PhD Dissertation

Effect of sea ice melt on growth and photophysiological performances of sea ice diatoms in the Sea of Okhotsk

オホーツク海における海氷融解が海氷珪藻類の増殖と光合成生理能力に及ぼす影響

by

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Abstract

Sea ice microalgal communities are dominated by diatoms and they play important roles in primary production at high latitudes. Growth of microalgae in ice-covered areas is primarily controlled by the seasonal light climate. In Arctic and subarctic seas, more light penetrates through sea ice in early spring than that during the winter time. This increase in light availability is a key driver of bloom in ice-covered seawaters. During ice melt in spring, ice algae are released from sea ice and could be exposed to changeable temperature, salinity and irradiance levels in surface water. Large variations of those environmental factors are likely to influence photophysiological performance of ice algae. Species with greater flexibility in photoacclimation are afforded a higher chance of survival and seed the following phytoplankton bloom.

The Sea of Okhotsk is the southernmost sea ice zone in the northern hemisphere with a sizeable seasonal ice cover, thus ice algae of the Sea of Okhotsk have a large potential to seed the early spring diatom bloom in the water column. However, little is known about the Okhotsk ice algal communities and their seeding effects. We investigated the dynamics of the composition and the photophysiological performances of an ice algal community in a 6-day laboratory incubation experiment that simulated the natural ice melt conditions. Centric diatoms, especially *Thalassiosira* spp., overwhelmingly dominated the ice algal community throughout the incubation, whereas pennate diatoms, mostly *Navicula* and *Nitzschia*, showed little growth with much higher mortality. The maximum photochemical efficiency of Photosystem II (F_v/F_m) was the lowest at the beginning of the ice melt, suggesting a suppressed photosynthetic functioning by changes in salinity. The cellular pigment contents decreased by 30% due to cellular damage, evidenced by deformed plastids under a microscope. The transcript level of the *rbcL* gene that encodes the large subunit of RubisCO was significantly higher during the ice melt and decreased in the no-ice period, suggesting an urgent need for osmoprotectants under the melt condition. Full recovery of the photosynthesis and growth was also made after complete ice melt. The results indicated high seeding potential of *Thalassiosira* to spring blooms owing to their photophysiological plasticity to dynamic salinity changes.

Saroma Lagoon is an embayment with two inlets leading to the Sea of Okhotsk. There is a seasonal development of sea ice in this lagoon. To investigate the living and photoprotective strategies of ice algae in such a coastal water system, we grew *Nitzschia* cf. *neglecta*, an ice diatom isolated from the sea ice of this lagoon, under irradiance levels of 30 and 100 μmol photons m^{-2} s^{-1}, and temperatures of 2 and 10 °C. Then the acclimated cells were exposed to high light in order to investigate the plasticity of their photosynthetic functioning. At 10 °C, cells grew faster and showed a weaker susceptibility to high light. Highly efficient photoprotection was achieved through the diadinoxanthin-diatoxanthin cycle-dependent non-photochemical quenching (NPQ). The wide tolerance to both temperature and light changes suggests that the thinning of sea ice and higher temperatures in a warmer climate will lead to more intense blooms in Saroma Lagoon.

Introduction

Sea ice in the polar and sub-polar regions provides a unique habitat for microorganisms, including numerous algae, bacteria, protists and viruses (Garrison, 1981; Garrison and Close, 1993; Wells and Deming, 2006; Comeau *et al.*, 2013; Arrigo, 2014; Hopes *et al.*, 2017). The microalgae living in sea ice are called ice algae. They form the base of the food web of the sea ice ecosystem and are the only source of fixed carbon for higher trophic levels in the ice cover (Garrison *et al.*, 1987). Algal abundance in sea ice can vary by up to six orders of magnitude, from 10^4 to 10^9 cells L^{-1} (Arrigo, 2016). The highest concentration of ice algae is usually found at the bottom of the ice near the ice-water interface, where the living condition is relatively favorable to cells (Arrigo et al., 1997; Juhl and Krembs, 2010).

Marine production in the Arctic and subarctic primarily comes from microalgae, including phytoplankton in the water column and ice algae associated with sea ice (Sakshaug, 2004). The Sea of Okhotsk is characterized by high biological productivity (Kim, 2012). Previous studies demonstrated that diatoms play a major role in the phytoplanktonic communities of the Sea of Okhotsk (Ohwada, 1956; Sorokin and Sorokin, 1999; Orlova *et al.*, 2004; Suzuki *et al.*, 2011; Kasai and Hirakawa, 2015). The sea of Okhotsk has many of the characteristics of polar seas, though it is located in temperate latitudes (Preller and Hogan, 1998). The most prominent one is the pronounced sea ice cover during the winter time. It is the southernmost sea that has a sizable sea ice cover in the northern hemisphere (Aota and Ishikawa, 1991). The Sea of Okhotsk is currently undergoing a period of sustained seasonal sea ice loss, putatively due to global warming (Kashiwase *et al.*, 2014; Ohshima *et al.*, 2014).There is a large amount of ice algae in the Okhotsk Sea ice. Brownish sea ice was commonly observed at the bottom of Okhotsk sea ice, especially in spring (Leonov *et al.*, 2007). The brownish color is from the algal cells and therefore is an indicator of high ice algal abundance. Chlorophyll *a* (Chl *a*) concentrations in the Okhotsk sea ice ranged between 0.2 to 3.5 mg m^{-2}, and was up to an order of magnitude higher than that in the under-ice seawater (Granskog *et al.*, 2015).

The bloom of diatoms after the melt of sea ice is a common phenomenon in the Sea of Okhotsk (Smirnova, 1959). In spring, a shallow pycnocline is formed due to the warming up of the melted sea ice at the surface. It is suggested that both the sufficient light and the stable vertical structure contributed to the beginning of the phytoplankton bloom in the Sea of Okhotsk (Sorokin and Sorokin, 1999). The annual primary production in the Sea of Okhotsk is largely derived from spring diatom bloom following sea ice melt (Ohwada, 1956; Zhang *et al.*, 2006). When ice breakup begins in spring, ice algae are rapidly washed out of the ice and dispersed into the water column, causing a significant increase in the downward flux of ice algal cells under the Okhotsk sea ice (Leonov *et al.*, 2007; Hiwatari *et al.*, 2008). Previous studies suggested that the release of ice algae into the water column initiated the spring development of phytoplankton (Garrison *et al.*, 1987; Garrison and Close, 1993). This release is enhanced toward the end of the melt season and some released ice algae may continue their growth in the water column to become the seed of the phytoplankton bloom after ice retreat, which is known as the seeding effect of ice algae.

Aim of the study

My PhD work investigated the survival and growth of ice algae under changing environmental conditions. Ice algal cells of the Sea of Okhotsk are released into seawater every spring as sea ice melts. The released ice algae are capable to seed the spring phytoplankton bloom. Despite the large biomass of the Okhotsk ice algae, the ecology and their seeding roles toward the spring bloom are not fully understood. Chapter 2 describes the investigation of the fates of ice algae during and after ice melt in the Sea of Okhotsk. A laboratory experiment simulating the field ice melt was conducted to measure the changes in the community composition during and after complete ice melt. The algal photosynthesis in the seawater was also measured to see the correlations with the dynamics of the community. Knowing the fate of ice algae under the ice melt

condition improves our understanding on the community dynamics and primary production of the Sea of Okhotsk in the changing climate. Chapter 3 describes an incubation work using a strain of the pennate diatom *Nitzschia* cf. *neglecta* isolated from the sea ice of Saroma Lagoon. Global warming poses a serious challenge to marine ecosystems (Petrou *et al.*, 2010). The multi-year ice in the Arctic is declining, specifically through changes in sea-ice thickness, the timing of advance and retreat, and associated shifts in overlying snow (Stroeve and Notz, 2018). Over the past five decades, there is a declining trend in the formation of sea ice in the Sea of Okhotsk due to global climate change (Ohshima *et al.*, 2014; Nihashi *et al.*, 2018). There exist conflicting views of how increased temperature affects microalgal productivity in sea ice-covered waters. The culture temperature and irradiance level were increased to investigate whether an ice alga can survive such conditions significantly different from that of sea ice. Then the cultured cells were exposed to high light to investigate the photoprotective strategies. The results of the experiments give us a better understanding of how ice algae survive under changing environments when sea ice melts and how the related ecological effects.

Results and discussion of Chapter 2

Both the light microscopy (LM) and the 18S rDNA data showed that centric diatoms dominated the communities. There was an overwhelming dominance of *Thalassiosira* of >70% revealed by both methods. *Chaetoceros* was the second most abundant genus in terms of cell number, around 10%, while it was much less abundant in the 18S rDNA community (0.3%). Overall, pennate diatoms were slightly more abundant in terms of 18S rDNA sequences (14.7%) than LM cell counts (8.9%). The Okhotsk sea ice could be unique that the discoid chain-forming centric diatoms *Thalassiosira* often occur with high abundance (McMinn et al. 2008; Ohwada 1956). The dominance of *Thalassiosira* during the no-ice period in our laboratory experiment is in consistence with that in the field that the early spring blooms of *Thalassiosira* is a common phenomenon occurring in the Sea of Okhotsk and the western subarctic Pacific, including the Oyashio region and the northwestern Sea of Japan near Russia when the water temperatures were still < 5°C (Ohwada 1956; Sorokin and Sorokin 1999; Motylkova and Konovalova 2010; Suzuki et al., 2011; Yoshida et al. 2018).

The specific growth rate for the no-ice period from day 2 to day 6 (μ_w) showed that the community growth (0.18 ± 0.02 day^{-1}) was mainly contributed by centric diatoms (0.18 ± 0.03 day^{-1}). While the growth rate of pennate diatoms was 3.5 times slower than that of the centric diatoms during the no ice period ($p < 0.01$) and the cell abundance of pennate diatoms remained low throughout the incubation. The percentages of dead cells in the diatom community were low. The highest percentage was around 5.89 ± 0.71% in the pennate diatoms under the ice melt condition. This percentage dropped to < 2% after ice melt ($p < 0.01$). The average percentage of dead cells of centric diatoms was 0.50 ± 0.17% during ice melt and increased to 1.68 ± 0.64% in the no-ice period ($p < 0.05$). This indicates that pennate diatoms have the potential to grow while the intrinsic growth rate might be slower than centric diatoms under similar environmental conditions. We thus suggest that such ice diatoms are possibly characterized by strong stenohalinity. On the other hand, the ecological success of *Thalassiosira* under stressful ice melt conditions could partly be attributed to their tolerance to salinity changes.

The cellular pigment contents showed a decreasing trend until each minimum value was reached after 10 h of incubation, and then they increased until the end. The sea ice melt significantly suppressed F_v/F_m. Overall, the transcript levels of the *rbcL* gene during the first 5 h were 8 times higher than the average level of the steady state from t_{79} to t_{127} ($p < 0.01$). We attribute this decrease to cell damage rather than acclimation due to the fact that abnormal cells were commonly observed at the beginning of the ice-melt period.

The diatom *Thalassiosira* was a major component of the Okhotsk sea ice diatom community and has a great potential to seed the spring phytoplankton bloom in the Sea of Okhotsk. The fast

acclimation to salinity changes ensured the ecological success of *Thalassiosira*. Pennate diatoms of *Navicula* and *Nitzschia* were abundant in sea ice while their contribution decreased and became negligible as the incubation progressed. Besides the inherently lower growth rates of *Navicula* and *Nitzschia*, the growth was hindered by the osmotic stress, indicated by higher mortality during the ice melt period. The inefficient photosynthesis and cell death under the melt stress possibly reduced their seeding potential.

Results and discussion of Chapter 3

On average, there was no significant difference between growth rates at different irradiance levels at 2 °C, while higher light enhanced growth by around 30 % at 10 °C ($p < 0.05$). Higher temperatures increased growth rate by around 40 and 30 % under the low and high irradiance levels, respectively ($p < 0.05$). Considering that the water temperature of Saroma Lagoon may rise up to around 20 °C in summer, this result suggests that despite being isolated from an ice core, the species we used here can tolerate a wide range of temperatures and can continue its growth throughout the year.

The high F_v/F_m and I_k values in high light, as well as the fast recovery of YII in the dark indicates a weak susceptibility to high light stress of this species. F_v/F_m values were above 0.65, indicating that the photosynthetic apparatus was functional under the culture conditions. Variations of $rETR_{max}$ and I_k between HL and LL were higher at 10 °C compared with 2 °C). These results suggest a larger flexibility of photoacclimation at the higher temperature.

Contents of the PSC Fxn (fucoxanthin) and the two chlorophylls were higher in LL than in HL, and the extent of the difference was similar for each temperature. The PPC pigment Dtx content at lower temperature was higher and showed a larger variation. These results suggest that cells grown at 2 °C used more Dtx for acclimation to the colder environment. This may also explain a phenomenon that only occurred at 2 °C, irrespective of light history. There was an immediate decrease of cellular pigment content when cells were transferred to the exposure condition. Here, we show that NPQ and cellular Dtx content during the E and R treatments were linearly correlated. Consequently, given a group of cells cultured in a certain condition, higher values of NPQ could be explained by higher content of Dtx.

Our investigation of photosynthetic responses of *N.* cf. *neglecta* to high light revealed that the photoacclimative plasticity of this species was largely shaped by temperature. Its survival and prevalence benefit from higher temperature due to more effective photoprotection. The weak shade adaptation may contribute to a rapid response to changing light when released into shallow coastal waters during ice melt. The rapid decrease of cellular pigment content in high light only occurred at 2 °C, indicating an intimate coupling of acclimation of photosynthesis to low temperatures and high light. The operation of the Ddx-Dtx cycle served as the main photoprotective strategy against high light while regulation of *psbA* and *rbcL* at transcription levels played a minor role. Despite its occurrence in sea ice, this species is not strictly psychrophilic, but has a wide temperature tolerance that can contribute to its ecological success in Saroma Lagoon ice algal and phytoplankton communities. We believe that these findings can apply to some other species in Saroma Lagoon. The wide tolerance to both temperature and light changes suggest that the thinning of sea ice and higher temperatures in a warmer world may cause more intense blooms in Saroma Lagoon, and potentially other coastal waters with seasonal ice cover.

Materials and methods

Light microscopy

These water samples were stored in the dark at 4°C before analysis under an inverted light microscope (CKX41-FI, Olympus, Japan). Diatoms in the water samples were identified and

counted within a few days after each sampling. Taxonomic identification followed Tomas (1997). Identifications were to the species level if possible but to the genus in most cases. To calculate the growth rate, cell abundance was natural log transformed before plotting against the day of incubation and the specific growth rate (μ, day^{-1}) was calculated as the slope of the linearly fitted curve.

Cell viability

To distinguish between live and dead cells, cells in water samples of 5 mL were stained with the LIVE/DEAD™ *Bac*Light™ stain (Thermo Fisher Scientific, Inc., USA) for 30 min in the dark. Then the sample was filtered onto black 25 mm polycarbonate membrane filters (0.2 μm in pore size, Whatman Nuclepore™, UK) and stored at –80 °C until analysis with an epifluorescence microscope (BZ-9000, KEYENCE, Japan) following Agusti et al. (2015).

Pigments

Samples of 5 or 10 mL were filtered onto GF/F glass fiber filters (25 mm in diameter, nominal pore size 0.7 μm, Whatman, UK) under gentle vacuum pressure (< 0.013 MPa). The filter samples were immediately frozen in a deep freezer at –80 °C for later analysis. Pigment extraction and analysis were conducted according to Suzuki et al. (2015) using the *N*,*N*-dimethylformamide (DMF) bead-beating technique and Ultra-High Performance Liquid Chromatography (UHPLC). The identified pigments were grouped into photosynthetic carotenoids (PSC = fucoxanthin (Fxn)), photoprotective carotenoids (PPC = diadinoxanthin + diatoxanthin + β-carotene (Car)) and total chlorophyll (TChl = Chl *c* + Chl *a*) (Roy *et al.*, 2011).

qPCR and RT-qPCR

For the DNA and RNA analysis, water samples were filtered onto polycarbonate nucleopore filters (pore size 2 μm, Whatman, UK) with gentle vacuum pressure (0.013 MPa). Then the filters were stored in a deep freezer at –80 °C until extraction. DNA and RNA extraction and the qPCR and RT-qPCR of the *rbcL* gene were conducted following Endo et al. (2013) and Endo et al. (2015), respectively. For the *psbA* gene, standards were generated from plasmid DNA (T-Vector pMD20, TaKaRa) with *psbA* gene fragments (105 bp in size) from the culture strain. The plasmid DNA standard was then linearized with *Hind*III (TaKaRa) and quantified using a NanoDrop spectrophotometer (ND-1000, ThermoFisher Scientific Inc.). The *psbA* gene sequence was obtained using the BigDye v.3.1 Terminator Cycle Sequencing technology (ThermoFisher Scientific Inc.). PCR products were analyzed on an Automated Capillary Electrophoresis Sequencer ABI 3130*xl* (ThermoFisher Scientific Inc.). The following primers were set using Primer Premier 6 (PREMIER Biosoft International, Palo Alto, CA, USA) for the amplification of the *psbA* gene: forward 5’ GGTTACAAATTCGGTCAAG 3', reverse 5’ AGCACGAGAGTTGTTGAATGA 3’. The thermal cycler conditions for the *psbA* gene were the same as for *rbcL* in Endo et al. (2015), except that the annealing temperature was 59 °C for the *psbA* gene.

Metabarcoding of diatom-derived 18S rRNA gene

The diatom-derived 18S rRNA gene (rDNA) including the V4 region was amplified and sequenced using the Ion Torrent sequencing technique following Endo et al. (2018). The target 18S fragment of the extracted DNA was amplified using the *TaKaRa Ex Taq™* Hot Start Version (TaKaRa). Each DNA sample was amplified in triplicates. The amplicon was checked by 1.2% agarose gel electrophoresis, purified using AMPure XP magnetic beads (Beckman Coulter) and quantified with an Agilent 2100 Bioanalyzer using a high sensitivity DNA Kit (Agilent Technologies). The PCR templates were then diluted to a final concentration of 13 pM and mixed for the emulsion PCR using the Ion OneTouch™ 2 system with the Hi-Q™ OT2 Kit (Thermo Fisher Scientific). The products of emulsion PCR were enriched using an Ion OneTouch™ ES (Thermo Fisher Scientific) and then loaded onto an Ion 318™ v2 chip. Sequencing of the

amplicon libraries was performed using an Ion Torrent PGM system with the Ion PGM™ Hi-Q™ sequencing kit (Thermo Fisher Scientific).

Signal processing, base calling, and quality filtering were conducted using the Torrent Suite™ (Thermo Fisher Scientific). Sequences with polyclonal and no match against the A-adapter were initially filtered. Further quality control was conducted using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). The reads without the trP1 adapter or the reverse primer sequence were removed. The remaining reads that have at least 75% of bases with a quality score of 26 were kept and trimmed so that only the V4 part between 18 and 270 bp of each read was used for further analysis. Ten thousand reads for each sample were used for the taxonomic assignment. The reads were aligned and classified on the SILVAngs web interface (https://www.arb-silva.de/ngs/) using a local nucleotide BLAST search against the non-redundant version of the SILVA SSU Ref dataset (release 132; http://www.arb-silva.de). Identical reads were clustered into OTUs of >98% identity. Reads of each OTU were used for taxonomic assignments. Singletons and the taxonomic assignments other than diatoms were not used for the taxonomy analysis.

Maximum photochemical efficiency of Photosystem II (F_v/F_m)

The maximum photochemical efficiency of Photosystem II (PSII) (F_v/F_m) was measured at each sampling time using a pulse amplitude modulated fluorometer (Water-PAM, Walz, Germany). Before measurement, samples need to be placed in the darkness for a period of time to make all PSII reaction centers open. The measurement is initiated by switching on the measuring light. This gives a measurement of a minimum fluorescence, F_o. Then a high intensity, short duration flash of light, also called a saturation pulse is applied. This allows the contribution of photochemical quenching to be transiently reduced to zero because it transiently closes all PSII reaction centers. Provided that this flash is short enough, no increase in heat dissipation occurs. Then we get the maximum fluorescence F_m. The maximum photochemical efficiency of PSII was calculated as $F_v/F_m = (F_m - F_o)/F_m$. F_v is the variable fluorescence. F_v/F_m reflects the proportion of efficiently working PSII among the total PSII population. Cell suspensions were placed in a quartz cuvette during the measurement. The saturating pulse was provided by a red-LED with a peak illumination at 620 nm and set at 4,000 μmol photons m^{-2} s^{-1} and lasted for 400 ms for all measurements (Yan *et al.*, 2019). Relative Electron Transport Rate (rETR, relative unit) was calculated as rETR = YII × PAR × 0.5, assuming that light was equally distributed between the two photosystems. The α-slope (α), minimum saturation irradiance for rETR (I_k), and maximal relative electron transport rate ($rETR_{max}$) were obtained by fitting the curves with a quadratic equation model following Eilers and Peeters (1988). During the exposure experiment, YII was determined with actinic light of around 450 μmol photons m^{-2} s^{-1}. To quantify the changes in YII and NPQ during exposure and recovery, YII or NPQ were plotted against exposure or recovery time and the curves were fitted with first-order exponential decay functions in the form of $y = y_0 + Ae^{-kx}$. The decay constants (k) were used to estimate the rates of change in YII and NPQ.

Statistics

Differences among different incubation time and periods were tested using one-way analysis of variance (ANOVA) and *t*-test after Levene's test for the homogeneity of variances. The significance level was set at 0.05.

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