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Author(s)	Matsumoto, Takuma; Matsuno, Kohei; Katakura, Seiji; Kasai, Hiromi; Yamaguchi, Atsushi
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Seasonal variability of the protist community and production in the southern
 Okhotsk Sea revealed by weekly monitoring.

Takuma Matsumoto<sup>a,\*</sup>, Kohei Matsuno<sup>a,b</sup>, Seiji Katakura<sup>c</sup>, Hiromi Kasai<sup>d</sup>, Atsushi
 Yamaguchi<sup>a,b</sup>

- 5 a Laboratory of Marine Biology, Graduate School of Fisheries Science, Hokkaido
- 6 University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041-0821, Japan
- 7 b Arctic Research Centre, Hokkaido University, Kita-21 Nishi-11 Kita-ku, Sapporo,
- 8 Hokkaido, 001-0021, Japan
- 9 c Kaiyo-Koryukan, Kaiyo-Koen 1, Mombetsu, Hokkaido 094-0031, Japan
- 10 d Fisheries Resources Institute, Japan Fisheries Research and Education Agency, 116
- 11 Katsurakoi, Kushiro, Hokkaido 085-0802, Japan
- 12 \*Corresponding author
- 13 E-mail address: foodfoot1995@eis.hokudai.ac.jp (T. Matsumoto)

## 14 Abstract

To evaluate seasonal changes in a protist community (diatoms, dinoflagellates 15and ciliates) and their production in the southern Okhotsk Sea, water sampling was 16 conducted approximately once a week from July 2016 to July 2017 at Mombetsu Port. 17Diatoms were the predominant category of the three taxa throughout the study period. 18The abundances of all taxa were high from March to September and were low from 19 20October 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. 21Group B showed the highest abundance  $(7.7 \times 10^4 \text{ cells } \text{L}^{-1})$ , with *Chaetoceros* spp. and 22Pseudo-nitzschia spp. being dominant. In group C, Odontella spp. and pennate diatoms 23were dominant and occurred from November to February. Group D was mainly observed 24in late October, and pennate diatoms were dominant. Seasonal changes in the predominant 25species of diatoms were explicitly observed, and these changes were associated with 26water mass exchanges. For dinoflagellates, abundance was highest in July 2016, and 2728biomass 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 29high chlorophyll *a* concentration and water temperature and a decrease in predation 30 pressure. The estimated primary production was lower than that reported in previous 31studies, which could reflect differences in the depth of the research areas and the methods 32

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.

36 Keywords: protist; diatoms; dinoflagellates; ciliates; seasonal change; Okhotsk Sea

#### 37 **1. Introduction**

The coastal area in the southern Okhotsk Sea off Hokkaido is an important region 38because it has high primary production and abundant fishery resources, including 39 Japanese scallop and salmon. In this area, the warm and saline Soya Warm Current and 40 41 cold and less-saline East Sakhalin Current are exchanged seasonally; the former can be 42observed from March to November, and the latter can be observed from November to 43March (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 44transported from the northern Okhotsk Sea. Because of the water mass exchanges and sea 4546ice 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). 47However, the information of dynamics for primary producers (e.g., diatoms) and 48mechanisms to high productivity is not sufficient for sustainable management of fisheries 4950resources.

51 Protists, which support marine ecosystems, consist of autotrophs, such as 52 diatoms; heterotrophs, such as ciliates and radiolarians; and mixotrophs, such as some 53 species of dinoflagellates. In the Okhotsk Sea, previous studies have reported that diatom 54 species are different in warm and cold waters (Kasai et al., 2015), and spring diatom 55 blooms are composed mainly of *Thalassiosira nordenskioeldii*, *Thalassiosira gravida*,

56	Thalassiosira eccentrica and Chaetoceros subsecundus in the coastal surface water
57	(Sorokin and Sorokin, 1999). Dinoflagellate species are represented by autotrophs,
58	heterotrophs and mixotrophs (Gaines and Elbrächter, 1987). In addition, many studies
59	have been conducted on Alexandrium tamarense, which causes paralytic shellfish poison
60	in the Okhotsk Sea (Shimada et al., 2010). Ciliates are heterotrophic protozooplankton
61	and are able to feed on bacteria and small phytoplankton, such as pennate diatoms
62	(Capriulo and Carpenter, 1983; Gast, 1985; Paranjape, 1987), and they also play an
63	important role in microbial loops (Pomeroy, 1974). Their growth rate is controlled not
64	only by the quality and quantity of their food but also by the water temperature, especially
65	under cold conditions, such as in polar regions; their growth rate increases in warmer
66	temperatures (Hansen and Jensen, 2000). In spite of their contribution to primary
67	production and their important role in microbial loops, protist communities have not been
68	well-studied along the coastal area of the Okhotsk Sea. Additionally, previous studies of
69	phytoplankton mainly revealed aspects of the chlorophyll $a$ (Chl $a$ ) concentration
70	(Hamasaki et al., 1998; Taguchi et al., 2000; Kasai et al., 2017). Although Sildever et al.
71	(2019) investigate the variability and the species composition of dinoflagellates in the
72	study area with a metagenomic approach, the compositions of the other protist
73	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.

#### 82 **2. Materials and Methods**

Surface water samples were collected with a plastic bucket at intervals of 83 approximately one week from 6 July 2016 to 3 July 2017 from a bridge that connects a 84 pier and the Okhotsk Tower in Mombetsu Port at 9 m depth (Fig. 1). Samples containing 85 500 mL of water were preserved with 1% glutaraldehyde. Approximately 100 mL of sea 86 87 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 88 a concentration was measured by a fluorometer (Turner Designs, Inc., 10-AU) according 89 to Welschmeyer (1994). The sea water from the surface was frozen to analyse the nutrients. 90 Nutrients were measured by an autoanalyser (Bran + Luebbe, AACS-III) according to 91

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 96 97 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 98 microscope slide with a micro pipet. Identification, counting and measuring of the cells 99 were conducted with an inverted microscope (Nikon, Eclipse-TE200) with 40-600× 100 magnification by the bright field observation. The genera of the diatoms were identified 101according to Hasle and Syvertsen (1997) and Hoppenerath et al. (2009). Concerning 102103 dinoflagellates, species identification was not conducted. Ciliates species were counted for two groups: loricate and aloricate ciliates. Counting was conducted for cells larger 104105than 10  $\mu$ m. The abundance of each taxon in the samples (cells L<sup>-1</sup>) was calculated using the number of cells in the sub-samples and the concentration. The cell volumes of each 106 taxon were  $(\mu m^3)$  calculated from the cell sizes, which were measured according to Sun 107108 and Liu (2003). Then, they were converted into carbon biomass using the conversion equation from Menden-Deuer and Lassard (2000). The production of diatoms and 109

110	dinoflagellates (mg C m <sup>-3</sup> day <sup>-1</sup> ) was estimated by the regression formula of Marañón
111	(2008): $\log_{10} P = b \log_{10} V + a (P: \text{ production (pg C cell^{-1} day^{-1})}; V: \text{ cell volume } (\mu \text{m}^3); a,$
112	<i>b</i> : constant for diatoms ( $a = -1.25$ and $b = 1.01$ ) and for dinoflagellates ( $a = -0.91$ and $b$
113	= 0.89)). Production of ciliates (mg C m <sup>-3</sup> day <sup>-1</sup> ) was estimated by multiplying the growth
114	rate (d <sup>-1</sup> ) calculated according to Montagnes et al. (1988) by the carbon biomass, as
115	described above. The growth rate was determined by the following formula: $\ln r = 0.1488$
116	$t - 0.3285 \ln (V \times 10^{-3}) - 1.3815$ ( <i>r</i> , growth rate (d <sup>-1</sup> ), <i>t</i> : water temperature (°C), and <i>V</i> : cell
117	volume ( $\mu$ m <sup>3</sup> )).

A cluster analysis was performed based on protist abundance data using software 118(PRIMER7, PRIMER-E Ltd.). The abundance (X: cells  $L^{-1}$ ) for all of the taxa were fourth 119root transformed  $(X^{1/4})$  prior to the analysis to reduce any bias in the abundance (Quinn 120and Keough, 2002). Similarities between the protist samples were calculated using the 121Bray-Curtis similarity index. To group the samples, the similarity indices were coupled 122123with hierarchical agglomerative clustering using a complete linkage method (Unweighted Pair Group Method using Arithmetic Mean: UPGMA) (Field et al., 1982). Non-metric 124Multidimensional Scaling (NMDS) ordination was performed to distribute the groups 125126onto a two-dimensional map. Pearson regression analyses were performed to clarify which environmental parameters (surface temperature, surface salinity, Chl a 127

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 132133controlling the variations in protist parameters (abundance, biomass and production) (Stomp et al., 2011). For SEM analysis, environmental parameters (rainfall, maximum 134wind speed, surface temperature, surface salinity and nitrate + nitrite concentration) and 135protist parameters were transformed into normalized values (average = 0, standard 136deviation = 1), and correlation coefficients between all parameters were calculated. For 137the path analysis, the parameters were grouped into the following three categories: 1) 138139atmospheric parameters (rainfall, maximum wind speed), 2) hydrographic parameters (surface temperature, surface salinity and nitrate + nitrite), and 3) protist parameters. Non-140significant interactions (p > 0.05) were deleted from the final model. The goodness-of-fit 141 index (GFI) and the adjusted goodness-of-fit index (AGFI) were used to evaluate the 142143fitting of the models. The standardized path coefficients were independent variables, 144which 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 145

146 (http://www.ohmsha.co.jp/data/link/978-4-274-06925-3/).

147 **3. Results** 

#### 148 *3.1. Hydrography*

149Soya 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 150151prevails 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 152153November 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 154it started to decrease. The surface sea temperature (SST) ranged from -1.7 to 22.3°C, and 155it had the maximum in August 2016 and the minimum in February 2017. From January 156to March 2017, it was approximately 0°C, and from July to August, it showed higher 157values (> 15°C) (Fig. 2a). The surface sea salinity (SSS) ranged from 20.85 to 33.59, and 158159it 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 160 161 from 0.24 to 6.1 µg L<sup>-1</sup>, 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 162µM. It increased from November 2016 to March 2017 and decreased sharply in late 163

164	March 2017. It also increased rapidly in April and June 2017 (Fig. 2b). The silicate
165	concentration ranged from 1.4 to 117.5 $\mu$ M and showed high values in September 2016
166	and from April to June 2017 (Fig. 2c). The phosphate concentration ranged from 0.063 to
167	1.2 $\mu$ M, and it increased from the end of October 2016 to the end of January 2017 and
168	declined thereafter to April 2017 (Fig. 2c). The air temperature (average) ranged from -
169	10.2 to 27.4°C, and it was highest on 21 July 2016 and was lowest on 11 January 2017
170	(Fig. 2d). Daily rainfall ranged from 0 to 75.5 mm, and it was highest during August 2016
171	(Fig. 2d). The daily maximum wind speed ranged 2.3 to 13.5 m s <sup>-1</sup> , and the frequency of
172	strong winds (> 5 m s <sup>-1</sup> ) was higher in September and October 2016 than that in spring
173	(March and April 2017) (Fig. 2e). In 2017, sea ice could be observed from 26 January to
174	11 April at Mombetsu, and the period of visible sea ice was 7 days longer than that of
175	normal years (cf. website of sea ice information of the City of Mombetsu: http://okhotsk-
176	mombetsu.jp/).

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# 177 *3.2. Seasonal variability of abundance*

The abundance of the diatoms ranged from  $1.55 \times 10^3$  to  $5.36 \times 10^5$  cells L<sup>-1</sup>, that of dinoflagellates ranged from N.D. (not detected) to  $5.28 \times 10^3$  cells L<sup>-1</sup>, and that of ciliates ranged from N.D. to  $5.92 \times 10^3$  cells L<sup>-1</sup> (Fig. 3a). The abundance of each taxon

181	was high from July to September 2016 and from March to July 2017, and it was low from
182	October 2016 to February 2017. In this study, diatoms were most dominant among the
183	three taxa. The species composition of diatoms showed seasonality, and had a summer-
184	autumn growth period (July-October), the minimum during late autumn to winter
185	(November-February), a spring bloom (March-April), and then recurrence of a summer
186	growth period (April/May-July). From July 2016 to the beginning of August 2016,
187	Chaetoceros spp. mainly dominated (58.3-88.7%), and Pseudo-nitzschia spp. were
188	mainly dominant from the middle of August 2016 to the middle of October 2017 (39.0-
189	87.7%), although other centric diatoms (mainly Skeletonema spp.) were temporarily
190	dominant (76.7%) at the end of August 2016. From the end of October 2016 to the
191	beginning of December 2017, other pennate diatoms (pennates excluding Pseudo-
192	nitzschia spp.) were mostly dominant (36.7-74.2%). Odontella spp. and other pennate
193	diatoms dominated from December 2016 to the beginning of February 2017 (3.7-58.2%
194	and 33.3-77.5%, respectively), and <i>Thalassiosira</i> spp. were mainly dominant from March
195	2017 to the beginning of April 2017 (64.9-95.0%). From the middle of April 2017 to the
196	beginning of July 2017, Chaetoceros spp. were mostly dominant again (29.3-97.7%).
197	Dinoflagellates showed the highest value in July 2016. Ciliates showed high values in
198	July and August 2016. Both of loricate and aloricate ciliates showed their highest values

#### 200 *3.3. Cluster analysis based on abundance*

Protist communities were divided into 4 groups (A-D) at 45% and 52% 201similarity by cluster analysis based on abundance (Fig. 4a). The environmental 202 203parameters 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 204occurred in March and April 2017, had high abundance  $(6.3 \times 10^4 \text{ cells } \text{L}^{-1})$  with a 205predominance of Thalassiosira spp. in March 2017 (Fig. 4c, d). Group B was observed 206 from July to October 2016 and from April to July 2017. This group had the highest 207abundance  $(7.7 \times 10^4 \text{ cells } \text{L}^{-1})$ , and *Chaetoceros* spp. and *Pseudo-nitzschia* spp. were 208 209 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 210end of October 2016 and February 2017. This group showed the lowest abundance 211 $(1.7 \times 10^3 \text{ cells } \text{L}^{-1})$ , and other pennate diatoms were dominant (Fig. 4c, d). 212

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.

## 223 3. 4. Seasonal changes of biomass and production

Carbon biomass of diatoms, dinoflagellates and ciliates ranged from 0.9 to 61 224mg C m<sup>-3</sup>, from N.D. to 21 mg C m<sup>-3</sup> and from N.D. to 33 mg C m<sup>-3</sup>, respectively (Fig. 5). 225Diatoms were dominant in biomass among the three taxa, which was the same as for the 226abundance. Seasonal changes occurred in diatom carbon biomass, which peaked in 227August 2016 and March 2017. Carbon mass in dinoflagellates peaked in September 2016 228(Fig. 5a), and that of ciliates peaked in July 2016. The composition in carbon biomass 229was dominated by mainly Chaetoceros spp. and Guinardia spp. from July to August 2016, 230231and 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 232

233	centric diatoms dominated, and from the middle of December 2016 to the beginning of
234	February 2017, Odontella spp. and other centric diatoms dominated. From the beginning
235	of February 2017 to the beginning of April 2017, Thalassiosira spp. dominated, and from
236	the middle of April 2017 to the beginning of July 2017, Chaetoceros spp. and other
237	pennate diatoms dominated.

The production of diatoms, dinoflagellates and ciliates ranged from 1.5 to 69 mg C m<sup>-3</sup> day<sup>-1</sup>, from N.D. to 9.2 mg C m<sup>-3</sup> day<sup>-1</sup> and from N.D. to 41 mg C m<sup>-3</sup> day<sup>-1</sup>, 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.

#### 243 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).

#### **4. Discussion**

### 254 4. 1. Seasonal change of Chl a concentration

255In 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 256257seasonally 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 258not appropriate to the classification of water masses in this study because due to the 259sampling location where was very close to the shore, the hydrographic conditions might 260be affected by terrestrial waters (e.g., river water inflow). Actually, in this study, sea 261surface salinity (SSS) did not excess 33.6 (the maximum was 33.58). Thus, water masses 262263should 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 264Okhotsk Sea, the effect of Soya Warm Current might exist from July to October 2016 and 265from April to July 2017, and that of East Sakhalin Current might exist from November 2662016 to April 2017 (cf. Fig. 2, Supplementary). 267

268	Many previous studies have reported on Chl a concentration in the study area
269	(Hamasaki et al., 1998; Taguchi et al., 2000; Kasai et al., 2010; Kasai et al., 2017), and
270	the results of this study were within the values of the previous studies. According to
271	Hamasaki et al. (1998), in Mombetsu Port, Chl a is common for three periods: June to
272	October (SWCW is dominant), November to February (ESCW is dominant)/April to May
273	(the transition from ESCW to SWCW), and March (sea ice period). They also report that
274	hydrographic conditions oscillate between two periods: June to October (SWCW) and
275	November to May (ESCW). Considering the succession of these periods correspond to
276	that of the major water masses, seasonal changes of Chl a and hydrographic conditions
277	such as nutrients in the study area may be related to the exchange of water masses.
278	Actually, those periods were also observed in this study. The peaks of Chl a concentration
279	were observed in July and September 2016 and March and June 2017. When the species
280	composition was compared at the time of these peaks, Chaetoceros spp. were dominant
281	at the peaks in July 2016 and June 2017, and Pseudo-nitzschia spp. were dominant in
282	September 2016. These species might contribute to the peak in the Chl $a$ concentration.
283	In March 2017, when sea ice retreat might occur, Thalassiosira spp. were dominant.
284	Because these species show high dominance in the ice algae communities of the study
285	area (Kasai et al., 2014), the peak in March might be partly caused by Thalassiosira spp.,

286	which are thought to be released from melting sea ice. Nutrient concentration began to
287	increase in November 2016, when high-nutrient East Sakhalin Current might arrive, and
288	this concentration decreased rapidly in March 2017, when the influence of East Sakhalin
289	Current might weaken. This decrease might also attribute to the consumption of nutrients
290	by diatom blooms because Chl a concentration increased at the same timing. Thus, major
291	seasonal changes in the nutrient concentration consisted of the exchange of water masses
292	observed in the area. In addition, nutrient concentration sporadically increased when the
293	rainfall was high. This might be caused by the inflow of fresh water from a river near the
294	sampling station. In addition, in the SEM analysis, rainfall had a positive effect on the
295	abundance of diatoms. This effect suggests that the rapid increase in river water from the
296	rainfall induced the input of high nutrients, which resulted in an increase in diatom growth.
297	However, the rapid growth of diatoms caused by the inflow of freshwater was likely a
298	temporal event, and its effect was limited relative to the seasonal changes in abundance.
299	In the study area, the Chl a concentration is generally higher in spring than in
300	autumn (Kasai et al., 1997; Shiomoto et al., 1998). However, the Chl a concentration in
301	autumn (September) of 2016 was higher than that in spring (March) of 2017 in this study.
302	This can be explained by the high frequency of strong wind (i.e., $> 5 \text{ m s}^{-1}$ ) events that
303	prevented stratification in the spring (March and April) of 2017. Spring plankton blooms

304	begin with stratification of the water column, and strong wind events prevent occurrences
305	of phytoplankton bloom (Niebauer et al., 1995; Hunt and Stabeno, 2002). Therefore, the
306	difference in the Chl <i>a</i> concentration between spring and autumn in this study is thought
307	to be relative to the difference in the frequency of strong winds. On the other hand,
308	predation by large zooplankton is one of the factors that affects the Chl $a$ concentration
309	(Kasai et al., 1997 Shiomoto et al., 1998). From winter to spring, when the East Sakhalin
310	Current Water is distinguished, large zooplankton are dominant and their productivity is
311	low, and from summer to autumn, smaller species are dominant and their productivity is
312	high (Hikichi et al., 2018). In addition, one small copepod, Pseudocalanus newmani,
313	which occurs in the study area, has a higher ingestion rate than a large copepod,
314	Neocalanus plumchrus (Nakagawa et al., 2016), and therefore, grazing impacts by
315	copepods might be high in summer due to smaller copepods being more dominant in
316	summer than in spring. However, as mentioned above, the Chl $a$ concentration in
317	September 2016 was higher than that in March 2017. Hence, the effect of winds on the
318	Chl $a$ concentration might be greater than that of grazing by zooplankton. Therefore,
319	bottom-up effects, such as water mass exchange, nutrient concentrations and wind,
320	greatly influenced seasonal changes of the Chl a concentration in the Okhotsk Sea off
321	Hokkaido.

#### 322 4. 2. Seasonal changes of diatoms

In this study, diatoms were the most dominant taxon, and the dominant species 323 324changed 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 325326 the species composition. In the coastal surface waters of the Okhotsk Sea, spring diatom 327 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 328 during the diatom bloom in March 2017, and then the abundance of *Thalassiosira* spp. 329 decreased remarkably from early April 2017, when sea ice retreated and Soya Warm 330 Current started to prevail. From July to October 2016, when warm SWCW might prevail 331332around 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 333 low temperature ESCW was dominant in the coastal area of the Okhotsk Sea off Hokkaido. 334 The results of cluster analysis and NMDS also suggest the effect of water mass exchange 335 on the seasonal succession of protist communities. Protist communities were divided into 336 337 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. 338

339	This result implies that the occurrence of those species in the study area is associated with
340	sea ice, corresponding to the dominance of Thalassiosira in ice algae communities in
341	Kasai et al. (2014). Group B mostly appeared from July to late October 2016 and from
342	the middle of April to July 2017, and these periods corresponded to the period when
343	SWCW might exist around the coastal regions of the southern Okhotsk Sea. This group
344	was characterized by high sea surface temperature (SST) and low nutrients, which may
345	be related to the water of Soya Warm Current. In contrast, group C appearing from
346	November 2016 to February 2017 when East Sakhalin Current might prevail was
347	characterized by low SST and high nutrients associated with ESCW. In group B,
348	Chaetoceros spp., and in group C, Odontella spp. was dominant. According to Kasai et
349	al. (2015), in the pelagic region and cold water mass of the Okhotsk Sea, Odontella aurita,
350	some Chaetoceros species (C. debilis, C. compressus, C. subsecundus), Thalassiosira
351	hyalina occur, whereas in the coastal region and in the warm water mass, Chaetoceros
352	species (C. affinis, C. contortus, C. didymus) are frequently observed. Although
353	identification was only conducted to genus in this study and the distinguishment between
354	neritic or pelagic species was not performed, it is assumed that Chaetoceros spp. and
355	Odontella spp. might be derived from Soya Warm Current and East Sakhalin Current,
356	respectively. However, Odontella aurita, which can be observed in cold water mass of

357	the Okhotsk Sea is also common along the coast of Hokkaido in winter, so that Odontella
358	spp.in this study probably originated from neritic water as well as ESCW. In this way, the
359	seasonal succession of dominant species might be mainly caused by the exchange of
360	ESCW and SWCW as mentioned above. Thus, the diatom communities in the coast of
361	the Okhotsk Sea off Hokkaido varied seasonally with the water mass exchange and sea
362	ice melting.

363 4. 3. Seasonal changes in ciliates

In this study, the biomass of the ciliates was greatest among the three taxa studied 364in July 2016. Ciliates play an important role in connecting microbial food webs, including 365bacteria to flagellates, with classical food chains (Levinsen and Nielsen, 2002). They are 366 preyed upon by mesozooplankton such as copepods (Campbell et al., 2009), and 367 predation pressure by the copepods decreases when the phytoplankton concentration is 368 369 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 370 they might have grazed on diatoms actively because of their high Chl a concentration in 371372 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 373

analysis showed that the abundance, biomass and production of ciliates had positive 374correlations with water temperature. In other regions, the biomass of ciliates was greatest 375in summer when the water temperature and Chl a concentration was high 376 377 (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 378peak, ciliates biomass decreased remarkably. When the decrease occurred, Chl a 379concentration was low. Because copepods are known to have a great feeding pressure on 380 ciliates (Fessenden and Cowles. 1994), the low Chl. a concentration led them to change 381their main prey from phytoplankton to ciliates. In addition, according to Campbell et al. 382(2009), copepods prefer microzooplankton to phytoplankton, when microzooplankton is 383 abundant. Therefore, a change in the prey of mesozooplankton, such as when copepods 384 385switch from phytoplankton to ciliates, may result in the rapid decrease in the ciliate biomass. 386

#### 387 *4. 4. Seasonal change in primary production*

<sup>13</sup>C or <sup>14</sup>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.

391 The advantage of this method is that we can estimate production from only cell size data392 without incubations.

The sum of the production of diatoms and dinoflagellates ranged from 1.6 to 67 393 mg C m<sup>-3</sup> day<sup>-1</sup>. Assuming that production was the same through the whole column (9 m 394depth), the water column production was converted to 14.4 to 603 mg C m<sup>-2</sup> day<sup>-1</sup>. 395According to Sorokin and Sorokin (1999), primary production in the summer (July to 396 August), when the abundance of phytoplankton was at the seasonal minimum, was more 397 than 60 mg C m<sup>-3</sup> day<sup>-1</sup> in the middle part of the Okhotsk Sea. The estimated values of 398 production in July and August of 2016 ranged from 1.8 to 38.3 mg C m<sup>-3</sup> day<sup>-1</sup>. In addition, 399 in a pelagic region of the southern Okhotsk Sea, the monthly average production from 400 spring to autumn, except for a period of phytoplankton bloom in early spring, was 401 approximately 500 mg C m<sup>-2</sup> day<sup>-1</sup> (Kasai et al., 2009). Production in the same season 402 was approximately 139 mg C m<sup>-2</sup> day<sup>-1</sup> in this study. The primary production estimated 403 in this study showed lower values than shown by previous studies. In the oligotrophic 404 condition (e.g., summer in this study), the production by nanoflagellates ( $< 2 \mu m$ ) is much 405 higher than that by micro-sized phytoplankton (Maita and Odate 1988). Noting that the 406 407 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 408

409	estimating production was determined under experimental incubations in stable
410	conditions. According to Marañón (2008), the number of taxa was less in experimental
411	incubations than in natural conditions, and these incubations did not reflect the variability
412	of the taxa in natural conditions, so discrete differences may exist between the estimated
413	values and the in situ values. Therefore, the production estimated by the method in this
414	study could be different from the actual values in the study area because of the differences
415	that existed between the estimated values and the reported values. However, even though
416	the estimated values may differ from the actual ones, few studies have reported on
417	seasonal changes in primary production in the study area, and the calculation of
418	production values is important, even if the values are estimates. Primary production
419	estimates in this study also showed relatively high values even during the winter, and the
420	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 426 growth associated with high Chl a concentration, high water temperatures and 427fluctuations in predation pressure from mesozooplankton. Identifications of species were 428not performed in this study, and such identification is needed to investigate more detailed 429seasonal changes of the protist community and the species composition and the factors 430controlling the changes. Determining the production values is also necessary, not only by 431estimating it from cell sizes but also by using incubation methods such as the <sup>13</sup>C method, 432to investigate the degree of difference between the estimated values based on cell sizes 433and the values obtained from incubations. Though the production estimated from cell 434sizes showed discrete differences from the reported values because of the differences in 435the methods, investigating seasonal changes in primary production is important for 436 understanding the mechanism required to promote high primary production and rich 437fishery resources in the study area. 438

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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.

	Groups								
Parameters	А	В	С	D	- one-way	Tuk	ey-Kı	ramer	test
	(6)	(33)	(15)	(3)	ANOVA				
SST	0.23	13.2	0.17	5.5	***	С	А	D	В
SSS	32.1	31.1	31.3	32	NS				
Chl.a	1.2	1.8	0.6	1.1	*	С	D	А	В
Nitrate + Nitrite	4.8	3.1	7.4	3.7	*	В	D	Α	С
SiO <sub>2</sub>	12.9	28.7	17	10.4	NS				
PO <sub>4</sub>	0.71	0.27	0.98	0.66	***	В	D	А	С
Air temperature	0.9	12.6	-3.6	0.6	***	С	D	А	В
Rainfall	1.1	2.4	0.4	0.2	NS				
Chaetoceros spp.	2268	22551	185	139	***	D	С	А	В
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	С	В	Α
Other centric diatoms	1855	16192	524	229	NS				
Other pennate diatoms	5098	11955	2623	944	*	D	С	Α	В
Total diatoms	63102	75755	5549	1620	*	D	С	Α	В
Dinoflagellates	62	813	80.2	80.4	**	А	С	D	В
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	С	A	В



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



at Mombetsu Port and (f) daily mean sea level difference (SBL) between Wakkanai and Abashiri (cf. Fig. 1) (black and surface, (d) daily averaged air temperature and daily amount of rainfall, (e) daily maximum wind speed and direction 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.



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 quantity of samples included in each group. Nonmetric multi-dimensional scaling plots of the four groups, with arrows indicating directions of environmental parameters (b). Air T: air temperature, Chl: chlorophyll *a*, Nut: nitrate and nitrite, SST: sea surface temperature. Abundance and species composition of each group (c). Seasonal changes of the occurrence for microplankton groups and the predominant water masses (d).



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.



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.



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 varied with path coefficient values. The overall fit of the model was evaluated using the goodness-of-fit index (GFI) and the adjunct goodness-of-fit index (AGFI). Rain: rainfall, Max. Wind: the maximum wind strength, SST: sea surface temperature, SSS: sea surface salinity, Nut: nitrate + nitrite.