

Seasonal variation in microplankton communities in Suttsu, Hokkaido, northern Japan, from 2020 to 2022

Kyosei Morimoto^{a,*}, Yusuke Hamao^a, Tomoyasu Yamazaki^b, Shoko Tatamisashi^c, Masahide Wakita^c, Kohei Matsuno^{a,d}

^a Faculty/Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041-0821, Japan

^b Shellfish Museum of Rankoshi, 1401, Minatocho, Rankoshi, Hokkaido 048-1341, Japan

^c Mutsu Institute for Oceanography, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 690 Kitasekine Sekine, Mutsu, Aomori 035-0022, Japan

^d Arctic Research Centre, Hokkaido University, Kita-21 Nishi-11 Kita-ku, Sapporo, Hokkaido 001-0021, Japan

ARTICLE INFO

Keywords:

Diatoms
Euglenoids
Warm-water species
Seasonal change
Subpolar Front Current

ABSTRACT

Southwestern Hokkaido faces intricate physiological and chemical changes. Subsequently, changes in lower trophic levels are of concern, but fully understanding the relationship between microplankton and hydrography in this region is required. To examine the relationship between the microplankton community (diatoms, dinoflagellates, ciliates, and euglenoids) and environmental factors in Suttsu, Hokkaido, water samples were collected at 4–15-day intervals from August 2020 to August 2022 at Yokoma Port. Diatoms were dominant in the study area, with an apparent seasonal change in species composition. Lower phytoplankton cell density was observed in the second year than in the first year during autumn and winter because of low temperatures and light intensity in the second year. A significant decrease in the number of attached diatoms (*Navicula* spp.) was observed during the winter of the second year, possibly due to calm conditions (low tide and wind speed), which prevent the detachment of weakly attached species. Large blooms of euglenoids (*Eutreptiella gymnastica* and *E. marina*) in April and May were caused by the exclusive use of nutrients from the inflow of river water during the snowmelt season. Warm-water species sporadically occurred, suggesting their transportation by the Tsushima Warm Current. Temperature, nutrients, and light intensity primarily control microplankton communities, which vary seasonally. These findings led to the prediction of lower trophic levels due to the impact of warm water inflow.

1. Introduction

Phytoplanktons are crucial organisms responsible for primary production in the ocean. Microsized phytoplanktons are mainly composed of diatoms, dinoflagellates, and euglenoids. Diatoms are classified as planktonic diatoms and benthic diatoms based on habitat differences. Planktonic diatoms are microalgae that are observed prominently in inner bays, coastal and offshore areas, and some species have chain-forming during spring bloom (Itakura, 2001). Benthic diatoms are a general term for diatoms that grow by attaching to various substrates in the water. Solitary cell is well observed with the ability of moving on the substrates. There is no clear boundary between planktonic diatoms and benthic diatoms, and some species have both floating and attached lives (Kawamura, 1994). In addition, diatoms are divided into two taxonomic groups: centric and pennate. Most planktonic diatoms are centric, and

most benthic diatoms are pennate (Kawamura, 1994). Diatoms have a rapid growth rate, with a maximum growth rate of $\mu_{\max} = 1.5 \text{ d}^{-1}$ when light conditions and nutrient concentrations are favorable (Sarthur et al., 2005). In addition, many diatoms form resting spores and can survive in environments that are unfavorable for growth (Itakura et al., 1992; Ishii et al., 2011; Matsubara, 2017). Euglenoids are widely distributed from freshwater to the ocean due to their high salinity tolerance, such as marine euglenoids *Eutreptiella gymnastica* (Lee and Lim, 2006; Xu et al., 2012). They can also grow in various temperatures (Xu et al., 2012). In Mexico, several hundred fish died from anoxia caused by *Eutreptiella marina* blooms (Cortés-Lara et al., 2010). In southern Hokkaido, a red tide occurrence of *Eutreptiella* sp. has been reported at Matsumae port (Shimada, 2021).

The Japan Sea of western Hokkaido is known to be a fishery for herring, Japanese flying squid, and walleye pollock (Nakata and Tanaka,

* Corresponding author.

E-mail address: ryoha19morimoto@eis.hokudai.ac.jp (K. Morimoto).

<https://doi.org/10.1016/j.rsma.2023.103322>

Received 24 July 2023; Received in revised form 29 November 2023; Accepted 4 December 2023

Available online 5 December 2023

2352-4855/© 2023 Elsevier B.V. All rights reserved.

2002). Sutttsu is located in the southwestern Hokkaido, facing the Sea of Japan, and the surrounding ocean area is characterized by low nutrients and productivity (Adachi and Osawa, 2005). In addition to the above species, scallops and oysters are caught in the waters surrounding Sutttsu. (Hokkaido Research Organization: http://www.fishexp.hro.or.jp/marinedb/internetdb/fishdb/fish_all.asp). This area is influenced by the Subpolar Front Current (SFC), which diverges from the Tsushima Warm Current. Seasonal variation in current speed of the Tsushima Warm Current, which flows around this western Hokkaido, is smaller (0.25 Sv) than the Tsugaru Warm Current (0.30 Sv) (Nakata and Tanaka, 2002; Nishida et al., 2003; Yabe et al., 2021). However, the Tsushima Warm Current in the Tsushima Strait has recently been increasing (Kida et al., 2021), followed by an increase in the SFC, which could change the oceanic environment around Hokkaido. In addition, in recent years, it is reported that sea surface temperatures in the waters surrounding Japan Sea have been rising. (e.g. <https://www.jma.go.jp/jma/en/Activities/cc.html>). As one example, ocean warming, acidification, and deoxygenation due to global change-driven may cause serious damage to calcifiers in Oshoro Bay, which also facing Japan Sea of the western Hokkaido (Fuji et al., 2021). In contrast, Sutttsu is seasonally affected by freshwater inflow from rivers (the Shiribetsu and Shubuto rivers). For example, a rapid decrease in salinity and nutrient supply in the

neighboring Ishikari Bay occurs with increased river water inflow in April during the snowmelt season (Adachi and Osawa, 2005). In addition, in Funka Bay, on the Pacific side of Hokkaido, nutrients in the river water support new phytoplankton production (Yoshimura and Kudo, 2003). Therefore, river water inflow is also an essential factor affecting phytoplankton composition and cell density.

Information on phytoplankton in the western side (Sea of Japan) of Hokkaido includes long-term monthly observations in Oshoro Bay that concluded that centric diatoms are dominant and that a little relationship exists between regime shifts and phytoplankton cell density (Fukui et al., 2010). The appearance of the harmful dinoflagellate *Karenia mikimotoi* (Shimada et al., 2016; Kakumu et al., 2018) and the northward expansion of the distribution area of *Heterocapsa circularisquama* (Kondo et al., 2012) have been reported in Hakodate Bay, potentially owing to an increase in the Tsushima Warm Current. However, the comprehensive dynamics of micro-sized phytoplankton communities are still unknown because of methodological issues (100 μm mesh net collection, monthly frequency) and the investigation of only specific species (in many cases, harmful species). A detailed analysis of the relationship between phytoplankton and hydrography is required to accurately evaluate the effects of changing environmental conditions (increased SFC) on marine ecosystems including fisheries production.

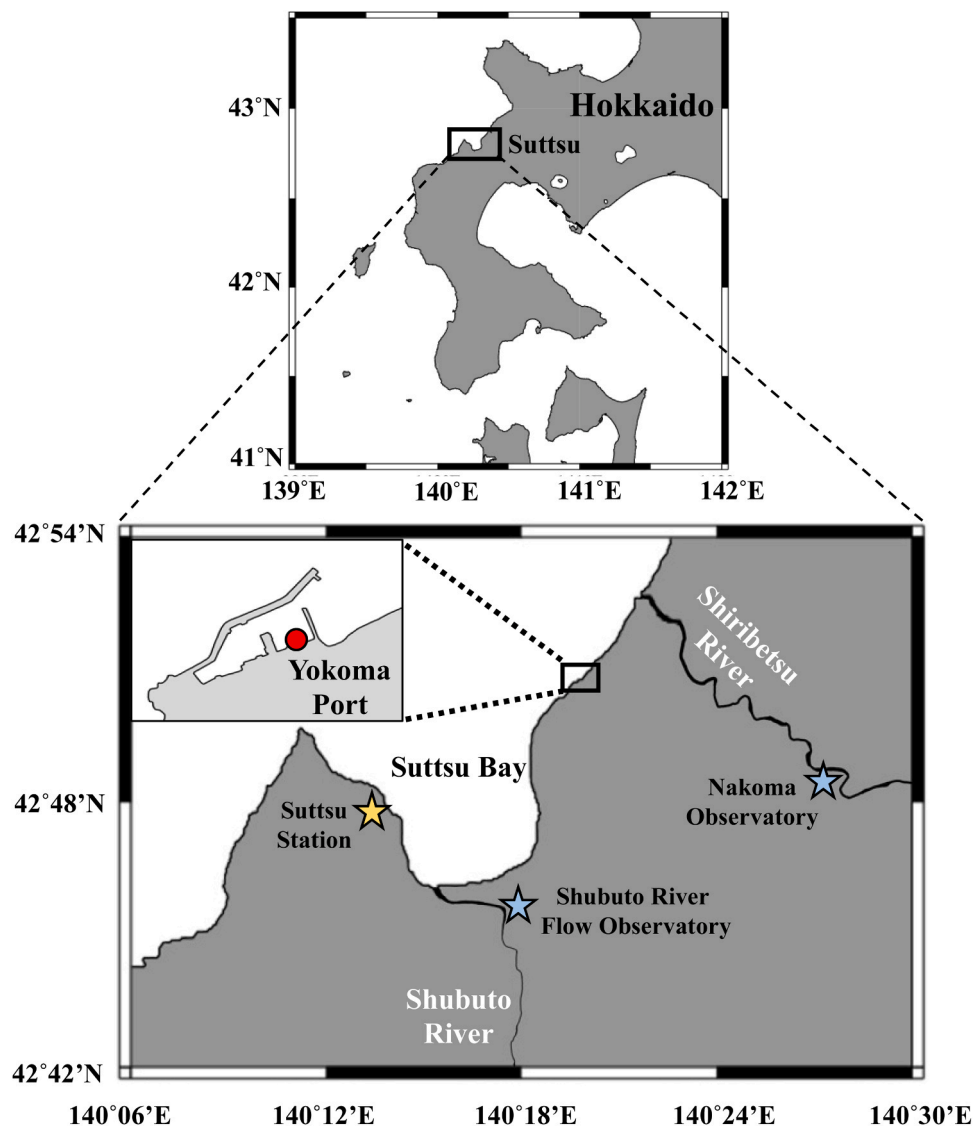


Fig. 1. Location of the sampling station at the Yokoma Port in southern Hokkaido. The red circle indicates a fixed station for water sampling, and the yellow star indicates a station for monitoring atmospheric conditions (temperature, rainfall, and wind). Light blue stars indicate the observatory of each river.

This study aimed to clarify seasonal changes in micro-sized phytoplankton and environmental factors by conducting high-frequency fixed-point observations over 2 years at Yokoma Port in Suttu, Hokkaido. Furthermore, we examined the relationship between micro-sized phytoplankton and environmental factors and discussed annual changes in phytoplankton, the mechanism of spring bloom formation, and the occurrence of warm-water species.

2. Materials and methods

2.1. Field sampling

A total of 80 samples were collected at the Yokoma Port of Suttu, Hokkaido (48.85° N, 140.33° E) at 4–15-day intervals from August 25, 2020, to August 15, 2022 (Fig. 1). Water samples were collected from the sea surface using plastic buckets. For salinity and nutrients (nitrate, nitrite, ammonium, phosphate, and silicate), water samples were stored in brown glass bottles and polyethylene bottles at 4 and – 20 °C until analyzed, respectively. For the phytoplankton samples, 500 mL of seawater was placed in a plastic bottle, and a fixative was added immediately. Note that we used two fixatives because of the availability of instruments: Lugol acid (final concentration 1%) from August 25, 2020, to January 11, 2022, and glutaraldehyde (final concentration 1%) from January 25, 2022, to August 15, 2022. Sea surface temperature (SST) was measured during water sampling. The study was conducted over a two-year period, with the first year defined as August 2020–August 2021 and the second year as September 2021–August 2022.

The average daily air temperature, total daily precipitation, average daily wind speed, maximum wind direction, tide level, and daylight hours were obtained from the Japan Meteorological Agency website at the Suttu Meteorological Station (<https://www.jma.go.jp/jma/index.html>). Data on the flow rate of the Shiribetsu River were obtained from the Ministry of Land, Infrastructure, Transport, and Tourism's Hydrologic and Hydrochemical Quality Database at the Nakoma Observatory, which is located in the middle reaches of the Shiribetsu River (<http://www1.river.go.jp>). Data on the flow rate of the Shubuto River was obtained from Hokkaido website at the Nakoma Observatory, which is located in the downstream area of the Shubuto River (<https://www.constr-dept-hokkaido.jp/ks/ikb/iji/ryuuryou/>).

2.2. Sample analysis

Salinity and nutrients were measured using a salinometer (Model 8400B AUTOSAL, Guildline Instruments Ltd.) and a continuous flow analyzer (QuAatro, BL TEC K.K.), respectively (Wakita et al., 2021).

In the laboratory, fixed samples (500 mL) were stored on a stone table for more than 1 day to allow the phytoplankton cells to settle at the bottom of the bottle. Subsequently, the samples were concentrated to 20 mL using a siphon. Subsamples (500 µL) of the concentrated sample were mounted on a glass slide using a micropipette. Microsized phytoplankton cells were identified and enumerated using an inverted microscope (ECLIPSE Ts2R, Nikon) at 40–600 × magnification. According to Fukuyo et al. (1997), Hasle and Syvertsen (1997), and Steidinger, Targen (1997), diatoms, dinoflagellates, and silicoflagellates were identified to the lowest possible levels (species or genus). Tinnid and oligotrich ciliates were counted separately. In addition to species and genus identification, the diatoms were classified as planktonic, benthic, and warm-water species. Cells were counted with a maximum of 300 cells per sample. After counting, we calculated the cell density (cells mL⁻¹).

2.3. Data analysis

The relationships between the cell density of planktonic, benthic, and warm-water diatom species and environmental factors (SST,

salinity, DIN [=nitrate+nitrite+ammonium], silicate, phosphate, air temperature, total daily precipitation, mean daily wind speed, tide level, and daylight hours) were analyzed using decision trees. Because cell densities were low for warm-water species, they were converted to presence/absence data and used in the analyses. These analyses were performed using the packages "rpart" and "partykit" in R (version 4.1.2, R Development Core Team, 2021).

Based on seasonal changes in the phytoplankton community based on cluster analysis and hydrography, this study defined August to October 2020 and September to October 2021 as autumn, November 2020 to March 2021 and November 2021 to March 2022 as winter, April to May 2021 and 2022 as spring, and June to August 2021 and 2022 as summer (Supplementary Fig. 1, Supplementary Tables 1 and 2). The Wilcoxon rank-sum test was used to identify annual changes in environmental factors during each season using StatView (SAS Institute Inc.).

3. Results

3.1. Environmental factors

SST ranged from 1.2 to 25.5°C and was high in August and low in February (Fig. 2). Salinity ranged from 22.1 to 34.0 and decreased rapidly from March to April in 2021 and 2022 and slightly decreased in winter and early summer (Fig. 2). DIN ranged from 0.56 to 12.2 µM, which tended to decrease during summer and increase during autumn and spring. Phosphate ranged from 0.061 to 1.18 µM, increased in March 2021 and 2022, and decreased during the summers (Fig. 2). Silicate ranged from 1.9 to 94.0 µM, and increased in March–April and May–June 2021, then decreased at 10–20 µM during the summer (Fig. 2). The DIN:P ratio exhibited fluctuations but no evident seasonal trend, with a distinct increase in November 2020 and January 2021. The Si:P ratio increased sharply in June in both years. Air temperatures were generally high in summer and low in winter, with a temporary decrease in August 2021 (Fig. 3a). The precipitation was high from summer to autumn (Fig. 3a). The wind speed showed high variability, and the direction was evidently northwest in winter and southeast in summer (Fig. 3b, c). The flow rate of the Shiribetsu River increased annually from March to April, and the flow rate of the Shubuto River increased annually in April (Fig. 3d, e).

3.2. Micro-sized plankton communities

Regarding cell density, diatoms (1.30–152 cells mL⁻¹), dinoflagellates (0–231 cells mL⁻¹), ciliates (0–156 cells mL⁻¹), and euglenoids (0–4921 cells mL⁻¹) were observed (Fig. 4a). Diatoms were the most dominant taxon among the four taxonomic groups, accounting for an average of 62.4% of the total microplankton cell density during the study period. Dinoflagellate and ciliate cell densities showed apparent seasonal variations, with high densities in spring and summer. Euglenoid cell densities were low in winter and high in spring, particularly from April to May, when a large euglenoid bloom was observed (Fig. 4a).

Seasonal changes in the species composition were observed. *Navicula* spp. were dominant in winter (from November 2020 to the end of March 2021), and euglenoids were dominant in April 2021 (Fig. 4b). During summer, dinoflagellates were dominant. Centric diatoms dominated in autumn, and euglenoids dominated in spring (Fig. 4b).

Planktonic, benthic, and warm-water diatom species increased in the summer and decreased in the winter (Fig. 5). Sporadic increases in planktonic diatoms *Chaetoceros* spp. were observed in the area in August to October 2020, June to July 2021, September to October 2021, March 2022, and July to August 2022 (Fig. 4b). The warm-water species *Chaetoceros didymus* was observed from August to September 2020. *Bacteriatrum delicatulum* increased rapidly from April to June 2021. Decision tree analysis showed that planktonic species had higher cell densities at ≥ 17.6 °C and < 0.207 µM in phosphate (Fig. 6a). Benthic

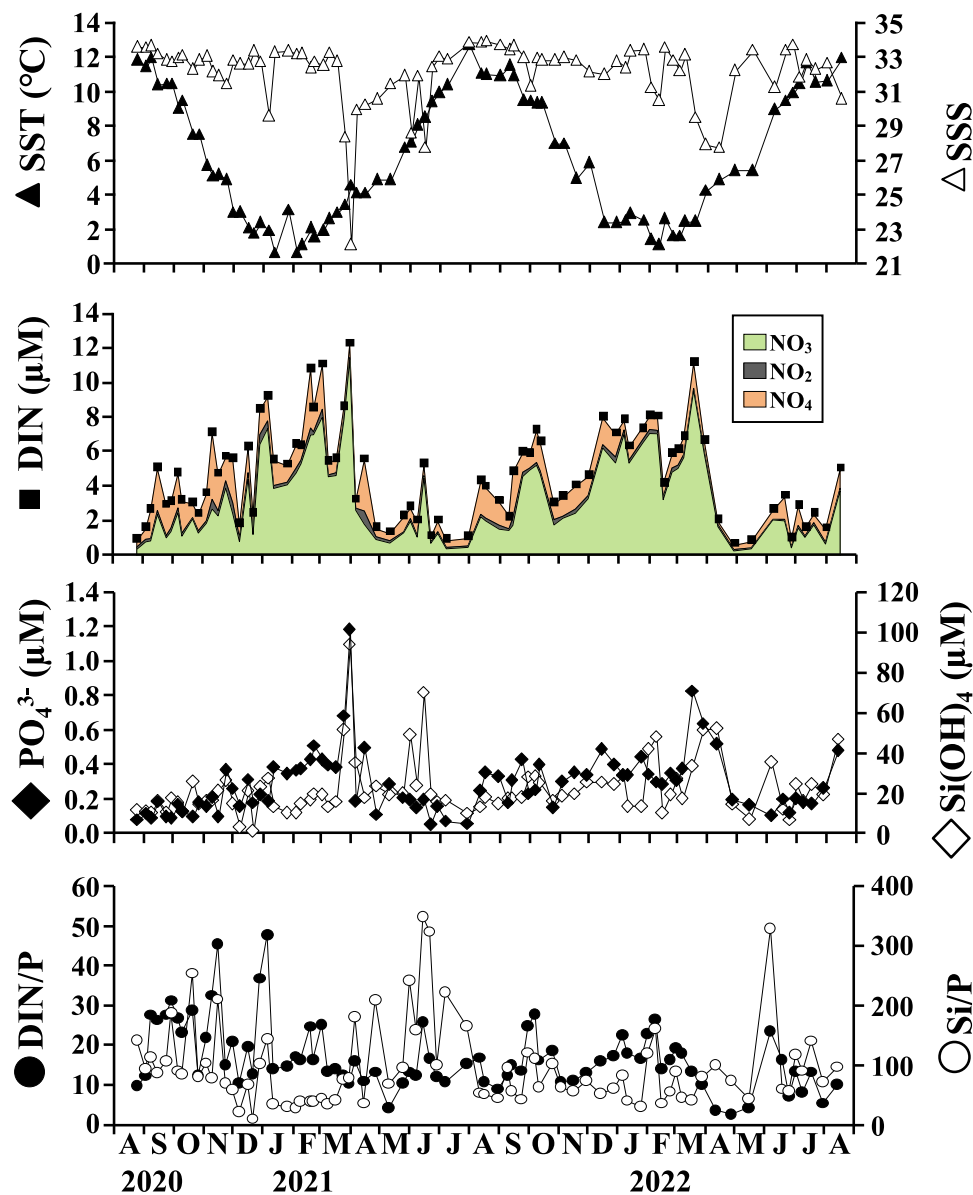


Fig. 2. Seasonal changes in environmental parameters surface temperature, salinity, nutrients (DIN, PO_4^{3-} , and $\text{Si}(\text{OH})_4$), DIN/P ratio, and Si/P ratio at the Yokoma Port from August 2020 to August 2022.

species had higher cell densities at < 0.8 h in daylight hours and < 6.68 μM DIN (Fig. 6b). Warm-water species occurred at $\text{SST} \geq 4.5$ $^\circ\text{C}$ and ≥ 17.3 μM in silicate or at $\text{SST} \geq 4.5$ $^\circ\text{C}$, < 17.3 μM in silicate and < 0.146 μM in phosphate (Fig. 6c).

Annual changes in phytoplankton cell density and environmental factors were compared for each season (Supplementary Fig. 1 and Supplementary Tables 1 and 2). In autumn, significantly lower cell densities were observed in planktonic diatoms ($p < 0.01$), warm diatoms ($p < 0.05$), and dinoflagellates ($p < 0.01$) in the second year (2021) than in the first year (2020), associated with lower SST ($p < 0.01$), higher nitrate ($p < 0.05$), higher DIN ($p < 0.05$), higher phosphate ($p < 0.05$), higher silicate ($p < 0.05$), lower air temperature ($p < 0.01$), and lower wind speed ($p < 0.05$) (Table 1). In winter, the cell densities of benthic diatoms ($p < 0.01$) and ciliates ($p < 0.05$) were significantly lower than those in the second year, with lower SST ($p < 0.05$), higher nitrate ($p < 0.01$), higher nitrite ($p < 0.05$), higher ammonium ($p < 0.05$), higher phosphate ($p < 0.01$), and lower tide levels ($p < 0.05$). No significant differences in the environmental factors were found during spring. In summer, benthic diatoms and dinoflagellates had significantly

lower cell densities ($p < 0.01$) and higher air temperatures ($p < 0.05$) in the first year than in the second year (Table 1).

4. Discussion

4.1. Environmental features at the sampling site

The Tsushima Warm Current can be classified into four main types: the Subpolar Front Current (SFC), Offshore North Current (ONC), Offshore South Current (OSC), and Coastal Current (CC) (Yabe et al., 2021). The SFC flows along the Japan Sea coast of Hokkaido (Yabe et al., 2021). The SFC has minimal seasonal variation, with the majority (83%) flowing northward along Hokkaido and the remaining 16% flowing into the Tsugaru Strait (Yabe et al., 2021). Yokoma Port, the site of this study, is located on the Japan Sea coast of Hokkaido and is expected to receive SFC inflow throughout the year.

Nutrient concentrations in the sea around southern Hokkaido showed a maximum (6 μM nitrate and nitrite, 0.4 μM phosphate, and 8 μM silicate) in March, and after the phytoplankton bloom in April,

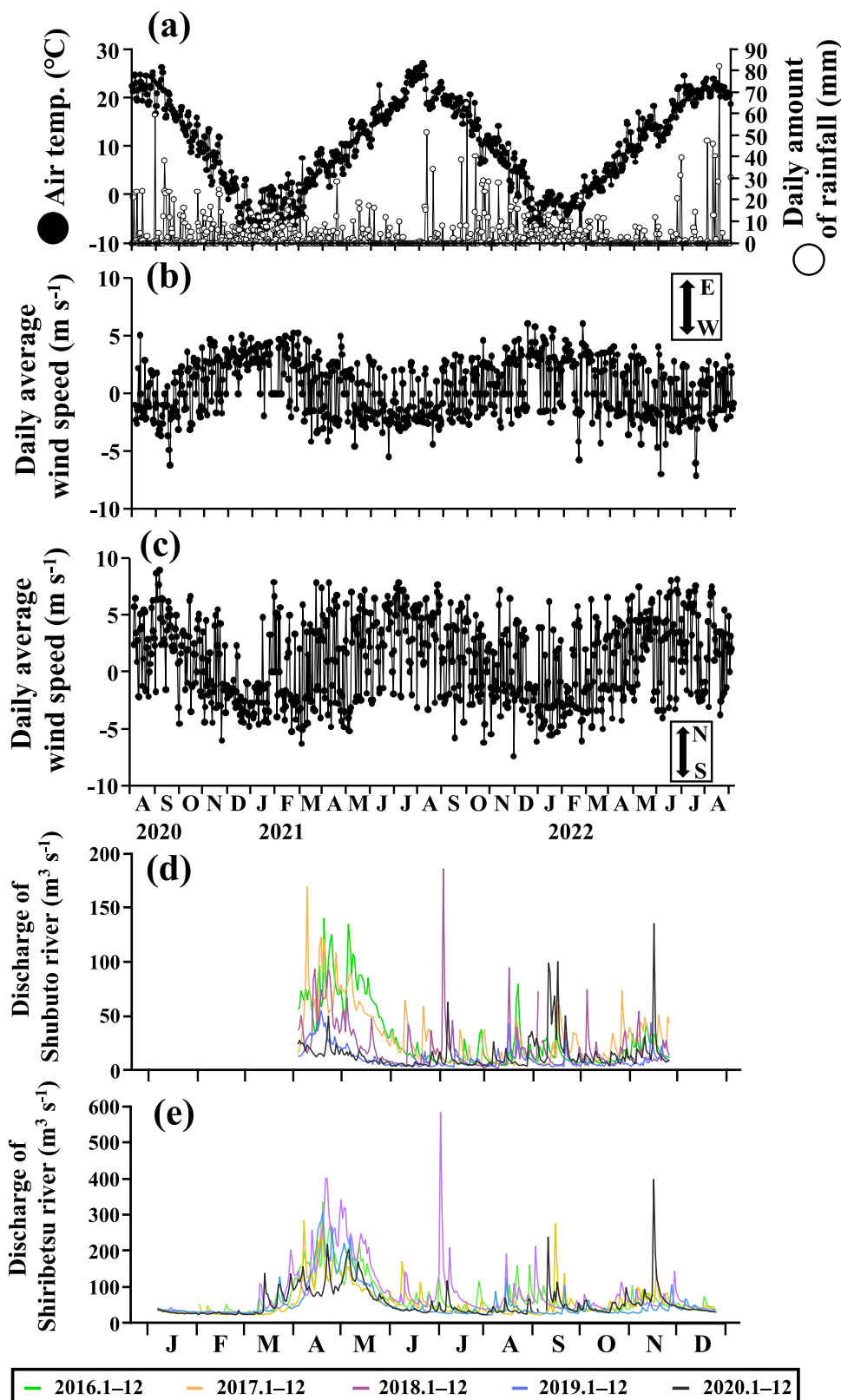


Fig. 3. Seasonal changes in atmospheric condition (air temperature, daily amount of rainfall, daily average wind speed, and direction) (a-c) were refereed at the Suttsu Station from August 2020 to August 2022. Seasonal changes on the discharge of Shubuto River (d) at the Shubuto River Flow Observatory and Shiribetsu River (e) at the Nakoma Observatory in the last 5 years. Note the X-axis was different between atmospheric data and river discharge.

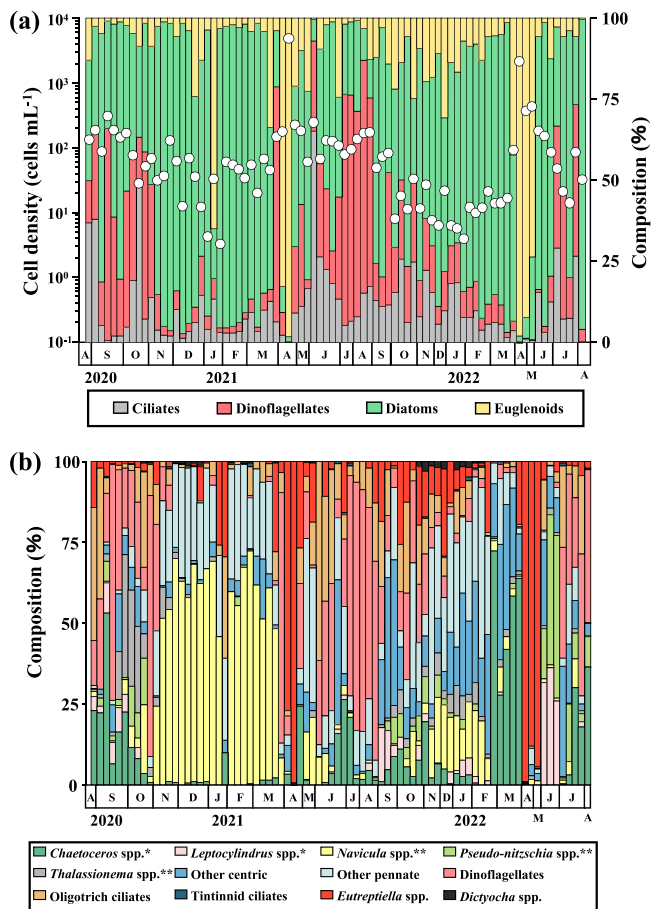


Fig. 4. Seasonal changes in microphytoplankton cell density and composition (a) and species composition (b) at the Yokoma Port from August 2020 to August 2022. *: Centric diatoms, **: Pennate diatoms.

nitrate and nitrite concentrations were 1–2 μM , phosphate was $< 0.1 \mu\text{M}$, and silicate was as low as 4 μM (Adachi and Osawa, 2005). In the offshore southern Hokkaido, seasonal variation from winter to summer in DIN and DIP are 0–6.6 μM and 0–0.64 μM , respectively (Kuribayashi et al., 2014). In the Oshoro Bay, DIN, DIP and silicate range 0.02–29.84 μM , 0.04–0.49 μM and 1.00–22.12 μM (Agboola et al., 2010). In this study, the maximum values of nitrate and nitrite (11.5 μM in 2021 and 9.68 μM in 2022) and phosphate (1.18 μM in 2021 and 0.83 μM in 2022) levels were observed during March in each year, and the minimum values of nitrate and nitrite (0.44 μM in 2021 and 0.32 μM in 2022) and phosphate (0.061 μM in 2021 and 0.11 μM in 2022) levels were observed during the spring and summer seasons. The silicate content in the present study remained in the range of 10–20 μM during spring and summer, which was higher than that in the offshore region around southern Hokkaido. Compared the variation ranges in nutrients with previous results, the present results showed a similar trend to coastal region rather than offshore region. This regional difference may be attributed to the nutrient inflow from the river (Yoshimura and Kudo, 2003).

The DIN:P ratio was generally below 16 as compared to the Redfield ratio, with the lowest values occurring from the end of April to early May (Adachi and Osawa, 2005). This suggests that DIN may be a limiting factor for phytoplankton growth in the waters around the Yokoma Port. The eastern area of Ishikari Bay is affected by water supplied by the Ishikari River, and salinity was below 20 at the surface in April, when discharge water increased due to snowmelt (Adachi and Osawa, 2005). In our case, the Shiribetsu River and Shubuto River flow increased around April, which was observed in Suttsu Bay, and was associated

with a decrease in salinity in April 2021 and 2022. During the same period, an increase in air temperature and low precipitation suggested snowmelt water inflow decreased salinity in Yokoma Port during April. Adachi and Osawa (2005) reported that rivers supply DIN, and vertical mixing in Ishikari Bay supplies phosphate during winter. DIN and phosphate concentrations at Yokoma Port showed maximum values from the end of March to early April when the river water increased. This suggests that the nutrients were supplied by rivers near the sampling site. Specifically, the environmental conditions around Yokoma Port were strongly influenced by river water.

The Tsushima Warm Current is a water mass with a salinity ≥ 33.7 (Higaki et al., 2008), which is higher than that around Yokoma Port. Salinity exceeding 33.7 was observed from the end of July to mid-September 2021 and at the end of June 2022. Nutrient concentrations in the coastal surface layer during July and September in the Tsushima Warm Current were reported to be 1–5 μM for DIN, 0.1 μM for phosphate, and 3–7 μM for silicate (Ikeuchi et al., 1998). Nutrient concentrations (DIN 0.97–4.77 μM , phosphate 0.06–0.37 μM , silicate 10.3–17.9 μM) in the Yokoma Port from July–September 2021 were at the same level, excluding silicate, as the previously reported Tsushima Warm Current water. This suggests that the temporal inflow of the Tsushima Warm Current occurred from July to September 2021.

4.2. Annual changes in phytoplankton and their factors

Diatoms are classified based on their ecological characteristics into planktonic diatoms, which live a planktonic life, and benthic diatoms, which adhere to the substrate. Comparing the results of decision trees between planktonic and benthic diatoms, SST was the most important factor to increase cell density for planktonic diatoms, but daylight hour was chosen as the factor for benthic diatoms. This difference could indicate their physiological differences, however the detail difference should be discussed individually based on incubation experiment because the boundary between the categories (planktonic vs benthic) is not clear (Kawamura, 1994).

Interannual changes in phytoplankton and environmental factors during autumn and winter, including lower phytoplankton cell density, lower SST, and higher nutrient levels, were observed in the second year. Decision tree analysis indicated that planktonic diatoms had the lowest cell densities when the temperature was below 17.6 $^{\circ}\text{C}$ and the number of daylight hours was below 9.35 h. In the second autumn (September–October 2021), as the average SST (17.1 $^{\circ}\text{C}$) and daylight hours (6.37 h) were consistent with the conditions, phytoplankton growth was limited by low SST and light conditions (Bissinger et al., 2008; Shikata et al., 2010), and the resulting planktonic diatoms decreased. Nutrients were higher in the second year, possibly due to reduced phytoplankton growth and suppressed nutrient absorption. On the other hand, sporadic increases in planktonic diatoms *Chaetoceros* spp. were observed from spring to fall during the study period. Most of these periods, with the exception of March 2022, had phosphate concentrations of about 0.2 μM and water temperatures of about 18 $^{\circ}\text{C}$ or higher. Based on the results of the decision tree analysis, cell densities were highest when phosphate was $< 0.207 \mu\text{M}$ and water temperature was $> 17.55 \text{ }^{\circ}\text{C}$, suggesting that the period of increased *Chaetoceros* spp. was a favorable environment for this species.

In winter, benthic diatoms decreased significantly in the second year. Decision tree analysis indicated that benthic diatoms decreased when daylight hours were between 0.8 and 6.95 h and SST was above 4.4 $^{\circ}\text{C}$. However, both of these environmental factors in the winter of the first and second years were within the conditions (0.8–6.95 daylight hours and $\geq 4.4 \text{ }^{\circ}\text{C}$ SST). The tide level should be discussed as an alternative factor because it was significantly lower in the second year than in the first year. The wind speed was also lower in the second year (but the difference was not significant). These results suggest that the physiological disturbances driven by wind and waves may weaken during the second year. Note that this is the tide level at the sampling time and not

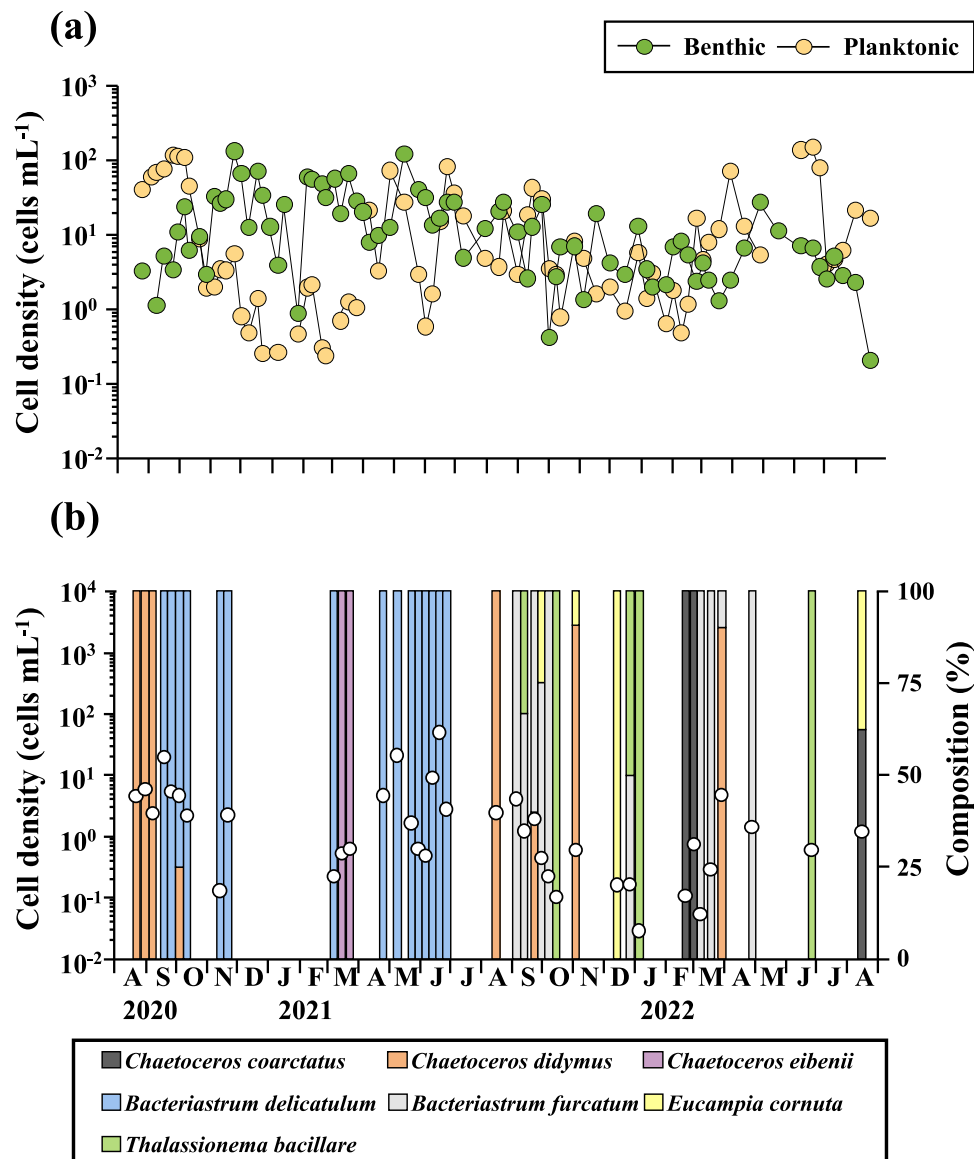


Fig. 5. Seasonal changes in cell density of planktonic and benthic species (a) and warm-water species (b) at the Yokoma Port from August 2020 to August 2022.

the wave height. Among benthic diatoms, *Navicula* spp. were significantly reduced in the second year. This species has a weaker attachment to the substrate than other benthic diatoms (i.e., *Cocconeis* spp.) (Kawamura, 1998). Therefore, it is possible that this species detached from the substrate and was suspended in the water under normal conditions. However, in the second year, detachment was difficult under low-tide and low-wind-intensity conditions, resulting in low cell density.

4.3. Euglenoid blooms in spring

Large euglenoid blooms (mean cell density: 2.0×10^3 cells mL⁻¹) were identified in four samples during April 2021 and April–May 2022. There are two species of marine euglenoids: *Eutreptiella gymnastica* and *E. marina*. *E. marina* also occurred from spring to summer in previous studies conducted in the Sea of Marmara, an inland sea connecting the Black Sea and the Mediterranean Sea, where blooms with cell densities reaching 30.2×10^3 cells mL⁻¹ were observed in spring (Taş, 2019). The DIN:P ratio decreased from April to May when euglenoid blooms were found at Yokoma Port, and there was a negative correlation between the DIN:P ratio and *E. marina* cell density ($r = -0.21$) (Taş, 2019). *E. gymnastica* has salinity tolerance and can survive at salinities ranging

from 10 to 30 (Xu et al., 2012) and 15–40 (Lee and Lim, 2006). Therefore, this species can proliferate in environments in which the inflow of river water reduces salinity. A harmful bloom of *E. marina* (2.21×10^3 cells mL⁻¹) was reported in the southern part of Banderas Bay, Jalisco, Mexico, where hundreds of dead fish were found (Cortés-Lara et al., 2010). It is considered that this is due to oxygen depletion in the water and that blooms of this species do not directly affect human health (Cortés-Lara et al., 2010). The euglenoid bloom in Suttsu (2.0×10^3 cells mL⁻¹) was comparable to the reported bloom in the southern part of Banderas Bay. The red tide of Euglenoids has been reported in southern Hokkaido (Shimada, 2021), so fish harm and death may be caused in the waters in southern Hokkaido, including Yokoma port.

Regarding dominant species during spring bloom, majority studies reports diatoms around Hokkaido. In Oshoro Bay, *Chaetoceros* spp., *Coscinodiscus* spp. and *Thalassionema* spp. are composed (Kanomata and Kotori, 2000; Fukui et al., 2010). In Funka Bay, *Thalassionema* spp., *Thalassiosira* spp. and *Chaetoceros* spp. are dominant (Nakata, 1982; Shinada et al., 1999). These previous studies were based on once- or twice-monthly observations and may have missed short-term changes in *Eutreptiella* spp. In Funka Bay, salinity at the center and mouth of the bay was about 32.0 at low salinity, which was higher than in spring Suttsu

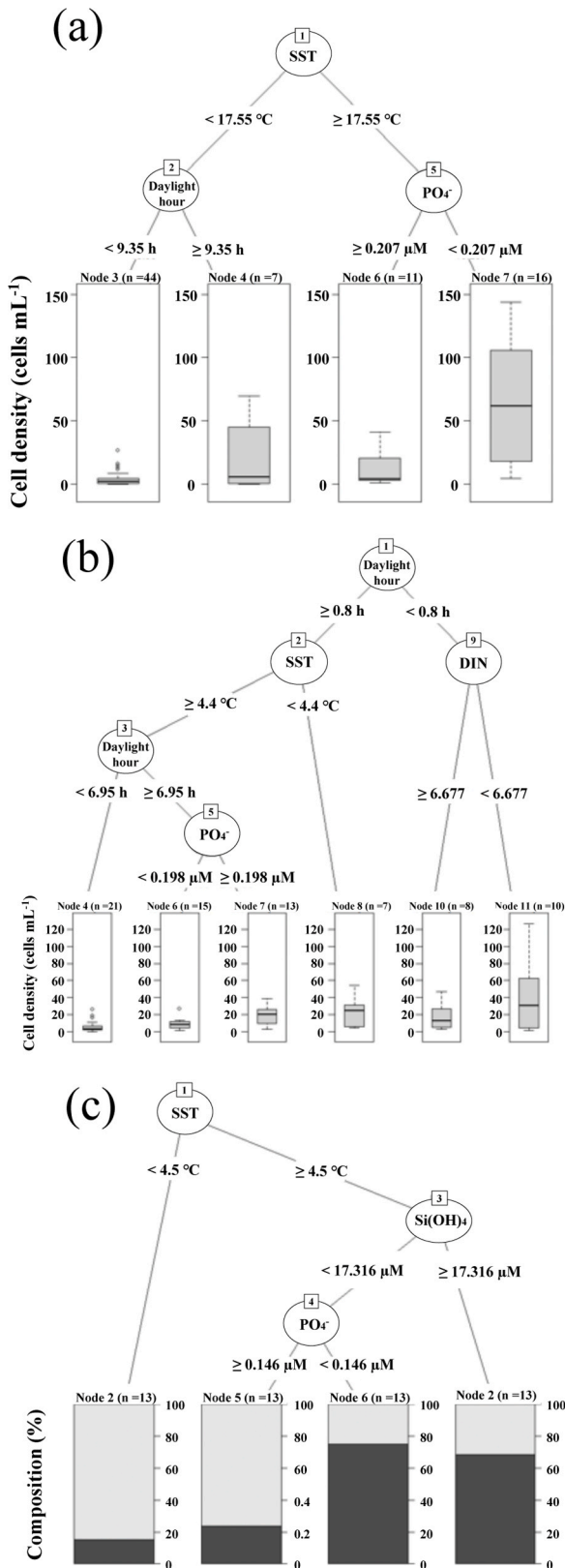


Fig. 6. Decision trees of planktonic (a), benthic (b), and warm-water species (c) in diatoms at the Yokoma Port from August 2020 to August 2022. Warm-water species were analyzed as presence /absence data.

(Nakata, 1982). Therefore, it is possible that phytoplankton other than *Eutreptiella* spp. did not dominate because they were not limited by low salinity and were able to proliferate.

4.4. Appearance trends of warm-water species

As for the occurrence of warm-water species, *Chaetoceros didymus* appeared in August-September and October 2020 but was not observed until the spring of the following year. Since the optimal temperature for the growth of warm-water species is 16–27 °C (Creswell, 2010), SST < 16 °C during mid-October 2020 restricted the growth of the warm-water species *C. didymus*. As an important life strategy, many species in the genus *Chaetoceros* form resting stage cells and germinate even after 12 months at 4 °C (Matsubara, 2017). Among the warm-water species, *C. didymus*, *C. coarctatus*, and *C. eibonii* have been identified, and *C. didymus* forms resting spores (Ishii et al., 2011). Therefore, *C. didymus* may have overwintered as a resting-stage cell in the study area. However, germination experiments of resting stages of *Chaetoceros* species suggest that it takes 10–13 days to germinate at 15 °C and 11 h of light, and more time is needed at lower temperatures (Matsubara, 2017). This study observed > 15 °C SST after May–June, with average daylight hours ranging from 6.6 to 10.4 h in spring and summer. Since phytoplankton cell density also increases around May–June, the growth of other phytoplankton (cold-water species) begins earlier than the germination of this species, even if the resting-stage cells of *C. didymus* survive until the following year.

Bacteriastrium delicatulum increased rapidly from April to June 2021. In the northern Adriatic Sea, a higher cell density of *B. delicatulum* (119 cells mL⁻¹) was observed from June to August (Godrižjan et al., 2012), consistent with our Yokoma Port results. In addition, the cell density of *B. furcatum*, which is classified in the same genus as *B. delicatulum*, was positively correlated with PO₄ concentration (Bosak et al., 2016). Phosphate was at its maximum level in April at Yokoma Port, suggesting that *B. delicatulum* could grow by taking advantage of the abundant phosphate present in this study. However, no warm-water species were observed in July–August 2021. This may be due to a decrease in nutrients caused by dinoflagellate blooms during the same period, which may have prevented the growth of warm-water species.

In the study area, warm-water species occurred sporadically, with significant seasonal variations in their species composition. The decision tree indicated that ≥ 4.5 °C SST was the most critical factor within the condition in all seasons except winter. Furthermore, as mentioned above, the seasonal variation in the Tsushima Warm Current is minimal (Yabe et al., 2021). Considering these facts, the warm-water species possibly had not been established in the area, but rather that a cell was transported by the Tsushima Warm Current. The establishment of warm-water species in the area is considered to be difficult because of the short period (4 months from June to September) at optimal temperature (16–27 °C) and the challenging competitive situation for acquiring daylight and nutrients with other phytoplankton (cold-water diatoms, dinoflagellates, and euglenoids) during the nutrient-rich spring season.

5. Conclusion

Diatoms were mainly dominant at Yokoma Port in Suttsubu, Hokkaido, and their species composition showed evident seasonal changes. As an annual change, phytoplankton cell densities in the autumn and winter of the second year were significantly lower than those of the same period in the first year. This may be due to the low SST and daylight hours, which prevent phytoplankton growth. A significant decrease in the number of benthic diatoms was also observed during the winter of the second year, suggesting that calm sea conditions (low tide levels and wind speed) decreased the detachment of benthic diatoms (*Navicula* spp.). Large euglenoid blooms were observed yearly from April to May. This may be because of their high salinity tolerance and ability to exclusively utilize

Table 1

Comparison of environmental factors between the years in each season at the Yokoma Port from August 2020 to August 2022. Differences between the groups were tested by Wilcoxon signed rank test. Values are mean (\pm S.D.). Wilco.: Wilcoxon test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Parameters	Autumn			Winter			Spring			Summer		
	1st year (2020)	2nd year (2021)	Wilco.	1st year (2020)	2nd year (2021)	Wilco.	1st year (2021)	2nd year (2022)	Wilco.	1st year (2021)	2nd year (2022)	Wilco.
Benthic diatoms (cells mL ⁻¹)	6.78 \pm 6.59	7.93 \pm 7.58		36.5 \pm 28.3	4.21 \pm 3.03	**	37.3 \pm 42.1	14.4 \pm 8.39		18.5 \pm 8.20	3.68 \pm 2.11	*
Planktonic diatoms (cells mL ⁻¹)	68.4 \pm 33.7	11.1 \pm 13.2	**	1.20 \pm 1.33	9.52 \pm 17.9		24.5 \pm 24.5	5.97 \pm 5.21		17.9 \pm 23.0	50.2 \pm 55.1	
Warm-water diatoms (cells mL ⁻¹)	4.56 \pm 5.12	0.22 \pm 0.61	*	3.77 \pm 9.91	0.78 \pm 1.12		0.07 \pm 0.07	0.00 \pm 0.00		0.68 \pm 1.30	0.20 \pm 0.38	
Dinoflagellates (cells mL ⁻¹)	53.2 \pm 66.2	4.15 \pm 2.81	**	8.81 \pm 26.0	0.29 \pm 0.17		20.3 \pm 21.8	0.55 \pm 0.15		62.2 \pm 34.9	10.4 \pm 11.9	*
Euglenoids (cells mL ⁻¹)	3.87 \pm 5.50	2.80 \pm 2.64		1.77 \pm 3.31	2.09 \pm 4.87		1028 \pm 1947	985 \pm 858		5.43 \pm 7.81	2.24 \pm 3.28	
Ciliates (cells mL ⁻¹)	18.4 \pm 26.6	1.83 \pm 2.69		14.8 \pm 32.6	8.51 \pm 8.87	*	0.97 \pm 0.99	0.28 \pm 0.19		0.63 \pm 0.86	8.73 \pm 10.5	
SST (°C)	20.6 \pm 2.75	17.1 \pm 4.15	**	6.09 \pm 3.48	4.64 \pm 1.53	*	9.90 \pm 1.94	10.5 \pm 0.52		19.9 \pm 3.19	21.0 \pm 1.93	
SSS	33.2 \pm 0.44	32.9 \pm 0.62		32.0 \pm 2.47	32.0 \pm 1.65		30.9 \pm 0.75	31.2 \pm 2.45		32.2 \pm 2.14	32.4 \pm 1.00	
NO ₃ ⁻ (μM)	1.37 \pm 0.72	3.21 \pm 1.42	*	4.66 \pm 2.60	6.04 \pm 1.44	**	1.46 \pm 0.68	0.75 \pm 0.64		1.59 \pm 1.14	1.65 \pm 0.97	
NO ₂ (μM)	0.18 \pm 0.05	0.22 \pm 0.10		0.31 \pm 0.16	0.23 \pm 0.05	*	0.26 \pm 0.27	0.09 \pm 0.03		0.13 \pm 0.05	0.11 \pm 0.04	
NH ₄ (μM)	1.41 \pm 0.65	1.31 \pm 0.54		1.47 \pm 0.86	0.89 \pm 0.32	*	1.00 \pm 0.98	0.25 \pm 0.06		0.86 \pm 0.56	0.74 \pm 0.33	
DIN (μM)	2.96 \pm 1.25	4.73 \pm 1.54	*	6.45 \pm 2.76	7.15 \pm 1.57		2.72 \pm 1.54	1.09 \pm 0.62		2.58 \pm 1.44	2.50 \pm 1.19	
PO ₄ ³⁻ (μM)	0.12 \pm 0.03	0.30 \pm 0.09	*	0.36 \pm 0.22	0.42 \pm 0.15	**	0.26 \pm 0.13	0.30 \pm 0.16		0.19 \pm 0.10	0.22 \pm 0.11	
Si(OH) ₄ (μM)	14.8 \pm 4.85	21.9 \pm 4.51	*	21.6 \pm 18.5	27.7 \pm 12.7		23.5 \pm 6.20	25.0 \pm 19.6		25.5 \pm 18.1	23.6 \pm 12.1	
DIN/P	23.8 \pm 7.14	16.4 \pm 5.49		20.7 \pm 10.6	17.8 \pm 4.15		11.0 \pm 3.92	3.66 \pm 0.68		14.4 \pm 4.55	12.3 \pm 5.44	
Si/P	129 \pm 54.0	79.2 \pm 25.7		65.7 \pm 44.4	69.4 \pm 36.1		119 \pm 66.2	73.0 \pm 22.9		171 \pm 106	121 \pm 83.0	
Air temperature (°C)	18.0 \pm 4.50	13.7 \pm 3.97	**	0.74 \pm 4.63	-0.32 \pm 2.82		7.96 \pm 2.63	11.2 \pm 1.93		17.9 \pm 3.27	20.8 \pm 3.64	*
Wind speed (m s ⁻¹)	5.24 \pm 1.97	2.85 \pm 0.99	*	4.37 \pm 1.43	3.72 \pm 1.40		3.88 \pm 2.33	3.23 \pm 0.87		4.73 \pm 1.58	3.45 \pm 0.94	
Rainfall (mm)	2.17 \pm 5.15	0.05 \pm 0.15		2.18 \pm 2.10	2.46 \pm 3.30		0.90 \pm 1.80	0.17 \pm 0.24		0.05 \pm 0.15	4.25 \pm 9.25	
Tide level (cm)	28.9 \pm 6.74	24.9 \pm 4.85		11.9 \pm 8.12	7.54 \pm 5.83	*	13.8 \pm 5.15	14.7 \pm 3.68		25.5 \pm 2.69	25.4 \pm 6.56	
Daylight hours (h)	4.77 \pm 3.53	6.37 \pm 2.56		1.75 \pm 2.85	2.05 \pm 2.90		8.04 \pm 4.12	10.4 \pm 3.08		8.05 \pm 2.76	6.60 \pm 4.26	

nutrients from the inflow of river water during the snowmelt season. Some euglenoids caused fish death at cell densities similar in scale to those of the blooms observed in this study, which is of concern in the vicinity of Suttsu Bay. Although several warm-water species were observed, they occurred sporadically in the area, suggesting that they were transported by the Tsushima Warm Current rather than by established or resting-stage cells. This study clarified the relationship between seasonal and annual changes in the phytoplankton community and environmental conditions in Suttsu. These findings will help predict the impact of climate change on warm water inflow areas. Suitable conditions can be established for warm-water species if sea surface temperatures increase due to global warming and the optimal water temperatures are prolonged. Monitoring the environment (temperature and nutrients) and phytoplankton communities should continue to evaluate the ecosystem in the Anthropocene climate.

CRediT authorship contribution statement

Morimoto Kyosei: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Hamao Yusuke:** Investigation, Methodology. **Wakita Masahide:**

Formal analysis, Funding acquisition, Methodology. **Matsuno Kohei:** Conceptualization, Methodology, Supervision, Visualization. **Yamazaki Tomoyasu:** Investigation. **Tatamisashi Shoko:** Formal analysis, Methodology.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgments

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI [grant numbers JP22H00374 (A) and JP21H02263 (B)].

Author statement

KM and KM designed the study; TY preformed field sampling; KM, YH, MW and ST analyzed the samples; KM and KM analyzed the data; and KM and KM wrote the paper with contributions from all authors.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2023.103322.

References

- Adachi, K., Osawa, Y., 2005. Variation in nutrient concentrations in the coastal water of southwestern Hokkaido. *Proc. Civ. Eng. Ocean* 21, 635–639 (in Japanese with English abstract).
- Agboola, J.I., Uchimiya, M., Kudo, I., Kido, K., Osawa, M., 2010. Dynamics of pelagic variables in two contrasting coastal systems in the western Hokkaido coast off Otaru port, Japan. *Estuar., Coast. Shelf Sci.* 86, 477–484.
- Bissinger, J.E., Montagnes, D.J., harples, J., Atkinson, D., 2008. Predicting marine phytoplankton maximum growth rates from temperature: improving on the Eppley curve using quantile regression. *Limnol. Oceanogr.* 53, 487–493.
- Bosak, S., Godrijan, J., Šilović, T., 2016. Dynamics of the marine planktonic diatom family Chaetocerotaceae in a Mediterranean coastal zone. *Estuar. Coast. Shelf Sci.* 180, 69–81.
- Cortés-Lara, M.C., Cortes-Altamirano, R., Alonso-Rodríguez, R., Cupul-Magana, A.L., 2010. *Eutreptiella marina* (Euglenophyceae) bloom causes significant fish kills in Banderas Bay, Jalisco, México. *Harmful Algae News: IOC Newsl. Toxic. Algae Algal Blooms* 42, 12–13.
- Creswell, L., 2010. Phytoplankton culture for aquaculture feed. *Southern Regional Aquaculture Center*.
- Fujii, M., Takao, S., Yamaka, T., Akamatsu, T., Fujita, Y., Wakita, M., Yamamoto, A., Ono, T., 2021. Continuous monitoring and future projection of ocean warming, acidification, and deoxygenation on the subarctic coast of Hokkaido, Japan. *Front. Mar. Sci.* 8, 590020.
- Fukui, D., Kitatsuji, S., Ikeda, T., Shiga, N., Yamaguchi, A., 2010. Long-term changes in the abundance and community structure of net-phytoplankton in Oshoro Bay, Hokkaido. *Bull. Plankton Soc. Jpn.* 57, 30–40 (in Japanese with English abstract).
- Fukuyo, Y., Inoue, H., Takayama, H., 1997. Division dinophyta. An Illustrated Guide to Marine Plankton in Japan. Tokai University Press (Ed), pp. 31–146.
- Godrijan, J., Marić, D., Imešek, M., Janeković, I., Schweikert, M., Pfannkuchen, M., 2012. Diversity, occurrence, and habitats of the diatom genus *Bacteriastrium* (Bacillariophyta) in the northern Adriatic Sea, with the description of *B. jadrantum* sp. nov. *Bot. Mar.* 55, 415–426.
- Hasle, G.R., Syvertsen, E.E., 1997. Marine diatoms. In: Tomas, C.R. (Ed.), *Identifying Marine Phytoplankton*. Academic Press, San Diego, pp. 5–385.
- Higaki, N., Isoda, Y., Isogai, Y., Yahaba, H., 2008. Seasonal variations of water system distributions and flow patterns off the west coast of Hokkaido. *J. Oceanogr.* 17, 223–240 (in Japanese with English abstract).
- Ikeuchi, H., Kamizono, M., Sugino, K., 1998. Distributions of nutrient and plankton in the Genkai-nada and the Eastern Tsushima Channel. *Bull. Fukuoka Fish. Mar. Technol. Res. Cent.* 8, 97–106.
- Ishii, K.I., Iwataki, M., Matsuoka, K., Imai, I., 2011. Proposal of identification criteria for resting spores of *Chaetoceros* species (Bacillariophyceae) from a temperate coastal sea. *Phycologia* 50, 351–362.
- Itakura, S., 2001. Ecological role of the resting stage cells of planktonic diatoms. *Bull. Plankton Soc. Jpn.* 48, 101–103.
- Itakura, S., Imai, I., Itoh, K., 1992. Morphology and rejuvenation of *Skeletonema costatum* (Bacillariophyceae) resting cells from the bottom sediments of Hiroshima Bay, the Seto Inland Sea, Japan. *Bull. Plankton Soc. Jpn.* 38, 135–145.
- Kakumu, A., Morita, K., Shimada, H., Yamaguchi, A., Imai, I., 2018. First detection of the noxious red tide dinoflagellate *Karenia mikimotoi* and bloom dynamics in 2015 and 2016 in Hakodate Bay, Hokkaido, northern Japan. *Bull. Plankton Soc. Jpn.* 65, 1–11.
- Kanomata, I., Kotori, M., 2000. Long-term changes in net-collected phytoplankton assemblage during spring blooms in Oshoro Bay, Hokkaido, Japan. *Bull. Plankton Soc. Jpn.* 47, 93–100.
- Kawamura, T., 1994. Taxonomy and ecology of marine benthic diatoms. *Mar. Fouling* 10, 7–25.
- Kawamura, T., 1998. Benthic diatoms: ecology and the role in the animal communities. *Sess. Org.* 15, 15–22.
- Kida, S., Takayama, K., Sasaki, Y.N., Matsuura, H., Hirose, N., 2021. Increasing trend in Japan Sea throughflow transport. *J. Oceanogr.* 77, 145–153.
- Kondo, S., Nakao, R., Iwataki, M., Sakamoto, S., Itakura, S., Matsuyama, Y., Nagasaki, K., 2012. *Heterocapsa circularisquama* coming up north - Mass mortality of Pacific oysters due to its blooming at Lake Kamo in Sado Island, Japan - . *Nippon Suisan Gakkaishi* 78, 719–725 (in Japanese with English abstract).
- Kuribayashi, T., Abe, T., Montani, S., 2014. Nutritional status of seaweed communities along the west coast of the Japan Sea off Hokkaido, Japan, from monitoring data and detecting $\delta^{15}N$ Records in Saccharina Specimens. *Bull. Mar. Sci.* 52, 75–81.
- Lee, C., Lim, W., 2006. Variation of harmful algal blooms in Masan-Chinhae Bay. *Sci. Asia* 32, 51–56.
- Matsubara, T., 2017. Influences of water temperature and photoperiod on germination/rejuvenation and growth of marine diatoms. *Bull. Plankton Soc. Jpn.* 64, 45–49.
- Nakata, A., Tanaka, I., 2002. Seasonal and year-to-year variability of baroclinic volume transport of the Tsushima Warm Current, off the west of Hokkaido. *Sci. Rep. Hokkaido Fish. Exp. Station* 63, 1–8.
- Nakata, K., 1982. Species composition of phytoplankton community of Funka Bay in the Spring Bloom, 1981. *Bull. Jpn. Soc. Fish. Oceanogr.* 41, 23–37.
- Nishida, Y., Kanomata, I., Tanaka, I., Sato, S., Takahashi, S., Matsubara, H., 2003. Seasonal and interannual variations of the volume transport through the Tsugaru Strait. *Oceanogr. Jpn.* 12, 487–499 (in Japanese with English abstract).
- Sarthou, G., Timmermans, K.R., Blain, S., Tréguer, P., 2005. Growth physiology and fate of diatoms in the ocean: a review. *J. Sea Res.* 53, 25–42.
- Shikata, T., Sakurada, K., Jomoto, Y., Onji, M., Yoshida, M., Ohwada, K., 2010. Effect of temperature, salinity and light irradiance on phytoplankton growth in the Yatsushiro Sea. *Nippon Suisan Gakkaishi* 76, 34–45 (in Japanese with English abstract).
- Shimada, H., 2021. Long-term fluctuation of red tide and shellfish toxin along the coast of Hokkaido (Review). *Sci. Rep. Hokkaido Fish. Res. Inst.* 100, 1–12.
- Shimada, H., Kanamori, M., Yoshida, H., Imai, I., 2016. First record of red tide due to the harmful dinoflagellate *Karenia mikimotoi* in Hakodate Bay, southern Hokkaido, in autumn 2015. *Nippon Suisan Gakkaishi* 82, 934–938 (in Japanese with English abstract).
- Shinada, A., Shiga, N., Ban, S., 1999. Structure and magnitude of diatom spring bloom in Funka Bay, southwestern Hokkaido, Japan, as influenced by the intrusion of Coastal Oyashio Water. *Plankton Biol. Ecol.* 46, 24–29.
- Steidinger, K.A., Targen, K., 1997. Dinoflagellates. In: Tomas, C.R. (Ed.), *Identifying Marine Phytoplankton*. Academic Press, San Diego, pp. 387–584.
- Taş, S., 2019. Microalgal blooms in a eutrophic estuary (Golden Horn, Sea of Marmara) following a remediation effort. *Bot. Mar.* 62, 537–547.
- Wakita, M., Sasaki, K., Nagano, A., Abe, H., Tanaka, T., Nagano, K., Sugie, K., Kaneko, H., Kimoto, K., Okunishi, T., Takada, M., Yoshino, J., Watanabe, S., 2021. Rapid Reduction of pH and CaCO₃ saturation state in the Tsugaru Strait by the intensified Tsugaru Warm Current during 2012–2019. *Geophys. Res. Lett.* 48, e2020GL091332.
- Xu, N., Pang, S., Shan, T., Liu, F., Zhao, X., Gao, S., 2012. Molecular identification and culture trials of *Eutreptiella gymnastica* (Eutreptiales, Euglenophyceae). *Chin. J. Oceanol. Limnol.* 30, 446–455.
- Yabe, I., Kawaguchi, Y., Wagawa, T., Fujio, S., 2021. Anatomical study of Tsushima warm current system: determination of principal pathways and its variation. *Prog. Oceanogr.* 194, 102590.
- Yoshimura, T., Kudo, I., 2003. Riverine nutrient loading and their impact on primary production in Funka Bay, Japan. *J. Oceanogr.* 12, 185–193 (in Japanese with English abstract).