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Winter-to-spring dynamics of phytoplankton in the southern Sea of Okhotsk: community structure, photosynthetic activity, and sinking characteristics
（オホーツク海南部における植物プランクトンの冬季から春季への動態：群集構造、光合成活性、および沈降特性）

Abstract

The southern Sea of Okhotsk (SSO) is a semi-enclosed marginal sea in the North Pacific and represents the southernmost seasonally ice-covered zone in the Northern Hemisphere. In winter, it is covered by extensive sea ice driven by the East Sakhalin Current (ESC) and northerly winds, while its spring ice melt triggers a large phytoplankton bloom that contributes significantly to annual primary production. Despite the ecological importance of phytoplankton, the winter-to-spring changes in their community structure, photosynthetic activity, and contributions to the biological carbon pump remain poorly understood.

To investigate the winter-to-spring dynamics of phytoplankton and their environmental drivers in the SSO, we applied microscopy, algal pigment signatures, and 18S rRNA gene (rDNA)-based metabarcoding to characterize predominant phytoplankton assemblages during 2022 and relate them to environmental variables. As a result, diatoms were the dominant group in winter, leading to a phytoplankton bloom in spring. Other phytoplankton also showed relative contribution during winter and at the lower Chlorophyll (Chl) *a* stations in spring. Co-occurrence network analysis using 18S rDNA sequences showed that temperature-sensitive, small-sized *Chaetoceros* and nutrient-dependent, large centric diatom *Thalassiosira* exhibited strong coexistence at coastal stations during winter, making the most significant contribution to the spring bloom with higher Chl *a* concentrations. The pennate diatom *Fragilariopsis* was considered an ice-related genus, and it occurred in seawater during winter and at lower Chl *a* stations in spring. We found that not only sea ice diatoms but also winter phytoplankton in ESC-affected coastal waters can contribute to the large spring bloom in the SSO, and that could be more prevalent in less sea ice conditions due to warming.

To investigate whether the photosynthetic activity of phytoplankton also contributes to the spring bloom, we compared the differences in the expression level of the *rbcL* gene, which encodes the large subunit of the photosynthetic carbon fixation

enzyme RuBisCO, among dominant diatom families in the SSO from winter to spring in 2022 and compared the results with environmental factors. Higher diatom *rbcL* transcription efficiency, as measured by the mRNA (cDNA)/DNA copy number ratio, was observed in winter-coastal and spring waters than in winter under-ice waters, suggesting that light availability is a key driver of their photosynthetic activity. Additionally, centric diatoms exhibited significantly higher *rbcL* transcription efficiency than pennate diatoms throughout the study period. The transcription efficiency of three dominant diatom families – Chaetocerotaceae, Thalassiosiraceae, and Bacillariaceae – showed strong positive correlations with both the maximum quantum yield of photochemistry in algal photosystem II (*Fv*/*Fm*) and Chl *a* concentrations. This suggests that light-harvesting capacity and carbon fixation activity (mediated by *rbcL*-encoded RuBisCO) jointly contribute to spring bloom in the SSO.

The diversity of diatoms, manifested in their morphological and physiological traits, directly influences their capacity to export organic carbon to the deep ocean. To investigate the sinking processes of phytoplankton before and after the ice-covered period, we deployed a sediment trap at 550 m depth on the continental slope of the SSO to collect sinking particles from December 2022 to June 2023. Using scanning electron microscopy (SEM) and molecular techniques, we analyzed changes in the flux and community composition of phytoplankton within sinking particles. The results revealed two distinct peaks in phytoplankton flux over the sampling period: the first occurred during the early stage of ice coverage, when sea ice was transported into the study area, and the second, more pronounced peak occurred during the ice-melt period. The community composition analysis indicated that *Fragilariopsis cylindrus* and *Thalassiosira nordenskioeldii*, the dominant diatoms associated with sea ice, contributed most to the biological carbon pump during the ice-melting peak. These findings highlight the seasonal succession in phytoplankton export composition and emphasize the critical role of sea ice in driving diatom-mediated carbon flux to the deep ocean.

Introduction

Phytoplankton encompasses a diverse array of photosynthetic protists and bacteria. This diversity includes large-celled taxa, such as diatoms and dinoflagellates, smaller eukaryotes, such as coccolithophores and prasinophytes, and key prokaryotic cyanobacteria, notably *Prochlorococcus* and *Synechococcus* (Bar-On et al., 2018; Pierella Karlusich et al., 2020). These organisms form the trophic base of marine food webs and play indispensable roles in essential biogeochemical cycles, including oxygen generation, nutrient recycling, and the sequestration of atmospheric CO_2 (De Vargas et al., 2015). In contrast to the water-column inhabitants, a unique group of photosynthetic microorganisms, collectively known as ice algae, thrives in the extreme conditions of sea ice. During ice formation, solutes are expelled, creating high-salinity brine channels where these algae reside, either through passive entrapment or subsequent colonization (Weeks and Ackley, 1982; Eicken, 2008; Arrigo, 2014). Unlike most marine phytoplankton, ice algae possess distinct physiological and molecular adaptations to tolerate the high salinity, low temperature, and highly variable light conditions of the brine channels. Their adaptive arsenal includes synthesizing osmolytes, such as dimethylsulfoniopropionate (DMSP), secreting protective extracellular polymeric substances (EPS), and forming resting stages (Kirst et al., 1991; Krembs et al., 2011). Furthermore, ice algae are notable for their capacity for photoacclimation to extremely low light levels, sustaining photosynthesis at irradiance levels as low as 0.02% of surface irradiance (Cota, 1985; Hancke et al., 2018). This efficient light utilization drives rapid biomass accumulation upon the return of light in spring, positioning them as crucial primary producers in Seasonal Ice Zone (SIZ) ecosystems. The contribution of ice algae to regional primary production is substantial and highly variable. In the seasonally ice-covered regions of the Arctic Ocean, they account for approximately 3–25% of annual NPP (Legendre et al., 1992), with their contribution soaring to over 60% in the permanently ice-covered central Arctic (Lee et al., 2008; Mundy et al., 2009). Temporally, their peak influence occurs during the early ice-melting period, where their release acts as a seeding mechanism for subsequent blooms, significantly contributing to seasonal primary production and carbon export (Tremblay et al., 2006; Leu et al., 2015). Thus, ice algae are not merely a supplement to phytoplankton production under challenging light and ice conditions; they represent a key driver of carbon fluxes in polar ecosystems. Given the ongoing and rapid decline of sea ice, a thorough understanding of ice algal productivity dynamics and its complex interaction with phytoplankton production is critically important for accurately predicting future changes in carbon cycling and ecosystem functioning across polar and subpolar oceans.

The southern Sea of Okhotsk (SSO) is the Northern Hemisphere's southernmost SIZ and shares characteristics with the Arctic Ocean. It is a highly productive region, supporting abundant fisheries (Kim, 2012; Kasai and Hirakawa, 2016). The mean primary production of the Sea of Okhotsk has been estimated at approximately 450 g C m^{-2} yr^{-1}, which is about four times the global average for the world's oceans (Field et al., 1998). Previous studies have pointed out that diatoms play a key role in the phytoplankton community of the Sea of Okhotsk (Sorokin and Sorokin, 1999; Suzuki et al., 2011; Kasai and Hirakawa, 2016; Matsumoto et al., 2021; Hamao et al., 2022; Yan et al., 2022). Previous studies show that the dominant diatom genera in the Sea of Okhotsk include centric diatoms such as *Thalassiosira* and *Chaetoceros*, and pennate diatoms like *Fragilariopsis* (Ohwada, 1957; McMinn et al., 2008; Kasai and Hirakawa, 2016; Matsumoto et al., 2021; Yan et al., 2022). In other polar and subpolar SIZ regions, pennate diatoms such as *Fragilariopsis*, *Nitzschia*, and *Navicula* are generally the dominant groups of the ice algal community (Poulin et al., 2011; Szymanski and Gradinger, 2016). In the Sea of Okhotsk, however, the dominant ice algae are commonly identified as *Fragilariopsis cylindrus* and *Thalassiosira nordenskioeldii*

(McMinn et al., 2008; Kasai et al., 2014; Yan et al., 2022). Among these, large chain-forming centric diatoms- *Thalassiosira* often occur with high abundance in the sea ice, sometimes reaching up to 50% of the total diatom population (McMinn et al., 2008; Yan et al., 2022).

The biological carbon pump (BCP) is one of the most important components of the oceanic carbon cycle. Through photosynthesis, phytoplankton fix atmospheric CO_2, and particulate organic carbon (POC) is subsequently transported to the deep ocean via sinking and remineralization processes (Guidi et al., 2016; Basu and Mackey, 2018). Tréguer et al. (2018) highlighted that diatom diversity—including morphological and physiological traits—significantly influences their capacity for carbon export, underscoring the importance of studying phytoplankton communities in sinking particles. Blooms of ice algae and under-ice phytoplankton in diatom-dominated polar and subpolar SIZs during the melting period drive substantial carbon fluxes, leading to the rapid sinking of carbon into the deep ocean. Few studies have analyzed the phytoplankton in sinking particles in the Sea of Okhotsk. For example, Nakamura et al. (2020) examined phytoplankton communities collected by sediment traps deployed at 280 m depth near Sakhalin Island from 1998 to 2000. They found that diatom fluxes peaked in spring and summer, but decreased by one to two orders of magnitude during periods of sea-ice cover. Similarly, Hiwatari et al. (2008) investigated sinking particles at a depth of 60 m near Mombetsu Harbor, Hokkaido, during the sea-ice covered period (January-March 2005) and reported that the particles were dominated by *Fragilariopsis* and *Thalassiosira*. In summary, our understanding of the role of phytoplankton sedimentation in sustaining the biological carbon pump in the Sea of Okhotsk remains very limited. Addressing this gap is essential for elucidating the mechanisms of carbon sequestration in the SIZs and their implications for the global ocean carbon cycle.

Scientific Questions and Aim of the Study

In the southern Sea of Okhotsk (SSO), key uncertainties continue to limit our understanding of winter–spring phytoplankton dynamics.

First, the sources seeding the spring bloom remain unclear. Although ice algae—dominated by Fragilariopsis and *Thalassiosira*—show much higher biomass than seawater phytoplankton (Yan et al., 2022), and melting experiments indicate that *Thalassiosira* survives and grows particularly well (Yan et al., 2020), this mechanism has not been verified under natural SSO conditions. The seasonal behavior of *Fragilariopsis* and the role of winter Chaetoceros, also abundant in coastal waters (Matsumoto et al., 2021; Yan et al., 2022), remain largely unknown. Second, while temperature, nutrients, and light are recognized as major drivers of primary production (Sugie and Kuma, 2008; Kishi et al., 2021), the effects of light availability and other environmental factors on the photosynthetic performance of dominant phytoplankton groups remain unexamined. Third, the processes governing phytoplankton sedimentation and carbon export—particularly the role of sea ice during the winter–spring transition—remain poorly resolved.

To address these gaps, Chapter 2 analyzes phytoplankton communities in winter and spring 2022 using microscopy, pigments, and molecular data to identify sources of bloom-forming taxa. Chapter 3 evaluates photophysiological traits to clarify how major groups respond to environmental variability. Chapter 4 uses sediment trap data (550 m, Dec 2022–Jun 2023) to characterize seasonal changes in export flux and assess the influence of sea ice on the biological carbon pump. Given the continued decline in Okhotsk sea-ice extent (Toyota et al., 2022), these results are essential for predicting future carbon-cycle responses in polar and subpolar oceans.

Results and discussion of Chapter 2

In chapter 2, I investigated the differences in phytoplankton assemblages and their controlling factors in the ice-covered SSO during winter and the open water in spring. The results showed that phytoplankton assemblages were influenced by different environmental factors in each season. In winter, phytoplankton communities were associated with ice-covered regions, whereas in spring, they were associated with bloom status. Diatoms were the dominant group in winter, leading to a phytoplankton bloom in spring. Other phytoplankton also showed relatively low contributions during winter and at the lower Chl *a* stations in spring. The large centric diatom *Thalassiosira* and the small *Chaetoceros* became widespread in coastal waters after melting of the ESC-transported sea ice, resulting in the spring bloom with higher Chl *a* concentrations. The pennate diatom *Fragilariopsis* is considered an ice-related genus (e.g., Yan et al., 2022), and it occurred in seawater during winter and at lower Chl *a* stations in spring.

Given the dominance of diatoms as commonly indicated by analytical techniques, we constructed a co-occurrence network based on diatoms' 18S rDNA sequencing and analyzed the ecological traits of the major diatom groups. We found that interactions among major diatom groups and their ecophysiological traits could significantly influence diatom assemblages. Temperature-sensitive *Chaetoceros* and nutrient-dependent *Thalassiosira* had a strong coexistence relationship at coastal stations during winter. As r-strategy-selected genera in the SSO, *Thalassiosira* and *Chaetoceros* could become dominant under optimal growth conditions. In contrast, *Fragilariopsis* emerged as a K-strategy-selected genus, allowing them to thrive under various environmental conditions. Although Yan et al. (2022) proposed the importance of *Thalassiosira* in sea ice for seeding the spring bloom in the SSO, our results suggest that winter diatom communities in ESC-influenced coastal waters closely resemble those during the spring bloom; hence, winter coastal waters contribute to the spring bloom, even in the late stage. The role of coastal diatoms in the spring bloom may become more pronounced under less sea ice conditions due to warming (Toyota et al., 2022). This study is the first to reveal the contribution of the sea ice diatom *Fragilariopsis* to the spring bloom in the SSO. Additionally, using algal pigment analysis and 18S rDNA sequencing, we quantitatively assessed and identified specific taxa of other phytoplankton groups, including cryptophytes and haptophytes. The multiple analytical approaches will help comprehensively determine the phytoplankton community changes in the SSO relevant to the decrease in sea ice extent due to warming.

Results and discussion of Chapter 3

In chapter 3, I investigated the transcriptional dynamics of the *rbcL* gene in diatoms during the transition from winter to spring in the SSO, focusing on differences among diatom taxa and their relationship to environmental factors. Our findings highlight the critical role of diatoms in driving primary production in this sub-Arctic region, particularly during spring blooms. The *rbcL* transcription efficiency of diatoms varied significantly across seasons and diatom families. In winter, under-ice seawater exhibited negligible *rbcL* transcription efficiency and low maximum photochemical efficiency (F_v/F_m) of algal photosystem II, suggesting that light availability was the primary limiting factor for photosynthetic activity. In contrast, spring bloom stations with high Chl *a* concentrations showed extremely high transcription efficiency and *Fv*/*Fm*.

Among diatom taxa, centric diatoms, particularly Chaetocerotaceae and Thalassiosiraceae, displayed higher *rbcL* transcription efficiency than pennate diatoms, such as Bacillariaceae. Chaetocerotaceae demonstrated the highest transcription efficiency during spring, particularly at bloom stations, and showed strong positive correlations with water temperature, F_v/F_m, and Chl *a* concentrations. These results underscore the adaptability of Chaetocerotaceae to changing environmental conditions and its pivotal role in sustaining high primary production during spring blooms. Conversely, pennate diatoms exhibited lower

transcription efficiency and were more negatively affected by environmental factors, such as salinity changes, limiting their contribution to phytoplankton blooms in open waters.

Furthermore, our analysis revealed that the cDNA copy number of the *rbcL* gene correlated more strongly with fucoxanthin than did DNA-based measurements. This suggests that transcriptional activity provides a more accurate representation of the functional dynamics of diatoms in response to environmental changes.

Results and discussion of Chapter 4

This study presents the first year-round analysis of sinking particles in the mesopelagic layer of the SSO from December 2022 to June 2023. Diatoms were the absolutely dominant phytoplankton group in the sinking particles throughout the deployment period. We identified two high-flux carbon export events, both linked to sea ice dynamics. The most significant diatom carbon export event occurred in early April, coinciding with the initial sea ice melt. This peak was driven by the sinking of ice algae, represented by *Fragilariopsis cylindrus* and *Thalassiosira nordenskioeldii*, which were released from melting ice. A certain contribution to this spring bloom was also made by winter coastal water species, mainly *Chaetoceros*. A second carbon export peak was observed in early February, at the onset of sea ice coverage. This event was caused by the rapid sinking of under-ice phytoplankton from the waters surrounding sea ice that was transported from the north by the East Sakhalin Current (ESC). Furthermore, we successfully amplified short DNA fragments from formalin-fixed settling particles using qPCR, and the results showed a strong positive correlation with the carbon flux estimated by microscopy. This validates the feasibility of using short-fragment molecular approaches for the quantitative analysis of such samples. However, their broader applicability, particularly for long-fragment sequencing, requires further validation. Therefore, future research on formalin-fixed samples should prioritize the use of short DNA fragments for molecular analysis and actively integrate this data with microscopic observations to get a more comprehensive understanding of the role of phytoplankton in the biological carbon pump.

Materials and methods

Scanning electron microscopy (SEM)

Seawater samples and sediment trap samples were mixed with 20% neutralized formalin (final concentration 2%) and stored in a refrigerator at 3°C until analysis. An aliquot of the samples was filtered onto Whatman polycarbonate membrane filters (pore size 2.0 μm) through a glass funnel under a vacuum pressure of <0.013 MPa. Then, the filters were rinsed with Milli-Q water to remove salts and dried at room temperature. The dried filters were coated with Pt-Pb alloy by vapor deposition using an MSP-1S magnetron sputter device (Vacuum Device, Japan). The samples were transferred to a scanning electron microscope (SEM, VE-8800, KEYENCE Corp. Japan) to identify and enumerate armored phytoplankton cells, including silicified phytoplankton (i.e., diatoms, silicoflagellates, and parmales) and thecal-plates-containing dinoflagellates. Species identification at the genus or species level was performed with references (Tomas, 1997; Von Quillfeldt, 2001; Nakamura et al., 2020). The cell biovolumes of armored phytoplankton were calculated from the dimensions and shapes (Hillebrand et al., 1999). The carbon biomass was evaluated using the empirical formula: $\mathrm{Log}_{10}C = \log_{10}A + B \times \log_{10}V$ (Menden-Deuer and Lessard, 2000), where A and B are constants based on phytoplankton taxa and cell volume, C represents carbon biomass (pg C cell^{-1}), and V indicates cell volume (μm^3).

Pigments

Water samples (1.2 L) were filtered onto 25 mm Whatman GF/F glass-fiber filters (0.7 μm pore size) under low vacuum pressure (<0.013 MPa). The obtained filters were blotted with qualitative filter papers and stored in a deep freezer (−80°C) or liquid nitrogen until analysis on land. Phytoplankton pigments were extracted with *N*,*N*-dimethylformamide (DMF) using the bead-beating method and analyzed by ultra-high-performance liquid chromatography (UHPLC) (Suzuki et al., 2015). The contributions of each phytoplankton group to Chl *a* concentration were estimated using pigment concentrations through the simulated annealing method of the R package "phytoclass" (Hayward et al., 2023). The default settings for the simulated annealing method by Hayward et al. (2023) were used, and the initial pigment ratio matrix was obtained from Suzuki et al. (2002).

qPCR and RT-qPCR

For DNA and RNA analyses, water samples were gently filtered onto polycarbonate Nuclepore filters (2 μm pore size; Whatman, UK) under low vacuum (0.013 MPa). Filters were immediately stored at −80 °C until nucleic acid extraction. DNA and RNA extraction, as well as qPCR and RT-qPCR targeting the *rbcL* gene, were performed according to the protocols of Endo et al. (2013, 2015). The abundance of diatom-specific 18S rRNA (rDNA) and *rbcL* gene fragments was quantified using qPCR with primers specific for diatoms, as described by Zimmermann et al. (2011) and John et al. (2007), respectively. Primer design and amplification procedures followed the general methods outlined in Endo et al. (2018).

For RNA analysis, filters were immersed in 600 μL of RNAlater solution (Thermo Fisher Scientific) in cryovials and stored either in liquid nitrogen or at −80 °C until extraction. Extracted RNA was reverse-transcribed into complementary DNA (cDNA) using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara). qPCR assays were performed in triplicate 10 μL reaction mixtures, each containing 1 μL of genomic DNA or cDNA, 5 μL of TB Green Premix Ex Taq™ II (Takara, Japan), 0.4 μL each of forward and reverse primers, and 3.2 μL of DNase-free Milli-Q water. Quantification of *rbcL* gene DNA and transcripts (cDNA) was carried out on a CFX Connect Real-Time PCR Detection System (Bio-Rad, USA) using the diatom-specific primers from John et al. (2007).

Metabarcoding of diatom-derived 18S rRNA gene and rbcL gene

The V4 region of the 18S rDNA was amplified using the primers V4_18SNext.For and V4_18SNext.Rev (Piredda et al., 2017) and KOD FX Neo polymerase (Toyobo), following the manufacturer's protocol with an annealing temperature of 50 °C. Libraries were prepared and sequenced at Bioengineering Lab Co., Ltd. on an Illumina MiSeq platform to obtain paired-end reads. Sequence processing was performed in QIIME2 (v2022.8) using the DADA2 plugin (Callahan et al., 2016). Reads were quality-filtered, denoised, dereplicated, and checked for chimeras using the "dada2 denoise-pyro" command, and rare ASVs were removed with "feature-table filter-features." Taxonomic assignment was conducted using a naïve Bayes classifier ("q2-feature-classifier") trained on the PR2 database (v5.0.1).

For *rbcL* metabarcoding, gene fragments were amplified from DNA or cDNA using the diatom-specific primers of John et al. (2007) and KOD FX Neo polymerase. Library construction and MiSeq paired-end sequencing were performed by Bioengineering Lab Co., Ltd. Raw reads were first demultiplexed with fastx_barcode_splitter (FASTX Toolkit v0.0.14), retaining only sequences with complete primer matches. Primer sequences were removed using fastx_trimmer, after which

low-quality reads (Q < 20) and reads ≤ 40 bp were removed with sickle (v1.33). Paired-end reads were merged using FLASH (v1.2.11) with a minimum 10 bp overlap. Merged reads were processed in QIIME2/DADA2, followed by taxonomic identification using BLASTN against the GenBank *rbcL* database. ASVs accounting for ≥0.2% of total reads and showing >95% similarity to reference sequences were assigned to diatom families; ASVs matching multiple families were treated as unidentified diatoms.

Maximum photochemical efficiency of Photosystem II (F_v/F_m)

The maximum photochemical quantum efficiency of photosystem II (PSII), expressed as Fv/Fm, was assessed at each sampling period using a pulse-amplitude-modulated fluorometer (Water-PAM, Walz, Germany) after 30 min of dark acclimation, following (Suzuki et al., 2014). The F_v/F_m ratios were determined using the formula $F_v/F_m = (F_m - F_o)/F_m$, where F_o represents the minimum fluorescence yield recorded after 30 min of dark acclimation, and F_m denotes the maximum fluorescence yield obtained under the saturating pulse by the fluorometer.

Statistics

Environmental and community patterns were analyzed using heatmaps, clustering, PCoA, and RDA in R. Diatom ASV-level *rbcL* transcription efficiency was estimated by combining qPCR-based DNA/cDNA copy numbers with ASV relative abundances and visualized with heatmaps and boxplots. Statistical tests, including Spearman's correlation, Mann–Whitney *U test*, Mantel tests, linear regression, and permutation-tested RDA, were used to evaluate relationships among diatom transcription activity, taxonomic groups, and environmental factors. All statistical analyses were performed in R.

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