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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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**Abstract**

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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 ($7.7 \times 10^4$ 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

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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.

## 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 et al., 2015), and spring diatom 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

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

6

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

7

110     dinoflagellates (mg C m$^{-3}$ day$^{-1}$) was estimated by the regression formula of Marañón

111     (2008): $\log_{10} P = b \log_{10} V + a$ ($P$: production (pg C cell$^{-1}$ day$^{-1}$); $V$: cell volume ($\mu$m$^3$); $a$,

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 (mg C m$^{-3}$ 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 (°C), and $V$: cell

117     volume ($\mu$m$^3$)).

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/).

**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 22.3ºC, and it had the maximum in August 2016 and the minimum in February 2017. From January to March 2017, it was approximately 0ºC, and from July to August, it showed higher values (> 15ºC) (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

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 27.4ºC, 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 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-

176    mombetsu.jp/).


177    *3.2. Seasonal variability of abundance*

178    The abundance of the diatoms ranged from $1.55\times10^3$ to $5.36\times10^5$ cells L$^{-1}$, that

179    of dinoflagellates ranged from N.D. (not detected) to $5.28\times10^3$ cells L$^{-1}$, and that of

180    ciliates ranged from N.D. to $5.92\times10^3$ 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

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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 ($6.3 \times 10^4$ 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 ($7.7 \times 10^4$ 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    ($1.7 \times 10^3$ 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

14

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

15

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).

16

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., $> 5$ 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.

## 4. 2. Seasonal changes of diatoms

322

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. nordenskioeldii*, *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　　　　$^{13}$C or $^{14}$C 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    mg C m$^{-3}$ 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 mg C m$^{-2}$ 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 mg C m$^{-3}$ 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 mg C m$^{-3}$ 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 mg C m$^{-2}$ day$^{-1}$ (Kasai et al., 2009). Production in the same season

403    was approximately 139 mg C m$^{-2}$ 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 μm) 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 $^{13}$C 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.

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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 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 |
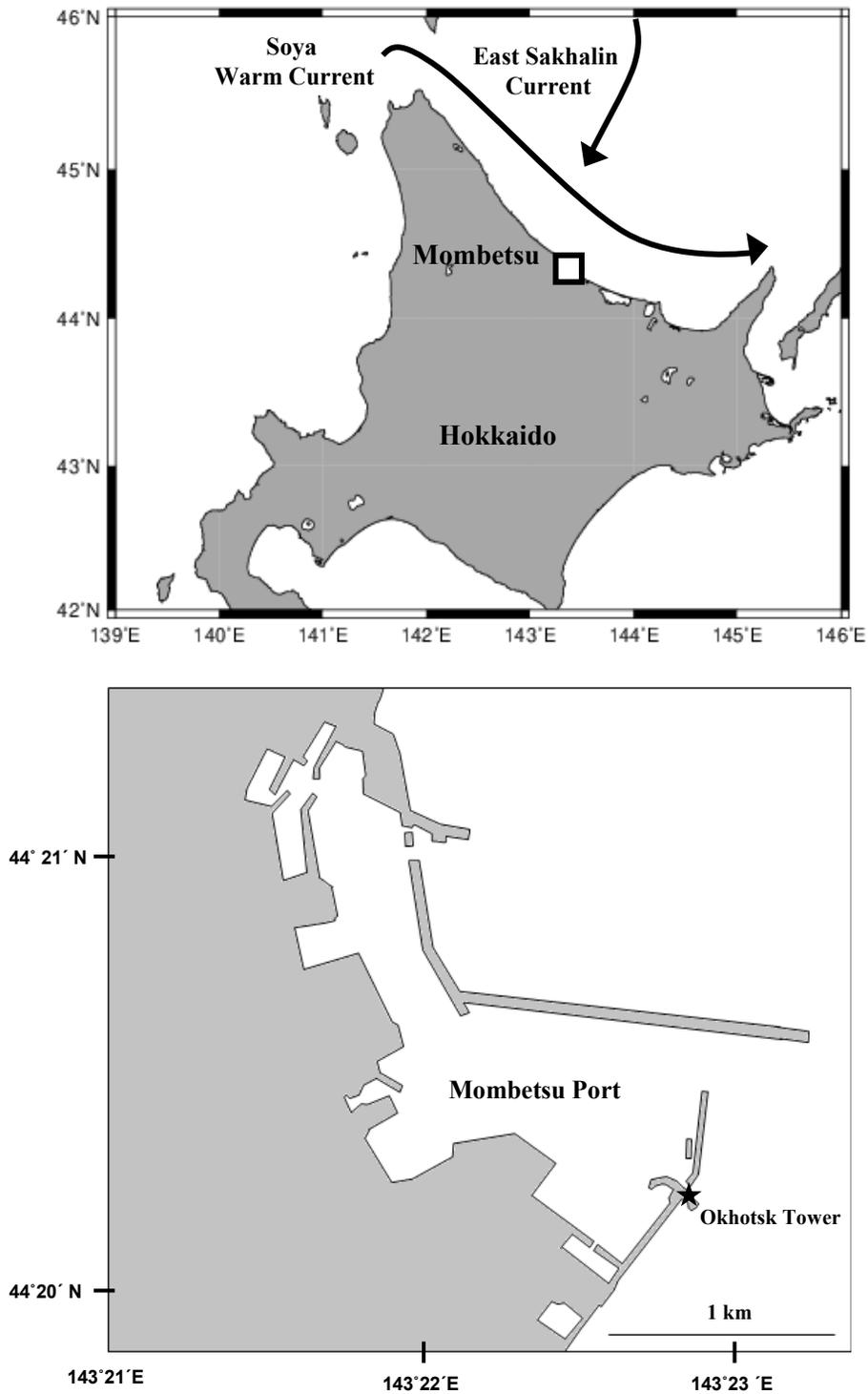
Fig. 1. Location of the sampling station (Okhotsk Tower) at Mombetsu Port, north-eastern Hokkaido. Arrows indicate the approximate direction of current flows.
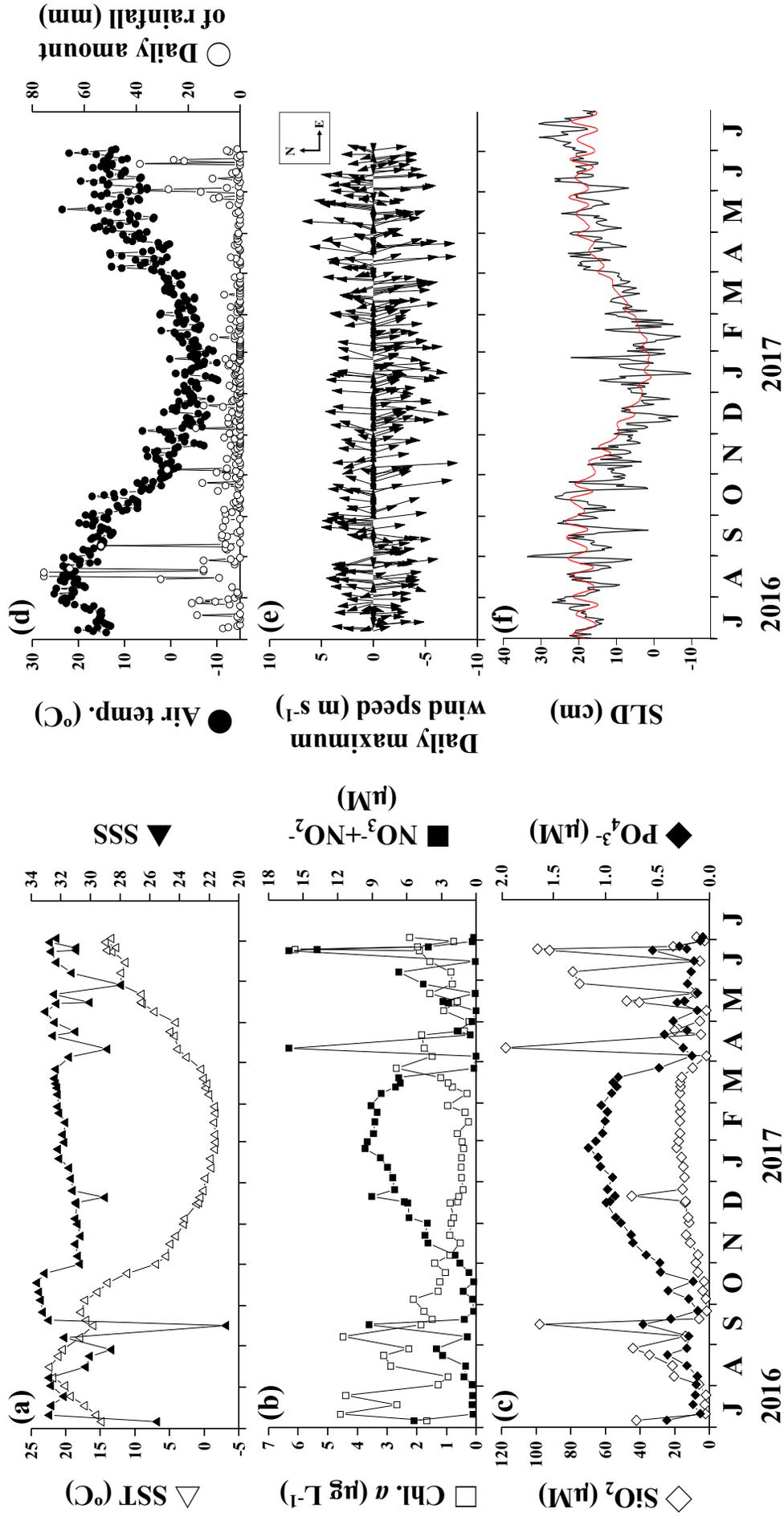
**Fig. 2.** Seasonal changes in environmental parameters. (a) Daily mean sea surface temperature (SST) and salinity (SSS), (b) chlorophyll *a* and nitrate and nitrite ($NO_3^+$ and $NO_2^-$) at sea surface, (c) silicate and phosphate at the sea surface, (d) daily averaged air temperature and daily amount 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.
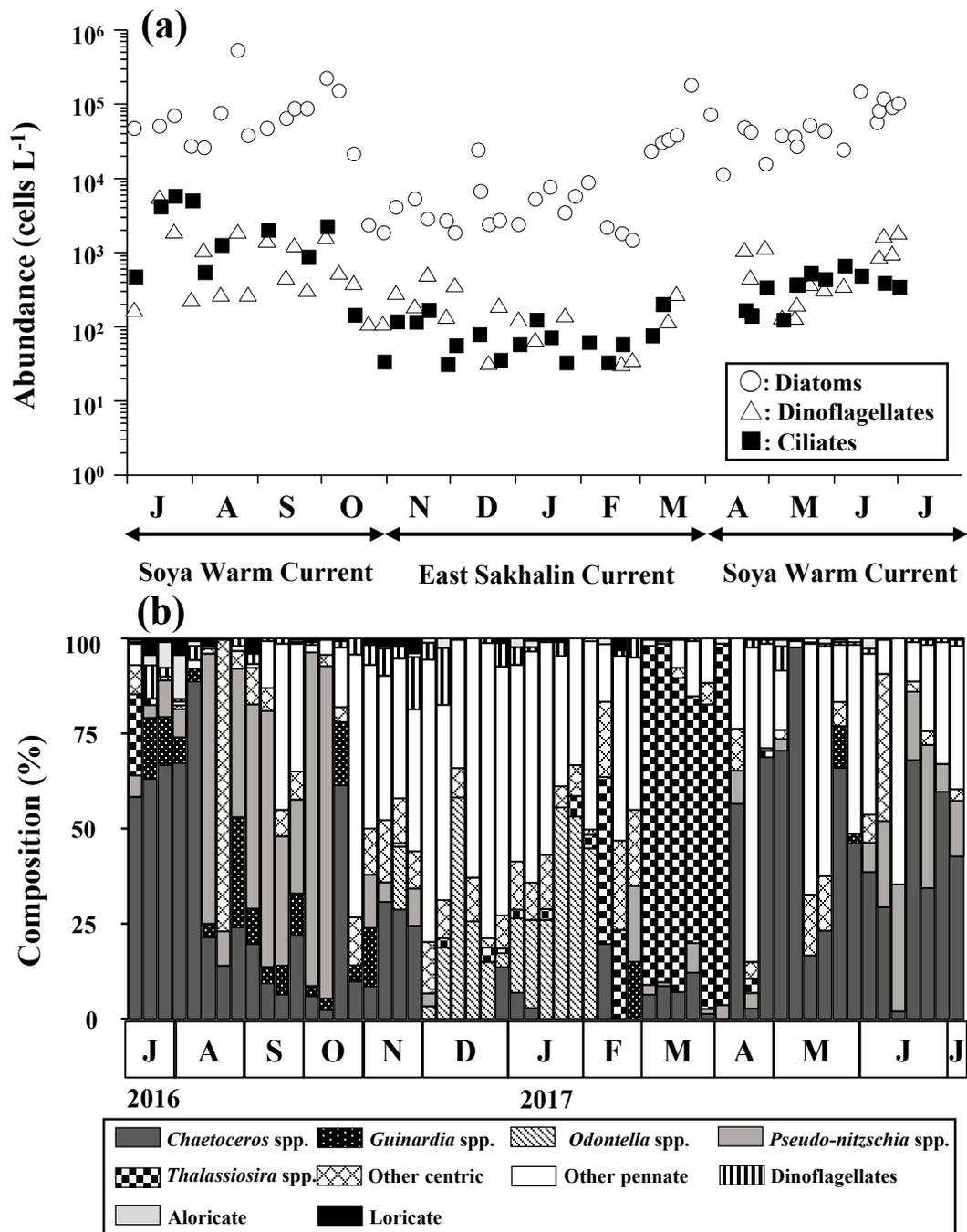
Fig. 3. Seasonal changes in protist abundance and the predominant water masses (a) and species composition (b) at Mombetsu Port from July 2016 to July 2017.
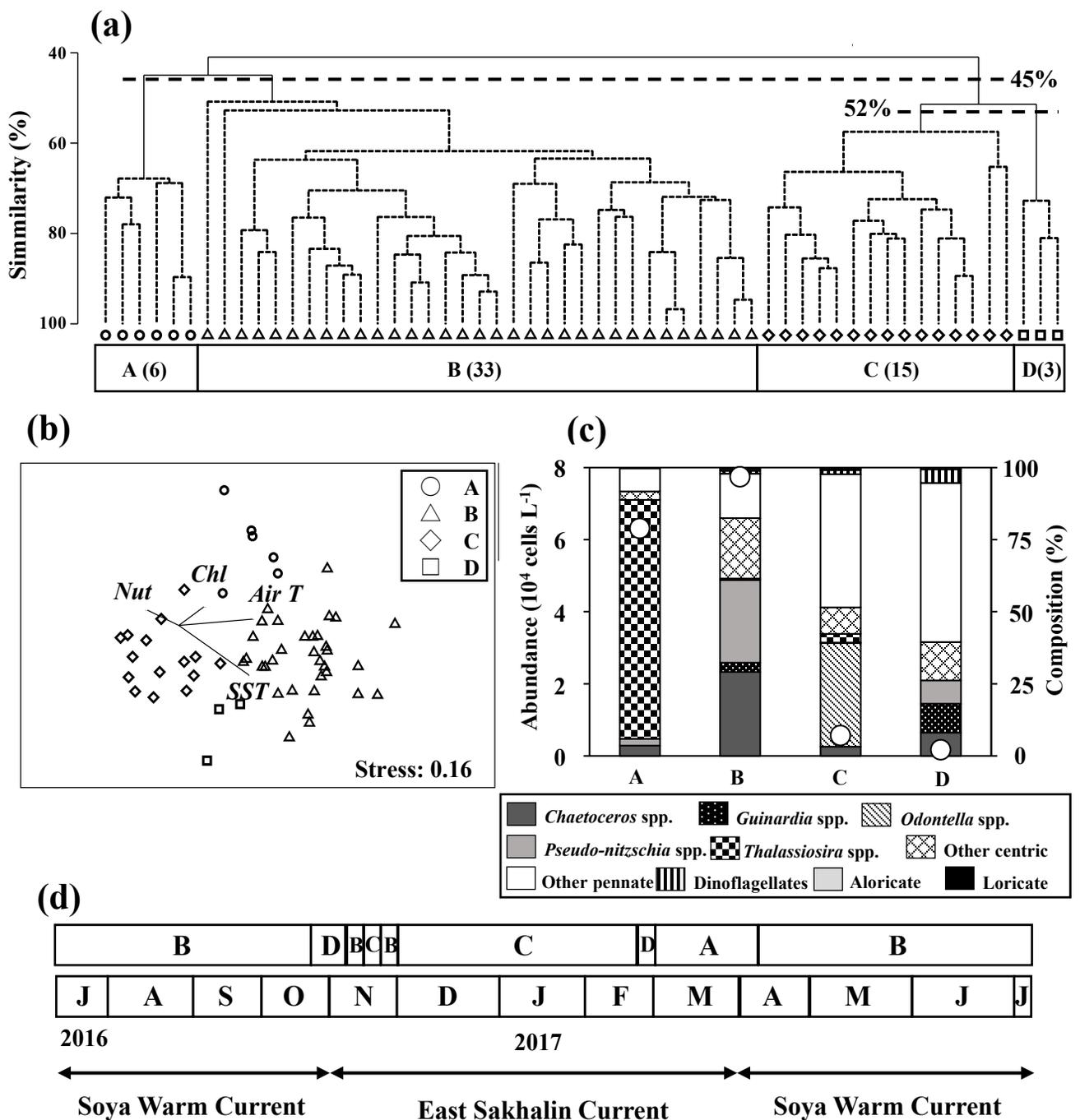
Fig. 4. Results of cluster analysis based on protist abundance by Bray-Curtis similarity connected with UPGMA. Four groups (A–D) were identified with the dissimilarity at 45 and 52%, respectively (dashed lines) (a). Numbers in the parentheses indicate quantity of samples included in each group. Nonmetric 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).
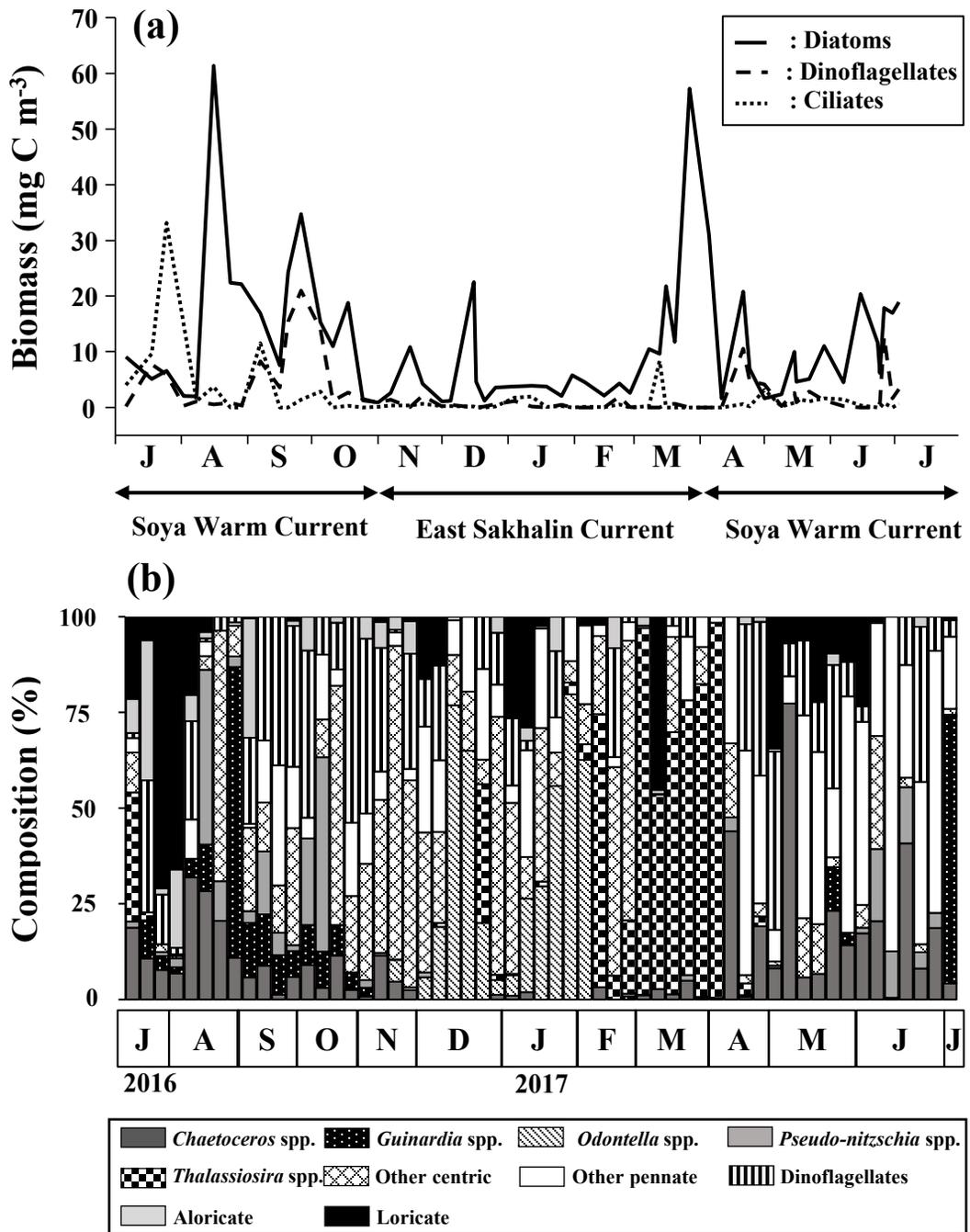
Fig. 5. Seasonal changes in protist biomass and the predominant water masses (a) and species composition (b) at Mombetsu Port from July 2016 to July 2017.
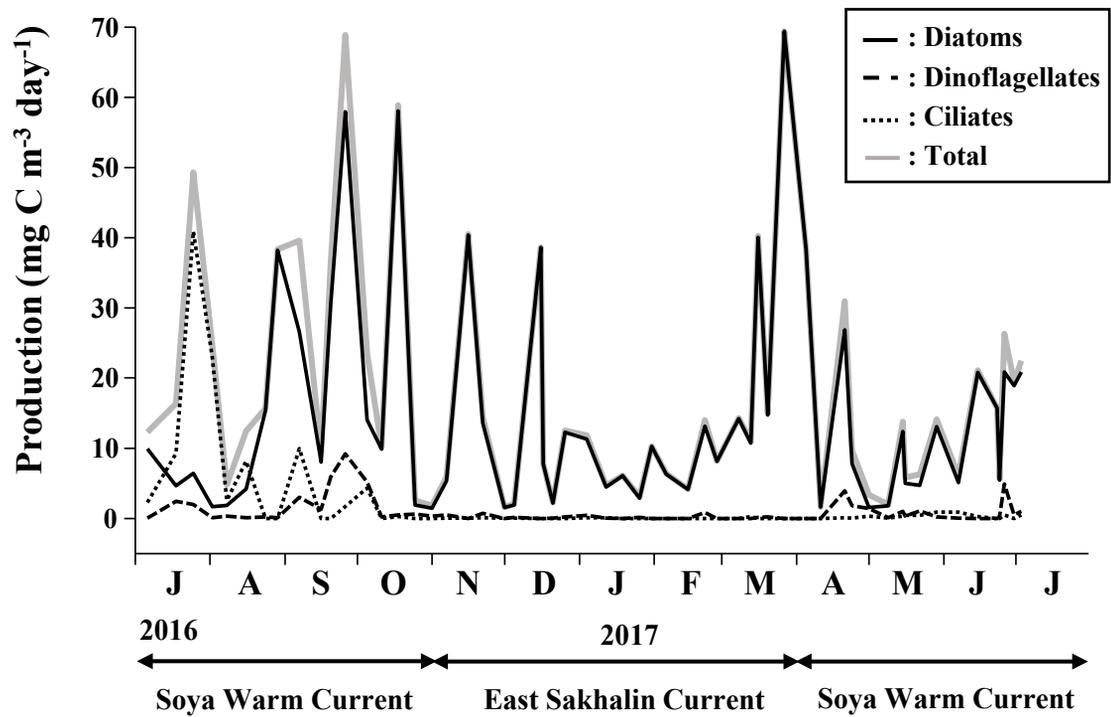
Fig. 6. Seasonal changes in protist production estimated from cell size and ambient temperature, and the predominant water masses at Mombetsu Port from July 2016 to July 2017.
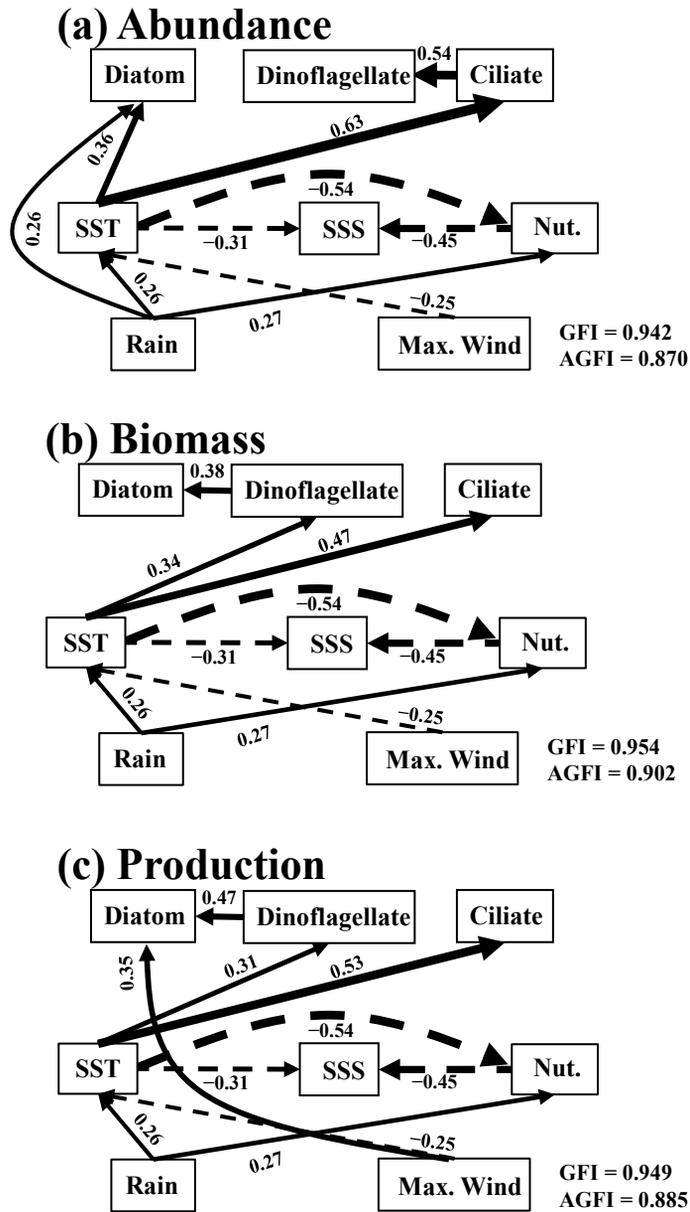
Fig. 7. Results of structural equation models (SEM) for protist parameters (abundance, biomass and production) with environmental factors. The values along the pathways represent standardized path coefficients. Arrows with solid or dashed lines indicate positive or negative effects. Thickness of arrows 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.