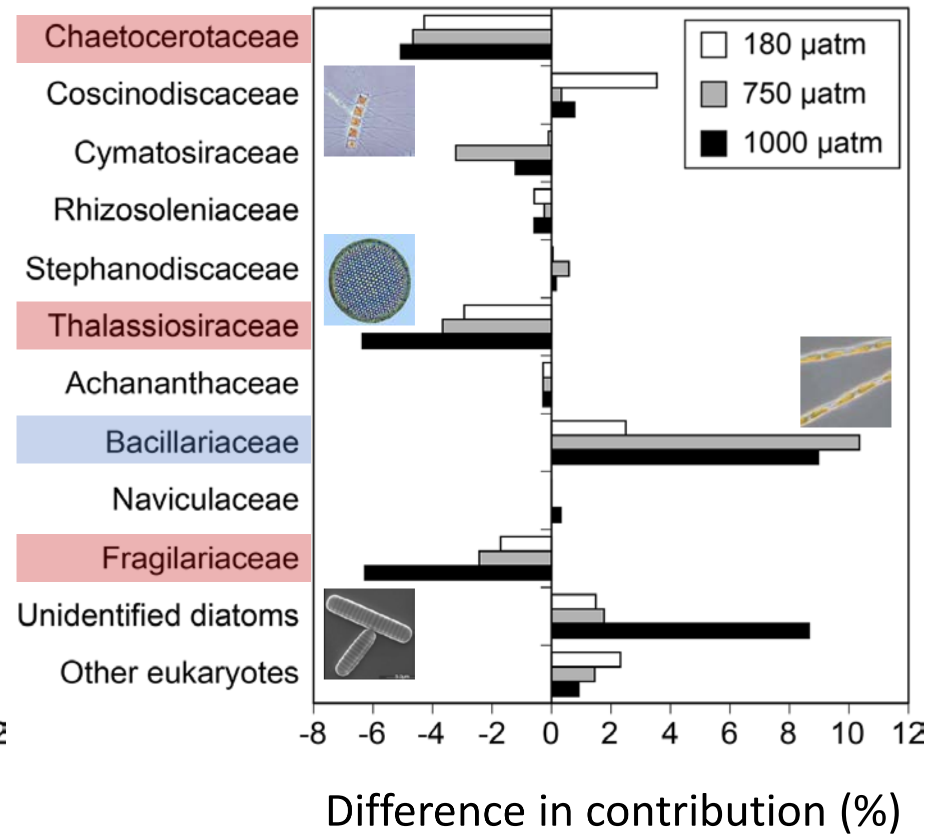
Pseudo-nitzschia seriata

Percent differences in *rbcL* contribution (%) between the control (350 µatm *p*CO_2) and other *p*CO_2 treatments in the diatom DNA or cDNA libraries

rbcL DNA library (abundance)



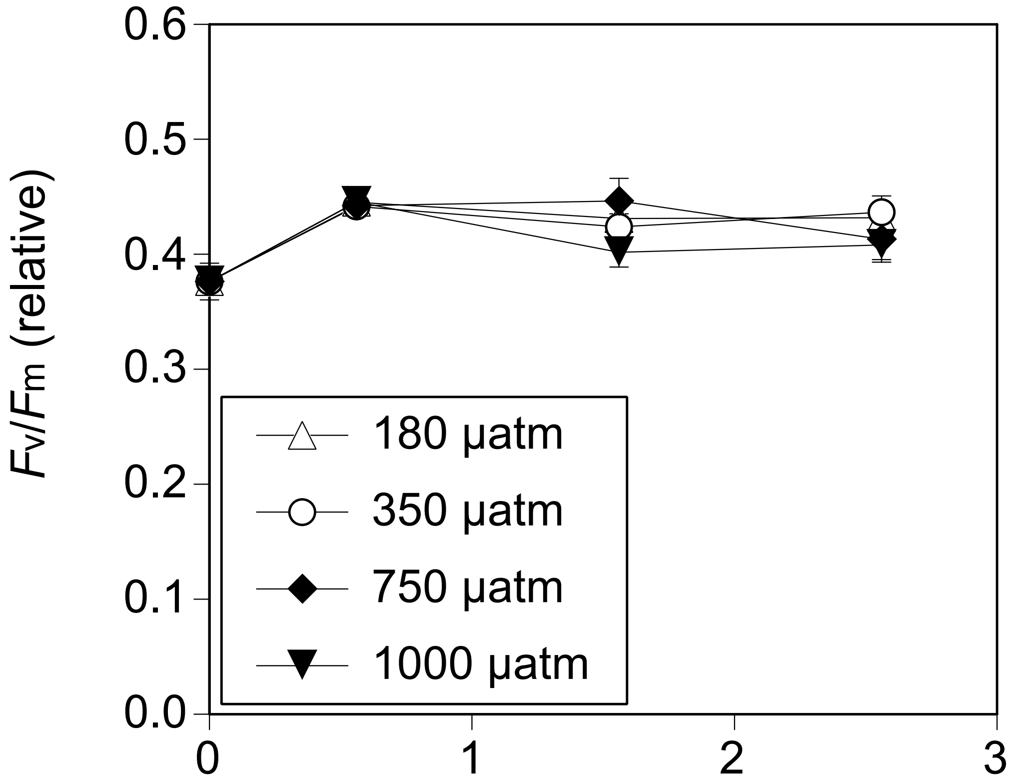
rbcL cDNA library (photosynthetic activity)



The vulnerable diatom families suggested

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



$p > 0.05$
Kruskal-Wallis ANOVA

The results indicate the photosystem II activity of the phytoplankton assemblages was little affected by CO_2 availability.

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!