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Photophysiological responses of marine phytoplankton and ice algae to temperature, iron and light availability in subpolar and polar regions

(亜極域・極域に生息する海洋植物プランクトンおよび海水微細藻類の温度・鉄・光利用度への光合成生理応答)

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要旨

Global warming increases sea temperatures and intensifies stratification of the water column, which directly and indirectly affects marine ecosystems and biogeochemistry (e.g., less nutrient supply due to the stratification). It has been suggested that the effects of the climate change may emerge earlier in subpolar and polar regions, which are highly productive from spring to summer and ecologically important (e.g., phytoplankton blooms and ice algal production). In these regions, an increase in temperature may benefit some algal primary producers, but contracts extent of sea ice, which is an inhabitant of ice-associated biota. Light is also a crucial factor for all photosynthetic organisms, and its availability can change dramatically by sea ice melt. Iron (Fe) as a micro-nutrient plays a key role in algal metabolic processes including photosynthesis. These environmental variables surely control primary productivity of marine phytoplankton in subpolar oceans and ice algae, sympagic microalgae inhabiting sea ice, in polar areas. This study thus aimed at clarifying the effects of temperature, light and Fe availability on the photophysiology of marine phytoplankton and ice algae in the changing subpolar and polar oceans.

Both marine phytoplankton and ice algae, of course, produce organic matter through photosynthesis. Photosynthesis consists of two major processes; light reaction and dark

reaction. Photosynthesis starts with light absorption by photosynthetic antennae consisting of photosynthetic pigments and associated proteins (i.e., light harvesting complex II). The absorbed solar energy by a photosynthetic antenna is transferred to its reaction centre in PSII (RCII), in turn, excites the RCII. When the excited RCII turns back to the ground state, an electron is emitted as a result of charge separation at RCII and phaeophytin. The electron travels along the electron transport chain, so-called Z scheme, and finally arrives at ferredoxin. The electron transportation results in production of a reductant NADPH and chemical energy ATP. These compounds are used in the following dark reaction processes. The dark reaction starts with carbon fixation by the carbon fixation enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). The fixed carbons are reduced with the use of 3NADPH and 3ATP, and an organic carbon compound is finally produced as a final product.

In the western subarctic Pacific, there are two productive cold currents; Oyashio off the north-east coast of Hokkaido and coastal Oyashio (COY) along the coast. Oyashio has been paid much attention, whereas COY has not been well-studied thus far although spring blooms in COY are more productive and longer than those in Oyashio due to the continuous upward nutrient supply from the shelf. The spring diatom blooms in COY waters are highly productive in spite of the low sea surface temperature (SST) ($-1-2^{\circ}\text{C}$ in early spring). It is well-known that low temperature inhibits growth and photosynthesis of phytoplankton because the enzymatic activity of RuBisCO is highly temperature sensitive. It is thus questionable and also important how phytoplankton can form the annual spring blooms in the cold but productive COY waters and who can form the extensive bloom. Effects of temperature on a spring diatom bloom in the coastal Oyashio (COY) were, therefore, investigated as a representative subpolar coastal spring bloom. This study hypothesized that low temperature is the main limiting factor for photosynthesis, particularly carbon fixation

processes and one or some specific diatoms have potentials to rapidly respond to the increase in temperature in spring. This study thus focused on the photophysiology and community composition of phytoplankton in COY waters in the pre-bloom and bloom periods from March to April 2015 during the KH-15-1 and AK15 expedition on board R/V *Hakuho Maru* and TR/V *Misago Maru*. Photophysiology of phytoplankton was holistically assessed using multiple photosynthetic measurements. The conventional photosynthesis versus irradiance curve (P - E curve) was used to assess the whole process of photosynthesis from light absorption to production of organic carbons, with the ^{13}C method, Variable chlorophyll a (Chl a) fluorescence, on the other hand, measures potential photochemical efficiency of PSII. In Chapter 2, we used both Pulse Amplitude Modulation (PAM) and Fast Repetition Rate (FRR) fluorometry. The dark reaction was also assessed as gene expression measurements on the *rbcL* gene coding the large subunit of RuBisCO (i.e., an indicator of synthesis rate of RuBisCO). Community composition also affects photosynthetic properties, we quantified chlorophylls and carotenoids of phytoplankton to estimate the community composition using diagnostic pigment signatures. Next-generation sequencing (NGS) was also used to deeply look at diatom community by targeting the diatom-specific 18S rRNA gene. The diagnostic pigment analysis revealed that diatoms formed blooms in COY regions. NGS revealed that the diatom *Thalassiosira* generally dominated the phytoplankton community in COY waters on the shelf (shelf COY). Additionally, the relative contribution of *Thalassiosira* to the total diatom assemblages showed a positive correlation with maximum photosynthetic rates ($P_{\text{max}}^{\text{B}}$) throughout this study, suggesting that the genus largely contributed to the bloom. We also conducted short-term on-deck incubation experiments to clarify the role of temperature in determining the photosynthetic functioning of phytoplankton. As a result, in the shelf COY, rising temperature (+7 °C) led an increase in $P_{\text{max}}^{\text{B}}$ and transcriptional levels of the diatom-specific *rbcL* gene coding the large subunit of RuBisCO. It suggested that the rising

temperature upregulated the diatom-specific *rbcL* gene and then triggered the higher $P_{\max}^B$ as well as the spring diatom bloom in the shelf COY waters. This dual effect by temperature benefits carbon fixation during spring diatom blooms. The genus *Thalassiosira*, particularly, has potentials to rapidly respond to an increase in temperature, they can thus outcompete the blooms.

In summer, Fe availability is, on the other hand, a crucial factor for the dynamics of spring diatom blooms in the western subarctic Pacific including the Oyashio region. The western subarctic Pacific is known as one of the largest High Nutrient Low Chlorophyll (HNLC) regions. At present, little is known about Fe deficiency in phytoplankton in neritic zones and its boundary region because of multiple Fe sources and strong vertical mixing along the Kuril Islands. Chapter 3 examined the effects of Fe on the community composition and photophysiology of phytoplankton in the western subarctic Pacific near the Kuril Islands and the eastern Kamchatka Peninsula in bloom and post-bloom phases during the Mu14 expedition from June to July 2014 on board R/V *Professor Mulanovskiy*. Photophysiology of phytoplankton was again assessed with *P-E* curve experiments as well as Variable Chl *a* fluorescence using PAM and Fluorescence Induction and Relaxation (FIRE) fluorometry. Chl *a* biomass was measured fluorometrically. Dissolved Fe concentrations at sampling stations were determined using the flow injection chemi-luminescence analysis. We defined a HNLC water as $D\text{Fe} < 0.1 \text{ nM}$, $\text{NO}_3 > 2 \text{ }\mu\text{M}$, and $\text{Chl } a < 1 \text{ mg m}^{-3}$. A HNLC water was observed outside of the shelf slope. High Chl *a* concentrations were observed in the coastal region in early June near Bussol' Strait, whereas spring diatom blooms seemed to be terminated in the coastal region near the Kamchatka Peninsula. Detailed scanning electron microscopy demonstrated a clear biogeographical difference in armoured plankton community composition between the coastal and offshore waters. The diatom *Chaetoceros* was abundant in the Fe-rich coastal waters, while the genera *Fragilariopsis* and *Pseudo-nitzschia* were

dominant in the HNLC water outside of the shelf slope. The redundancy analysis (RDA) was performed to evaluate relationships between physicochemical properties and community composition of diatoms at the level of genus after the Hellinger normalization. The RDA results also suggest that the two dominant species (i.e., *Chaetoceros* and *Fragilariopsis*) had almost opposite vectors. In addition, DFe and *Chaetoceros* only showed similar vectors, indicating that DFe could be the statistically important factor controlling diatom community composition. Light availability was also important for primary productivity of phytoplankton, we thus conducted on-deck Fe enrichment bottle incubation experiments at stations with the second shallowest and the deepest surface mixed layer depths (MLD) (i.e., stations off Bussol' Strait and near the Kamchatka Peninsula, respectively). Fe addition at the station with the second shallowest station did not affect biomass and photochemical efficiency at photosystem II (F_v/F_m), whereas Chl *a* biomass and F_v/F_m were increased at the deepest MLD station. These results suggest the Fe-light interaction at the station with the deepest MLD, also supported by the results of photophysiological parameters (E_k : light saturation index of photosynthesis versus irradiance curve). Functional absorption cross section of PSII (σ_{PSII}) measured with a FRe fluorometer also supported the high-light and low-light acclimation at the second shallowest and the deepest MLD stations. At the study area, it was indicated that light availability can significantly affect Fe deficiency in the phytoplankton assemblages (i.e., Fe-light co-limitations).

Sea-ice algae contribute 10–25% of the annual primary production of polar seas, and seed large-scale ice-edge blooms. Large fluctuations in temperature, salinity, light and Fe availability, associated with freezing and melting of sea ice, can significantly change the photophysiology of ice algae. Therefore, effects of multiple co-stressors (i.e., freezing temperature and high brine salinity in a freezing event; and sudden high light exposure during ice melting as well as Fe starvation) on the photophysiology of ice algae were investigated in

a series of ice tank experiments in Chapters 4 and 5. The polar diatom *Fragilariopsis cylindrus* was incubated under different light intensities (150 and 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively) as well as different Fe availability (1.5 nM and 4.6 pM as inorganic Fe species (Fe^{3+}), respectively) in the Aquil medium buffered with ethylenediaminetetraacetic acid to control Fe^{3+} concentration. The ice tank replicates *in situ* pack ice environments by forming artificial sea ice in an ice tank chamber and enables incubation of ice algae for an extended period and monitoring their photophysiology continuously in sea ice. During the ice tank incubation experiments, seawater samples were obtained before ice formation to assess the initial state of algal photosynthesis, and then the freezing cycle was switched on to form artificial ice in the ice tank. Ice samples were collected every 5 days for 20 days. After the 20-day ice sampling, ice samples were melted at 2.5 °C, and high light (800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to the melted samples to reproduce the environment where released ice algal cells would experience when they are released from sea ice to open waters. Photophysiology of ice algae was assessed with FRR fluorometry, pigment analysis using Ultra High Performance Liquid Chromatography (UHPLC), and gene expression measurements on photosynthesis-related gene; the *psbA* and *rbcL* genes. The *psbA* gene codes the central protein of PSII (i.e., D1 protein), upregulation of the gene thus enhance repair of damaged PSII such as by photoinhibition. When algal cells were frozen into the ice, F_v/F_m decreased regardless of the treatments, possibly due to the damage of PSII reaction centres or high brine salinity stress suppressing the reduction capacity downstream of PSII (e.g., Q_A and PQ pool). The suppression on reduction capacity of downstream components, however, seemed to be more likely because σ_{PSII} was invariant when they were frozen in. When RCII is damaged, σ_{PSII} can also change as the ratio of photosynthetic antennae to a reaction centre becomes larger. Non photochemical quenching (NPQ_{NSV}), a safety valve of photosynthesis under stress, was, on the other hand, upregulated possibly for protecting themselves from excess excitation

pressure caused by suppression of downstream components. Also, gene expression of the *rbcL* gene was highly upregulated when algal cells were frozen into the ice, suggesting a survival strategy for acclimating to the cold ice environment in the ice. The frozen algae within the ice showed almost the same levels of F_v/F_m regardless of the treatments, indicating that sea ice is a good and stable platform for ice algal photosynthesis. At the same time during the frozen period, NPQ_{NSV} was also stable at a high level, indicating that F_v/F_m and NPQ_{NSV} behaved oppositely. These results suggested that ice algae maintained and optimized their photosynthesis in the ice. When the ice melted and the cells were exposed to the high light, F_v/F_m sharply decreased, NPQ_{NSV} was upregulated to dissipate the excess light energy (i.e., photoinhibition). Interestingly, the *psbA* gene encoding the D1 protein of PSII was upregulated under high Fe conditions and vice versa. These results suggested Fe repletion accelerated *de novo* synthesis of the D1 protein, whereas Fe starvation inhibited repair of the PSII damaged by the high light. Our results imply that Fe-starved cells cannot well regulate their photosynthetic plasticity to the environmental changes during ice melting, and would little contribute to ice-edge blooms.