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Citation	Regional Studies in Marine Science, 43, 101683 https://doi.org/10.1016/j.rsma.2021.101683
Issue Date	2021-03
Doc URL	http://hdl.handle.net/2115/89241
Rights	https://www.elsevier.com/journals/heliyon/2405-8440/open-access-journal
Rights(URL)	http://creativecommons.org/licenses/by-nc-nd/4.0/
Type	article (author version)
File Information	Manuscript.pdf



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

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14 **Abstract**

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

33 used for estimating production. In the coastal area of the southern Okhotsk Sea, where
34 production is high, not only the species composition but also the protist community varied
35 clearly with season because of variations in the water mass.

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

37 **1. Introduction**

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

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 nordenskiöldii*, *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

74 communities vary in accordance with the seasonal exchange of water masses (Hikichi et
75 al., 2018), but a relationship between the variability in protist communities and the
76 exchange of water masses is still unknown.

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

82 **2. Materials and Methods**

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

92 Parsons et al. (1984). Water temperature and salinity were measured by CTD (JFE
93 Advantech Inc., ASTD102). Data on air temperature, rainfall, the maximum wind speed,
94 and wind direction were obtained from the web site of the Japanese Meteorological
95 Agency (<https://www.data.jma.go.jp/obd/stats/etrn/index.php>).

96 The fixed protist samples were stored on a flat table for more than 1 day to allow
97 the microprotist cells to settle to the bottom of the bottle. Then, the samples were
98 concentrated down to 20 mL using a siphon. Subsamples (1 mL) were mounted on a glass
99 microscope slide with a micro pipet. Identification, counting and measuring of the cells
100 were conducted with an inverted microscope (Nikon, Eclipse-TE200) with 40–600×
101 magnification by the bright field observation. The genera of the diatoms were identified
102 according to Hasle and Syvertsen (1997) and Hoppenerath et al. (2009). Concerning
103 dinoflagellates, species identification was not conducted. Ciliates species were counted
104 for two groups: loricate and aloricate ciliates. Counting was conducted for cells larger
105 than 10 μm . The abundance of each taxon in the samples (cells L^{-1}) was calculated using
106 the number of cells in the sub-samples and the concentration. The cell volumes of each
107 taxon were (μm^3) calculated from the cell sizes, which were measured according to Sun
108 and Liu (2003). Then, they were converted into carbon biomass using the conversion
109 equation from Menden-Deuer and Lassard (2000). The production of diatoms and

110 dinoflagellates ($\text{mg C m}^{-3} \text{ day}^{-1}$) was estimated by the regression formula of Marañón
111 (2008): $\log_{10} P = b \log_{10} V + a$ (P : production ($\text{pg C cell}^{-1} \text{ day}^{-1}$); V : cell volume (μm^3); a ,
112 b : constant for diatoms ($a = -1.25$ and $b = 1.01$) and for dinoflagellates ($a = -0.91$ and b
113 $= 0.89$)). Production of ciliates ($\text{mg C m}^{-3} \text{ day}^{-1}$) was estimated by multiplying the growth
114 rate (d^{-1}) 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 $t - 0.3285 \ln (V \times 10^{-3}) - 1.3815$ (r : growth rate (d^{-1}), t : water temperature ($^{\circ}\text{C}$), and V : cell
117 volume (μm^3)).

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

128 concentration, nitrate + nitrite concentration, phosphate concentration, silicate
129 concentration, air temperature and rainfall) had significant relationships with the groups.
130 Inter-group differences in the major taxa and environmental parameters were tested by
131 using a one-way ANOVA, followed by Tukey-Kramer post hoc test.

132 Structural Equation Modelling (SEM) was performed to evaluate the factors
133 controlling the variations in protist parameters (abundance, biomass and production)
134 (Stomp et al., 2011). For SEM analysis, environmental parameters (rainfall, maximum
135 wind speed, surface temperature, surface salinity and nitrate + nitrite concentration) and
136 protist parameters were transformed into normalized values (average = 0, standard
137 deviation = 1), and correlation coefficients between all parameters were calculated. For
138 the path analysis, the parameters were grouped into the following three categories: 1)
139 atmospheric parameters (rainfall, maximum wind speed), 2) hydrographic parameters
140 (surface temperature, surface salinity and nitrate + nitrite), and 3) protist parameters. Non-
141 significant interactions ($p > 0.05$) were deleted from the final model. The goodness-of-fit
142 index (GFI) and the adjusted goodness-of-fit index (AGFI) were used to evaluate the
143 fitting of the models. The standardized path coefficients were independent variables,
144 which indicate the relative contributions of the different paths within the SEM (Stomp et
145 al., 2011). The SEM analysis was performed using add-in software for MS-Excel

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

147 **3. Results**

148 *3.1. Hydrography*

149 Soya Warm Current is driven by the sea level difference (SLD) between Japan
150 Sea and Okhotsk Sea (Aota, 1975; Fukamachi et al., 2008). When East Sakhalin Current
151 prevails in the Okhotsk Sea, this SLD becomes small, resulting in decrease in the volume
152 of Soya Warm Current Water into the Okhotsk Sea. The SLD started to decline from
153 November 2016 and showed the minimum in the middle of January 2017 (Fig. 2f). It kept
154 increasing thereafter until late April 2017 and returned to the same level as that of when
155 it started to decrease. The surface sea temperature (SST) ranged from -1.7 to 22.3°C, and
156 it had the maximum in August 2016 and the minimum in February 2017. From January
157 to March 2017, it was approximately 0°C, and from July to August, it showed higher
158 values (> 15°C) (Fig. 2a). The surface sea salinity (SSS) ranged from 20.85 to 33.59, and
159 it declined sporadically due to rain fall through the study period. Low salinity in April
160 2017 might be attributed to melting of sea ice (Fig. 2a). The Chl *a* concentration ranged
161 from 0.24 to 6.1 $\mu\text{g L}^{-1}$, and there were large peaks in July 2016, September 2016 and
162 March 2017 (Fig. 2b). The total nitrate and nitrite concentration ranged from 0 to 16.2
163 μM . It increased from November 2016 to March 2017 and decreased sharply in late

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 μM 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 μM , 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^{-1} , and the frequency of
172 strong winds ($> 5 \text{ m s}^{-1}$) 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-](http://okhotsk-mombetsu.jp/)
176 [mombetsu.jp/](http://okhotsk-mombetsu.jp/)).

177 3.2. Seasonal variability of abundance

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

199 in July 2016.

200 3.3. Cluster analysis based on abundance

201 Protist communities were divided into 4 groups (A-D) at 45% and 52%
202 similarity by cluster analysis based on abundance (Fig. 4a). The environmental
203 parameters that had a significant relationship with the NMDS of each group were Chl *a*,
204 air temperature, sea surface temperature, and nitrate + nitrite (Fig. 4b). Group A, which
205 occurred in March and April 2017, had high abundance (6.3×10^4 cells L⁻¹) with a
206 predominance of *Thalassiosira* spp. in March 2017 (Fig. 4c, d). Group B was observed
207 from July to October 2016 and from April to July 2017. This group had the highest
208 abundance (7.7×10^4 cells L⁻¹), and *Chaetoceros* spp. and *Pseudo-nitzschia* spp. were
209 dominant. Group C was observed in winter (November 2016 to February 2017), with a
210 predominance of *Odontella* spp. and other pennate diatoms. Group D was observed at the
211 end of October 2016 and February 2017. This group showed the lowest abundance
212 (1.7×10^3 cells L⁻¹), and other pennate diatoms were dominant (Fig. 4c, d).

213 The environmental parameters that had an inter-group difference were surface
214 temperature, Chl *a*, nitrate + nitrite, phosphate and air temperature (Table 1). Group B
215 was characterized by high air temperature, high sea surface temperature, low nutrient

216 concentration and high Chl *a* concentration. In contrast to group B, group C was
217 characterized by low air temperature, low surface temperature, high nutrient
218 concentration and low Chl *a* concentration. Groups A and D had the middle values in the
219 environmental parameters between groups B and C. Protist abundance was high in groups
220 A and B and was low in groups C and D. In addition, group A was characterized by a
221 predominance of *Thalassiosira* spp., and group B was characterized by predominance of
222 *Chaetoceros* spp.

223 3. 4. Seasonal changes of biomass and production

224 Carbon biomass of diatoms, dinoflagellates and ciliates ranged from 0.9 to 61
225 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).
226 Diatoms were dominant in biomass among the three taxa, which was the same as for the
227 abundance. Seasonal changes occurred in diatom carbon biomass, which peaked in
228 August 2016 and March 2017. Carbon mass in dinoflagellates peaked in September 2016
229 (Fig. 5a), and that of ciliates peaked in July 2016. The composition in carbon biomass
230 was dominated by mainly *Chaetoceros* spp. and *Guinardia* spp. from July to August 2016,
231 and ciliates were dominant at the end of July 2016 and the beginning of August 2016 (Fig.
232 5b). From September 2016 to the middle of December 2016, dinoflagellates and other

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.

238 The production of diatoms, dinoflagellates and ciliates ranged from 1.5 to 69 mg
239 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,
240 respectively (Fig. 6). The production of diatoms showed a pulse-like variation, with
241 dinoflagellates showing a peak in late September 2016 but with a low value compared to
242 that of the other taxa. The production of ciliates peaked in July 2016.

243 3. 5. SEM analysis

244 From the SEM analysis on the protist parameters (abundance, biomass and
245 production) of each taxon, high values were observed for GFI (0.94–0.95) and AGFI
246 (0.87–0.90) (Fig. 7). The abundance of diatoms had positive correlations with water
247 temperature and rainfall, and both the biomass and production of diatoms had a positive
248 correlation with dinoflagellates. Regarding diatom production, a positive correlation with
249 the maximum wind speed was observed. For dinoflagellates, ciliates had a positive effect

250 on the abundance, and water temperature had a positive effect on the biomass and the
251 production. Every parameter for the ciliates had a strong positive correlation with water
252 temperature (path coefficients: $pc = 0.47$ to 0.63).

253 **4. Discussion**

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

255 In the coastal region of the southern Okhotsk Sea, two of the major water masses,
256 Soya Warm Current Water (SWCW) and East Sakhalin Current Water (ESCW), are
257 seasonally exchanged. According to previous studies, salinity of SWCW and ESCW is >
258 33.6 and < 32.0, respectively (e.g., Itoh and Ohshima, 2000). However, this definition is
259 not appropriate to the classification of water masses in this study because due to the
260 sampling location where was very close to the shore, the hydrographic conditions might
261 be affected by terrestrial waters (e.g., river water inflow). Actually, in this study, sea
262 surface salinity (SSS) did not excess 33.6 (the maximum was 33.58). Thus, water masses
263 should not be defined strictly in this study by the definition reported in the previous
264 studies. However, considering the seasonal change of the SLD and SSS, in the southern
265 Okhotsk Sea, the effect of Soya Warm Current might exist from July to October 2016 and
266 from April to July 2017, and that of East Sakhalin Current might exist from November
267 2016 to April 2017 (cf. Fig. 2, Supplementary).

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; Shiimoto 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 *a* 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*

323 In this study, diatoms were the most dominant taxon, and the dominant species
324 changed seasonally. Though there is no previous data about abundance of diatoms in the
325 study area for comparison with this study's data, seasonal changes have been reported in
326 the species composition. In the coastal surface waters of the Okhotsk Sea, spring diatom
327 blooms were dominated by *T. nordenskiöldii*, *T. gravida*, *T. eccentrica*, *C. subsecundus*
328 and others (Sorokin and Sorokin, 1999). In this study, *Thalassiosira* spp. were dominant
329 during the diatom bloom in March 2017, and then the abundance of *Thalassiosira* spp.
330 decreased remarkably from early April 2017, when sea ice retreated and Soya Warm
331 Current started to prevail. From July to October 2016, when warm SWCW might prevail
332 around the study area, the abundance of *Chaetoceros* spp. was high. *Odontella* spp. were
333 dominant in abundance from December 2016 to the beginning of February 2017, when
334 low temperature ESCW was dominant in the coastal area of the Okhotsk Sea off Hokkaido.
335 The results of cluster analysis and NMDS also suggest the effect of water mass exchange
336 on the seasonal succession of protist communities. Protist communities were divided into
337 4 groups in this study. Group A appeared from March to early April 2017, when sea ice
338 existed around the study area., and in this group, *Thalassiosira* spp. were predominant.

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*

364 In this study, the biomass of the ciliates was greatest among the three taxa studied
365 in July 2016. Ciliates play an important role in connecting microbial food webs, including
366 bacteria to flagellates, with classical food chains (Levinsen and Nielsen, 2002). They are
367 preyed upon by mesozooplankton such as copepods (Campbell et al., 2009), and
368 predation pressure by the copepods decreases when the phytoplankton concentration is
369 high (Levinsen et al., 2000). Therefore, the high biomass of ciliates observed in July 2016
370 possibly can be attributed to the low predation pressure on ciliates by copepods, since
371 they might have grazed on diatoms actively because of their high Chl *a* concentration in
372 July 2016. This phenomenon is referred to as regulation windows and is known to occur
373 in Disco Bay along southwestern Greenland (Levinsen and Nielsen, 2002). The SEM

374 analysis showed that the abundance, biomass and production of ciliates had positive
375 correlations with water temperature. In other regions, the biomass of ciliates was greatest
376 in summer when the water temperature and Chl *a* concentration was high
377 (Godhantaraman, 2002), so that ciliates in this study were also able to grow actively in
378 the period with high Chl *a* concentration and water temperature. However, soon after this
379 peak, ciliates biomass decreased remarkably. When the decrease occurred, Chl *a*
380 concentration was low. Because copepods are known to have a great feeding pressure on
381 ciliates (Fessenden and Cowles. 1994), the low Chl. *a* concentration led them to change
382 their main prey from phytoplankton to ciliates. In addition, according to Campbell et al.
383 (2009), copepods prefer microzooplankton to phytoplankton, when microzooplankton is
384 abundant. Therefore, a change in the prey of mesozooplankton, such as when copepods
385 switch from phytoplankton to ciliates, may result in the rapid decrease in the ciliate
386 biomass.

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

388 ¹³C or ¹⁴C incubation methods are usually used (Sorokin and Sorokin, 1999;
389 Kasai et al., 2009) when measuring the photosynthesis rates of phytoplankton, whereas
390 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 data
392 without incubations.

393 The sum of the production of diatoms and dinoflagellates ranged from 1.6 to 67
394 $\text{mg C m}^{-3} \text{ day}^{-1}$. Assuming that production was the same through the whole column (9 m
395 depth), the water column production was converted to 14.4 to 603 $\text{mg C m}^{-2} \text{ day}^{-1}$.
396 According to Sorokin and Sorokin (1999), primary production in the summer (July to
397 August), when the abundance of phytoplankton was at the seasonal minimum, was more
398 than 60 $\text{mg C m}^{-3} \text{ day}^{-1}$ in the middle part of the Okhotsk Sea. The estimated values of
399 production in July and August of 2016 ranged from 1.8 to 38.3 $\text{mg C m}^{-3} \text{ day}^{-1}$. In addition,
400 in a pelagic region of the southern Okhotsk Sea, the monthly average production from
401 spring to autumn, except for a period of phytoplankton bloom in early spring, was
402 approximately 500 $\text{mg C m}^{-2} \text{ day}^{-1}$ (Kasai et al., 2009). Production in the same season
403 was approximately 139 $\text{mg C m}^{-2} \text{ day}^{-1}$ in this study. The primary production estimated
404 in this study showed lower values than shown by previous studies. In the oligotrophic
405 condition (e.g., summer in this study), the production by nanoflagellates ($< 2 \mu\text{m}$) is much
406 higher than that by micro-sized phytoplankton (Maita and Odate 1988). Noting that the
407 nanoflagellates were not enumerated in this study, taking no account of their production
408 is one of the potential reasons for the low productivity. In addition, the expression for

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.

421 *4. 5. Conclusion*

422 Seasonal variability in the protist community at Mombetsu Port was determined
423 by frequent observations. Bottom-up effects by the exchange of water masses, nutrients
424 and winds seemed to have great influence on the seasonal variability of Chl *a*
425 concentration. In particular, the exchange of water masses had a great effect on diatom

426 communities. Ciliates showed a dramatic variability governed by an enhancement of
427 growth associated with high Chl *a* concentration, high water temperatures and
428 fluctuations in predation pressure from mesozooplankton. Identifications of species were
429 not performed in this study, and such identification is needed to investigate more detailed
430 seasonal changes of the protist community and the species composition and the factors
431 controlling the changes. Determining the production values is also necessary, not only by
432 estimating it from cell sizes but also by using incubation methods such as the ¹³C method,
433 to investigate the degree of difference between the estimated values based on cell sizes
434 and the values obtained from incubations. Though the production estimated from cell
435 sizes showed discrete differences from the reported values because of the differences in
436 the methods, investigating seasonal changes in primary production is important for
437 understanding the mechanism required to promote high primary production and rich
438 fishery resources in the study area.

439 **Acknowledgements**

440 This study was financially supported by the Arctic Challenge for Sustainability
441 (ArCS) Project (Programme Grant Number JPMXD1300000000) and Arctic Challenge
442 for Sustainability II (ArCS II) (Programme Grant Number JPMXD1420318865). Part of

443 this study was supported by a Grant-in-Aid for Challenging Research (Pioneering)
444 20K20573, and Scientific Research 20H03054 (B), 19H03037(B), 18K14506 (Early
445 Career Scientists), and 17H01483 (A) from the Japanese Society for the Promotion of
446 Science (JSPS). This study was supported partly by the Grant for Joint Research Program
447 of the Japan Arctic Research Network Center.

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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; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Parameters	Groups				one-way ANOVA	Tukey-Kramer test
	A (6)	B (33)	C (15)	D (3)		
SST	0.23	13.2	0.17	5.5	***	C A <u>D</u> B
SSS	32.1	31.1	31.3	32	NS	
Chl. <i>a</i>	1.2	1.8	0.6	1.1	*	C <u>D</u> A B
Nitrate + Nitrite	4.8	3.1	7.4	3.7	*	B <u>D</u> 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	
<i>Chaetoceros</i> spp.	2268	22551	185	139	***	D C A B
<i>Guinardia</i> spp.	0	2487	0	172	*	Not detected
<i>Odontella</i> spp.	0	0	2039	0	**	Not detected
<i>Pseudo-nitzschia</i> spp.	1500	22150	10	137	NS	
<i>Thalassiosira</i> 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 <u>A</u> B
Total diatoms	63102	75755	5549	1620	*	D C <u>A</u> B
Dinoflagellates	62	813	80.2	80.4	**	A C <u>D</u> B
Aloricate ciliates	17	477	25	0	NS	
Loricated ciliates	30	363	26	12	NS	
Total ciliates	47	840	52	12	NS	
Whole taxa	63121	77409	5681	1712	*	D C <u>A</u> B

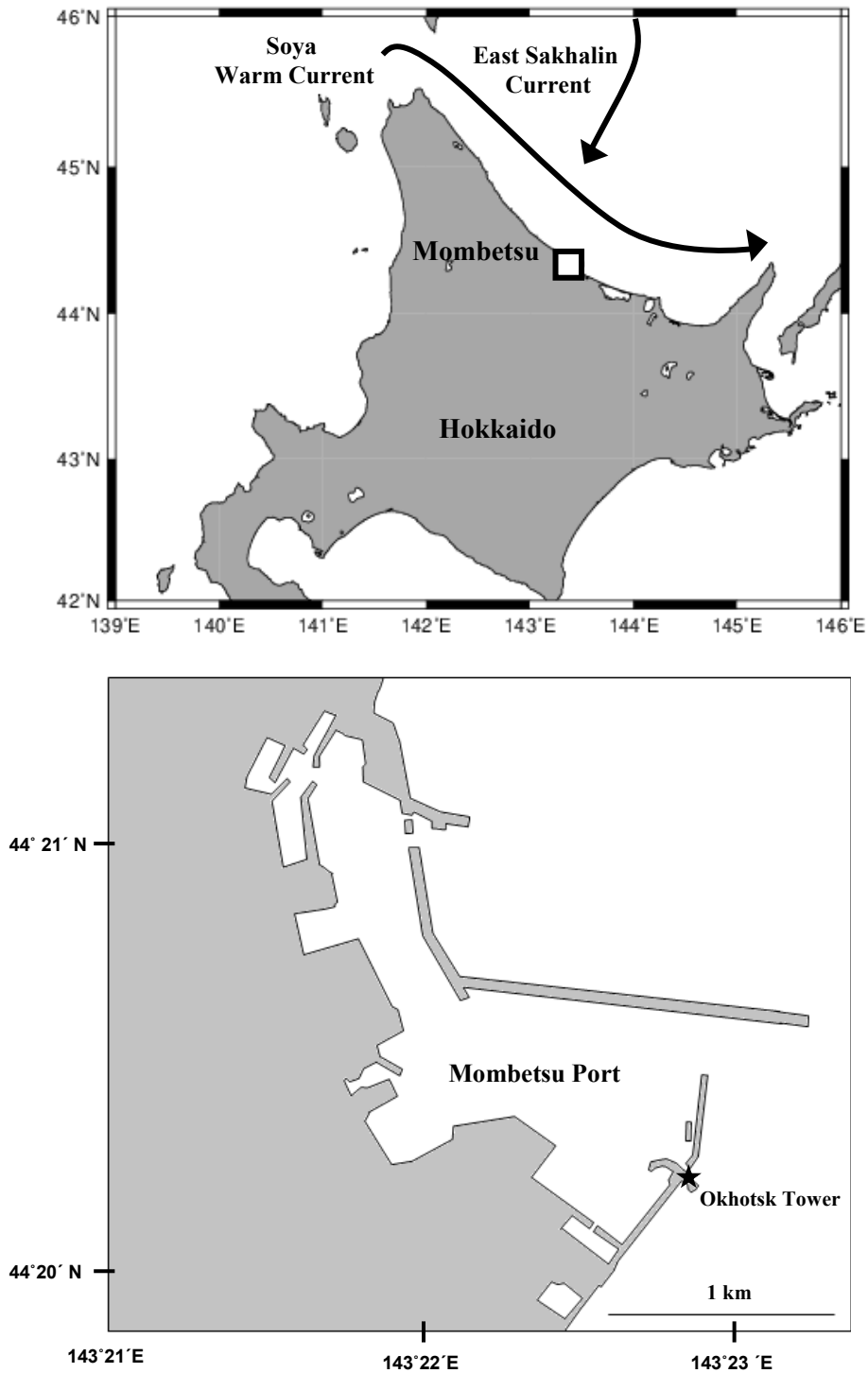


Fig. 1. Location of the sampling station (Okhotsk Tower) at Mombetsu Port, north-eastern Hokkaido. Arrows indicate the approximate direction of current flows.

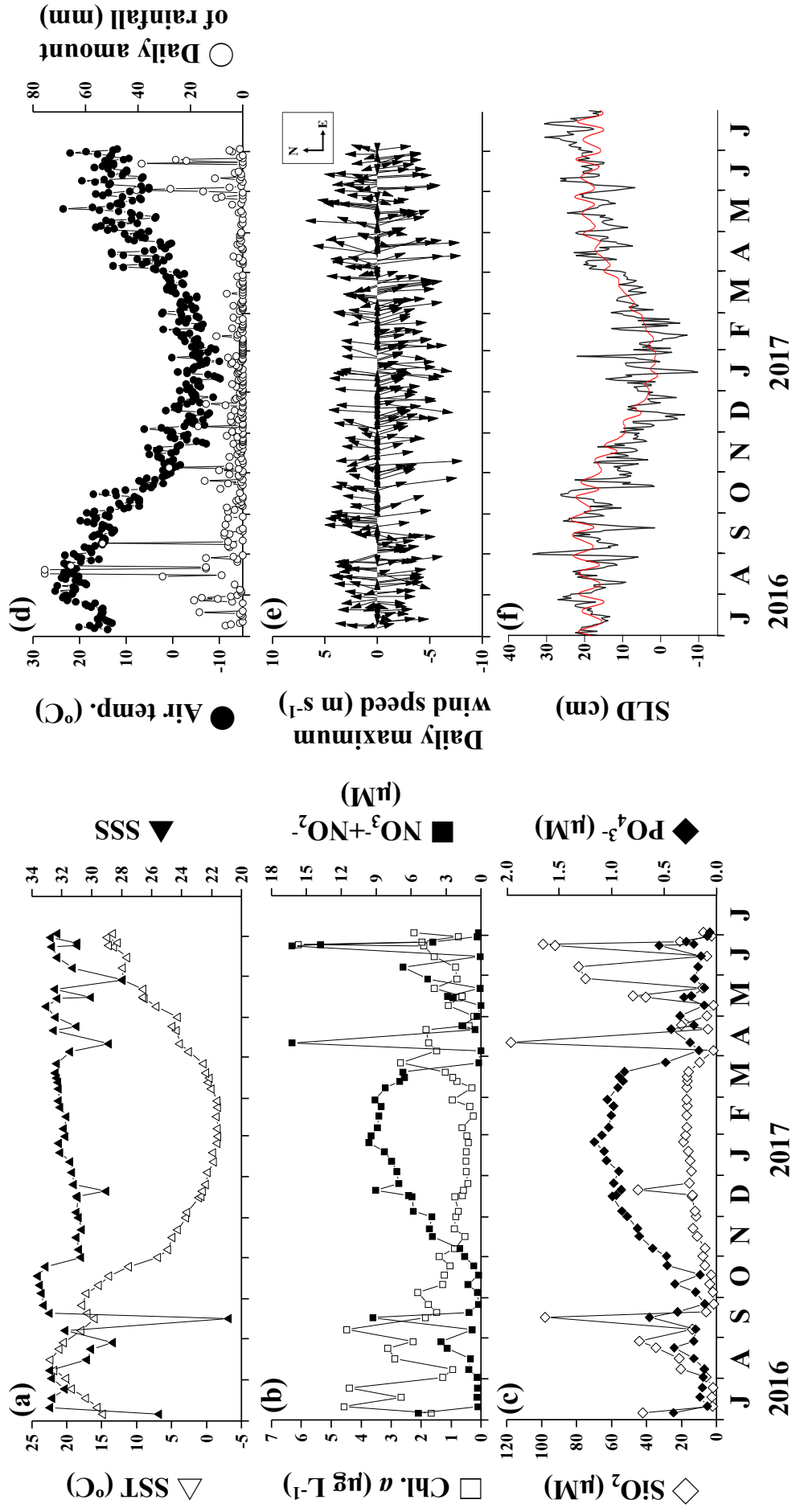


Fig. 2. Seasonal changes in environmental parameters. (a) Daily mean sea surface temperature (SST) and salinity (SSS), (b) chlorophyll *a* and nitrate and nitrite (NO_3^- and NO_2^-) at sea surface, (c) silicate and phosphate at the sea surface, (d) daily averaged air temperature and daily amount of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

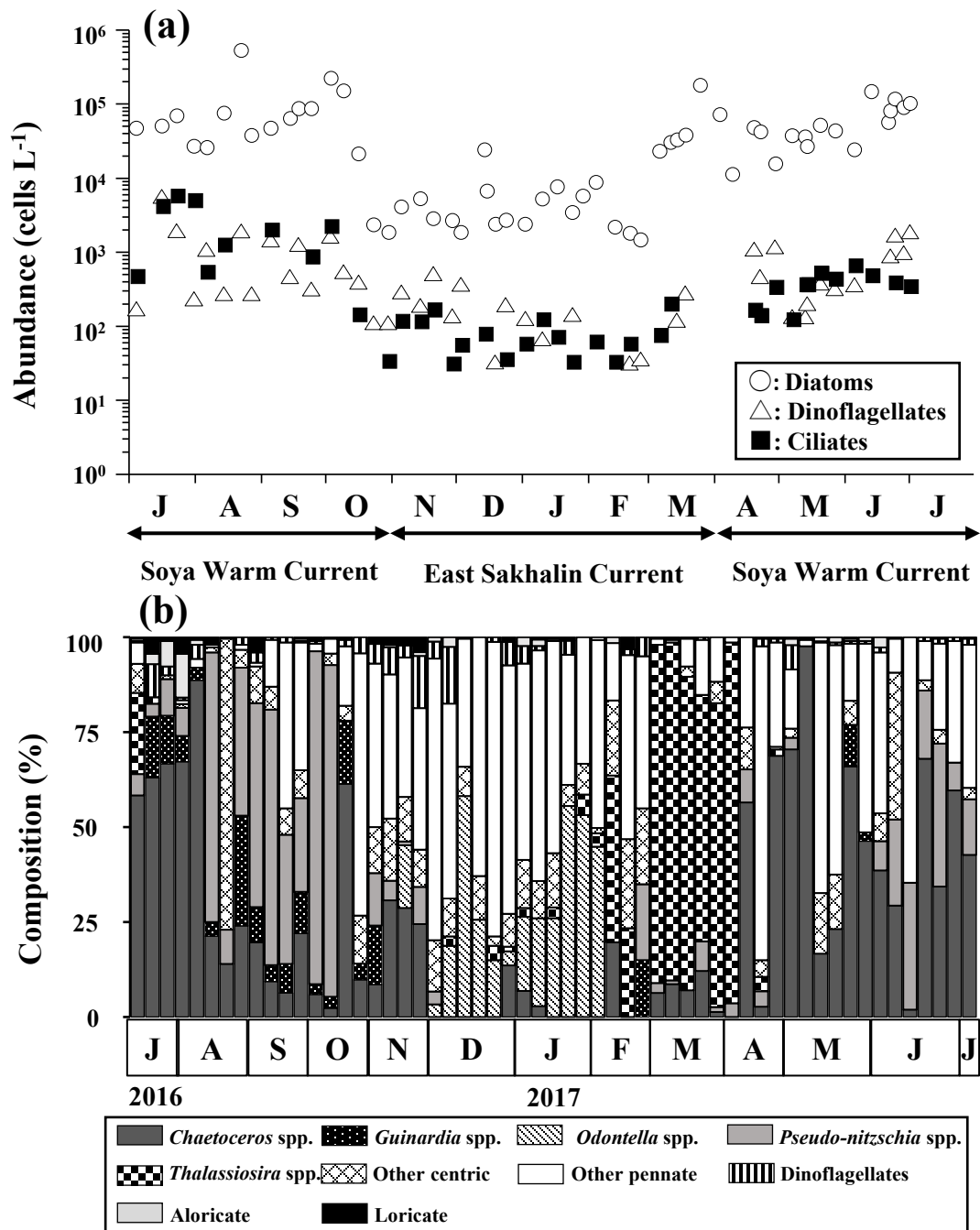


Fig. 3. Seasonal changes in protist abundance and the predominant water masses (a) and species composition (b) at Mombetsu Port from July 2016 to July 2017.

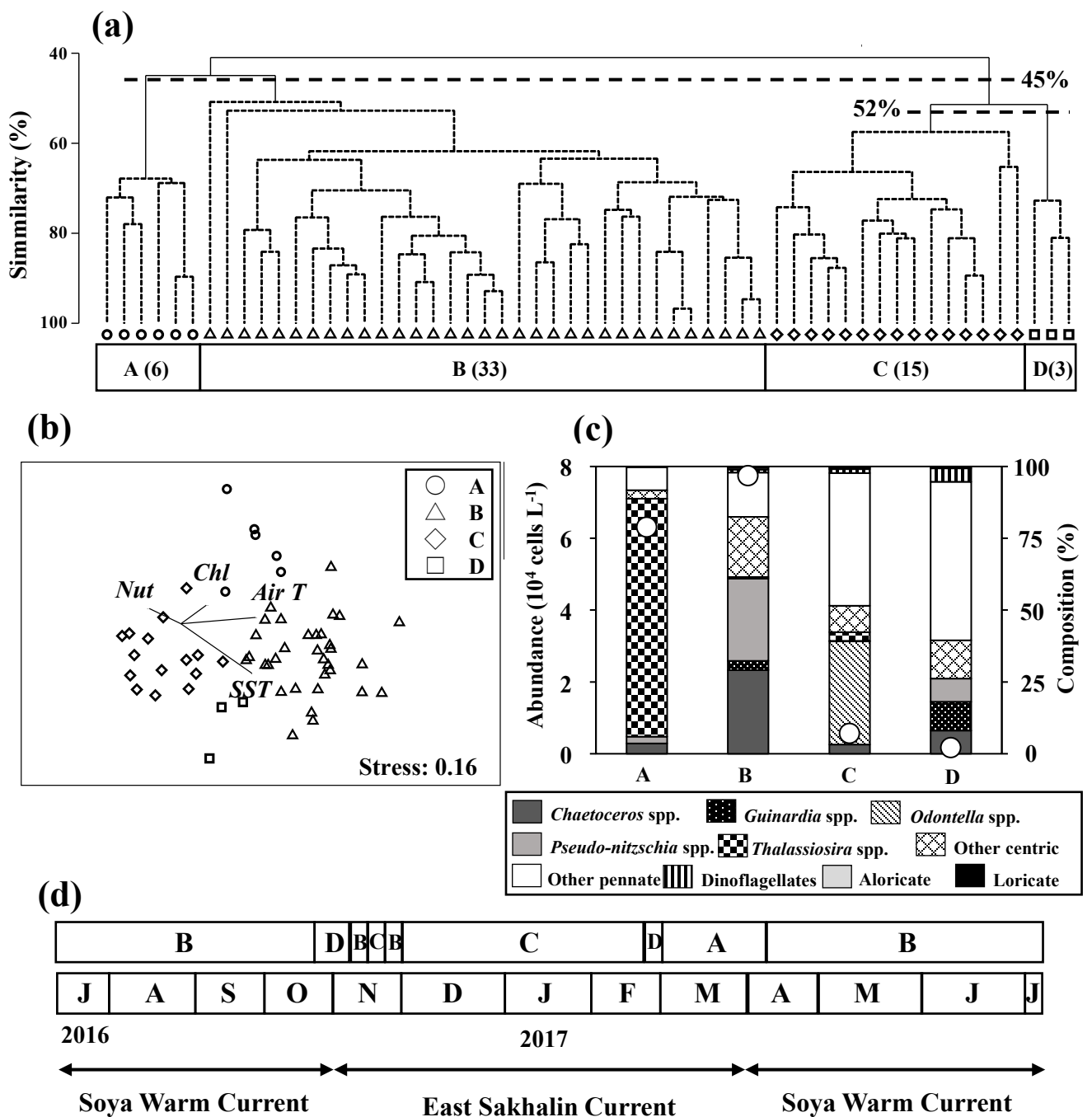


Fig. 4. Results of cluster analysis based on protist abundance by Bray-Curtis similarity connected with UPGMA. Four groups (A–D) were identified with the dissimilarity at 45 and 52%, respectively (dashed lines) (a). Numbers in the parentheses indicate quantity of samples included in each group. Nonmetric 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).

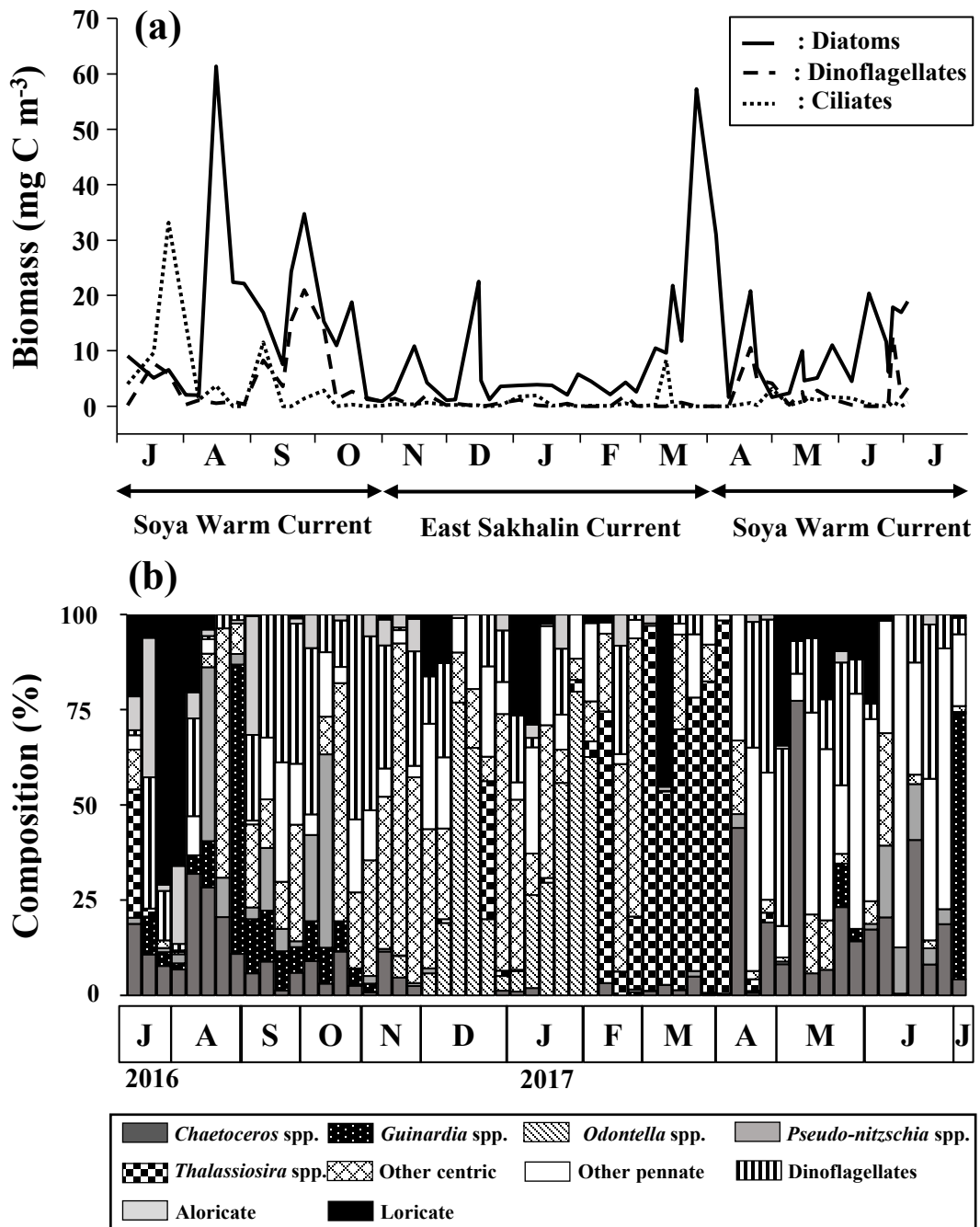


Fig. 5. Seasonal changes in protist biomass and the predominant water masses (a) and species composition (b) at Mombetsu Port from July 2016 to July 2017.

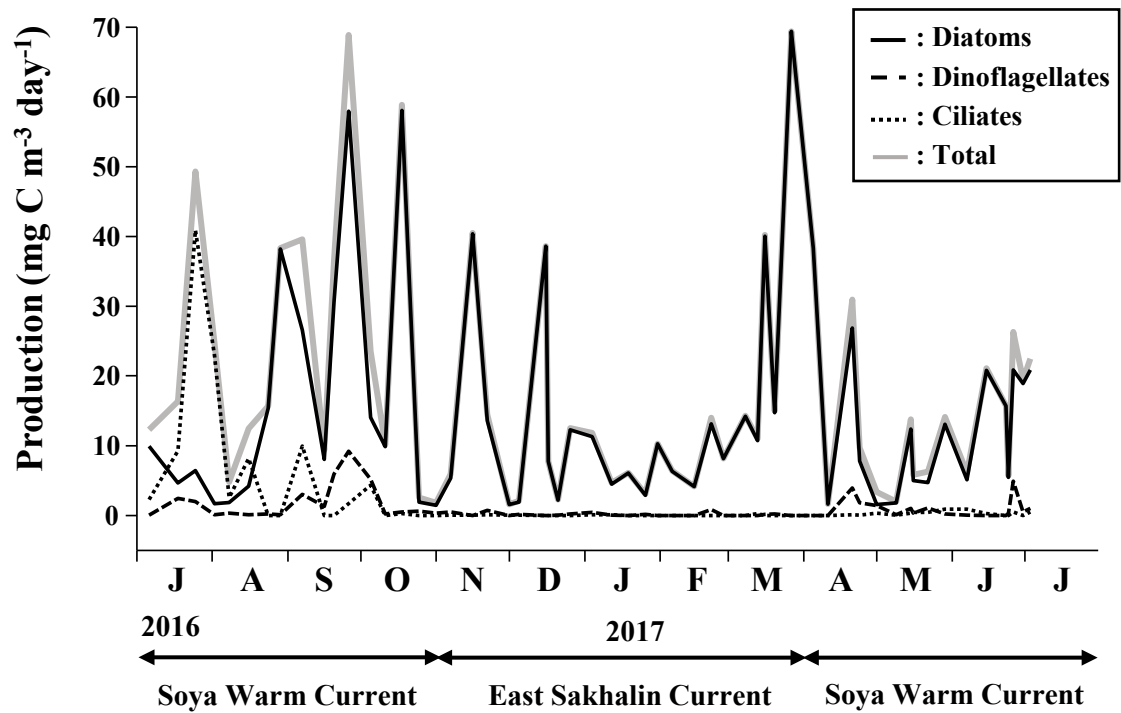


Fig. 6. Seasonal changes in protist production estimated from cell size and ambient temperature, and the predominant water masses at Mombetsu Port from July 2016 to July 2017.

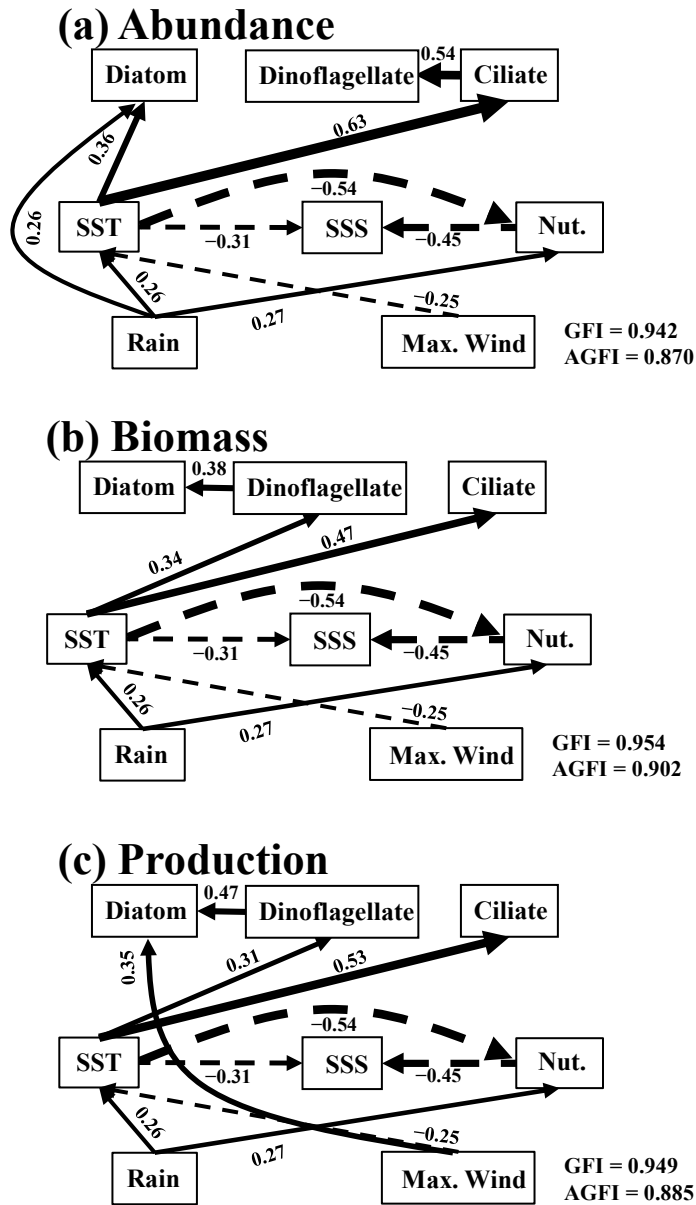


Fig. 7. Results of structural equation models (SEM) for protist parameters (abundance, biomass and production) with environmental factors. The values along the pathways represent standardized path coefficients. Arrows with solid or dashed lines indicate positive or negative effects. Thickness of arrows 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.