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Seasonal variability of the protist community and production in the southern Okhotsk Sea revealed by weekly monitoring

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ABSTRACT

To evaluate seasonal changes in a protist community (diatoms, dinoflagellates and ciliates) and their production in the southern Okhotsk Sea, water sampling was conducted approximately once a week from July 2016 to July 2017 at Mombetsu Port. Diatoms were the predominant category of the three taxa throughout the study period. The abundances of all taxa were high from March to September and were low from October to February. Four groups (A–D) were identified by cluster analysis according to abundance. Group A, of which *Thalassiosira* spp. were dominant, occurred in March. Group B showed the highest abundance (7.7×10^4cells L^{-1}), with *Chaetoceros* spp. and *Pseudo-nitzschia* spp. being dominant. In group C, *Odontella* spp. and pennate diatoms were dominant and occurred from November to February. Group D was mainly observed in late October, and pennate diatoms were dominant. Seasonal changes in the predominant species of diatoms were explicitly observed, and these changes were associated with water mass exchanges. For dinoflagellates, abundance was highest in July 2016, and biomass and production were highest in September. Large fluctuations in the ciliate biomass in July 2016 might have been induced by the high growth rate, which reflects the high chlorophyll *a* concentration and water temperature and a decrease in predation pressure. The estimated primary production was lower than that reported in previous studies, which could reflect differences in the depth of the research areas and the methods used for estimating production. In the coastal area of the southern Okhotsk Sea, where production is high, not only the species composition but also the protist community varied clearly with season because of variations in the water mass.

1. Introduction

The coastal area in the southern Okhotsk Sea off Hokkaido is an important region because it has high primary production and abundant fishery resources, including Japanese scallop and salmon. In this area, the warm and saline Soya Warm Current and cold and less-saline East Sakhalin Current are exchanged seasonally; the former can be observed from March to November, and the latter can be observed from November to March (Aota, 1975; Takizawa, 1982; Fukamachi et al., 2008; Mustapha et al., 2009). In addition, this area is covered by sea ice from February to March, and this sea ice is transported from the northern Okhotsk Sea. Because of the water mass exchanges and sea ice coverage, marine ecosystems in the coastal area of the southern Okhotsk Sea vary greatly with season (Taguchi et al., 2000; Kasai et al., 2010; Hikichi et al., 2018). However, the information of dynamics for primary producers (e.g., diatoms) and mechanisms to high productivity is not sufficient for sustainable management of fisheries resources.

Protists, which support marine ecosystems, consist of autotrophs, such as diatoms; heterotrophs, such as ciliates and radiolarians; and mixotrophs, such as some species of dinoflagellates. In the Okhotsk Sea, previous studies have reported that diatom species are different in warm and cold waters (Kasai and Hirakawa, 2015), and spring diatom blooms are composed mainly of *Thalassiosira nordenskioeldii*, *Thalassiosira gravida*, *Thalassiosira eccentrica* and *Chaetoceros subsecundus* in the coastal surface water (Sorokin and Sorokin, 1999). Dinoflagellate species are represented by autotrophs, heterotrophs and mixotrophs (Gaines and Elbrächter, 1987). In addition, many studies have been conducted on *Alexandrium tamarense*, which causes paralytic shellfish poison in the Okhotsk Sea (Shimada et al., 2010). Ciliates are heterotrophic protozooplankton and are able to feed on bacteria and

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small phytoplankton, such as pennate diatoms (Capriulo and Carpenter, 1983; Gast, 1985; Paranjape, 1987), and they also play an important role in microbial loops (Pomeroy, 1974). Their growth rate is controlled not only by the quality and quantity of their food but also by the water temperature, especially under cold conditions, such as in polar regions; their growth rate increases in warmer temperatures (Hansen and Jensen, 2000). In spite of their contribution to primary production and their important role in microbial loops, protist communities have not been well-studied along the coastal area of the Okhotsk Sea. Additionally, previous studies of phytoplankton mainly revealed aspects of the chlorophyll *a* (Chl *a*) concentration (Hamasaki et al., 1998; Taguchi et al., 2000; Kasai et al., 2017). Although Sildever et al. (2019) investigate the variability and the species composition of dinoflagellates in the study area with a metagenomic approach, the compositions of the other protist communities still have not been clarified in this region. It is reported that zooplankton communities vary in accordance with the seasonal exchange of water masses (Hikichi et al., 2018), but a relationship between the variability in protist communities and the exchange of water masses is still unknown.

In this study, seasonal variability in the abundance and community structure of protists (diatoms, dinoflagellates and ciliates) was investigated to clarify the relationship among the protists and environmental factors. We also used cell volumes and water temperature to estimate protist production so that we could discuss seasonal changes in this production.

2. Materials and methods

Surface water samples were collected with a bucket at intervals of approximately one week from 6 July 2016 to 3 July 2017 from a bridge that connects a pier and the Okhotsk Tower in Mombetsu Port at 9 m depth (Fig. 1). Samples containing 500 mL of water were preserved with 1% glutaraldehyde. Approximately 100 mL of sea surface water was filtered through a GF/F filter to measure the Chl *a* concentration. The filter samples for Chl *a* were immersed in N,N-dimethylformamide (DMF), and the Chl *a* concentration was measured by a fluorometer (Turner Designs, Inc., 10-AU) according to Welschmeyer (1994). The sea water from the surface was frozen to analyse the nutrients. Nutrients were measured by an autoanalyser (Bran + Luebbe, AACS-III) according to Parsons et al. (1984). Water temperature and salinity were measured by CTD (JFE Advantech Inc., ASTD102). Data on air temperature, rainfall, the maximum wind speed, and wind direction were obtained from the web site of the Japanese Meteorological Agency (https://www.data.jma.go.jp/obd/stats/etrn/index.php).

The fixed protist samples were stored on a flat table for more than 1 day to allow the microprotist cells to settle to the bottom of the bottle. Then, the samples were concentrated down to 20 mL using a siphon. Subsamples (1 mL) were mounted on a glass microscope slide with a micro pipet. Identification, counting and measuring of the cells were conducted with an inverted microscope (Nikon, Eclipse-TE200) with 40–600× magnification by the bright field observation. The genera of the diatoms were identified according to Hasle and Syvertsen (1997) and Hoppenrath et al. (2009). Concerning dinoflagellates, species identification was not conducted. Ciliates species were counted for two groups: loricate and aloricate ciliates. Counting was conducted for cells larger than 10 μm. The abundance of each taxon in the samples (cells L^{-1}) was calculated using the number of cells in the sub-samples and the concentration. The cell volumes of each taxon were (μm^3) calculated from the cell sizes, which were measured according to Sun and Liu (2003). Then, they were converted into carbon biomass using the conversion equation from Menden-Deuer and Lessard (2000). The production of diatoms and dinoflagellates (mg C m^{-3} day^{-1}) was estimated by the regression formula of Marañón (2008): $\log_{10} P = b \log_{10} V + a$ (P: production (pg C $cell^{-1}$ day^{-1}); V: cell volume (μm^3); a, b: constant for diatoms ($a = -1.25$ and $b = 1.01$) and for dinoflagellates ($a = -0.91$ and $b = 0.89$)). Production of ciliates (mg C m^{-3} day^{-1}) was estimated by multiplying the growth rate (d^{-1}) calculated according to Montagnes et al. (1988) by the carbon biomass, as described above. The growth rate was determined by the following formula: $\ln r = 0.1488\ t - 0.3285 \ln (V \times 10^{-3}) - 1.3815$ (r, growth rate (d^{-1}), t: water temperature (°C), and V: cell volume (μm^3)).

A cluster analysis was performed based on protist abundance data using software (PRIMER7, PRIMER-E Ltd.). The abundance (X: cells L^{-1}) for all of the taxa were fourth root transformed ($X^{1/4}$) prior to the analysis to reduce any bias in the abundance (Quinn and Keough, 2002). Similarities between the protist samples were calculated using the Bray–Curtis similarity index. To group the samples, the similarity indices were coupled with hierarchical agglomerative clustering using a complete linkage method (Unweighted Pair Group Method using Arithmetic Mean: UPGMA) (Field et al., 1982). Non-metric Multidimensional Scaling (NMDS) ordination was performed to distribute the groups onto a two-dimensional map. Pearson regression analyses were performed to clarify which environmental parameters (surface temperature, surface salinity, Chl *a* concentration, nitrate + nitrite concentration, phosphate concentration, silicate concentration, air temperature and rainfall) had significant relationships with the groups. Inter-group differences in the major taxa and environmental parameters were tested by using a one-way ANOVA, followed by Tukey–Kramer post hoc test.

Structural Equation Modelling (SEM) was performed to evaluate the factors controlling the variations in protist parameters (abundance, biomass and production) (Stomp et al., 2011). For SEM analysis, environmental parameters (rainfall, maximum wind speed, surface temperature, surface salinity and nitrate + nitrite concentration) and protist parameters were transformed into normalized values (average = 0, standard deviation = 1), and correlation coefficients between all parameters were calculated. For the path analysis, the parameters were grouped into the following three categories: (1) atmospheric parameters (rainfall, maximum wind speed), (2) hydrographic parameters (surface temperature, surface salinity and nitrate + nitrite), and (3) protist parameters. Non-significant interactions (p>0.05) were deleted from the final model. The goodness-of-fit index (GFI) and the adjusted goodness-of-fit index (AGFI) were used to evaluate the fitting of the models. The standardized path coefficients were independent variables, which indicate the relative contributions of the different paths within the SEM (Stomp et al., 2011). The SEM analysis was performed using add-in software for MS-Excel (http://www.ohmsha.co.jp/data/link/978-4-274-06925-3/).

3. Results

3.1. Hydrography

Soya Warm Current is driven by the sea level difference (SLD) between Japan Sea and Okhotsk Sea (Aota, 1975; Fukamachi et al., 2008). When East Sakhalin Current prevails in the Okhotsk Sea, this SLD becomes small, resulting in decrease in the volume of Soya Warm Current Water into the Okhotsk Sea. The SLD started to decline from November 2016 and showed the minimum in the middle of January 2017 (Fig. 2f). It kept increasing thereafter until late April 2017 and returned to the same level as that of when it started to decrease. The surface sea temperature (SST) ranged from −1.7 to 22.3 °C, and it had the maximum in August 2016 and the minimum in February 2017. From January to March 2017, it was approximately 0 °C, and from July to August, it showed

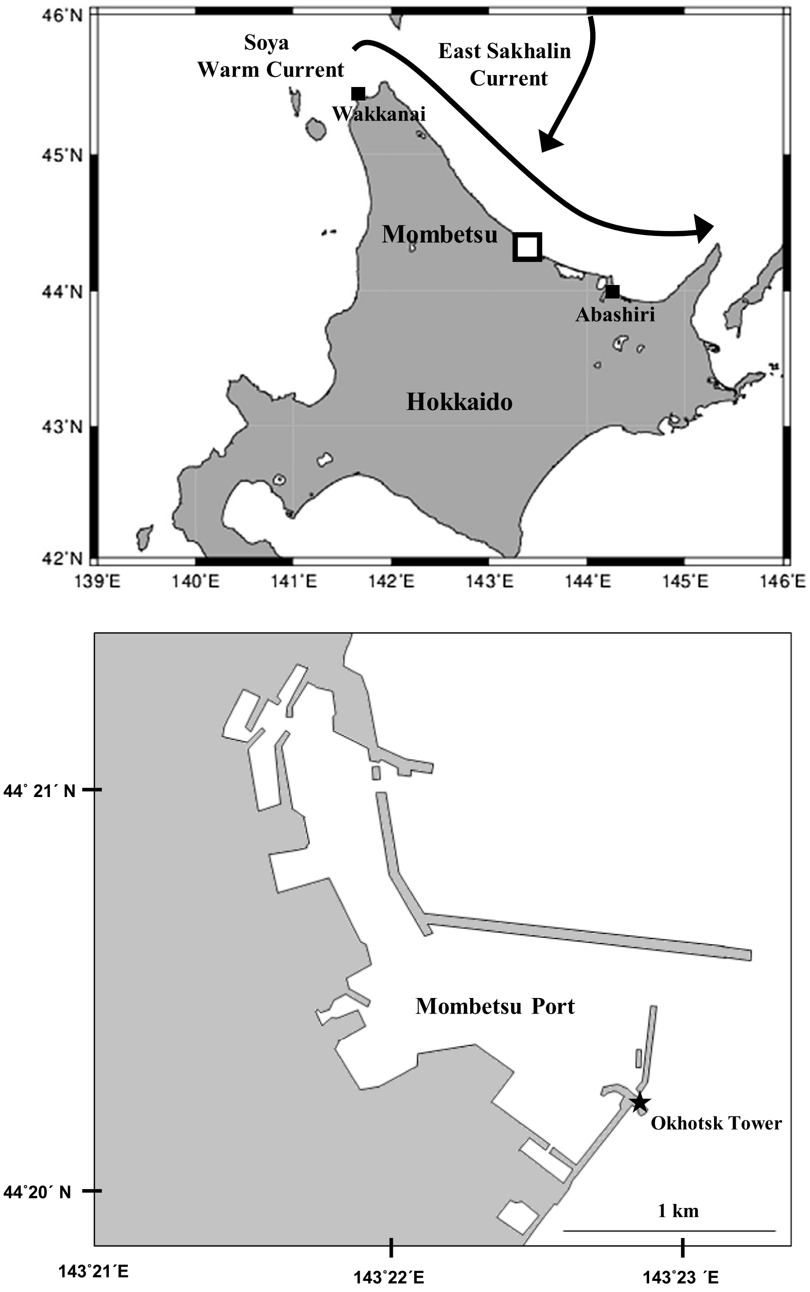

Fig. 1. Location of the sampling station (Okhotsk Tower) in Mombetsu Port in north-eastern Hokkaido. Arrows indicate the approximate direction of the current flows.

higher values (> 15 °C) (Fig. 2a). The surface sea salinity (SSS) ranged from 20.85 to 33.59, and it declined sporadically due to rain fall through the study period. Low salinity in April 2017 might be attributed to melting of sea ice (Fig. 2a). The Chl *a* concentration ranged from 0.24 to 6.1 μg L^{-1}, and there were large peaks in July 2016, September 2016 and March 2017 (Fig. 2b). The total nitrate and nitrite concentration ranged from 0 to 16.2 μM. It increased from November 2016 to March 2017 and decreased sharply in late March 2017. It also increased rapidly in April and June 2017 (Fig. 2b). The silicate concentration ranged from 1.4 to 117.5 μM and showed high values in September 2016 and from April to June 2017 (Fig. 2c). The phosphate concentration ranged from 0.063 to 1.2 μM, and it increased from the end of October 2016 to the end of January 2017 and declined thereafter to April 2017 (Fig. 2c). The air temperature (average) ranged from −10.2 to 27.4 °C, and it was highest on 21 July 2016 and was lowest on 11 January 2017 (Fig. 2d). Daily rainfall ranged from 0 to 75.5 mm, and it was highest during August 2016 (Fig. 2d). The daily maximum wind speed ranged 2.3 to 13.5 m s^{-1}, and the frequency of strong winds (> 5 m s^{-1}) was higher in September and October 2016 than that in spring (March and April 2017) (Fig. 2e). In 2017, sea ice could be observed from 26 January to 11 April at Mombetsu, and the period of visible sea ice was 7 days longer than that of normal years (cf. website of sea ice information of the City of Mombetsu: http://okhotsk-mombetsu.jp/).

3.2. Seasonal variability of abundance

The abundance of the diatoms ranged from 1.55×10^3 to 5.36×10^5 cells L^{-1}, that of dinoflagellates ranged from N.D. (not detected) to 5.28×10^3 cells L^{-1}, and that of ciliates ranged from N.D. to 5.92×10^3 cells L^{-1} (Fig. 3a). The abundance of each

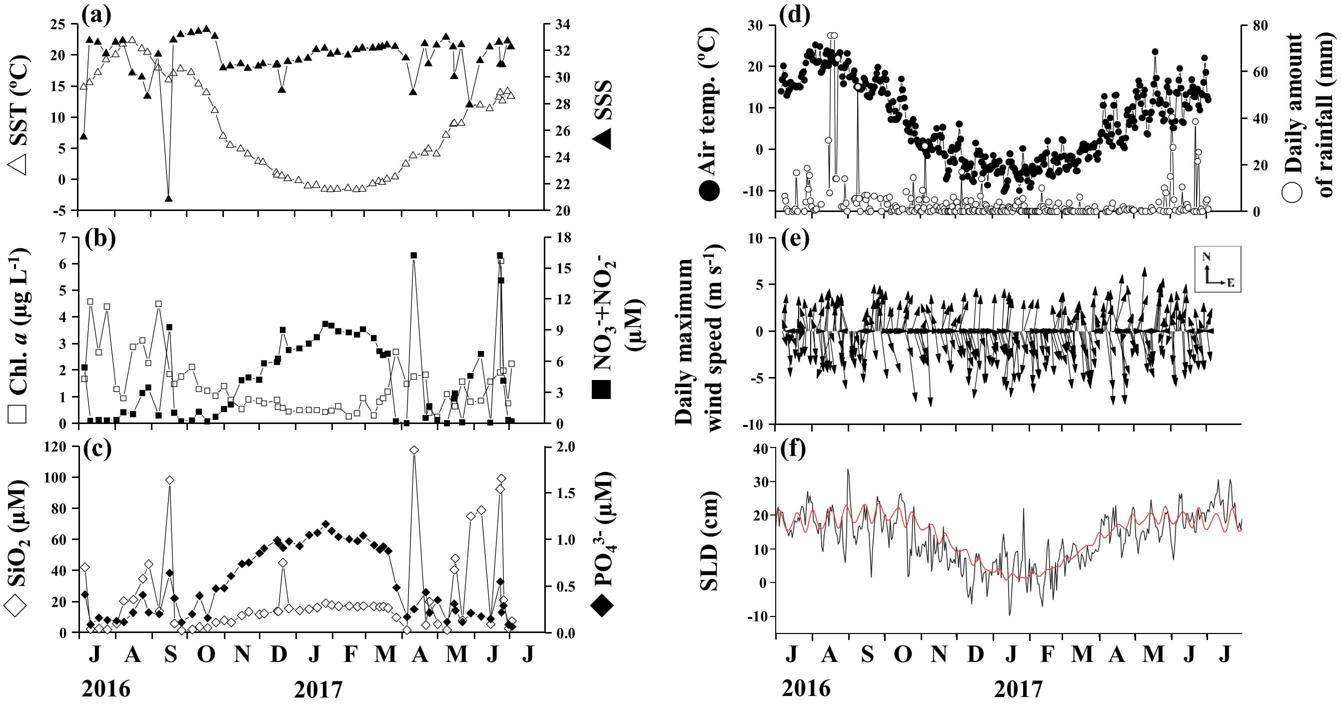

Fig. 2. Seasonal changes in environmental parameters. (a) Daily mean sea surface temperature (SST) and salinity (SSS), (b) chlorophyll *a* and nitrate and nitrite (NO_3^+ and NO_2^-) at sea surface, (c) silicate and phosphate at the sea surface, (d) daily averaged air temperature and daily amount of rainfall, (e) daily maximum wind speed and direction at Mombetsu Port and (f) daily mean sea level difference (SLD) between Wakkanai and Abashiri (cf. Fig. 1) (black and red lines indicate SLD observed and SLD calculated, respectively) from July 2016 to July 2017.

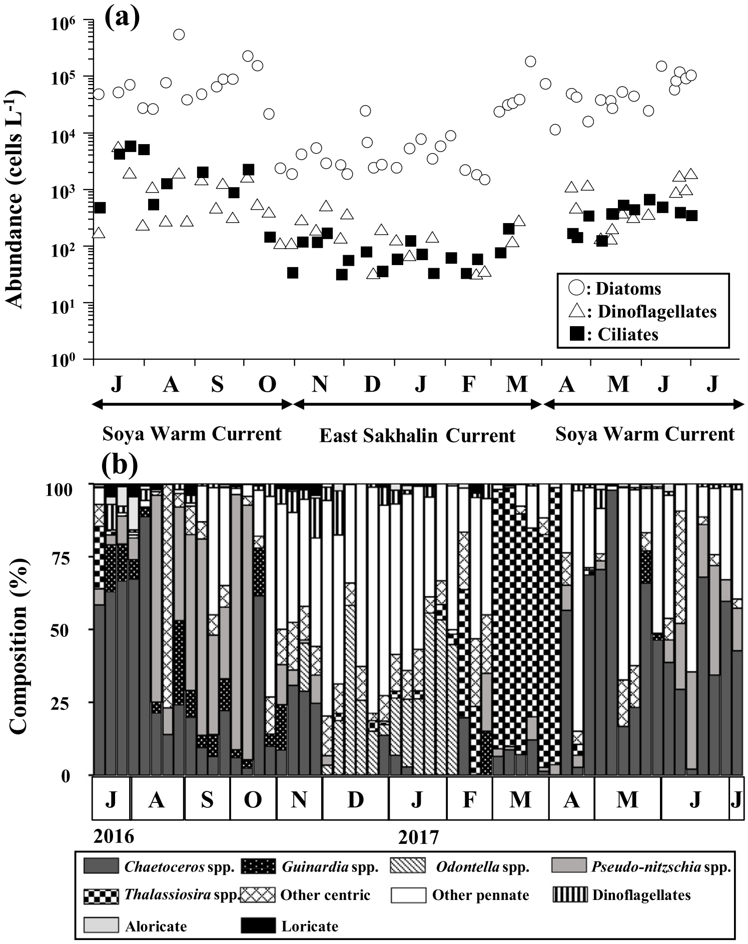

Fig. 3. Seasonal changes in protist abundance and the predominant water masses (a) and species composition (b) at Mombetsu Port from July 2016 to July 2017.

Table 1
Comparisons of environmental variables (temperature, salinity, chlorophyll *a*, nitrate + nitrite, phosphate, silicic acid, air temperature and rainfall) and microplankton species for the four groups (A-D) at Mombetsu Port from July 2016 to July 2017. The four groups were identified from Bray-Curtis similarity based on microplankton abundance (cf. Fig. 4a). Results are shown as mean. Differences between groups were tested by one-way ANOVA with the Tukey-Kramer HSD post-hoc test. Any groups not connected by underlines are significantly different ($p < 0.05$). Numbers in parentheses indicate the number of stations included in each group. NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Parameters	Groups A (6)	 B (33)	 C (15)	 D (3)	one-way ANOVA	Tukey-Kramer test
SST	0.23	13.2	0.17	5.5	***	C A D B
SSS	32.1	31.1	31.3	32	NS	
Chl.*a*	1.2	1.8	0.6	1.1	*	C D A B
Nitrate + Nitrite	4.8	3.1	7.4	3.7	*	B D A C
SiO_2	12.9	28.7	17	10.4	NS	
PO_4	0.71	0.27	0.98	0.66	***	B D A C
Air temperature	0.9	12.6	-3.6	0.6	***	C D A B
Rainfall	1.1	2.4	0.4	0.2	NS	
Chaetoceros spp.	2268	22551	185	139	***	D C A B
Guinardia spp.	0	2487	0	172	*	Not detected
Odontella spp.	0	0	2039	0	**	Not detected
Pseudo-nitzschia spp.	1500	22150	10	137	NS	
Thalassiosira spp.	52301	420	167	0	***	D C B A
Other centric diatoms	1855	16192	524	229	NS	
Other pennate diatoms	5098	11955	2623	944	*	D C A B
Total diatoms	63102	75755	5549	1620	*	D C A B
Dinoflagellates	62	813	80.2	80.4	**	A C D B
Aloricate ciliates	17	477	25	0	NS	
Loricate ciliates	30	363	26	12	NS	
Total ciliates	47	840	52	12	NS	
Whole taxa	63121	77409	5681	1712	*	D C A B

taxon was high from July to September 2016 and from March to July 2017, and it was low from October 2016 to February 2017. In this study, diatoms were most dominant among the three taxa. The species composition of diatoms showed seasonality, and had a summer–autumn growth period (July–October), the minimum during late autumn to winter (November–February), a spring bloom (March–April), and then recurrence of a summer growth period (April/May–July). From July 2016 to the beginning of August 2016, *Chaetoceros* spp. mainly dominated (58.3–88.7%), and *Pseudo-nitzschia* spp. were mainly dominant from the middle of August 2016 to the middle of October 2017 (39.0–87.7%), although other centric diatoms (mainly *Skeletonema* spp.) were temporarily dominant (76.7%) at the end of August 2016. From the end of October 2016 to the beginning of December 2017, other pennate diatoms (pennates excluding *Pseudo-nitzschia* spp.) were mostly dominant (36.7–74.2%). *Odontella* spp. and other pennate diatoms dominated from December 2016 to the beginning of February 2017 (3.7–58.2% and 33.3–77.5%, respectively), and *Thalassiosira* spp. were mainly dominant from March 2017 to the beginning of April 2017 (64.9–95.0%). From the middle of April 2017 to the beginning of July 2017, *Chaetoceros* spp. were mostly dominant again (29.3–97.7%). Dinoflagellates showed the highest value in July 2016. Ciliates showed high values in July and August 2016. Both of loricate and aloricate ciliates showed their highest values in July 2016.

3.3. Cluster analysis based on abundance

Protist communities were divided into 4 groups (A–D) at 45% and 52% similarity by cluster analysis based on abundance (Fig. 4a). The environmental parameters that had a significant relationship with the NMDS of each group were Chl *a*, air temperature, sea surface temperature, and nitrate + nitrite (Fig. 4b). Group A, which occurred in March and April 2017, had high abundance (6.3×10^4 cells L^{-1}) with a predominance of *Thalassiosira* spp. in March 2017 (Fig. 4c, d). Group B was observed from July to October 2016 and from April to July 2017. This group had the highest abundance (7.7×10^4 cells L^{-1}), and *Chaetoceros* spp. and *Pseudo-nitzschia* spp. were dominant. Group C was observed in winter (November 2016 to February 2017), with a predominance of *Odontella* spp. and other pennate diatoms. Group D was observed at the end of October 2016 and February 2017. This group showed the lowest abundance (1.7×10^3 cells L^{-1}), and other pennate diatoms were dominant (Fig. 4c, d).

The environmental parameters that had an inter-group difference were surface temperature, Chl *a*, nitrate + nitrite, phosphate and air temperature (Table 1). Group B was characterized by high air temperature, high sea surface temperature, low nutrient concentration and high Chl *a* concentration. In contrast to group B, group C was characterized by low air temperature, low surface temperature, high nutrient concentration and low Chl *a* concentration. Groups A and D had the middle values in the environmental parameters between groups B and C. Protist abundance was high in groups A and B and was low in groups C and D. In addition, group A was characterized by a predominance of *Thalassiosira* spp., and group B was characterized by predominance of *Chaetoceros* spp.

3.4. Seasonal changes of biomass and production

Carbon biomass of diatoms, dinoflagellates and ciliates ranged from 0.9 to 61 mg C m^{-3}, from N.D. to 21 mg C m^{-3} and from N.D. to 33 mg C m^{-3}, respectively (Fig. 5). Diatoms were dominant in biomass among the three taxa, which was the same as for the abundance. Seasonal changes occurred in diatom carbon biomass, which peaked in August 2016 and March 2017. Carbon mass in dinoflagellates peaked in September 2016 (Fig. 5a), and that of ciliates peaked in July 2016. The composition in carbon biomass was dominated by mainly *Chaetoceros* spp. and *Guinardia* spp. from July to August 2016, and ciliates were dominant at the end of July 2016 and the beginning of August 2016 (Fig. 5b). From September 2016 to the middle of December 2016, dinoflagellates and other centric diatoms dominated, and from the middle of December 2016 to the beginning of February 2017, *Odontella* spp. and other centric diatoms dominated. From the beginning of February 2017 to the beginning of April 2017, *Thalassiosira* spp. dominated, and from the middle of April 2017 to the beginning of July 2017, *Chaetoceros* spp. and other pennate diatoms dominated.

The production of diatoms, dinoflagellates and ciliates ranged from 1.5 to 69 mg C m^{-3} day^{-1}, from N.D. to 9.2 mg C m^{-3} day^{-1} and from N.D. to 41 mg C m^{-3} day^{-1}, respectively (Fig. 6). The production of diatoms showed a pulse-like variation, with dinoflagellates showing a peak in late September 2016 but with a low value compared to that of the other taxa. The production of ciliates peaked in July 2016.

3.5. SEM Analysis

From the SEM analysis on the protist parameters (abundance, biomass and production) of each taxon, high values were observed for GFI (0.94–0.95) and AGFI (0.87–0.90) (Fig. 7). The abundance of diatoms had positive correlations with water temperature and rainfall, and both the biomass and production of diatoms had a positive correlation with dinoflagellates. Regarding diatom production, a positive correlation with the maximum wind speed was observed. For dinoflagellates, ciliates had a positive effect on the abundance, and water temperature had a positive effect on the biomass and the production. Every parameter for the ciliates had a strong positive correlation with water temperature (path coefficients: pc = 0.47 to 0.63).

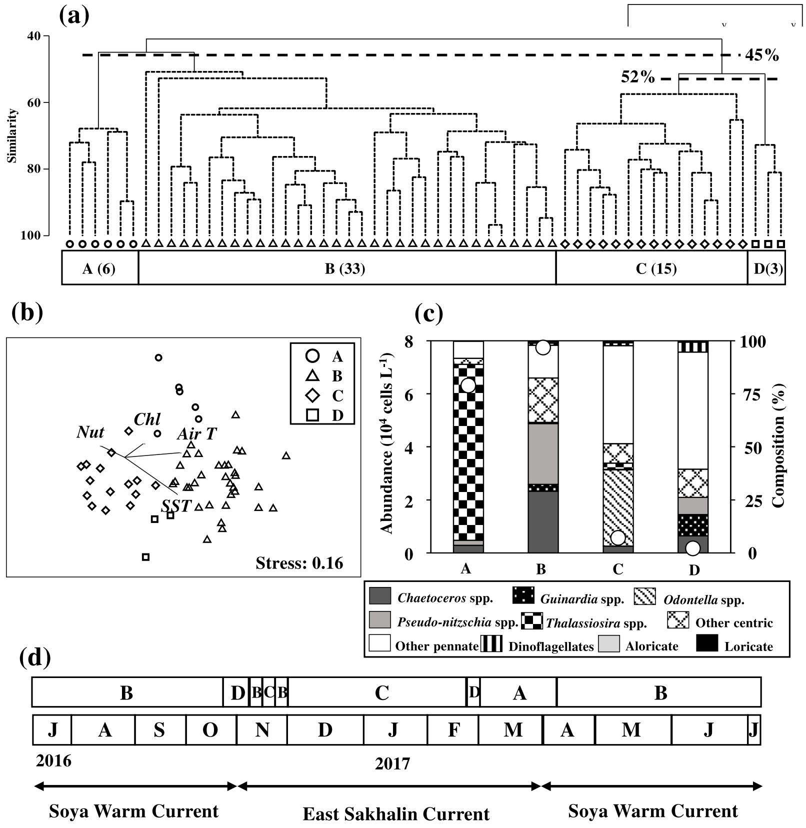

Fig. 4. Results of cluster analysis based on protist abundance by Bray–Curtis similarity connected with UPGMA. Four groups (A–D) were identified with the dissimilarity at 45 and 52%, respectively (dashed lines) (a). Numbers in the parentheses indicate the quantity of samples included in each group. Non-metric multi-dimensional scaling plots of the four groups, with arrows indicating directions of environmental parameters (b). Air T: air temperature, Chl *a*: chlorophyll *a*, Nut: nitrate and nitrite, SST: sea surface temperature. Abundance and species composition of each group (c). Seasonal changes of the occurrence for protist groups and the predominant water masses (d).

4. Discussion

4.1. Seasonal change of Chl a concentration

In the coastal region of the southern Okhotsk Sea, two of the major water masses, Soya Warm Current Water (SWCW) and East Sakhalin Current Water (ESCW), are seasonally exchanged. According to previous studies, salinity of SWCW and ESCW is > 33.6 and < 32.0, respectively (e.g., Itoh and Ohshima, 2000). However, this definition is not appropriate to the classification of water masses in this study because due to the sampling location where was very close to the shore, the hydrographic conditions might be affected by terrestrial waters (e.g., river water inflow). Actually, in this study, sea surface salinity (SSS) did not excess 33.6 (the maximum was 33.58). Thus, water masses should not be defined strictly in this study by the definition reported in the previous studies. However, considering the seasonal change of the SLD and SSS, in the southern Okhotsk Sea, the effect of Soya Warm Current might exist from July to October 2016 and from April to July 2017, and that of East Sakhalin Current might exist from November 2016 to April 2017 (cf. Fig. 2, Supplementary).

Many previous studies have reported on Chl *a* concentration in the study area (Hamasaki et al., 1998; Taguchi et al., 2000; Kasai et al., 2010, 2017), and the results of this study were within the values of the previous studies. According to Hamasaki et al. (1998), in Mombetsu Port, Chl *a* is common for three periods: June to October (SWCW is dominant), November to February (ESCW is dominant)/April to May (the transition from ESCW to SWCW), and March (sea ice period). They also report that hydrographic conditions oscillate between two periods: June to October (SWCW) and November to May (ESCW). Considering the succession of these periods correspond to that of the major water masses, seasonal changes of Chl *a* and hydrographic conditions such as nutrients in the study area may be related to the exchange of water masses. Actually, those periods were also observed in this study. The peaks of Chl *a* concentration were observed in July and September 2016 and March and June 2017. When the species composition was compared at the time of these peaks, *Chaetoceros* spp. were dominant at the peaks in July 2016 and June 2017, and *Pseudo-nitzschia* spp. were dominant in September 2016. These species might contribute to the peak in the Chl *a* concentration. In March 2017, when sea ice retreat might occur, *Thalassiosira* spp. were dominant. Because these species show high dominance in the ice algae communities of the study area (Kasai et al., 2014), the peak in March might be partly caused by *Thalassiosira* spp., which are thought to be released from melting sea ice. Nutrient concentration began to increase in November 2016, when high-nutrient East Sakhalin Current might arrive, and this concentration decreased rapidly in March 2017, when the influence of East Sakhalin Current might weaken. This decrease might also attribute to the consumption of nutrients by diatom blooms because Chl *a* concentration increased at the same timing. Thus, major seasonal changes in the nutrient concentration consisted of the exchange of water masses observed in the area. In addition, nutrient concentration sporadically increased when the rainfall was high. This might be caused by the inflow of fresh water from a river near the sampling station. In addition, in the SEM analysis, rainfall had a positive effect on the abundance of diatoms. This effect suggests that the rapid increase in river water from the rainfall induced the input of high nutrients, which

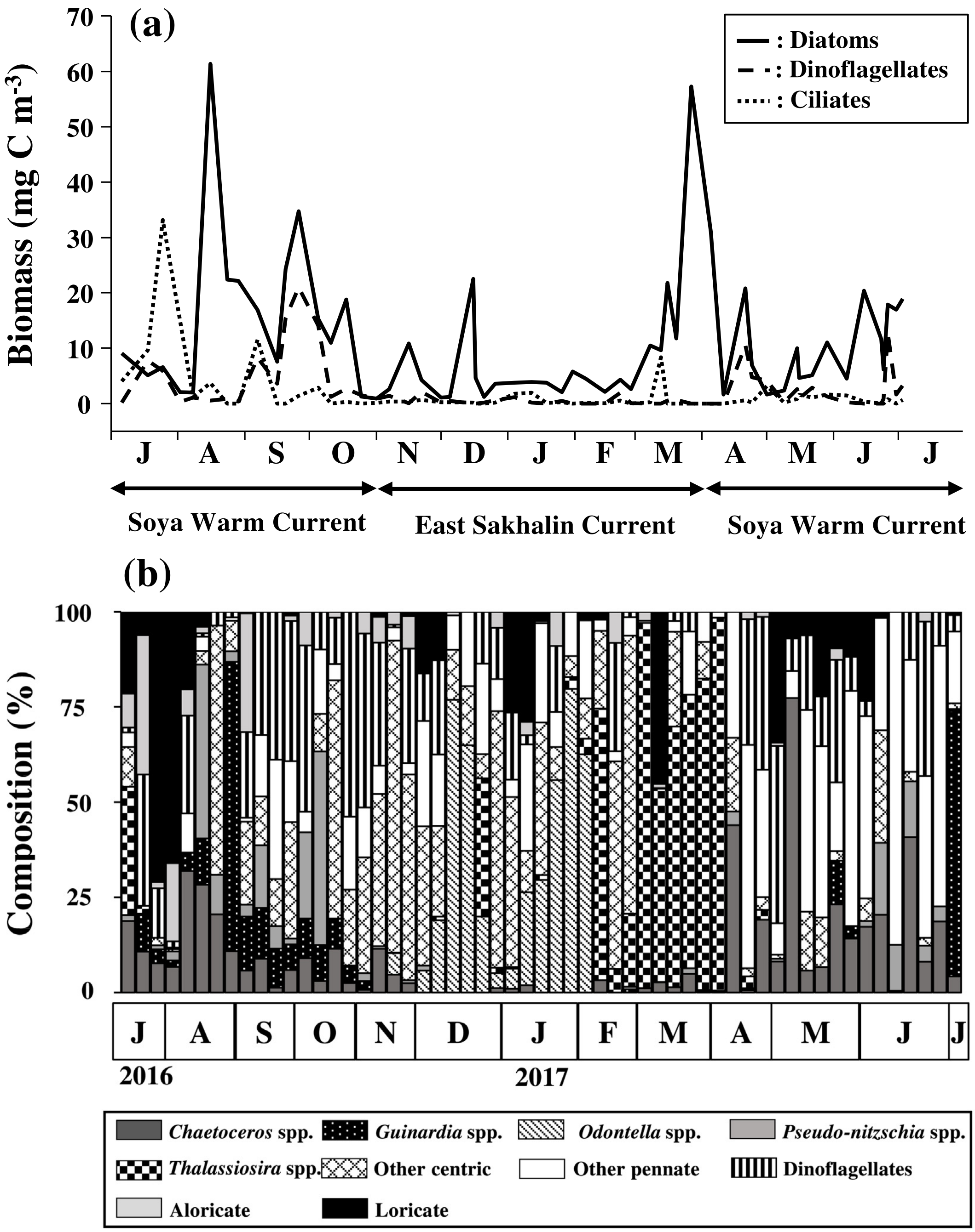

Fig. 5. Seasonal changes in protist biomass and the predominant water masses (a) and species composition (b) at Mombetsu Port from July 2016 to July 2017.

resulted in an increase in diatom growth. However, the rapid growth of diatoms caused by the inflow of freshwater was likely a temporal event, and its effect was limited relative to the seasonal changes in abundance.

In the study area, the Chl *a* concentration is generally higher in spring than in autumn (Kasai et al., 1997; Shiomoto et al., 2002). However, the Chl *a* concentration in autumn (September) of 2016 was higher than that in spring (March) of 2017 in this study. This can be explained by the high frequency of strong wind (i.e., > 5 m s^{-1} events that prevented stratification in the spring (March and April) of 2017. Spring plankton blooms begin with stratification of the water column, and strong wind events prevent occurrences of phytoplankton bloom (Niebauer et al., 1995; Hunt and Stabeno, 2002). Therefore, the difference in the Chl *a* concentration between spring and autumn in this study is thought to be relative to the difference in the frequency of strong winds. On the other hand, predation by large zooplankton is one of the factors that affects the Chl *a* concentration (Kasai et al., 1997; Shiomoto et al., 2002). From winter to spring, when the East Sakhalin Current Water is distinguished, large zooplankton are dominant and their productivity is low, and from summer to autumn, smaller species are dominant and their productivity is high (Hikichi et al., 2018). In addition, one small copepod, *Pseudocalanus newmani,* which occurs in the study area, has a higher ingestion rate than a large copepod, *Neocalanus plumchrus* (Nakagawa et al., 2016), and therefore, grazing impacts by copepods might be high in summer due to smaller copepods being more dominant in summer than in spring. However, as mentioned above, the Chl *a* concentration in September 2016 was higher than that in March 2017. Hence, the effect of winds on the Chl *a* concentration might be greater

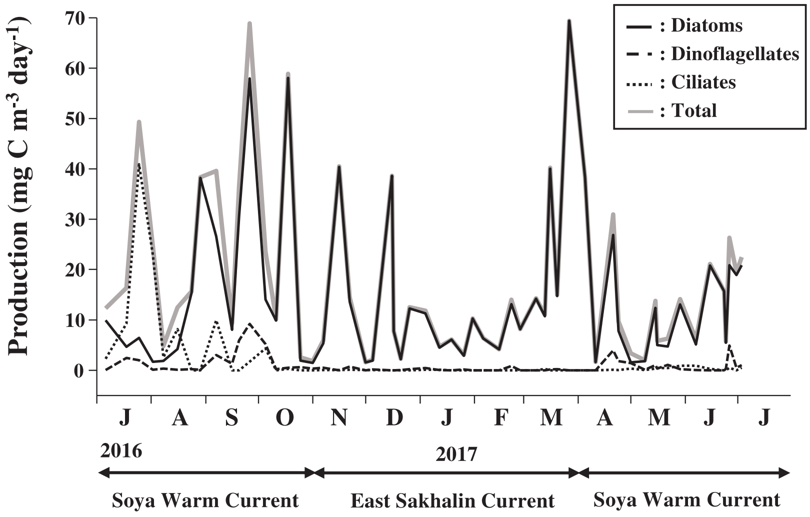

Fig. 6. Seasonal changes in protist production estimated from cell size and ambient temperature, and the predominant water masses at Mombetsu Port from July 2016 to July 2017.

than that of grazing by zooplankton. Therefore, bottom-up effects, such as water mass exchange, nutrient concentrations and wind, greatly influenced seasonal changes of the Chl *a* concentration in the Okhotsk Sea off Hokkaido.

4.2. Seasonal changes of diatoms

In this study, diatoms were the most dominant taxon, and the dominant species changed seasonally. Though there is no previous data about abundance of diatoms in the study area for comparison with this study's data, seasonal changes have been reported in the species composition. In the coastal surface waters of the Okhotsk Sea, spring diatom blooms were dominated by *T. nordenskioeldii*, *T. gravida*, *T. eccentrica*, *C. subsecundus* and others (Sorokin and Sorokin, 1999). In this study, *Thalassiosira* spp. were dominant during the diatom bloom in March 2017, and then the abundance of *Thalassiosira* spp. decreased remarkably from early April 2017, when sea ice retreated and Soya Warm Current started to prevail. From July to October 2016, when warm SWCW might prevail around the study area, the abundance of *Chaetoceros* spp. was high. *Odontella* spp. were dominant in abundance from December 2016 to the beginning of February 2017, when low temperature ESCW was dominant in the coastal area of the Okhotsk Sea off Hokkaido. The results of cluster analysis and NMDS also suggest the effect of water mass exchange on the seasonal succession of protist communities. Protist communities were divided into 4 groups in this study. Group A appeared from March to early April 2017, when sea ice existed around the study area., and in this group, *Thalassiosira spp.* were predominant. This result implies that the occurrence of those species in the study area is associated with sea ice, corresponding to the dominance of *Thalassiosira* in ice algae communities in Kasai et al. (2014). Group B mostly appeared from July to late October 2016 and from the middle of April to July 2017, and these periods corresponded to the period when SWCW might exist around the coastal regions of the southern Okhotsk Sea. This group was characterized by high sea surface temperature (SST) and low nutrients, which may be related to the water of Soya Warm Current. In contrast, group C appearing from November 2016 to February 2017 when East Sakhalin Current might prevail was characterized by low SST and high nutrients associated with ESCW. In group B, *Chaetoceros* spp., and in group C, *Odontella* spp. was dominant. According to Kasai and Hirakawa (2015), in the pelagic region and cold water mass of the Okhotsk Sea, *Odontella aurita*, some *Chaetoceros* species (*C. debilis*, *C. compressus*, *C. subsecundus*), *Thalassiosira hyalina* occur, whereas in the coastal region and in the warm water mass, *Chaetoceros* species (*C. affinis*, *C. contortus*, *C. didymus*) are frequently observed. Although identification was only conducted to genus in this study and the distinguishment between neritic or pelagic species was not performed, it is assumed that *Chaetoceros* spp. and *Odontella* spp. might be derived from Soya Warm Current and East Sakhalin Current, respectively. However, *Odontella aurita*, which can be observed in cold water mass of the Okhotsk Sea is also common along the coast of Hokkaido in winter, so that *Odontella* spp. in this study probably originated from neritic water as well as ESCW. In this way, the seasonal succession of dominant species might be mainly caused by the exchange of ESCW and SWCW as mentioned above. Thus, the diatom communities in the coast of the Okhotsk Sea off Hokkaido varied seasonally with the water mass exchange and sea ice melting.

4.3. Seasonal changes in ciliates

In this study, the biomass of the ciliates was greatest among the three taxa studied in July 2016. Ciliates play an important role in connecting microbial food webs, including bacteria to flagellates, with classical food chains (Levinsen and Nielsen, 2002). They are preyed upon by mesozooplankton such as copepods (Campbell et al., 2009), and predation pressure by the copepods decreases when the phytoplankton concentration is high (Levinsen et al., 2000). Therefore, the high biomass of ciliates observed in July 2016 possibly can be attributed to the low predation pressure on ciliates by copepods, since they might have grazed on diatoms actively because of their high Chl *a* concentration in July 2016. This phenomenon is referred to as regulation windows and is known to occur in Disco Bay along southwestern Greenland (Levinsen and Nielsen, 2002). The SEM analysis showed that the abundance, biomass and production of ciliates had positive correlations with water temperature. In other regions, the biomass of ciliates was greatest in summer when the water temperature and Chl *a* concentration was high (Godhantaraman, 2002), so that ciliates in this study were also able to grow actively in the period with high Chl *a* concentration and water temperature. However, soon after this peak, ciliates biomass decreased remarkably. When the decrease occurred, Chl *a* concentration was low. Because copepods are known to have a great feeding pressure on ciliates (Fessenden and Cowles, 1994), the low Chl. *a* concentration led them to change their

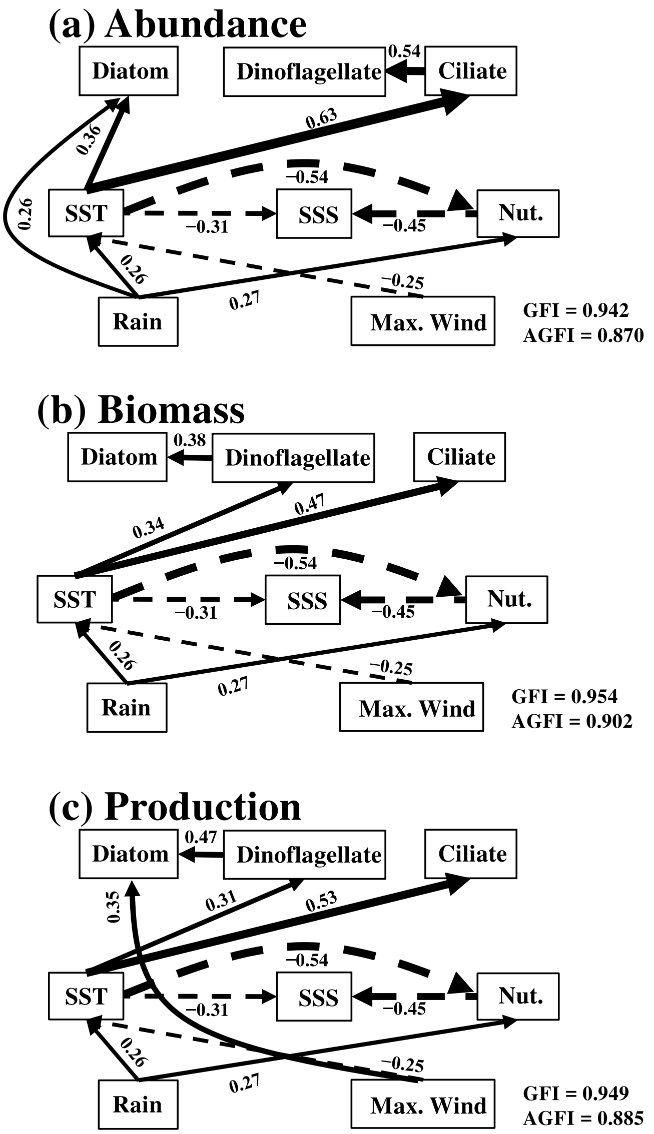

Fig. 7. Results of structural equation models (SEM) for protist parameters (abundance, biomass and production) with environmental factors. The values along the pathways represent standardized path coefficients. Arrows with solid or dashed lines indicate positive or negative effects. Thickness of arrows varies with path coefficient values. The overall fit of the model was evaluated using the goodness-of-fit index (GFI) and the adjusted goodness-of-fit index (AGFI). Rain: rainfall, Max. Wind: the maximum wind strength, SST: sea surface temperature, SSS: sea surface salinity, Nut: nitrate + nitrite.

main prey from phytoplankton to ciliates. In addition, according to Campbell et al. (2009), copepods prefer microzooplankton to phytoplankton, when microzooplankton is abundant. Therefore, a change in the prey of mesozooplankton, such as when copepods switch from phytoplankton to ciliates, may result in the rapid decrease in the ciliate biomass.

4.4. Seasonal change in primary production

^{13}C or ^{14}C incubation methods are usually used (Sorokin and Sorokin, 1999; Kasai et al., 2009) when measuring the photosynthesis rates of phytoplankton, whereas we estimated production using a relational expression of production rates and cell sizes. The advantage of this method is that we can estimate production from only cell size data without incubations.

The sum of the production of diatoms and dinoflagellates ranged from 1.6 to 67 mg C m^{-3} day^{-1}. Assuming that production was the same through the whole column (9 m depth), the water column production was converted to 14.4 to 603 mg C m^{-2} day^{-1}. According to Sorokin and Sorokin (1999), primary production in the summer (July to August), when the abundance of phytoplankton was at the seasonal minimum, was more than 60 mg C m^{-3} day^{-1} in the middle part of the Okhotsk Sea. The estimated values of production in July and August of 2016 ranged from 1.8 to 38.3 mg C m^{-3} day^{-1}. In addition, in a pelagic region of the southern Okhotsk Sea, the monthly average production from spring to autumn, except for a period of phytoplankton bloom in early spring, was approximately 500 mg C m^{-2} day^{-1} (Kasai et al., 2009). Production in the same season was approximately 139 mg C m^{-2} day^{-1} in this study. The primary production estimated in this study showed lower values than shown by previous studies. In the oligotrophic condition (e.g., summer in this study), the production by nanoflagellates ($< 2\ \mu$m) is much higher than that by micro-sized phytoplankton (Maita and Odate, 1988). Noting that the nanoflagellates were not enumerated in this study, taking no account of their production is one of the potential reasons for the low productivity. In addition, the expression for estimating production was determined under experimental incubations in stable conditions. According to Marañón (2008), the number of taxa was less in experimental incubations than in natural conditions, and these incubations did not reflect the variability of the taxa in natural conditions, so discrete differences may exist between the estimated values and the in situ values. Therefore, the production estimated by the method in this study could be different from the actual values in the study area because of the differences that existed between the estimated values and the reported values. However, even though the estimated values may differ from the actual ones, few studies have reported on seasonal changes in primary production in the study area, and the calculation of production values is important, even if the values are estimates. Primary production estimates in this study also showed relatively high values even during the winter, and the reliability of this method should be tested by following up with studies in the future.

4.5. Conclusion

Seasonal variability in the protist community at Mombetsu Port was determined by frequent observations. Bottom-up effects by the exchange of water masses, nutrients and winds seemed to have great influence on the seasonal variability of Chl *a* concentration. In particular, the exchange of water masses had a great effect on diatom communities. Ciliates showed a dramatic variability governed by an enhancement of growth associated with high Chl *a* concentration, high water temperatures and fluctuations in predation pressure from mesozooplankton. Identifications of species were not performed in this study, and such identification is needed to investigate more detailed seasonal changes of the protist community and the species composition and the factors controlling the changes. Determining the production values is also necessary, not only by estimating it from cell sizes but also by using incubation methods such as the ^{13}C method, to investigate the degree of difference between the estimated values based on cell sizes and the values obtained from incubations. Though the production estimated from cell sizes showed discrete differences from the reported values because of the differences in the methods, investigating seasonal changes in primary production is important for understanding the mechanism required to promote high primary production and rich fishery resources in the study area.

CRediT authorship contribution statement

Takuma Matsumoto: Formal analysis, Investigation, Writing - original draft, Visualization. **Kohei Matsuno:** Conceptualization, Formal analysis, Writing - review & editing, Supervision, Project administration. **Seiji Katakura:** Investigation, Resources. **Hiromi Kasai:** Investigation, Writing - review & editing. **Atsushi Yamaguchi:** Conceptualization, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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