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Multi-annual comparisons of summer and under-ice phytoplankton communities of a mountain lake

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Abstract Little is known on the dynamics of under-ice phytoplankton communities. We investigated phytoplankton communities in the upper (0–20 m) and lower (30–35 m) layer of oligotrophic Lake Tovel, Brenta Dolomites (Italy) over 6 years during summer and under ice. Winter conditions were different from one year to another with respect to ice thickness and snow cover. Proxies for light transmission (Secchi disc transparency, light attenuation) were similar between seasons, even though the incident solar radiation was lower in winter. Algal richness and chlorophyll-*a* were not different between seasons while biomass was higher during summer. In four of the 6 years, Bacillariophyta dominated during summer and Miozoa (class Dinophyceae) under ice while in 2 years Bacillariophyta also dominated under ice. Generally, a shift to larger size classes from summer to under ice was observed for Bacillariophyta, Chlorophyta, and Ochrophyta (class Chrysophyceae) while Dinophyceae showed the opposite pattern. No strong links between phytoplankton community composition and abiotic factors (under-ice convective mixing, snow on ice, under-ice light) were found. We suggest that inter-species relationships and more precise indicators of under-ice light should be considered to better understand under-ice processes.

Keywords Size structure · Under-ice light · Ice cover · Mixotrophy · Indicator species · Lake Tovel

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Introduction

Traditionally, field studies on plankton in high latitude and altitude lakes mainly focus on summer. The winter period is neglected because of theoretical assumptions (i.e. summer is considered the main growth season while winter is considered a period of reduced activity) and practical constraints (i.e. sampling on ice presents logistical and safety challenges; Block et al., 2019). Recently, the perception of winter as an inactive period has been challenged (Hampton et al., 2017), and many studies (e.g. Felip et al., 2002; Bashenkhaeva et al., 2015; Ardyna et al., 2020; Dory et al., 2021; Hazuková et al., 2021; Hrycik &

Stockwell, 2021; Patriarche et al., 2021) show that plankton is adapted to harsh environmental conditions during winter and under ice.

The harsh environmental conditions are mainly linked to low water temperature that leads to stiffening of cell membranes, cell damage through ice crystal formation, and slowed down physiological processes. Adaptive mechanisms to low temperature include the evolution of cold shock and antifreeze proteins, the modulation of the kinetics of key enzymes, and the development of more fluid biological membranes through the accumulation of polyunsaturated fatty acyl chains (Morgan-Kiss et al., 2006). The concentration of reactive oxygen species increases at low temperature (De Maayer et al., 2014), and mycosporine-like amino acids, furthermore, are used as antioxidants (Flaim et al., 2014).

Apart from cold adaptation, adaptation to reduced under-ice light availability with respect to summer is important. Snow on ice substantially reduces light transmittance (Bolsenga et al., 1991; Petrov et al., 2005) while clear ice can transmit up to 95% of incoming light. However, ice cracks, gas bubbles, and particles within the ice decrease light transmittance by light scattering, and light absorption by ice increases from blue to red wavelengths (Warren, 2019). Nevertheless, under-ice irradiance can be high if snow cover is low because of snow melting, snow transport by wind, and/or no precipitation.

The under-ice light conditions affect algal photosynthesis. Phytoplankton can adapt to low light availability by strategies such as denser pigment packing, higher photosynthetic efficiency, and increased chlorophyll-a content that are used to maximise light absorption and photosynthetic capacity (Palmer et al., 2013; Lewis et al., 2019); furthermore, often mixotrophy is the main algal feeding strategy (Søgaard et al., 2021). In addition, incident irradiance can lead to under-ice convective mixing that keeps plankton in suspension and close to the under-ice lit area (Kelley, 1997; Vehmaa & Salonen, 2009; Yang et al., 2017, 2021). Thus, because of different adaptation capacities, different algae occur under ice. Experimental incubations of natural Arctic freshwater protist communities show that dinoflagellates dominate under low-light conditions whereas chrysophytes under high-light conditions (Charvet et al., 2014). Diatoms often dominate in marine and sea ice habitats because of heterotrophic carbon acquisition (Morgan-Kiss et al., 2006) and their capacity to withstand prolonged periods of darkness (van de Poll et al., 2020) also contributes to their success with respect to flagellates. Using ice thickness as a proxy for winter severity, motile mixotrophic species are favoured during severe winters and phototrophic species during mild winters (Özkundakci et al., 2016). Also thick ice decreases the abundance of cyanobacteria and diatoms while it increases the abundance of chrysophytes (Kalinowska & Grabowska, 2016; Özkundakci et al., 2016). Apart from studies on temporarily frozen lakes, studies on permanently frozen lakes show that despite a huge ice cover (3–5 m), photosynthetically active radiation (PAR) is present under ice, and phytoplankton (mainly chlorophytes and cryptophytes) thrive in the water column and show seasonal succession (Li et al., 2016; Patriarche et al., 2021). All these studies indicate that the under-ice phytoplankton communities are dynamic and adapted to their physical environment.

Summer and winter environmental conditions are different, especially with respect to temperature and light and these differences are partly reflected in algal parameters. Comparing summer to the winter and under-ice period, total phytoplankton biomass and chlorophyll-a (chl-a) as proxy for phytoplankton biomass are generally lower in winter than in summer, even though exceptions exist (Dokulil et al., 2014; Hampton et al., 2017). Furthermore, small-sized algae (<100 μm^3) generally dominate during winter because of reduced mixing compared to summer (Dokulil et al., 2014). A large-scale analysis of data from over 100 lakes, however, did not find general trends in phytoplankton community composition from summer to winter except for a tendency of more cyanobacteria and less chlorophytes during summer than during winter (Hampton et al., 2017).

With climate change, the tendency of enhanced warming at high elevations coupled with reduced precipitation in the form of snow may deplete reserves of mountain snow and ice (Pepin et al., 2021); these patterns will have effects on a lake's snow and ice cover as well. Northern European lakes experience a shortening of ice-cover length with important implications for lake physics, chemistry, and biology (Sharma et al., 2020). For example, autumn mixing reaches deeper water layers with later ice-in (Flaim et al., 2020), lake surface water temperature is driven by winter ice cover (O'Reilly et al., 2015),

phytoplankton composition changes in response to winter severity (Beall et al., 2016), and bacterio-plankton composition responds to changes in under-ice conditions (Obertegger, 2022).

While the number of studies focusing on winter and under-ice communities is increasing in recent years, only few studies are multi-annual permitting a comprehensive understanding of the many facets of summer (no ice) to winter changes in the lake habitat and algal communities. Here, we used a 6-year data set from mountain Lake Tovel, an oligotrophic and cold-water lake that freezes during winter. The lake's phytoplankton community is characterised by diatoms and dinoflagellates during the ice-free period (Cellamare et al., 2016) but little is known on its under-ice community. The objectives of this study were to assess differences between summer and under-ice phytoplankton communities from the upper (0–20 m) and lower, near-bottom (30–35 m) layer. Environmental conditions are important for phytoplankton, and thus, we first compared summer and under-ice environmental conditions; secondly, we specifically described winter environmental conditions influencing the under-ice environment, and finally, we investigated phytoplankton communities for their seasonal differences and under-ice characteristics. We hypothesised that (i) phytoplankton communities during summer and under ice are different because of the specific adaptations required to thrive in the cold, low-light, and low-nutrient under-ice environment; while diatoms generally dominate during summer (Cellamare et al., 2016), we expected less under-ice dominance of diatoms because of light limitation; furthermore, we hypothesised that (ii) under-ice communities show inter-year differences, and (iii) under-ice communities from the upper and lower layers are different. We expected for (ii) that ice thickness and snow cover, factors that influence under-ice light, would be the drivers of community differences, and for (iii), we expected lower light availability determining community differences with depth.

Material and methods

Lake Tovel (LTSER site IT09-005-A; 46.261 N, 10.949 E; 1177 m above sea level) is a dimictic, glacial lake (area: 0.4 km^2; maximum depth: 39 m; mean depth: 19 m; volume: 7.4 * 10^6 m^3) surrounded by the Brenta Dolomites (Trentino, Italy). The lake is ice-covered from December to mid-April (2009–2021: mean duration of ice cover = 126 days). During winter, topographical shadowing limits direct sunlight on the sampling site from half an hour around midday on the first day of the year to 4 h (11:00 to 15:00) on 31 January. Total phosphorus is low in Lake Tovel (< 10 μg l^{-1}; Cellamare et al., 2016), typical of oligotrophic conditions (Carlson & Simpson, 1996).

Phytoplankton samples were taken monthly during summer (July, August, September) and once a year under ice (January or February) from an upper (0–20 m; years 2012, 2015, 2017, 2018, 2019, 2020) and lower layer (30–35 m; years 2015, 2017, 2018, 2019, 2020). In summer, 1% of light reaches a depth of approximately 23 m (Cellamare et al., 2016). Chl-*a* fluorescence profiles indicate that algae are widely distributed along the water column under ice (Obertegger, 2022) as postulated by the lake ice continuum concept (Cavaliere et al., 2021). Therefore, we were confident that the upper layer reflects the habitat of most phytoplankton, both during summer and under ice.

Samples from the upper layer were taken with a 20-m long tube (weighted at the bottom) and from the lower layer with a Ruttner bottle (30 and 35 m). The summer samples from one calendar year were considered together with the under-ice samples of the following calendar year; jointly they are referred to as 'sampling period' and denoted p1–p6 (Table 1). The dates of winter sampling were dictated by logistic and safety considerations, and therefore days since ice-in and under-ice conditions differed from year to year (Table 1).

Phytoplankton samples (250 ml) were fixed immediately after sampling with acid Lugol solution. Quantitative phytoplankton analysis was performed using an inverted microscope (LEICA DMIRB) according to the Utermöhl method (Lund et al., 1958). For each sample, at least 400 algal units (filament, colony, and single-celled organisms) were counted using the phytoplankton counting and measuring software PlanktoMetrix (Zohary et al., 2016). The wet-weight biomass (mg m^{-3}) of each taxon was estimated from abundance and species-specific biovolume, obtained by geometrical approximations (Hillebrand et al., 1999) from the measurements of 25 individuals. Species identification was based on the updated phytoplankton taxonomic literature, and nomenclature was

Table 1 The number of phytoplankton samples collected from the upper (up, 0–20 m) and lower (low, 30–35 m) layers in summer and under-ice each period (p1–p6), the years of sampling of those periods, number of days from ice-in till the date of sampling under-ice), precipitation (precip; mm) since ice-in, and ice thickness (ice; cm); the summer months (July to September; 3 months) of 1 year and the under-ice sampling of the following year were grouped into one period; depth of 1% light (m) as single value for p1–p2 and as seasonal mean for p3–p6; single values are based on a PAR profile taken during the sampling and seasonal means are based on daily values over three weeks before the sampling; no light profile was taken for under-ice p2

Period	Summer			Under ice			Days since ice-in	Precip	Ice	Depth of 1% light (m)	
	Year	Up	Low	Year	Up	Low				Summer	Under ice
p1	2011	3	0	2012	1	0	76	36.2	40	24.7 ± 1.0	13.9
p2	2014	3	3	2015	1	1	33	49.8	38	22.8 ± 2.2	
p3	2016	2	1	2017	1	1	26	0	35	25.3 ± 1.4	34.5 ± 2.8
p4	2017	2	2	2018	1	1	51	108	40	28.3 ± 2.0	25.4 ± 7.5
p5	2018	3	3	2019	1	1	39	1.6	40	32.1 ± 4.2	20.7 ± 3.2
p6	2019	3	3	2020	1	1	54	40.7	40	26.6 ± 2.4	22.5 ± 2.1

according to Guiry & Guiry (2014). Taxa that could not be identified to species level were allocated to the lowest taxonomic level possible, and then further subdivided into taxonomic units by cell shape and size. For example, some unidentified Bacillariophyta were subdivided by shape (pennate or centric) and size. These taxonomic units were regarded at the same level as species for considerations on biodiversity (e.g. species richness; multivariate ordination).

Cell or colony size is an algal master trait because it impacts sinking rates, susceptibility to grazing, nutrient, and light utilisation (Litchman & Klausmeier, 2008) and shows temperature dependence (Zohary et al., 2020). Algal taxonomic units were allocated to four size classes based on their biovolume (< 100, 100–1000, 1000–10,000, and $> 10,000$ μm^3 alga $unit^{-1}$; Cellamare et al., 2016); for each sampling, the biomass of each size class was reported for each phylum/class, expressed as percentage of the total biomass per sample.

To characterise the summer and under-ice habitat, water temperature, mean % saturation of dissolved oxygen (% DO), conductivity, and pH were measured with a multiparametric probe (Idronaut Ocean Seven 316 Plus) at 1-m intervals. Water samples were taken with a bottle at 5-m intervals for nutrients (total nitrogen, total phosphorus, silica, nitrate, hydrogen carbonate) and other chemical parameters (ions) that were analysed according to APHA (2017). Water samples for chl-*a* determinations were taken with the tube (0–20 m), and chl-*a* was extracted from 1 L with 90% acetone and determined spectroscopically

according to the trichromatic method (Rice et al., 2017). Secchi disc depth was recorded as a proxy for light transparency. The attenuation coefficient of downwelling photosynthetically active radiation (kd_{PAR}) was assessed as the average for the central daylight hours (10:30 to 14:00) from readings of high-frequency sensors (HOBO by Onset UA-002–08 Pendant Temperature/Light Data Logger; 30-minutes recording frequency), deployed at discrete depths (1, 2, 5, 10, 15, 20 m) from 2016 onwards (p3–p6). The average kd_{PAR} for three weeks before sampling was reported to account for any legacy effect of light on phytoplankton. No continuous under-water light data were available for p1 and p2, and therefore, vertical profiles of under-water light taken monthly with an under-water quantum sensor (LI 192 LICOR, Inc., Lincoln, NE, USA) at 1-m intervals were used to calculate kd_{PAR}. Kd_{PAR} is a summary index of the under-water light attenuation that can be the driver (photosynthesis) and/or the consequence (light shading and scattering) of algal growth. From kd_{PAR}, the depth of 1% light ($\ln(100)/kd_{PAR}$) was calculated. To tentatively investigate under-ice mixing, temperature sensors (HOBO water temperature Pro v2 Data logger – U22-001; 30-minutes recording frequency) deployed under ice at different depths (surface, 0.25 m, 0.5 m, 1 to 9 m at 1-m intervals) were used to visually inspect under-ice temperature patterns from ice-in to sampling. An under-ice increase of daily mean water temperature during daylight (10 to 14.30 am) for sensors deeper than 0.25 m was interpreted as heating by solar radiation that could cause

convective mixing. Sensors deeper than 9 m were not considered because temperature differences between sensors deeper than 2 m were already undistinguishable for the periods of interest. Precipitation and air temperature from the date of ice-in to the date of winter sampling were recorded by an onshore meteorological station; missing data were imputed using the close-by Cles weather station (46.361 N, 11.040 E; 656 m above sea level; 13 km from Lake Tovel) and the randomForest algorithm (Liaw & Wiener, 2002). We reported daylight air temperature (11 am to 15 pm) to investigate any snow or ice melting potentially occurring during the warmest hours of the day. Environmental data were available for the upper and lower layer for all summer and under-ice sampling dates while some phytoplankton data were missing.

Statistical analyses

All statistical analyses were done using R 4.1.3 (R Core Team, 2022). The nonparametric Mann–Whitney U test that is appropriate for small sample size and does not rely on balanced samples (Sokal & Rohlf, 1995) was used to compare mean values (Secchi disc depth, kd_{PAR}, richness, biomass) between seasons. We tested for differences between (i) seasons independent of layer differences, and (ii) seasons specific for layers. We refrained from performing two-way ANOVA to test for seasonal and layer differences because of our unbalanced sample size, and any comparison of variance with low sample size is not meaningful.

As a measure of beta-diversity (Anderson et al., 2006), the variation of distances between phytoplankton communities and their season centroid (summer versus under ice; R function *betadisper* with bias.adjust = TRUE to consider the unequal sample size) was tested.

A principal correspondence analysis (PCA) with standardised environmental variables was performed and the function *factorfit* of package vegan (Oksanen et al., 2020) was used to investigate seasonal differences; the function *envfit* was used to link environmental variables to the ordination.

Non-metric multidimensional scaling (NMDS) analyses were performed with algal phyla using biomass weighted Bray–Curtis dissimilarity as distance. Complementary to function *factorfit*, an analysis of similarity (ANOSIM) of phytoplankton communities

was performed to investigate seasonal differences; in ANOSIM, R values close to 0 indicate similarity while values close to 1 dissimilarity.

Indicator species analysis for seasons was performed with package indicspecies (De Caceres & Legendre, 2009). Algal taxonomic units as indicators for summer or the under-ice period were determined based on merging samples from the upper and lower layers and also considering each layer separately. Only algal taxonomic units with both probabilities A and B > 0.5 were considered. Probability A is the probability that the sample is a summer (or under-ice) sample given the fact that a summer (or under-ice) indicator species has been found; probability B is the probability of finding the indicated summer (or under-ice) indicator species in a summer (or under-ice) sample.

Results

Summer versus under-ice environmental conditions

Mean water temperature in the upper layer was higher ($P < 0.001$) during summer than under ice (mean$_{summer}$ ± 1 standard deviation = 9.3 ± 1.5 °C; mean$_{under\ ice}$ = 3.8 ± 0.17 °C). Mean water temperature in the lower layer was always low even though it was slightly lower ($P < 0.05$) under ice (mean$_{summer}$ = 5.0 ± 0.3 °C; mean$_{under\ ice}$ = 4.5 ± 0.5 °C).

Mean % DO in the upper layer was higher ($P < 0.001$) during summer than under ice (mean$_{summer}$ = $116 \pm 5\%$ DO; mean$_{under\ ice}$ = $79 \pm 5\%$ DO) while mean % DO in the lower layer was not different ($P > 0.05$) between seasons (mean$_{summer}$ = $38 \pm 13\%$ DO; mean$_{under\ ice}$ = $42 \pm 13\%$ DO).

Mean Secchi disc depth (Fig. 1) was not different ($P > 0.05$) between summer and under ice (mean$_{summer}$ = 12.1 ± 3.2 m; mean$_{under\ ice}$ = 11.2 ± 4.3 m). Highest Secchi disc depths were observed in summer p4 and under-ice p3.

Chl-*a* concentrations were always low (< 2.5 mg l^{-1}; Fig. 1) and were not different ($P > 0.05$) between seasons (mean$_{summer}$ = 1.4 ± 0.5 mg m^{-3}; mean$_{under\ ice}$ = 1.7 ± 0.6 mg m^{-3}). The lowest chl-*a*

Fig. 1 Boxplots of seasonal values for (**a**) Secchi disc depth and (**b**) chl-*a* concentration; shown are also the observed values, colour-coded by period (p1–p6), and shape-coded for sampling month (numbers indicate months)
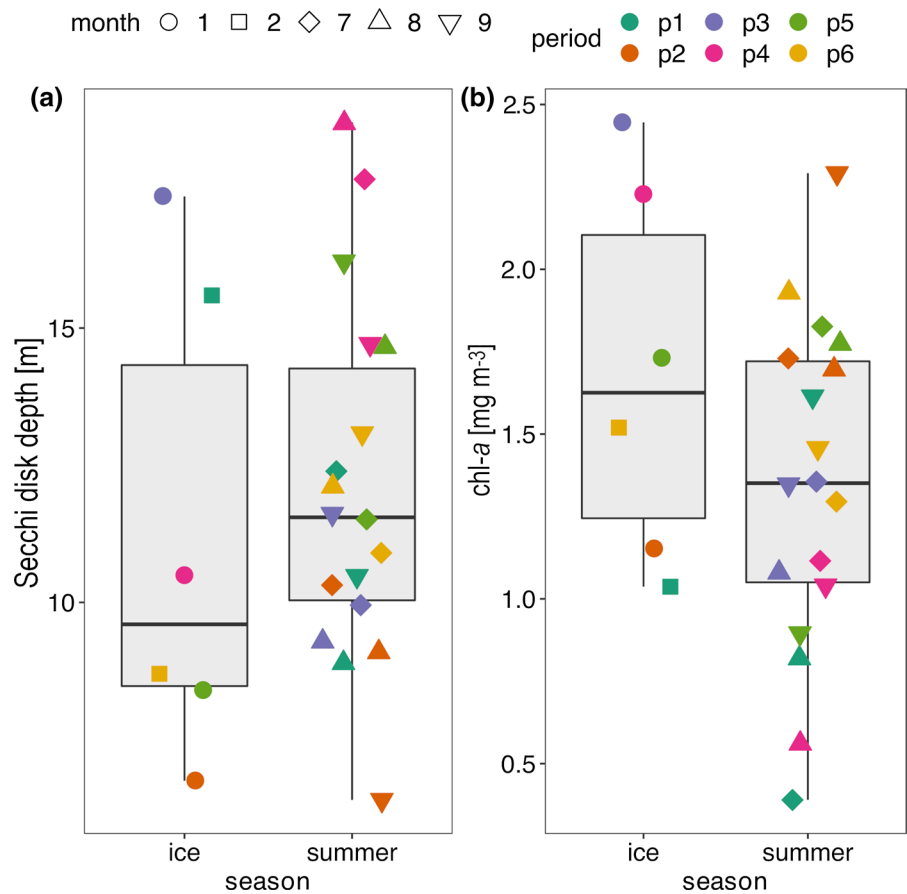
was observed in summer p1 and the highest under-ice p3.

Based on continuous measurements, light attenuation over three weeks preceding sampling was lower ($P < 0.001$) during summer than under ice (kd_{PAR} $mean_{summer} = 0.17 \pm 0.02$ m^{-1}; $mean_{under\ ice} = 0.19 \pm 0.10$ m^{-1}). Considering single periods, the depth of 1% light was deeper under ice than during summer for p3 ($P < 0.001$), did not show seasonal differences for p4 ($P > 0.05$), and was shallower for p5 ($P < 0.001$) and p6 ($P < 0.001$) (Table 1).

Focusing on the main nutrients, total phosphorus was always low (< 10 µg l^{-1}) and did not show seasonal differences, both in the upper and lower layers. Only in the upper layer, total nitrogen and silica showed higher values under ice than during summer (Table 2).

A PCA based on standardised summer and under-ice environmental variables (PCA-axis 1 = 40% explained variability, PCA-axis 2 = 13%) separated

Table 2 Mean concentration and standard deviation for total phosphorus (TP; µg l^{-1}), total nitrogen (TN; µg l^{-1}), and silica (Si; mg l^{-1}) for seasons and layers statistically significant differences between seasons are indicated by * ($P < 0.05$), ** ($P < 0.01$)

	Upper layer		Lower layer	
	Summer	Under ice	Summer	Under ice
TP	4.6 ± 2.3	4.5 ± 1.8	4.9 ± 1.0	5.8 ± 2.6
TN	381 ± 62*	449 ± 64*	450 ± 25	437 ± 74
Si	0.5 ± 0.2**	1.5 ± 1.2**	2.0 ± 0.6	1.5 ± 0.6

summer samples of the upper and lower layers while most under-ice samples clustered with summer samples of the lower layer (Fig. 2). However, the under-ice sample p2 of the upper layer was similar to summer samples because of similar high % DO values, attributable to a carry-over effect of a deep mixing event in late autumn bringing oxygen to the lower
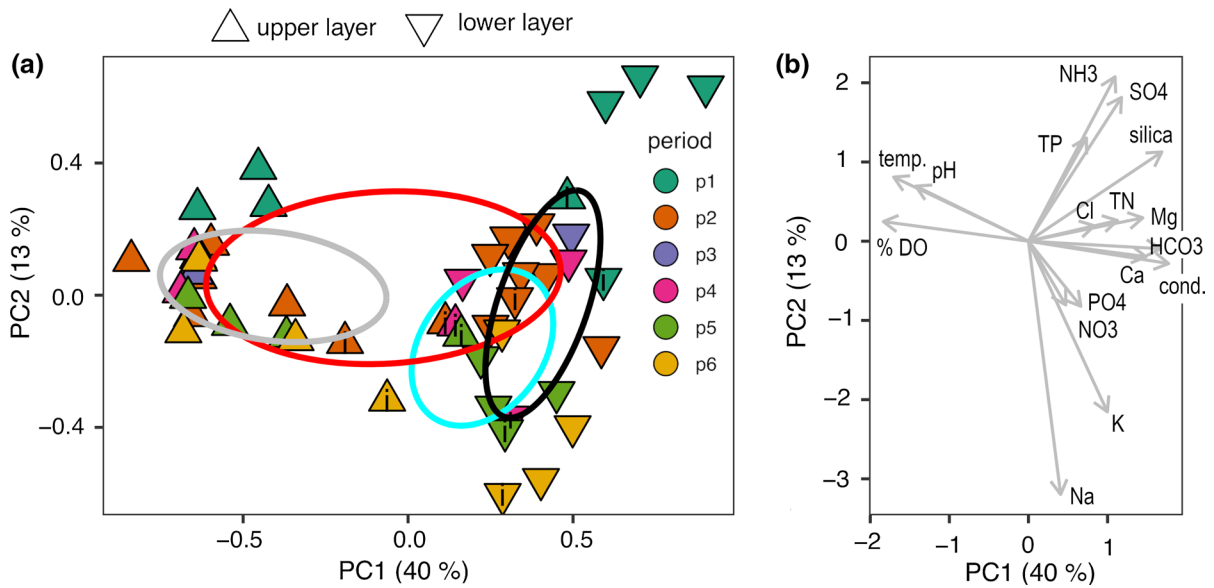
Fig. 2 PCA with standardised environmental variables. (**a**) ordination of samples, (**b**) environmental variables. The 11 under-ice samples are labelled 'i', the rest are summer samples. The 95% confidence envelopes of centroids are colour-coded:

grey is upper layer, black is lower layer, red is summer, and cyan is under ice; conductivity (cond.), temperature (temp.), total phosphorous (TP), total nitrogen (TN)

layer (Flaim et al., 2020). The under-ice p6 sample showed similar high pH as summer samples of the upper layer and thus did also not cluster with lower layer samples. Accordingly, layer centroids were clearly different (factorfit: $P < 0.001$) while season centroids were weakly different (factorfit: $P < 0.05$). Comparing layers, the upper layer was characterised by higher % DO, higher temperature and pH and the lower layer by higher nutrient concentrations (Fig. 2).

Environmental conditions during different winters

Winter incident solar radiation during daylight over three weeks preceding sampling was on average half the summer incident radiation ($\text{mean}_{\text{summer}} = 627 \pm 180$ W m^{-2}; $\text{mean}_{\text{winter}} = 298 \pm 110$ W m^{-2}). Snow and ice conditions determine the amount of light passing through the ice cover of a frozen lake. Light attenuation through the ice cover was measured only for p3 and showed that 31% of the incident light was transmitted. Winter p1 showed relatively little precipitation with continuously sub-zero daylight air temperature (Fig. 3). Winter p4 showed the highest cumulative

precipitation until sampling, winter p2 showed the second highest (Table 1; Fig. 3), and daylight air temperature was sub-zero most of the time in both periods (Fig. 3). Winters p3 and p5 had no or very little precipitation, and winter p6 showed early precipitation concomitantly to above zero daylight air temperature implying snow melting during the day and ice formation during the colder night hours. Based on these observations, p3 and p5 were grouped into low precipitation, p1 and p6 into intermediate precipitation, and p2 and p4 into high precipitation winters (Table 3).

The surface, 0.25 m, and 0.5 m temperature sensors showed the greatest temperature variations under ice (supplementary figure S1). Because the surface and 0.25 m sensor were often stuck in the ice, we focused on the 0.5 m temperature sensor. The under-ice periods p1 and p4 showed indications of under-ice mixing (i.e. temperature increase of the 0.5 m sensor) more than 30 days before the sampling while for p2 and p6 five and ten days, respectively, passed from the end of potential mixing (i.e. stable water temperature) until sampling; under-ice samplings of p3 and p5 occurred right at the end of possible mixing (i.e. end of temperature changes; Table 3).
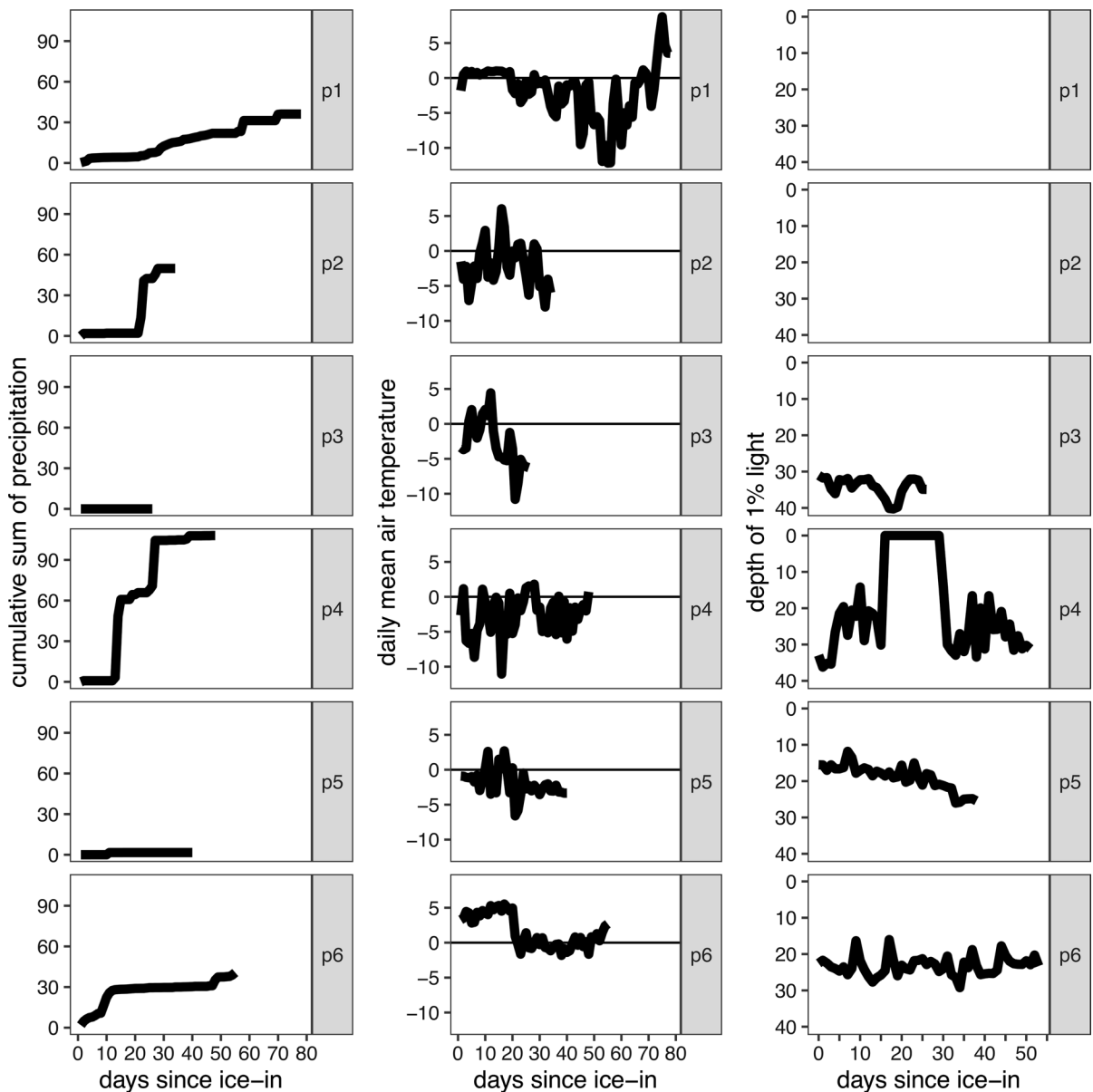
Fig. 3 Meteorological and under-ice conditions from ice-in to sampling for periods p1–p6; left panel: cumulative precipitation from ice-in (day 0) until the day of sampling; mid panel: mean air temperature (°C) from 11:00 to 15:00 of each day; the horizontal line indicated 0 °C; right panel: under-ice depth of 1% light

Under-ice light for p3, p5, and p6 was continuously measurable from ice-in to sampling while for winter p4 during two weeks (i.e. from day 34 to day 21 before the under-ice sampling) under-ice light was zero (Fig. 3). The mean depth of 1% light over three weeks before the sampling was highest for p3 and around 20 m for p4, p5, and p6 (Table 1). The single PAR profile of the under-ice sampling p1 showed

the lowest depth of 1% light. Based on the depth of 1% of under-ice light, p1 and p4 were grouped into low-light and p3, p5, and p6 into high-light periods. P2 was also regarded as a low-light period based on highest cumulative precipitation and because of a snowfall event a few days before the sampling.

Only under-ice periods p3, p4, and p5 followed expectations based on concurrent occurrence of

Table 3 Summary of environmental variables and phyla/class dominance

p	Precipitation			Pot. mix	Under-ice light		Secchi disc depth		chl-*a*		Dom. upper layer		Dom. lower layer			
	Low	High	Inter		Low	High	Low	High	Low	High	Dino	Dia	Dino	Dia	Chloro	Crypto
p1			x		x				x		x					
p2		x		x	x		x		x		x				x	x
p3	x			x		x		x		x	x					x
p4		x			x					x	x			x		
p5	x			x		x						x		x		x
p6			x	x		x						x	x			

(dom.; > 50% biomass; dinoflagellates–dino; diatoms–dia.; chlorophytes–chloro; cryptophytes–crypto) in the upper, lower, or composite layer for the six under-ice periods (p; p1–p6); potential mixing (pot. mix.); under-ice light based on summer to winter changes in the depth of 1% light; chl-*a* is for 0–20 m; the classification of precipitation in low, high, and intermediate is based on visual inspection of air temperature and the temporal pattern of cumulative sum of precipitation (see text); x indicates that the respective condition holds

potential mixing, precipitation, and light conditions (i.e. p4: no potential mixing with high precipitation and low light; p3 and p5: potential mixing with low precipitation and high light; Table 3). Highest and lowest under-ice Secchi disc depth and chl-*a* concomitantly occurred with low-light (lowest Secchi disc depth and chl-*a*: p2 and p1, respectively) and high-light (highest Secchi disc depth and chl-*a*: p3) periods (Fig. 1; Table 3).

A PCA based on standardised under-ice environmental variables (PCA-axis 1 = 28%, PCA-axis 2 = 23%) separated the upper from the lower layer (statistically significant layer centroids: $P < 0.001$) with mostly higher pH and % DO linked to the upper layer while higher temperature and nutrient concentrations to the lower layer (Fig. 4). Only the upper layer sample p1 showed higher silica and nutrient concentrations than the other upper layer samples.

Summer versus under-ice biodiversity

Nine phyla were found with Chlorophyta showing highest diversity (29 taxonomic units), and Cyanobacteria, Miozoa (Class Dinophyceae), Bacillariophyta, Cryptophyta, and Ochrophyta (classes Chrysophyceae and Eustigmatophyceae) having more than 10 taxonomic units each. Phyla Haptophyta (4 taxonomic units), Charophyta (2 taxonomic units), and Euglenozoa (1 taxonomic unit) showed reduced diversity.

Under-ice richness was higher (e.g. p1) and lower (e.g. p2) than summer richness (Fig. 5),
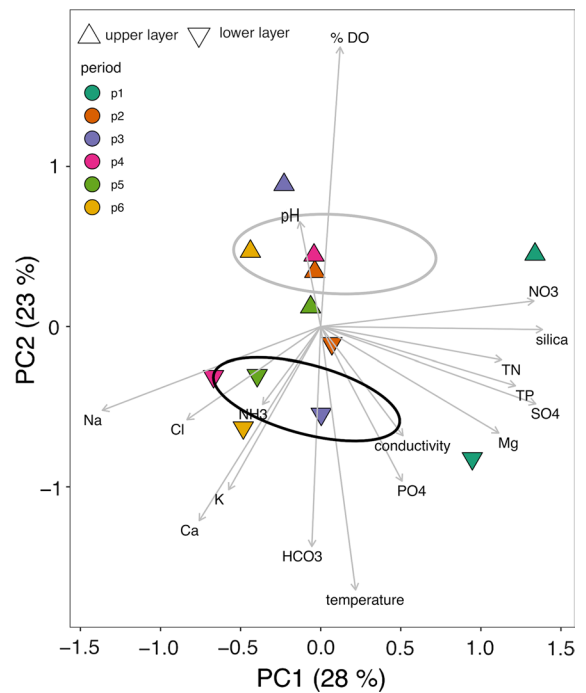

Fig. 4 PCA on standardised environmental variables based on under-ice data for the upper and lower layer over the six periods, p1–p6 (colour-coded). The 95% confidence intervals for layer centroids are shown: grey is upper layer; black is lower layer; total phosphorous (TP), total nitrogen (TN)

and accordingly, no seasonal difference in mean richness (mean$_{summer}$ = 36 ± 9 algal units; mean$_{under\ ice}$ = 39 ± 7 algal units) was found

Fig. 5 Boxplots of seasonal values for richness of algal taxonomic units of the upper layer **(a)** and lower layer **(b)**; shown are also the observed values, colour-coded by period (p1–p6), and shape-coded for sampling month (numbers indicate months)
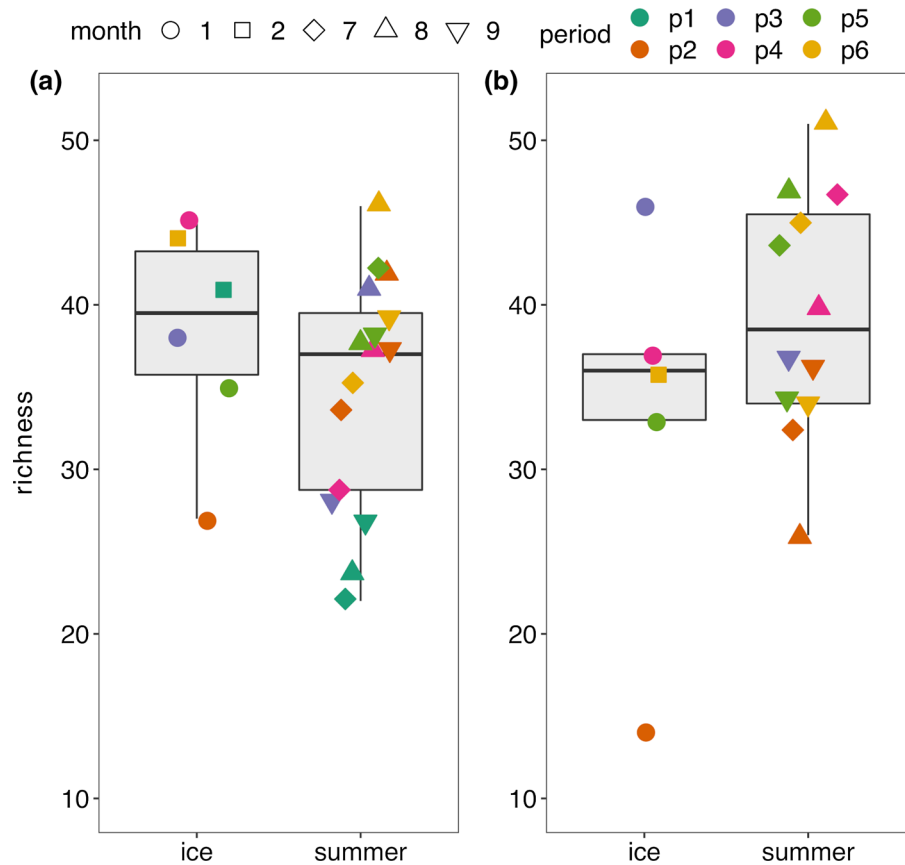
(P > 0.05), when merging richness for both layers and when considering each layer separately.

Total phytoplankton biomass was always low (range: 0.08 – 2.27 mg m^{-3}) and was usually higher during summer than under ice (mean$_{summer}$ = 0.85 ± 0.47 mg m^{-3}; mean$_{under ice}$ = 0.53 ± 0.23 mg m^{-3}), except for p4 in the upper layer and p2 in the lower layer (Fig. 6); accordingly, mean summer biomass was higher (P < 0.05) than mean under-ice biomass, when merging layers and when considering each layer separately

In the upper layer, % phylum/class contribution to total biomass showed a dominance of Bacillariophyta (mainly centric and small pennate diatoms) during summer and Miozoa (Dinophyceae) under ice for periods p1, p2, p3, and p4, while in periods p5 and p6, Bacillariophyta dominated also under ice (Fig. 7; Table 3). In addition, Ochrophyta (Chrysophyceae; p1, p3, p4) and Cryptophyta (p2) also increased from summer to under ice

In the lower layer, Bacillariophyta generally dominated during summer, similar to the upper layer; however, under-ice dominance was more diverse with Chlorophyta and Cryptophyta dominating in p2 (low light), Cryptophyta in p3 and p5 (both high light), Bacillariophyta in p4 (low light) and p5 (high light), and Dinophyceae in p6 (high light) (Table 3). The under-ice community of p2 and p3 was very different from the summer community of those periods, as well as from each other (Fig. 7).

Apart from seasonal differences of phyla and classes, some taxa only occurred under ice or more than doubled their biomass from summer to under ice: for example, in the upper layer, Miozoa (Dinophyceae) in p1 (low-light period; *Apocalathium aciculiferum* (Lemmermann) Craveiro, Daugbjerg, Moestrup & Calado, *Borghiella dodgei* Moestrup, Gert Hansen & Daugberg occurred only under ice), p2 (low light; *Gymnodinium* cf. *mirabile* Penard, *G. uberrimum* (G.J.Allman) Kofoid & Swezy, *Gyrodinium helveticum* (Penard) Y.Takano &

Fig. 6 Boxplots of seasonal values for algal total biomass [mg m^{-3}] of the upper layer (**a**) and lower layer (**b**); shown are also the observed values colour-coded by period (p1–p6), and shape-coded for sampling month (numbers indicate months)
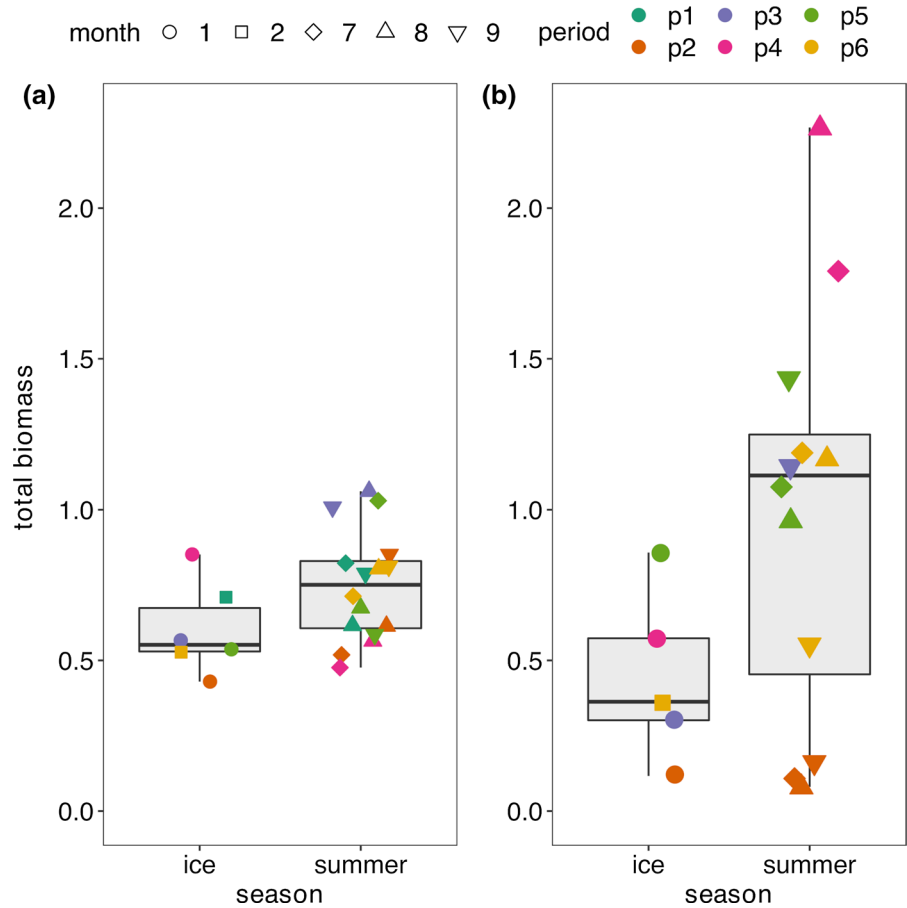
T.Horiguchi increased), and p3 (high light; *G. uberrimum* increased), Chrysophyceae in p4 (low light; *Stokesiella* sp. occurred only under ice), and Cryptophyta in p2 (low light; *Cryptomonas marssonii* Skuja, *C. reflexa* (M.Marsson) Skuja, and *C. rostratiformis* Skuja increased; *C. platyuris* Skuja increased). Similarly in the lower layer, some taxa increased from summer to under ice or only occurred under ice: for example, Chlorophyta in p2 (low light; *Tetraselmis* sp., *Oocystis* sp., *Tetraedron triangulare* Korshikov, *Dictyosphaerium subsolitarium* Van Goor increased) and Cryptophyta in p3 (high light; *C. rostratiformis* occurred only under ice; *C. marssonii* increased).

Multivariate ordination

Taxonomic units of Charophyta and Euglenozoa were found only once in all samples and were thus not considered further in NMDS based on phyla and classes. In NMDS with biomass of Bacillariophyta, Chlorophyta, Cyanobacteria, Cryptophyta, Miozoa (Class Dinophyceae), Haptophyta, and the two classes Chrysophyceae and Eustigmatophyceae of phylum Ochrophyta (stress = 0.10; Fig. 8), summer and under-ice communities showed a weak difference between season centroids (factorfit: $P = 0.03$; ANOSIM: $R = 0.26$, $P < 0.01$) and no difference in beta-diversity between summer and under-ice communities ($P > 0.05$). Relating environmental variables to the ordination, pH was mostly related to summer and under-ice p5 and p6 communities, and silica, conductivity, and nitrate were mostly related to the other under-ice communities and summer p2.

Considering biomass of algal phyla and classes from the upper and deeper layer separately (NMDS$_{upper\ layer}$: stress = 0.11; NMDS$_{lower\ layer}$: stress = 0.05; Fig. 8; supplementary Figure S2), a higher beta-diversity ($P < 0.01$) for the under-ice than summer communities and weak difference between
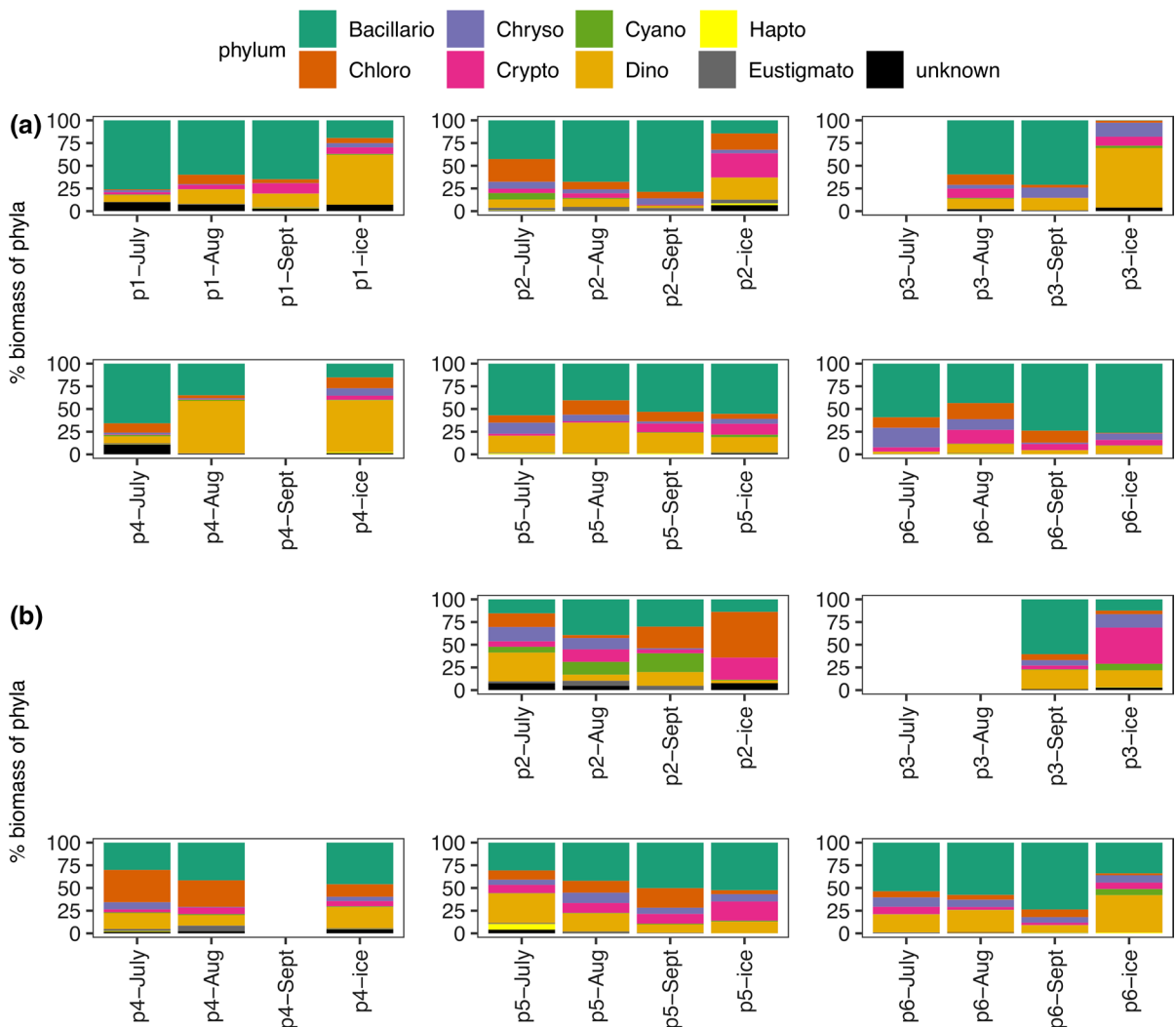
Fig. 7 Barplots of % total biomass of phyla per sample; (**a**) upper layer; (**b**) lower layer; empty columns indicate missing samples and the coding indicates the period and sampling month/season (August–Aug; September–Sept; under ice–ice). Cyanobacteria (Cyano), Miozoa (Class Dinophyceae–Dino), Bacillariophyta (Bacillario), Cryptophyta (Crypto), Ochrophyta (classes Chrysophyceae–Chryso; Eustigmatophyceae–Eustigmato), Chlorophyta (Chloro), Haptophyta (Hapto); Charophyta and Euglenozoa had biomass values < 2% and were not visible in the plot

season centroids (factorfit: $P = 0.04$; ANOSIM: $R = 0.47$, $P < 0.01$) were found for the upper layer; in contrast, non-significant season centroid differences (factorfit and ANOSIM: $P > 0.05$) and non-significant beta-diversity differences between summer and under-ice communities were found for the lower layer. Relating environmental variables to NMDS$_{upper\ layer}$, pH was mainly related to summer communities and the under-ice community p3, while silica and conductivity were related to under-ice communities and

August p4 and p5 and July p2. No variables were related to NMDS$_{lower\ layer}$.

Indicator species analysis

Three indicator species were indicated for summer and six for under ice when both layers were considered jointly (Table 4). Considering single layers, three of those species were indicator species for the upper layer (summer: *Sphaerellopsis* sp.; under ice: *Cryptomonas rostratiformis*) and the lower layer (under
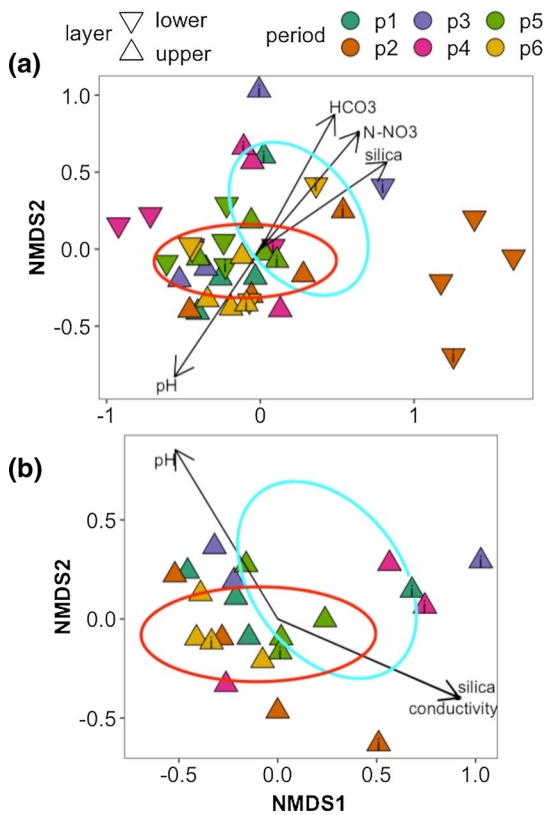
Fig. 8 NMDS of phytoplankton communities for (**a**) the upper and lower layer and (**b**) for only the upper layer. The under-ice samples are labelled 'i', the rest are summer samples. The 95% confidence intervals for layer centroids are shown: cyan is under ice and red is summer; periods are colour-coded

ice: *Stokesiella* sp.). In addition, *C. marssonii* was an under-ice indicator for the upper layer, and one Bacillariophyta taxonomic unit, one Cyanobacteria taxonomic unit, and *Pseudotetraëdriella kamillae* E.Hegewald & J.Padisák were summer indicator species for the lower layer.

Biovolume size class patterns

For Bacillariophyta (Fig. 9) and Chlorophyta (supplementary Figure S3), the distribution of size classes was similar in the upper and lower layer with generally the two middle size classes (100–1000 and 1000–10,000 μm^3) jointly dominating in all samples, even though exceptions were observed (Chlorophyta: dissimilarity in size distribution of layers: p3 and p6). For Cyanobacteria (supplementary Figure S4), only the lowest size class (< 100 μm^3) was observed

Table 4 Indicator algal units for summer and under ice (ice)

	All samples				Upper layer				Lower layer			
	Algal taxonomic unit	A	B	Sign	Algal taxonomic unit	A	B	Sign	Algal taxonomic unit	A	B	Sign
Summer	*Sphaerellopsis* sp.	1	0.5	*	*Sphaerellopsis* sp.	1	0.6	*	centric diatoms (Ø: 10 μm)	0.9	0.8	**
	centric diatoms (Ø: 6 μm)	0.9	0.6	*					*Pseudotetraëdriella kamillae*	0.9	1	**
	Tovellia sanguinea	1	0.5	*					Cyanophyta cells Ø <2 μm	0.9	1	**
Ice	*Cryptomonas rostratiformis*	0.9	0.6	*	*Cryptomonas rostratiformis*	0.9	0.8	**	*Stokesiella* sp.	0.9	0.6	*
	Stokesiella sp.	0.9	0.5	**	*Cryptomonas marssonii*	0.8	0.8	*				

A is the probability that the sample is a summer (or under-ice) sample given the fact that a summer (or under-ice) indicator species has been found

B is the probability of finding the indicated summer (or under-ice) indicator species in a summer (or under-ice) sample

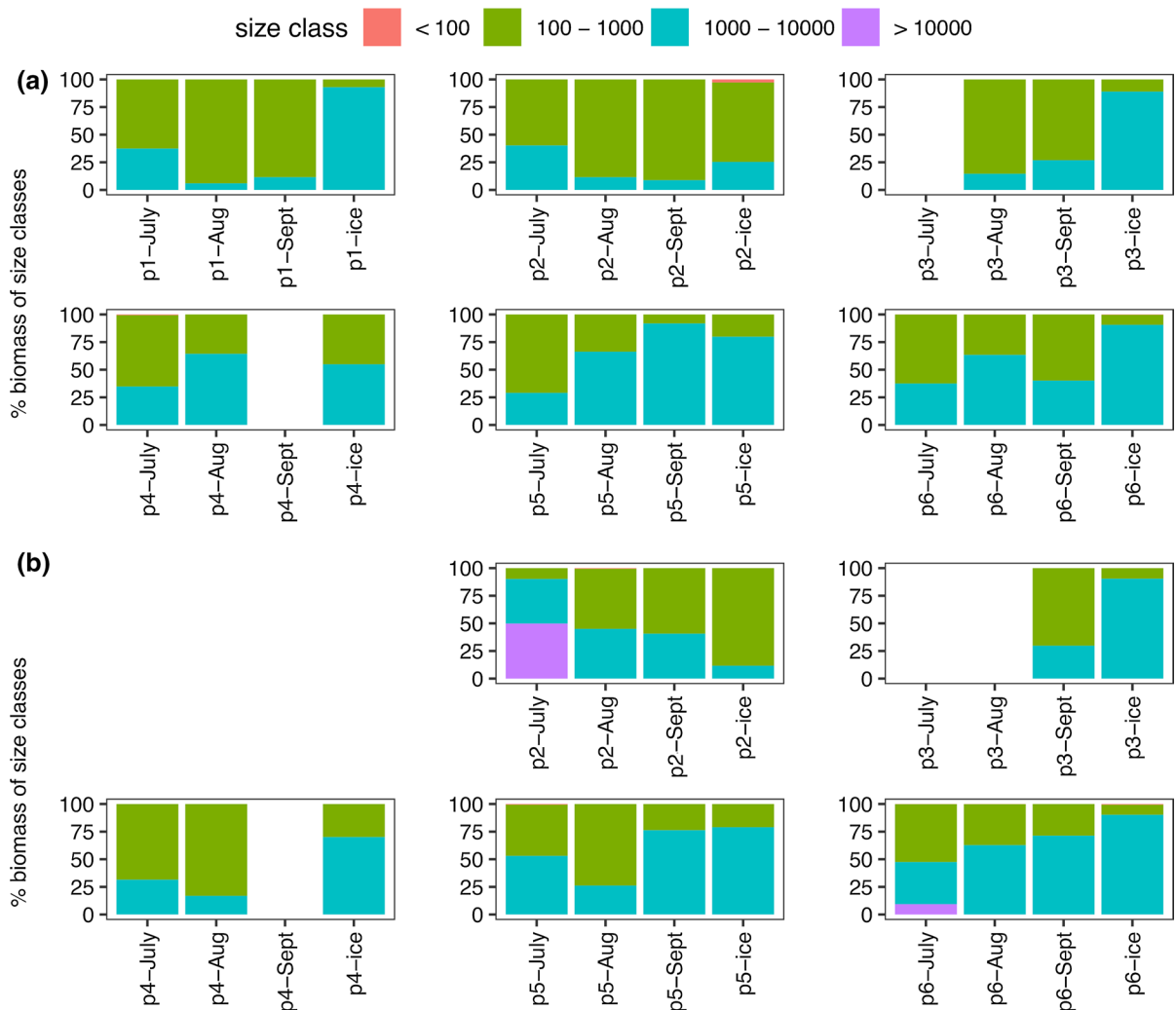Significance (sign.; $P < 0.05$ *; $P < 0.01$ **) is given

Fig. 9 Barplots of % total biomass in each of four size classes of Bacillariophyta in each sample; (**a**) upper layer; (**b**) lower layer; empty columns indicate missing samples and the coding states the period and sampling month/season (August–Aug; September–Sept; under ice–ice). Size class unit is μm^3 ind^{-1}

with generally no difference between the upper and lower layer. The distribution of size classes of Miozoa (Dinophyceae; Fig. 10) and Cryptophyta (supplementary Figure S5) was quite distinct between the upper and lower layer, with the largest size class (> 10,000 μm^3 ind^{-1}) prevailing more in the upper than the lower layer. The distribution of size classes of Ochrophyta (Chrysophyceae; supplementary Figure S6) was similar between the upper and lower layer only for p3 and p6; while in the upper layer mostly the smallest (< 100 μm^3) and second size class (100–1000 μm^3) dominated, in the lower layer also the third size class (1000–10,000 μm^3) dominated.

Considering shifts in size classes within periods (i.e. from summer to under ice), Bacillariophyta (Fig. 9), Chlorophyta, and Ochrophyta (Chrysophyceae) showed a shift from a smaller to a larger size class for the same (p1, p2, p4, p6) and different periods in the upper layer (Table 5); Miozoa (Dinophyceae) showed a shift from a large to a smaller size class (Fig. 10) and Cyanobacteria and Cryptophyta did not show any changes. In the lower layer, Chlorophyta showed a shift from a large to a smaller size class, Bacillariophyta showed a shift to a larger size class or did not change their size class pattern from summer to under ice (Fig. 9).
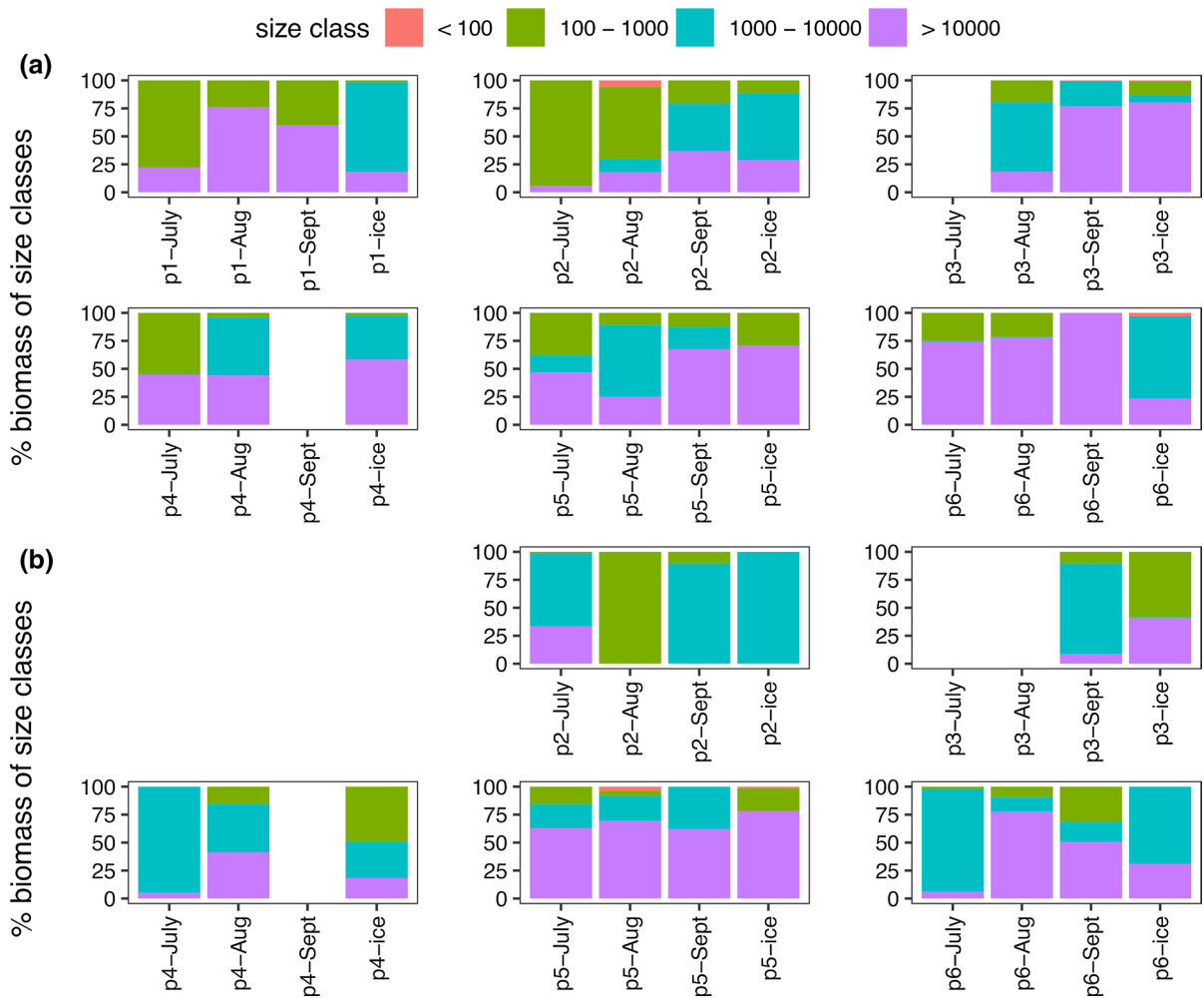
Fig. 10 Barplots of per cent total biomass in each of four size classes of Miozoa (Class Dinophyceae) in each sample; (**a**) upper layer; (**b**) lower layer; empty columns indicate missing samples and the coding states the period and sampling month/season (August–Aug; September–Sept; under ice–ice). Size class unit is μm^3 ind^{-1}

Discussion

Considering that no year is like another, under-ice samples of this study were not comparable with respect to timing and environmental conditions; therefore, statistical testing was limited. However, sampling at different time points and during different years also was advantageous because it showed how diverse phytoplankton communities were. Generally, abiotic environmental conditions such as light, nutrients, temperature, water column stability, and biotic, inter-species interactions such as grazing and competition determine phytoplankton community composition (Litchman & Klausmeier, 2008). Here, seasonal

environmental differences were observed for water temperature in the lower and upper layers and % DO and nutrients in the upper layer. The phytoplankton community in Lake Tovel is considered cold-water adapted during the ice-free season (Cellamare et al., 2016). In fact, the mean temperature in the summer upper layer was < 10 °C; furthermore, temperature differences between summer and under-ice periods were very slight in the lower layer. Consequently, we suggest that water temperature might generally play a minor role for this lake community. Proxies for light transmission (Secchi disc transparency, kd_{PAR}) were similar between seasons, even though the absolute amount of light reaching the ice cover was less during

Table 5 Observed shift (indicated by the arrow) in biovolume distribution from summer to under ice for different algal phyla/classes.

	Bacill	Chloro	Chryso	Crypto	Cyano	Dinophyceae
Upper layer						
p1	2 → 3	2 → 3	1 → 2	No discernible pattern from summer to under ice	Only smallest size class 1	4 + 2 → 3
p2	dom 2	2 → 3	1 → 2			4 + 2 → 3
p3	2 → 3	2 → 4				
p4	2 → 3		1 → 2 + 3			
p5	dom 3					
p6	2 → 3	2 → 3				4 → 3
Lower layer						
p1	No data					
p2	dom 2			2 → 3	2 → 3	No discernible pattern from summer to under ice
p3	2 → 3	2 + 3 → 1				
p4	2 → 3	2 → 1				
p5	dom 3					
p6	dom 3					

Bacillariophyta–Bacill., Chlorophyta–Chloro.; Chrysophyceae–Chryso.; Cryptophyta–Crypto.; Cyanobacteria–Cyano.; for the lower layer, no samples for p1 were available; size class 1: <100 μm^3 algal taxonomic unit^{-1}; size class 2: 100–1000; size class 3: 1000–10,000; size class 4:> 10,000 μm^3; dominance in summer and under ice (dom.); empty cells indicate no discernible size change.

winter. Chl-*a* and algal richness did not show seasonal differences, while biomass was lower under ice. Most studies find lower chl-*a* under ice compared to summer concentrations (Dokulil et al., 2014; Hampton et al., 2017; Hazuková et al., 2021; Hrycik et al., 2022) while phytoplankton biomass was found to be either lower under ice (Dokulil et al., 2014; Hampton et al., 2017; Hrycik et al., 2022) or similar (Hazuková et al., 2021). Thus, our observations contrasted (chl-*a*, biomass) or agreed (biomass) with those studies. Chl-*a* as a proxy for under-ice algal biomass is debated because of the persistence of chl-*a* of nonviable phytoplankton during darkness (van de Poll et al., 2020) and increased cellular content of photopigments as a photoacclimation strategy (Morgan-Kiss et al., 2016; Lewis et al., 2019) might influence chl-*a* estimates and any inferences from it. Thus, we suggest that the interplay of low-light adaptation and algal growth is responsible for these different observations and thus hampers general statements on chl-*a* and biomass seasonal patterns across lakes and studies. For example, complete darkness was observed four to three weeks before the under-ice sampling in p4 that was characterised by second highest under-ice chl-*a*. Mixotrophic chrysophytes and dinophytes (Litchman & Klausmeier, 2008; Mitra et al., 2016) dominated under-ice p4 and might have been responsible for the high observed chl-*a*.

In the PCA with environmental conditions, environmental differences were stronger for layers than for seasons. Remarkably, mainly summer samples showed the layer difference while under-ice samples were similar to summer samples of the lower layer, and a similar result for phytoplankton communities was expected (i.e. under-ice communities of the upper and lower layer similar to summer communities of the lower layer). However, in NMDS, summer and under-ice phytoplankton communities were similar, irrespective of considering layers jointly or separately. Therefore, our first hypothesis of seasonally different phytoplankton communities was rejected based on NMDS, a multivariate analysis. These results were in contrast to Obertegger (2022) finding with NMDS different bacterial communities during summer and under-ice in the upper layers of Lake Tovel. In contrast to community composition, phytoplankton beta-diversity was higher under ice than during summer, confirming our first hypothesis of seasonal differences. This, again, was in contrast

to bacterial communities that showed no seasonal differences in beta-diversity over 6 years in Lake Tovel (Obertegger, 2022). Phytoplankton and heterotrophic bacteria are linked in multiple ways such as competition for nutrients, use of algal exudates, decomposition of decaying algae, or mixotrophic predation of algae on bacteria (Bižić-Ionescu et al., 2014; Salcher, 2014; Unrein et al., 2014; Ivanova et al., 2018). While prokaryotes and protists are in temporal synchrony in Lake Tovel during the ice-free period (Obertegger et al., 2019), this study indicated temporal differences. Small environmental differences in under-ice conditions can have huge impacts on phytoplankton composition (Vehmaa & Salonen, 2009), and we suggest that year-to-year variability in under-ice environmental conditions and biotic interactions (e.g. competition, facilitation; Picoche & Barraquand, 2020) were responsible for the observed algal patterns.

Seasonal differences were found for phytoplankton beta-diversity but not for community composition, and a closer look at changes in community composition revealed the underlying cause. While there are general seasonal patterns in Lake Tovel with diatoms generally dominating during summer (Cellamare et al., 2016; this study) and dinoflagellates under ice, summer and under-ice communities of p5 and p6 were similar showing under-ice dominance of diatoms in the upper layer. These inter-season similarities hampered any statistics-based statement on seasonal differences.

Inspecting phyla/class biomass, inter-year differences according to our second hypothesis were observed. Dinoflagellates dominated over diatoms in three of four low-light under-ice samplings and this indicated light limitation of diatoms. Under ice, low nutrient concentrations and light availability are constraining factors. Nutrient uptake by algae is light dependent (Litchman et al., 2004): at low light, diatoms, dinoflagellates, and Cyanobacteria can utilise light more efficiently than other taxa (Litchman & Klausmeier, 2008). Furthermore, both dinoflagellates and diatoms can be mixotrophic (Mitra et al., 2016; Villanova et al., 2017), and various mixotrophic dinoflagellates have been described for Lake Tovel (Flaim et al., 2010, 2012, 2014). Mixotrophy supplements the carbon budget and the acquisition of inorganic nutrients (Laybourn-Parry, 2002) and is an adaptation during low-light, under-ice conditions (Søgaard et al., 2021). The under-ice periods p5 and p6 were

characterised by different snow cover and light transmission, with p5 having potentially more light than p6. Under-ice convective mixing possibly occurred in p5 and p6 as well as in p2 and p3 that did not show diatom dominance. Traditionally, diatom occurrence has been linked to vertical mixing and reduced stratification (Reynolds, 1997) but this perception is changing (Kemp & Villareal, 2018). In Lake Tovel, vertical mixing did not seem to be an important factor for diatoms. Some diatoms can perform buoyancy regulation that is energy dependent (Kemp & Villareal, 2018), can store nutrients enabling them to withstand prolonged periods of darkness (van de Poll et al., 2020), and can adjust their sinking rates in response to temperature and light (Behrenfeld et al., 2021). Thus, the under-ice conditions considered were not strictly linked to dominance of diatoms or dinoflagellates in Lake Tovel. Similarly, Kauko et al. (2018) have shown for sea ice algae that due to morphological and physiological adaptations, environmental conditions were not decisive for community composition. In the Baltic Sea, dinoflagellates can outcompete diatoms by allelopathy and therefore prevent their proliferation as shown experimentally (Suikkanen et al., 2011). Therefore, we suggest that inter-species relationships such as diatom-cyanobacteria symbiosis for nitrogen fixation (Kemp & Villareal, 2018) or allelopathy (Suikkanen et al., 2011) determined the under-ice dominance of specific algal phyla/classes. The NMDS, furthermore, indicated that silica, nitrate, and hydrogen carbonate were reduced for p5 and p6 in the upper layer. The transport of hydrogen carbonate and conversion to CO_2 is an important carbon-concentrating mechanism for phytoplankton (Raven, 1991), nitrate is the preferred nitrogen source at low water temperature for diatoms (Lomas & Glibert, 1999), and diatom blooms reduce silica concentrations before ice-out in spring (Twiss et al., 2012). Thus, the reduction of these nutrients corroborated diatom dominance in p5 and p6, while the underlying reasons remained unclear. Thus, our first hypothesis on diatom decline under ice was not completely confirmed while our second hypothesis of inter-year differences in phytoplankton communities can be accepted. Still, our underlying suggested reason (under-ice light availability) was not fully confirmed.

Merging phyla and classes to morphologically based functional groups (MBFG; Kruk et al., 2010) in the under-ice upper layer, cryptophytes, and dinoflagellates (algae of MBFG V) dominated in p1–p4 and diatoms (MBFG VI) in p5 and p6. Similarly in the lower layer, mostly *Tetraselmis* sp. (MBFG V) dominated in p2, p3, and p6 while diatoms (MBFG VI) in p4 and p5. This functional grouping indicated that unicellular flagellates of medium to large size alternated with diatoms under ice, similar to Özkundakci et al. (2016). Remarkably, the alternation between unicellular flagellates and diatoms was not temporally the same in the upper and lower layer according to our third hypothesis of under-ice layer differences.

Algal size is a master trait influencing algal adaptation to the environment. The under-ice environment constitutes a trade-off between optimising light (the higher in the water column, the better) and nutrient availability (the lower in the water column, the better). At low availability of light and nutrients, smaller phytoplankton have generally higher acquisition and growth rates (Charalampous et al., 2018) while among mixotrophs, cells < 20 μm dominate in nutrient-poor conditions while larger cells dominate in light-limited conditions (Leles et al., 2018). Dinoflagellates such as *Heterocapsa triquetra* (Ehrenberg) F.Stein and *Borghiella dodgei* Moestrup, Gert Hansen & Daugbjerg increase in cell volume at low water temperature (Baek et al., 2011; Flaim et al., 2012). Thus, different environmental conditions determine different size strategies in different algae. Here, Cyanobacteria and cryptophytes did not show any change, diatoms, chlorophytes, and chrysophytes shifted to larger biovolume from summer to under ice while dinoflagellates showed the opposite pattern; furthermore, these general shifts were not observed in all periods. These results corroborated our hypothesis on seasonal differences and once again indicated how variable the under-ice phytoplankton community was with no apparent relationship to light or under-ice mixing (Table 3). Nevertheless, the shift to smaller mixotrophic dinoflagellates might indicate the importance of nutrients under ice while the shift to larger diatoms the importance of light. Especially for diatoms, the observed biovolume increase indicated deviations from general expectations (i.e. sinking of large-sized diatoms) as already pointed out by Kemp & Villareal (2018). While we acknowledge that our approach of summing biovolume per phylum/class neglected any taxon-specific patterns, it nevertheless indicated community-wide trends.

In indicator species analysis, algal taxonomic units specific for summer and under ice were found. The dinoflagellate *Tovellia sanguinea* Moestrup, Gert Hansen, Daugbjerg, G.Flaim & d'Andrea, was an indicator of summer irrespective of layers while other taxonomic units were layer-specific, further corroborating their validity as indicators. *Tovellia sanguinea* is considered a cold-stenotherm occurring in oligotrophic or oligo-mesotrophic cold-water lakes, in which the average summer water temperature is not higher than 15 °C (Moestrup et al., 2006). We suggest that *Tovellia*'s non-layer specificity determined its general indicator status independent of layers. Under-ice indicator taxa for the upper layer belonged to chrysophytes and for the lower layer to cryptophytes, both considered mixotrophic (Mitra et al., 2016). *Cryptomonas* species show low-light tolerance (deNoyelles Jr et al., 2016), and motile taxa seem to resist convective mixing (Vehmaa & Salonen, 2009). Thus, these taxa point to mixotrophic feeding, low-light adaptation, and also swimming capacities as under-ice adaptations. This corroborated our earlier consideration based on MBFGs. Summer indicator taxa for the upper layer were a flagellated chlorophyte and for the lower layer a small, centric diatom, a small cyanobacterium, and *Pseudotetraëdriella kamillae*. The latter, a non-motile and cold-water species, generally occurring in cold seasons (autumn–winter-spring) (Hegewald et al., 2007), is the only representative of class Eustigmatophyceae occurring in Lake Tovel. In Lake Tovel, *P. kamillae* showed highest biomass in May 2013 after ice-out in the upper layer (Cellamare et al., 2016), but they did not consider the lower layer. May 2013 showed low water temperature over most of the water column (below a depth of 10 m water temperature is < 5 °C), and therefore, we suggest that this species is a summer species of the lower layer where temperature is low (< 5 °C) even in summer. The indication of small centric diatoms as summer indicator species was in agreement with the observation that mostly the second smallest cell biovolume size class dominated during summer; furthermore, a study on competition between pennate and centric diatoms in Lake Tovel indicated that small centric ones are better competitors for nutrients than large pennate species (Tolotti et al., 2007).

Apart from indicator species, several algal taxa (e.g. *Apocalathium aciculiferum*, *Tetraselmis* sp.) showed an absolute increase of biomass from summer to under ice indicating that these taxa are adapted to the under-ice environment and can proliferate under ice. *Apocalathium aciculiferum* is known to be a psychrophilic dinoflagellate that increases the cellular content of unsaturated fatty acids as adaptation to cold-water temperature (Flaim et al., 2014), and the chlorophyte *Tetraselmis* is mixotrophic (Penhaul Smith et al., 2021). Lower total nitrogen and silica concentrations during summer indicated higher resource use during summer than under ice. Diatoms and chrysophytes depend on silica, and while both showed an increase in % total biomass from summer to under ice in different periods, only chrysophytes showed an absolute biomass increase; this indicated that diatoms may have been silica limited and competitive exclusion between both phyla led to the increase of chrysophytes in different periods than that of diatoms. Other studies (Hazuková et al., 2021; Hrycik & Stockwell, 2021) similarly find a diverse under-ice phytoplankton community composed of dinoflagellates, chlorophytes, and mixotrophic chrysophytes.

Phytoplankton show vertical zonation (Klausmeier & Litchman, 2001; Karpowicz & Ejsmont-Karabin, 2017), also under ice (Li et al., 2019). Here, layer differences were observed for nutrients, and according to our third hypothesis layer, differences were also observed for phytoplankton composition, size classes, and beta-diversity. The biomass of taxa of different phyla increased from summer to under ice in the upper (dinoflagellates) and lower layer (chlorophytes), and only taxa from cryptophytes showed biomass increase in both layers. Furthermore, diatoms dominated during summer in both layers while the summer to under-ice biomass decrease was observed in different layers during different periods (upper layer: p1, p4; lower layer: p6) even though also in both layers during the same periods (p2, p3). Periods p2 and p3 were characterised by potential mixing and different under-ice light climate and precipitation (Table 3). While an under-ice high-light condition does not exclude some light in the lower layer, the underlying factors for the layer similarity remained unknown and was only indicated by algal composition. Beta-diversity showed seasonal differences for the upper layer but not for the lower layer, and this might be related to the environmental stability of the lower layer as already suggested for bacterial communities (Obertegger, 2022). Also, the distribution of size classes showed similarities and differences

between layers, indicating that statements were phylum/class and year dependent.

Summary

The under-ice period determines environmental processes of the following seasons (Hampton et al., 2017), and knowledge on phytoplankton community patterns under ice is integral for a complete understanding of ecosystem functioning. Here, inter-layer and inter-year differences and similarities were found with no strict link between phytoplankton and abiotic factors (potential mixing, snow on ice, under-ice light), and this indicates how limited and case-specific our understanding of under-ice biological processes is. Terminology such as severe and mild winters (Özkundakci et al., 2016) and winter indices such as ice thickness (Kalinowska & Grabowska, 2016; Özkundakci et al., 2016) might not fully describe the under-ice conditions. We suggest that more precise indicators are needed such as wavelength composition of under-ice light and inter-species relationships should be considered.

Declarations

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