Phytoplankton community composition during contrasting winter conditions in a shallow bay in the northern Baltic Sea

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Abstract

During 2019–20, the most severe wintertime marine heatwave since 2000–01 swept through the Baltic Sea. Sea ice was practically non-existent during this time, which is unusual for the northern Baltic Sea. The consequences for the phytoplankton community due to winter-time heatwaves are still unknown. However, it is likely that not just biomass, but also community composition during winter and subsequent spring are altered. For example, dinoflagellates may receive a head start during spring blooms after warm winters and their relative abundance could increase compared to the abundance of diatoms. During cold winters, sea ice has a strong influence on the pelagic phytoplankton community in spring. Seeding from the ice would give the dominating sympagic phytoplankton group a head start during spring blooms. Dinoflagellate cysts are better at binding carbon than diatom spores. In the long-term dinoflagellates could potentially alleviate eutrophication if they are not resuspended and germinated during cold winters.

The aim of the study was to compare the pelagic phytoplankton community during the ice-free cold-water period 2019–20 to the ice-covered cold-water period 2021–22, as well as investigate the potential seeding effect of the sympagic to the pelagic. Sampling occurred in the shallow bay Sjöviken, in the northwest of Åland. Phytoplankton were identified through microscopy using an inverted phase-contrast microscope. Environmental and biotic variables between the two cold-water periods were modelled using a generalized additive model.

Total community biomass was significantly higher during 2021–22. Ice cover and a significantly higher total phosphorus concentration in the pelagic during 2021–22 might be the reason for this. The biomass was strongly dominated by dinoflagellates during 2021–22, while

the low biomass of 2019–20 consisted of virtually no dinoflagellates. Diatoms dominated during 2019–20. Results are in contradiction to earlier studies, where warmer winters resulted in a dominance of dinoflagellates during nutrient-poor conditions and cold, nutrient-rich conditions favoured diatoms. The shift to dinoflagellate-dominance during spring blooms could potentially diminish benthic coupling, as sedimenting diatom biomass decreases and secondary production increases in the pelagic. Only one sympagic species, *S. hangoei,* seemed to have a seeding effect on the pelagic. This species has the potential to act as a carbon sink and alleviate eutrophication, as it sediments as carbon-rich cysts. However, how far-reaching this effect is, depends on the different sub-basins of the Baltic Sea, but this is yet to be studied extensively.

Key words: benthic coupling, cold-water period, the northern Baltic Sea, diatoms, dinoflagellates, ice cover, pelagic community, phytoplankton, primary production, spring bloom, sympagic community

Table of Contents

1 Introduction

1.1 Heatwaves

Temperatures have risen in the oceans during the last century, as a result of global climate change. The Baltic Sea is, from a global standpoint, a sea where surface temperature is currently rising the quickest (Cederberg, 2020; Meier et al., 2022), and setting heat records in the form of marine heatwaves in the process. Marine heatwaves are extreme events that affect the physiology, growth, reproduction and survival of living organisms, the result of which can be changes in species composition and biodiversity, or even structural and functional changes in the whole ecosystem (Cederberg, 2020; Viitasalo & Bonsdorff, 2022). Mesocosm experiments have indicated that warmer temperatures and changed light conditions lead to a decline in peak phytoplankton biomass and promote smaller cells during accelerated spring blooms. Small cells are favoured either directly or by increased copepod grazing (Sommer et al., 2012; Winder et al., 2012). These projections, however, are still uncertain and can depend on many different factors (Viitasalo & Bonsdorff, 2022).

The winter 2019–20 saw the highest ocean heat content in the Baltic Sea during December–February, since the winter 2000–01. These high pelagic temperatures prevented sea ice formation in most of the northern Baltic Sea, the Bothnian Bay being the exception. In the northern Baltic Sea, the cold-water season extends from October to May and the warm-water season from June to September (Enberg et al., 2018). The ice extent is usually the highest during February and March, with approximately 44% of the whole of the Baltic Sea covered in ice annually during the period 1961–2010. In the recent century however, the ice season of the Baltic Sea has shortened by 14–44 days due to milder climate (Thomas & Dieckmann, 2010; Thomas et al., 2017). The sea ice of winter 2019–20 was the recorded lowest since 1720, with an ice cover of less than 10% of the whole Baltic Sea area (von Schuckmann et al., 2022), a dramatic decrease as Baltic Sea ice coverage and its thickness are both already projected to continually decrease (Spilling et al., 2018; Meier et al., 2022). A negative correlation between the heat content anomaly and maximum sea ice extent and the sea ice volume anomalies has been found (von Schuckmann et al., 2022). Meier et al. (2022) noted that during the last decade only (2012–2022) an accelerated decline in ice cover in the Baltic Sea was observed, compared to the last century and that this is a direct result of milder winters. Phytoplankton spring blooms could begin earlier as a result of the abnormally high ocean heat content during winter and the

smaller ice cover. Oxygen consumption during oxidation of organic matter increases as water temperature in winter remains high, and the following summer and autumn are consequently exposed to worsened oxygen conditions (Enberg et al., 2018; Hjerne et al., 2019; von Schuckmann et al., 2022), something which would affect mainly higher trophic levels.

The increasing water temperature has consequences for the phytoplankton community, causing earlier and extended spring blooms (Spilling et al., 2018; Hjerne et al., 2019). A study by Klais et al. (2011) showed that the proportion of dinoflagellates to diatoms increased in the ice-covered areas of the northern Baltic Sea from 1995 onwards. The authors hypothesise this is due to a head start of dinoflagellates during spring blooms, even though diatoms grow faster than dinoflagellates. The hypothesis is that benthic dinoflagellate cysts are resuspended during wintertime mixing, then grows relatively quickly in the stratified waters under melting ice. This successfully seeds dinoflagellates and causes great competition for diatom populations (Klais et al., 2011). The elemental fluxes of the ecosystem are highly affected by this shift in dominant spring bloom algal groups, and thus also the functions of the Baltic Sea ecosystems (Spilling et al., 2018). Wasmund et al. (2017) developed a Diatom/Dinoflagellate index, which can be used to identify ecosystem changes. Diatoms and dinoflagellates contribute to the food web in different ways. Dinoflagellates stay in the euphotic zone longer, providing food for pelagic grazers, while rapidly sinking diatoms remineralise as food for zoobenthos (Wasmund et al., 2017; Spilling et al., 2018). Diatoms may also form resting stages, mainly in the form of spores, however these are often less efficient at binding carbon than dinoflagellates (Spilling et al., 2018). Therefore, Spilling et al. (2018) hypothesised that some species of dinoflagellates with high encystment rates, but low germination success could act as a biological sink, as burial of organic carbon increases. Available nutrients would thus diminish. In this way a shift to cyst-producing dinoflagellate dominance could mitigate eutrophication in the Baltic Sea (Klais et al., 2011; Spilling et al., 2018).

1.2 Phytoplankton in the Baltic Sea

Microalgae make up about 85% of the total sympagic (ice-associated) biomass in the Baltic Sea ice during winter (Thomas et al., 2017), of which the sympagic algae contribute about 0.4% to the annual primary production in the Baltic Sea (Enberg et al., 2018). According to molecular studies by Majaneva et al. (2012), diatoms, dinoflagellates, chlorophytes, and cryptophytes are

the most common phototrophic groups in Baltic Sea ice. Ciliates, cercozoa, dinoflagellates, and choanoflagellates are the most common heterotrophic protists. However, due to the large cells of diatoms, they are more represented in light-microscopic studies (Majaneva et al., 2012). Sympagic autotrophs are limited by light, salinity, and nutrients, while sympagic heterotrophs are more dependent on salinity and organic matter. Heterotrophs are thus benefitted by the high particulate and dissolved organic matter loading of the Baltic Sea (Granskog et al., 2006; Kuparinen et al., 2007). Kuparinen et al. (2007) noted that dissolved inorganic phosphate concentrations are generally lower in the ice than in the under-ice water, whereas nitrogenous inorganic nutrients are higher in the ice. Enberg et al. (2018) noted that as an ice-covered winter progressed, both ice assemblage and water column assemblage changed to diatom dominance.

Phytoplankton are highly governed by abiotic environmental variables, for instance the nutrients silicate and phosphorus as shown by Sommer (1985) in a classical laboratory experiment. The author categorised phytoplankton into three types of groups according to their nutrient utilisation strategy: storage specialists, affinity specialists, and velocity specialists. Storage specialists build an intra-cellular storage pool using temporarily high nutrient concentrations. Their growth rates remain at a moderate maximum, while their uptake rates are high. Affinity specialists are effective users of low nutrient concentrations and have minimal nutrient requirements. The uptake and growth rate of velocity specialists are high. They also use temporarily high nutrient concentrations, but for rapid growth instead of nutrient storage (Sommer, 1985; Grover, 1991). Depending on which nutrient phytoplankton are competing for, storage and velocity strategies have different potential importance, since the yield variations of different nutrients are greater or lesser (Grover, 1991).

Wasmund et al. (2011) found that phytoplankton taxa also fluctuate through the seasons with environmental variables. During spring, high salinity, high inorganic nutrients but low temperature were associated with the taxa Diatomophyceae, Dictyochophyceae and Cryptophyceae. The opposite conditions were associated with most other taxa, such as Dinophyceae. The high temperature, salinity, phosphate and silicate concentrations and low concentrations of dissolved inorganic nutrients in autumn, were associated with increases in Dinophyceae (Wasmund et al., 2011). There is therefore a recurring theme in phytoplankton succession, namely the alternating cycles of diatoms and dinoflagellates (Wasmund et al., 2011; Wasmund et al., 2017; Thomas et al., 2017; Enberg et al., 2018; Spilling et al., 2018; Hjerne et al., 2019). The spring and autumn blooms in the Baltic Sea are well-researched, but

little is known about the winter community composition, its succession and influence on the spring bloom.

1.3 Baltic Sea ice

The maximum ice extent in the Baltic Sea varies greatly due to the mild winters, and rain and freeze-melt cycles are common. Large parts of the ice may be formed from snow-ice, which forms as flooded snow layers freeze. Up to half of the total ice thickness consists of this snow-ice and superimposed ice, which is ice formed from melted snow that has frozen (Thomas et al., 2017). The ice formation itself is more dependent on temperature than salinity and begins as soon as the water column has cooled to the freezing point. The pelagic layer then stratifies, and warmer water stays underneath. This is why the ice growth rate is faster in brackish waters. Ice formation starts in the shallower, less saline water. This allows for land-fast ice to be formed, a type of ice anchored to islands and shoals making it robust against wind and waves. Brackish water is also stratified, with less mixing and is quite warm at the bottom layers (Thomas & Dieckmann, 2010; Thomas et al., 2017).

The seasonal ice cover in the Baltic Sea is quite unique in its characteristics, owing mainly to the brackish water, which causes both low bulk salinities and porosities (Granskog et al., 2006; Thomas et al., 2017). Organisms are restricted to the bottommost layers of the ice, the permeable layer, which makes up about 2–10% of the total ice volume (Thomas & Dieckmann, 2010). The ice is permeable only when brine volumes are big enough, which is when the temperature is –1°C and the bulk salinity is 1. A brown colouration marks the internal habitat, as the other parts of the ice are generally of low biological activity (Thomas et al., 2017). Since the extent of the permeable layer is a function of temperature and brine salinity, it varies in the different Baltic Sea basins. For instance, the permeable layer is therefore smaller in the northern Baltic Sea (Thomas et al., 2017). Nonetheless, Baltic Sea ice does not act as freshwater, even if salinities are as low as 2 in some regions. Ice in the far south of the Baltic Sea appears only during harsh winters but supports more sympagic organisms (Granskog et al., 2006). Enberg et al. (2018) observed that a thin ice cover (< 0.5 meters) leads to minimal contribution to primary production by ice algae.

1.4 Spring bloom and seeding effect

A stable ice cover during winter leads to lower primary production and biomass in the pelagic. As a consequence, concentrated and short-lived pelagic blooms are common as the ice melts (Mikkelsen et al., 2008). Sea ice may have a strong influence on the pelagic phytoplankton community in spring. Sympagic algae may remain in the water column after ice retreat. More likely, though, they disappear over time due to rapid cell sedimentation, predation-induced cell death, or necrosis due to shock from the quick environmental change. However, some algae may stay in the water column and set the "seed" for the phytoplankton bloom after ice melt (Haecky & Andersson, 1998; Enberg et al., 2018; Yan et al., 2020).

This "seeding theory" of ice algae to the spring bloom is highly debated and may only hold true on a case-to-case basis. Haecky & Andersson (1998) found that the pelagic spring bloom composition is different after ice-free winters compared to after ice-covered winters. Centric diatoms such as *Skeletonema marinoi* and *Thalassiosira baltica* generally dominate the spring bloom in ice-free locations. Blooms in ice-covered locations are dominated by diatoms *Pauliella taeniata, Chaetoceros wighamii* and *Nitzschia frigida*, of which *Chaetoceros* and *Nitzschia* are thought to originate from the sympagic community (Piiparinen et al., 2010). Enberg et al. (2018), however, found that there were differences in ice and spring bloom assemblages, and consequently little evidence of an ice algal seeding effect to the spring bloom. There is also some ambiguity about if it is even possible to establish from where the spring bloom species originate. Piiparinen et al. (2010) showed the importance of sampling not only the ice, but also the water column at different depths, in their study of the potential seeding effect of *P. taeniata.* The authors found high biomasses of the diatom both in the water column and in near-bottom waters after ice melt. *P. taeniata* is a cyst-forming species and the individuals in the water column could in this case have originated from both the ice and the sediment (Piiparinen et al., 2010). Enberg et al. (2018) also found that shallow areas are more prone to incorporation of dinoflagellate cysts into the ice, as cysts are generally abundant in the pelagic sediments of the Baltic Sea.

The two dominating species groups diatoms and dinoflagellates show some differences, even though they can both exhaust the inorganic nutrient reserves and contribute to the vertical export of carbon through high bloom levels. The groups exhibit different sedimentation patterns, and the potential of the settled biomass to mineralize at the seafloor is variable in different parts of the Baltic Sea. Diatoms disappear from the euphotic zone quickly, whereas

dinoflagellates either slowly lyse in the water column or sink as inactive resting cysts, both contributing to settling phyto-detritus (Spilling et al., 2018). Studies indicate, however, that cyst-formation in dinoflagellates is an inferior survival strategy in the low salinity water conditions of the northern Baltic Sea (Enberg et al., 2018).

Diatoms are generalists that thrive in most habitats, whereas dinoflagellates are more specialized in their habitat utilisation and are especially receptive to niche changes. Dinoflagellates experience geographic range expansion, competitive dominance, and bloom expansions as a result of changes in niche structure and community ecology. Diatoms have the upper hand during spring blooms when nutrients are abundant, while dinoflagellates have lower light saturation level, and compete better in low nutrient conditions. The competition between diatoms and dinoflagellates in cold-water conditions is explained by r/K selection theory. Small diatom species grow fast, and prosper in turbulent conditions, like typical r-strategists. Dinoflagellates, in comparison, grow slowly, become large and motile. They require specific niches for bloom formation, which allows them a competitive head start in biomass, like K-strategists. It is, however, still not understood just which factors and mechanisms select for which of these groups and their species during blooms (Klais et al., 2011). It has been noted, though, that bloom formation and subsequent dinoflagellate dominance is affected by the size of the inoculum dinoflagellate population and co-occurring diatoms (Enberg et al., 2018).

To be able to identify the changes in the phytoplankton community due to heatwaves, we must first identify the differences between the sympagic community and the water column community, which admittedly can be tricky in itself. Most studies are additionally centred on ice in the Arctic Ocean and then applied to the Baltic Sea ice, which can be deceiving as the two systems differ extremely (Thomas & Dieckmann, 2010; Thomas et al., 2017).

1.5 Purpose and problem statement

The problem statements are the following:

1) Are there variations in the phytoplankton community composition during ice-free winters and ice-covered winters? Environmental variables are expected to have an influence on which species groups dominate during a specific time frame. The community composition

during the ice-covered cold-water period 2021–22 is expected to differ from the ice-free cold-water period 2019–20.

2) Is a seeding effect from the sympagic community to the water column possible during the spring bloom? At least some sympagic species are expected to be able to seed to the water column after ice melt and thereby influence the phytoplankton spring bloom community compared to an ice-free winter. The seeding species will be species that are fairly dominating in the sympagic community during winter.

The purpose of the study is to examine the species composition of the phytoplankton community and its seasonal variation in species, their cell abundance, and biomass. The following environmental variables were sampled: chlorophyll *a*, oxygen concentration, pH, salinity, and nutrients, in the form of total phosphorus concentration (tot-P) and total nitrogen concentration (tot-N). Their connection to the phytoplankton community composition during the months October–May were investigated. These data were compared to the data collected in the thesis *"Ett växtplanktonsamhälles succession och cellstorleksstruktur under en kallvattenperiod i en grund havsvik på nordvästra Åland"* on the phytoplankton community at the same sampling site, but during an ice-free cold-water period from October to May 2019–20 (Lanndér, 2022).

2 Materials and methods

2.1 Sampling area

The sampling area (60°18.271 N, 19°48.451 E) is located in the northwest of Åland. Sjöviken is a bay in the inner archipelago, in the northwestern part of the island Bergö (fig. 1). The depth is less than 30 meters, with a maximum depth of 9 m at the sampling site. To the north, Sjöviken is connected to Ivarskärsfjärden through a wide opening. Ivarskärsfjärden is surrounded by settlement and agricultural land. The water quality of the bay has hence been low since the 1990s and shows a weak trend toward more shallow Secchi depth (Cederberg, 2018). In 2013 the oxygen levels in bottom waters were found to be well below the limit for acute hypoxia (2 ml oxygen/l) at 1.3 ml/l in Ivarskärsfjärden. The bay has since recovered, measuring healthy oxygen levels of 4.95 ml/l in 2017. The last few years both total nitrogen and total phosphorus levels have been fairly stable (Cederberg, 2018).
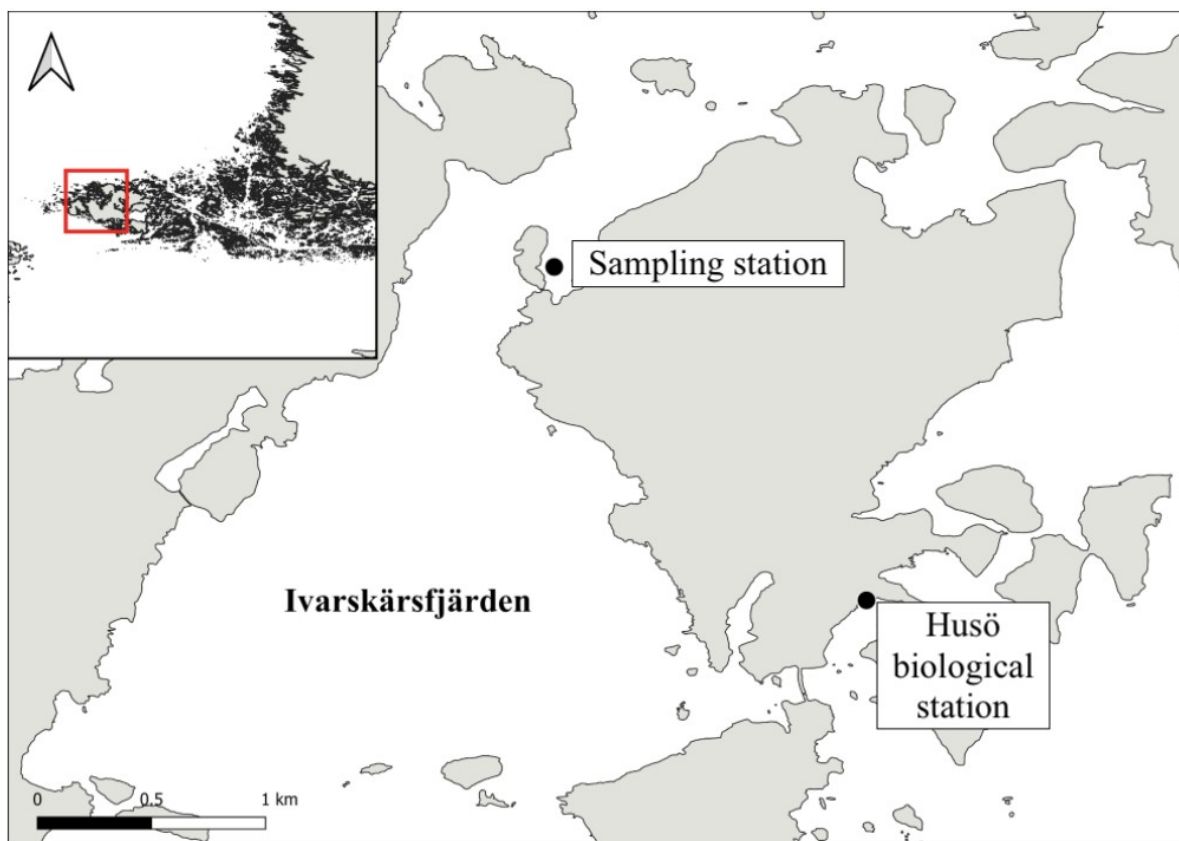

Figure 1. The sampling station Sjöviken in relation to Husö biological station and Ivarskärsfjärden (Lanndér, 2022; edited).

2.2 Sampling

Water samples were collected between 29.9.2021 and 3.5.2022. The bay was sampled once a month, with the exceptions twice in March and four times in April. Ice was sampled monthly between 9.12.2021 and 12.4.2022, with the exceptions twice in March and April. Ice samples were gathered by sawing the ice into chunks, which were then slowly melted in a cold room and later analyzed for chlorophyll *a*. A Limnos watersampler (Limnos, Turku) was used to take water samples at the depths 0, 1, 2, 4, 6 and 8 meters, of which plankton samples were taken at the depths 0, 1 and 8 meters. The plankton samples were fixed with Lugol's iodine solution. Temperature was measured at every depth. The samples were used for analysis of chlorophyll *a*, oxygen concentration, pH, salinity, total phosphorus concentration, total nitrogen concentration, and phytoplankton.

Oxygen, pH, and conductivity were measured the same day samples were taken. Chlorophyll *a* samples were filtered through a fibreglass filter (Whatman GF/C), after which they were dried in darkness. The chlorophyll *a* concentration was then determined by ethanol extraction and subsequent spectrophotometric analysis. Oxygen samples were analysed using the Winkler method (Winkler, 1888). pH was measured using a Metrohm 691-pH-meter and conductivity using a Metrohm 660-conductometer. Conductivity values were used to obtain the salinity using the formula:

$$Y = 0.6701 \times X - 0.3723$$

where Y = salinity (‰) and X = the measured conductivity.

Oxygen, pH, and salinity were established using the same methods and devices for both 2019–20 and 2021–22. However, nutrient analyses differed between the two cold-water periods. Total nitrogen and total phosphorus concentrations during the ice-free year were determined through persulphate oxidation, according to Grasshoff et al. (1999).

Nutrient samples from the ice-covered year were analysed using a Seal Analytical AQ300 in May 2023 at Husö biological station. Both total nitrogen and total phosphorus concentration were determined through simultaneous persulphate oxidation, according to Grasshoff et al. (1983).

2.3 Plankton analysis

The plankton samples were analysed during the autumn of 2022. The sample bottles were rotated at least 15 times to allow for an even distribution of cells, before settling either 25ml or 50ml of the sample in Utermöhl chambers. The amount settled was determined based on the chlorophyll α level of the samples, a higher chlorophyll α level meaning a smaller sample settled. In order for all plankton to sink to the bottom of the chamber, samples were settled for at least 24 hours before microscopy. The samples were then analysed according to Järvinen et al. (2011) instructions for plankton counting. Plankton were identified, to species level when possible, and counted, and their cell size measured using a Nikon Diaphot inverted phase-contrast microscope. Using the carbon content of taxa in Järvinen et al. (2011) and the biovolumes of species in Olenina et al. (2006), the plankton sizes and abundances were converted to the carbon biomasses of all the different plankton.

2.4 Statistical analysis

Results from both the ice-covered cold-water period 2021–22 and the ice-free cold-water period 2019–20 from Lanndér (2022) were utilised in statistical analyses. As 0 m and 1 m samples were very similar, these samples were merged and averaged to one pelagic sample for both environmental variables and phytoplankton data. The following results include the averaged 0m and 1m samples as one pelagic sample, as 8 m samples are not included in the analyses.

Statistical analyses were performed using R (version 4.0.5). Shannon diversity index was used to compare interannual species diversity. Shannon diversity index was calculated using the *vegan* package v2.5-7. For the calculation of Shannon diversity index cell abundance of the species were used.

The non-linear relationships of variables between years were modelled using a generalized additive model (GAM) using the *mgcv* package v1.8-34. The only months included in the model were December–March, as these were the months with the presence of ice-cover in 2021–22. Ice was also present during half of April during the ice-covered year, but these data were not included since no data were available from April of the ice-free year for comparison. October and November data were not included since these were not influenced by the presence

or absence of ice-cover. Data from after ice melt in May were also omitted, as this would reflect a completely different community than the ice-covered pelagic community. Data from the ice was not used in the GAMs. The function gam.check was used to assess the fit of the model and minimize oversmoothing. As the biggest difference between years was the presence or absence of ice, this was used as predictor variable. The numbers of months were used as smoothing factor. The model was applied to response variables total nitrogen, total phosphorus, chlorophyll α, cell abundance, biomass, and Shannon index. The function anova.gam was used to identify statistical differences between the two cold-water periods.

3 Results

3.1 Hydrography

Comparing the ice-free cold-water period of 2019–20 to the ice-covered cold-water period of 2021–22 illustrates clear differences. On average the temperature was 1.2 °C colder during December–March 2021–22, resulting in the presence of sea ice during the period December–mid-April, but never in 2019–20 (fig. 2).

The cold-water period 2021–22 started warmer than that of 2019–20, with a high water temperature of 12.5 ºC (fig. 2). In December 2021 the temperature sank lower than 2019, as the ice first appeared. The temperature dropped to 0 ºC during January 2022, as the ice kept thickening. The temperature started increasing slowly during March 2022, while the ice thickened to its seasonal maximum of 29 cm in late March. While temperature remained low in beginning of April 2022, the ice decreased to 18 cm. Thereafter the temperature increased steadily, but never exceeding the temperatures observed in 2020. By the end of April, the ice had melted completely, as the temperature rapidly increased.
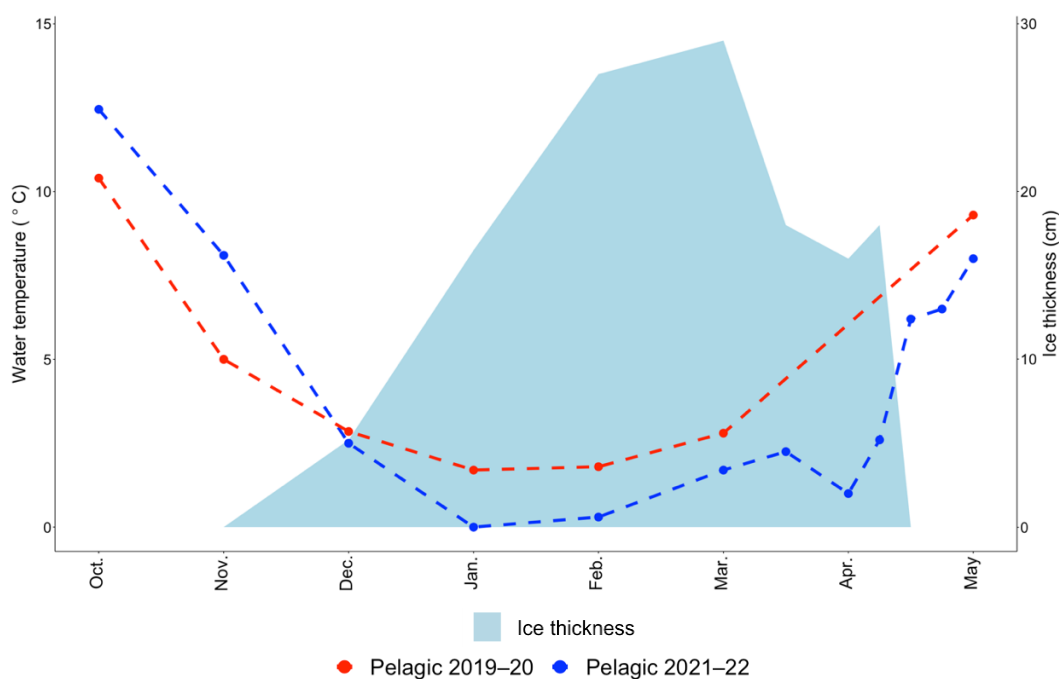

Figure 2. Water temperature in °C in the pelagic 2019–20 (red dashed line) and the pelagic 2021–2022 (blue dashed line) with ice thickness during 2021–22 in cm (blue area).

The dynamics of tot-N were not significantly different between 2021-22 and 2019-20 cold-water periods (ANOVA: $F_{4.474} = 0$, p = 0.993). There was a peak in tot-N during December-March during both winters, but the ice cover delayed the tot-N peak and slightly increased its intensity and duration (fig. 3a). More precisely, tot-N was lowest in October of 2019 and continuously increased until peaking in January 2020, at 599.08 µg l^{-1}. After peaking, the tot-N concentration during 2020 drastically decreased and in March it measured around the same level as after the spring bloom in May. In contrast to this, the tot-N concentration in 2022 peaked in February and remained at the post-spring-level for longer time. With a concentration of 627.53 µg l^{-1} the peak in tot-N concentration was 4.7% higher during 2022 compared to the 2020 and stayed at a similarly high level before dropping in late March. During April and May 2022 tot-N was stable.

Tot-P was significantly higher during 2021–22 compared to 2019–20 (ANOVA: $F_{5.301} = 210.3$, p = 0.00123), with some monthly variations (fig. 3b). Overall, 2021–22 experienced similar delays in tot-P concentration as in tot-N concentration, compared to 2019–20. An increase in tot-P concentration occurred in November 2019, as the tot-P concentration dropped
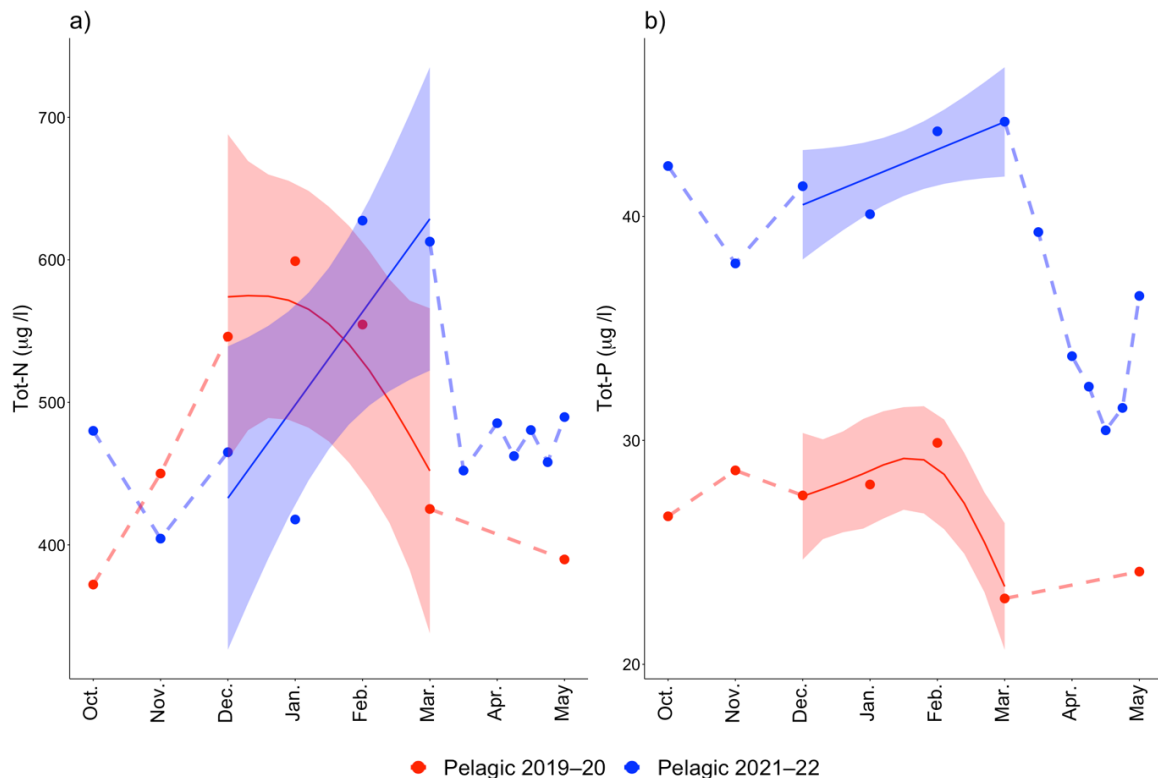

Figure 3. Average a) tot-N and b) tot-P in the pelagic 2019–20 (red dashed line) and in the pelagic 2021–2022 (blue dashed line). The solid line represents the GAM prediction and the shaded area the confidence intervals for the period that was ice-covered during 2021–22.

in 2021. Tot-P peaked at 44.23 µg l^{-1} during March 2022, a 47.9% difference to the highest tot-P peak of 29.89 µg l^{-1} during February 2020. Similarly to tot-N, the tot-P concentration remained longer at a higher level in 2022. The tot-P concentration during 2020 declined to its lowest concentration in March, about a month before the same drop in 2022. Even as the season 2021–22 hit its lowest tot-P concentration of 30.45 µg l^{-1} in April, it never dropped below even the highest tot-P concentrations of 2019–20.

3.2 Pelagic community

3.2.1 Chlorophyll α, cell abundance, biomass, and diversity index

Chlorophyll α as well as cell abundance showed similar fluctuations over the two cold-water periods. However, there were clear variations between ice-free and ice-covered pelagic community cell abundance magnitude and biomass fluctuations and magnitude. The community biomass was much higher and experiences bigger fluctuations during 2021–22.

Chlorophyll α measured slightly higher during the ice-covered year than the ice-free year overall, but not significantly (ANOVA: $F_{7.426} = 10.45$, $p = 0.319$). Both 2019–20 and 2021–22 displayed similar seasonal patterns of chlorophyll α concentrations (fig. 4a). During both 2020 and 2022 chlorophyll α peaked in early March, at 15.82 µg l^{-1} and 19.36 µg l^{-1}, respectively. The biggest differences in chlorophyll α were observed during December and January; December 2019 chlorophyll α measured 3.42 µg l^{-1}, while 2021 measured 8.63 µg l^{-1} and January 2019 measured 2.23 µg l^{-1}, compared to 7.08 µg l^{-1} in 2022.

There was no statistical significance in cell abundance between 2019–20 and 2021–22 (ANOVA: $F_{4.000} = 6.692$, $p = 0.0609$). Both October–December 2019–20 and 2021–22 communities shared a declining trend in cell abundance (fig. 4b). However, in January 2022 the community reached one of its lowest cell abundances. The community cell abundance peaked in mid-April 2022 at 112.1 million cells l^{-1}, followed by a considerable decline throughout the rest of April and beginning of May. During 2019–20 however, the cell abundance peak was potentially missed, as no sampling could be performed during the spring bloom in April. Despite a clear difference in cell abundance between the years (on average
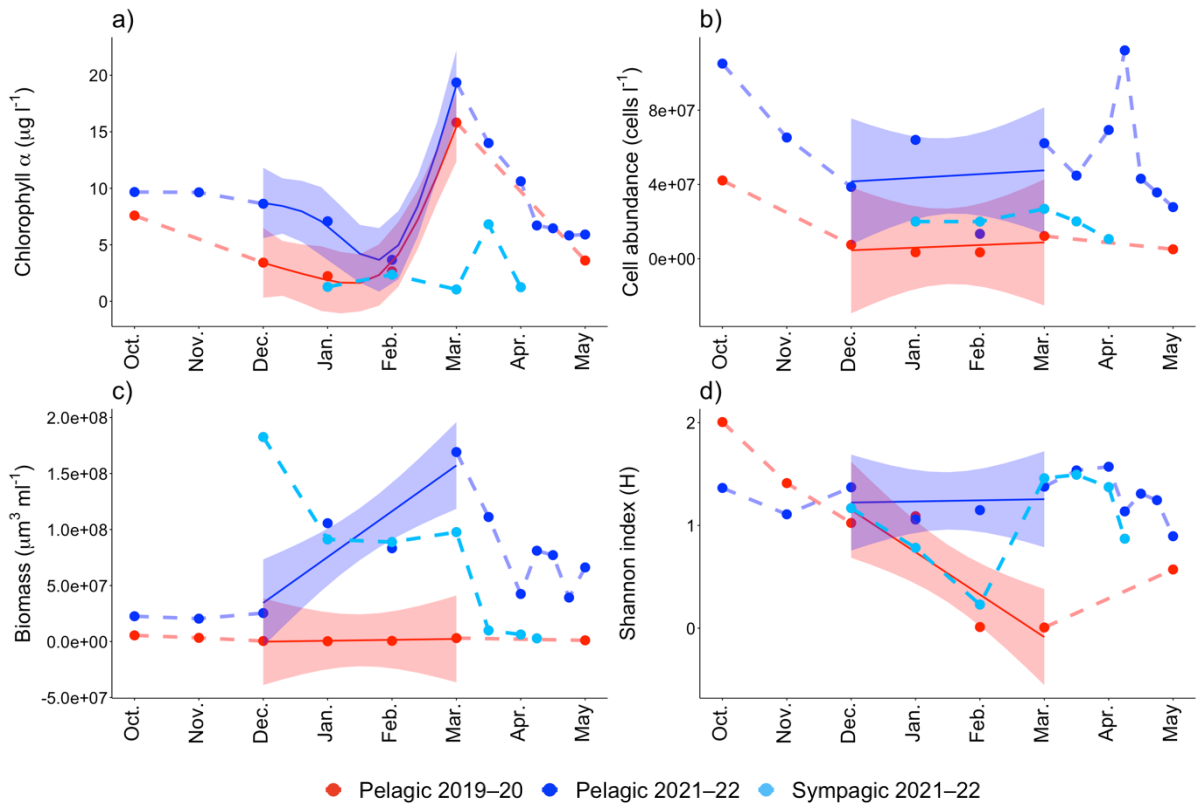
Figure 4. Biotic pelagic community. Average a) chlorophyll α, b) cell abundance, c) biomass and d) Shannon index in the pelagic 2019–20 (red dashed line), in the pelagic 2021–22 (blue dashed line) and in the sympagic 2021-22 (light blue dashed line). The solid line represents the GAM prediction and the shaded area the confidence intervals for the period that was ice-covered during 2021–22.

329% higher in 2021-22, fig. 4b), the GAM-analysis did not capture a statistically significant difference.

Total biomass was higher in 2021–22 compared to 2019–20 (fig. 4c). The biggest differences could be seen during December–April. Total biomass of 2021–22 was significantly higher (ANOVA: $F_{4.000} = 32.17$, $p = 0.00477$). Biomass was stable from October throughout December 2021 but increased in January 2022 to 105.638 million $\mu m^3\, ml^{-1}$. The biomass declined somewhat before reaching its highest values of 169.165 million $\mu m^3\, ml^{-1}$ in early March 2022. January–April 2022 represented the highest recorded biomasses in the entire data set. However, the opposite was true for 2019, with October and November showing the highest recorded biomasses of 5.477 million $\mu m^3\, ml^{-1}$ and 3.271 million $\mu m^3\, ml^{-1}$, respectively. The biomass peak during 2022 was therefore 308% higher than during 2019.

There was a significant difference in Shannon index between the two cold-water periods (ANOVA: $F_{4.000} = 12.29$, $p = 0.0248$). The Shannon index for 2021–22 was higher. The

Shannon index was represented by a decline for, essentially, the whole cold-water period up until May 2020, with a small increase during January. However, the highest Shannon index was observed in October 2019 (H = 2.00). In contrast, the Shannon index was seemingly more stable over the whole cold-water period 2021–22, with smaller monthly fluctuations. The highest Shannon index was detected in early April 2022 (H = 1.57). These clear differences in community cell abundance and biomass between years, that is, the variation in community carbon content and its distribution, stem in part from the compositional differences between the two communities.

3.2.2 Pelagic community taxa

Already during the early cold-water period October–November there was a noticeable difference between taxa dominating cell abundance and biomass between the two years 2019–20 and 2021–22 (fig. 5a–e). Dinoflagellates were an important component of the community in 2021–22. They drastically increased in both cell abundance and biomass in January and dominated until March 2022. In contrast, dinoflagellates disappeared almost completely in December 2019, not to be observed in detectable quantities for the rest of the cold-water period 2019–20.

Green algae were more abundant in cells than in biomass in both communities during November and October, however their cell abundance was much higher in 2019 than in 2021. In October 2021 nearly half the total biomass consisted of diatoms. As the ice-cover first appeared in December 2021, over half of the total cell abundance was dominated by diatoms, while the biomass in both communities was dominated by ciliates. In December 2019, 56.89% of the total biomass consisted of ciliates, while the same percentage was 60.50% in December 2021. During January the biggest distinction in total biomass between the two communities started to arise: Dinoflagellate cell abundance and biomass drastically increased in 2022, reducing ciliate biomass dominance. These differences intensified during February, when 91.61% of the total biomass was dominated by diatoms in 2020, while 97.79% of the total biomass consisted of dinoflagellates in 2022. During the last ice-covered months March and early April 2022, the total biomass was still heavily dinoflagellate-dominated, while the community of March 2020 was dominated mainly by diatoms, both in cell abundance and biomass. As both cold-water periods progressed, the cell abundance of cryptophytes and ciliate biomass increased in both communities. Both the cell abundance and biomass of diatoms

increased in 2022 during the end of the cold-water period. In May 2020 98.76% of the cell abundance and 64.12% of the biomass consisted of green algae. The cell abundance of dinoflagellates never exceeded 5% of the total cell abundance and at most only 13.72% of the total biomass during cold-water period of 2019–20, while dinoflagellates comprised a maximum of 58.9% of the total cell abundance and 97.78% of the total biomass during 2021–22.
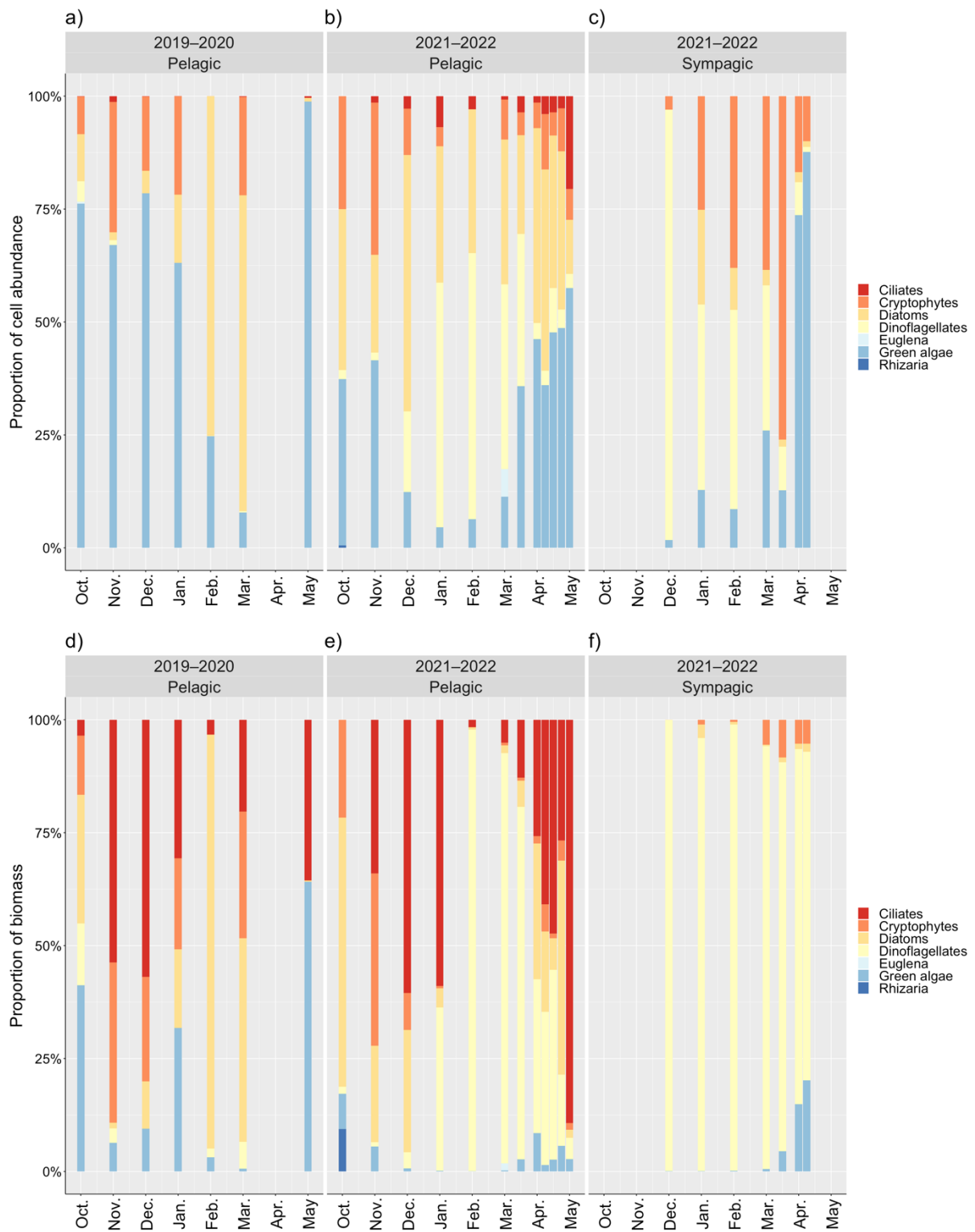
Figure 5a–f. Grouped cell abundance and biomass. Proportions of cell abundance in a) the pelagic 2019–20, b) the pelagic 2021–22 and c) the sympagic 2021–22. Proportions of biomass in d) the pelagic 2019–20, e) the pelagic 2021–22 and f) the sympagic 2021–22.

3.2.3 Pelagic community dinoflagellates

The dinoflagellate biomass during 2019–20 consisted of few species, and no dinoflagellates were present at all during December, January, or May. The dinoflagellate biomass of 2021–22 was more diverse in species and their seasonal distribution, driven largely by the presence of the ice-cover (fig. 6a–b).

During the early cold-water period October–November 2019, the dinoflagellate biomass was dominated by *Amphidinium* sp., and *Dinophysis acuminata*. As ice first appeared in 2021, the dinoflagellate biomass was comprised of only *Heterocapsa rotundata*. However, as the ice-cover matured, a shift in dinoflagellate species was observed in the pelagic community. The newcomer *Scrippsiella hangoei* made up 30.22% of the community biomass in January 2022. In February 2022, 100% of the dinoflagellate biomass consisted of of *Peridiniella catenata*, while *S. hangoei* dominated 98.94% of the dinoflagellate biomass in February 2020. In early March 2022 during the highest ice thickness, *S. hangoei* biomass declined, and *Heterocapsa triquetra* dominated the dinoflagellate biomass. The ice-cover experienced a melting event in late March and early April, after which the *S. hangoei* biomass decreased again after increasing in late March. However, after ice melt in mid-April, the dinoflagellate biomass consisted mainly of *S. hangoei.* The second-highest biomass of *S. hangoei* was observed in mid-April at 96.46%, after which its biomass started declining and *Protoperidinium bipes* increased slightly in biomass.

The dinoflagellate biomasses during 2019–20 and 2021–22 were quite different, owing mainly to the presence of *S. hangoei* in 2021–22. Its biomass measured a maximum of 98.94% of the total dinoflagellate community during 2021–22, while it never occurred in any quantifiable biomass during 2019–20.
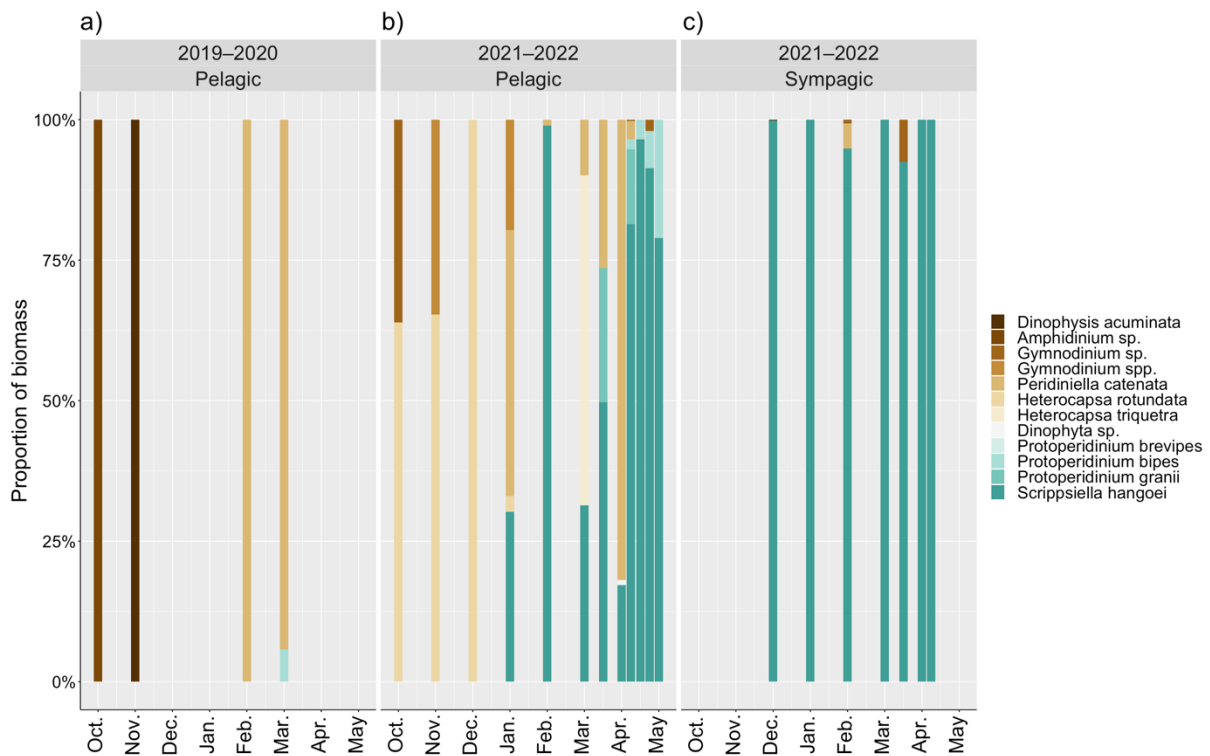
Figure 6a–c. Dinoflagellate biomass. Proportions of dinoflagellate species biomass in a) the pelagic 2019–2020, b) the pelagic 2021–2022 and c) the sympagic 2021–2022.

3.2.4 Pelagic community diatoms

The diatom biomasses of both 2019–20 and 2021–22 were largely defined centric diatoms, mainly by *Skeletonema marinoi,* and to a great extent also different species of *Chaetoceros* spp., mainly after ice melt in 2022 (fig. 7a–b).

During the early cold-water period 2019 the diatom biomass consisted of only *Eupodiscales* sp*.,* whereas the diatom biomass consisted of 57.68% *Bacillaria paxillifera* in 2021. The diatom biomass changed drastically during November 2019, to consisting of 58.93% *Chaetoceros* spp. and 41.07% *S. marinoi.* As ice cover formed in December 2021, the diatom biomass was dominated by *S. marinoi*. *S. marinoi* also dominated the diatom biomass of December 2019. This dominance was maintained in the diatom biomass throughout December–May 2019–20 and December–January 2021–22. During February 2022, however, a dominance of *Chaetoceros* spp. appeared, further increasing until early March, when 99.94% of diatom biomass consisted of *Chaetoceros* spp. During April and May 2022, the diatom biomass experienced the biggest increases in *S. marinoi*, *Pauliella taeniata*, *Thalassiosira levanderi*, and *Thalassiosira baltica*. Interestingly, however, *Chaetoceros* spp. was still the dominating diatom in the diatom biomass throughout spring. In May 2020 43.61% of the diatom biomass

was identified as *Chaetoceros wighamii* specifically, making it the second most abundant single species in the diatom community during 2019–20.

Over the whole cold-water period, the 2021–22 community saw almost double the diversity in diatom species. However, the contribution of *S. marinoi* and *Chaetoceros* spp. to the diatom biomass were very similar during both 2019–20 and 2021–22.
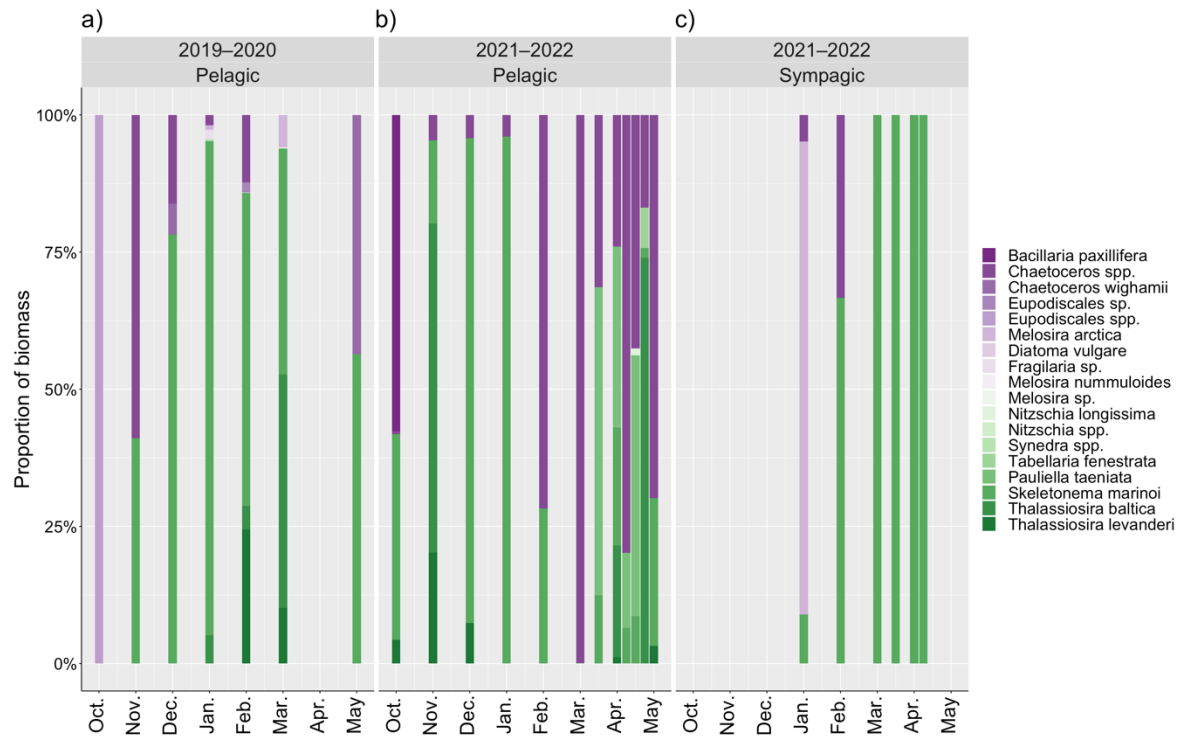
Figure 7a–c. Diatom biomass. Proportions of diatom species biomass in a) the pelagic 2019–2020, b) the pelagic 2021–2022 and c) the sympagic 2021–2022.

3.3 Sympagic community

3.3.1 Chlorophyll α, cell abundance, biomass, and diversity index

The sympagic community was seemingly less productive than the pelagic community. Chlorophyll α was generally quite low in the ice, with the pelagic chlorophyll α staying on average 400% higher during the ice-covered months (fig. 4a). Aside from the magnitude, a similar pattern in chlorophyll α was observed in the ice as in the pelagic water, with a somewhat delayed peak. Chlorophyll α peaked at 6.81 µg l^{-1} in the ice, about a month after the pelagic. Cell abundance was highest in early March at 26.8 million cells l^{-1}, but this was still incredibly low compared to the cell abundance peak of the pelagic community in mid-April (112.1 million cells l^{-1}; fig. 4b). The sympagic biomass, however, measured higher after ice formation in

December (182.587 million $\mu m^3\,ml^{-1}$) than the highest peak of the pelagic community in early March (169.165 million $\mu m^3\,ml^{-1}$; fig. 4c). Biomass was lowest during the second sampling in April 2.832 million $\mu m^3\,ml^{-1}$, after a big melting event during March. The Shannon index (fig. 4d) declined the two first months after ice formation in December to its lowest value in February (H = 0.23). In March, the Shannon index of the sympagic community peaked at 1.49.

3.3.2 Sympagic community taxa

As indicated by the Shannon index, the sympagic community was generally less diverse than the pelagic community. It was defined by fewer and less abundant taxa than the pelagic community (fig. 5c). Just after ice formation, the sympagic community was heavily dominated by dinoflagellates, both by cell abundance and biomass. As the ice season progressed, however, the fraction of cryptophyte, diatom and green alga cell abundance grew. Dinoflagellates were no longer the sole contributor to the sympagic cell abundance, as cryptophytes had increased to 25.19%. Diatoms also started occurring monthly after their first appearance in January. By mid-March, as the ice cover reached its maximum thickness, cryptophytes reached their highest cell abundance of 76.06%. However, by early April green algae were the new dominating taxa at 73.63% of the sympagic cell abundance. Nearing ice melt, green algae reached dominance at 87.66% of the sympagic cell abundance. The sympagic biomass, however, was almost entirely dominated of dinoflagellates throughout the whole ice season (fig. 5f). Up until mid-March, dinoflagellates never declined below 93.67% of the community biomass. In mid-March, dinoflagellates dominated 83.02% of the community biomass, with cryptophytes at their highest biomass of 8.36%. Green algae increased slightly over the following weeks and reached a final of 20.20% of the sympagic biomass before ice melt. Dinoflagellates were, however, still dominating the community at 72.74% of the total biomass. Neither ciliates, rhizaria nor euglenophyta were ever present in the sympagic community, not by cell abundance or biomass.

3.3.3 Sympagic dinoflagellates, diatoms, and green algae

The biggest contributor to the sympagic community was undoubtedly dinoflagellates. However, the diversity in dinoflagellates was extremely low in the sympagic community. The

sympagic dinoflagellate biomass was undoubtedly *S. hangoei*-dominated throughout the whole ice season (fig. 6c), apart from a small increase in *P. catenata* in February and slightly bigger increase in *Gymnodinium* sp. in late March.

The diversity of diatoms was also low, with only 3 different species found in quantifiable biomass (fig. 7c). In January 86.21% of sympagic diatom biomass consisted of *Melosira arctica*. The biggest diatom contributor, however, was by far *S. marinoi,* dominating the sympagic diatom biomass from February until ice melt. From early March through April, *S. marinoi* dominated 100% of the diatom biomass.

The sympagic green algae community composition was quite different from the pelagic green algae community (fig. 8a–c). As the ice formed, *Chlamydomonas* sp. dominated 100% of the sympagic green algae biomass, whereas a month later *Monoraphidium contortum* dominated 100% of it. *M. contortum* biomass fluctuated throughout the rest of the ice season. *Chlorogonium minimum* first appeared in great dominance in February, at 85.41% of the sympagic green algae biomass and sustaining a big part of the green algae biomass throughout the rest of the ice season. During the end of the ice season, *Binuclearia lauterbornii* strongly dominated the green algae community biomass.
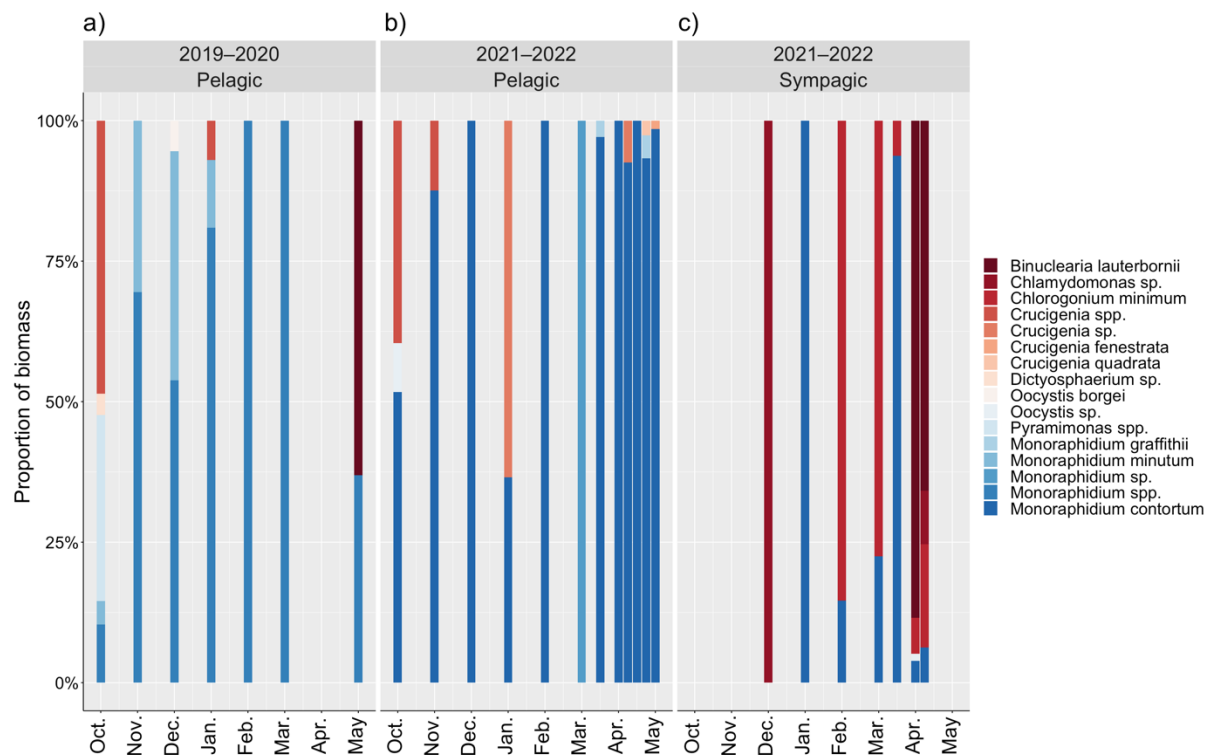
Figure 8a–c. Green algae biomass. Proportions of green algae species biomass in a) the pelagic 2019–2020, b) the pelagic 2021–2022 and c) the sympagic 2021–2022.

The sympagic community harboured fewer species indeed, but many of these were ice-specific species. The diatom *M. arctica*, the dinoflagellate *Dinophysis acuminata*, and the green algae *Binuclearia lauterbornii*, *Chlamydomonas* sp., *Chlorogonium minimum* and *Oocystis* sp. were species to occur uniquely in the ice in both cell abundance and quantifiable biomass during the ice-covered sampling period of 2021–22. Even though they are closely connected, the sympagic habitat clearly promotes different species to the pelagic community.

4 Discussion

4.1 Pelagic community composition

This thesis compared a phytoplankton community during a warm, ice-free winter to a cold, ice-covered winter, and to some degree, investigated the seeding effect of the sympagic community. During the colder winter of 2021–22, dinoflagellates were much more prevalent in the phytoplankton community, while the warmer temperature and subsequent lack of ice cover in the winter of 2019–20 promoted a higher abundance of diatoms and green algae. The dinoflagellate *Scrippsiella hangoei* clearly dominated during 2021–22. This species has a potential to act as a carbon sink as it sediments in large quantities as cysts on the seafloor during spring (Kremp & Heiskanen, 1999; Klais et al., 2011; Spilling et al., 2018).

The most striking contrast between the investigated cold-water periods is the dominance of dinoflagellates versus diatoms in the phytoplankton communities. During the ice-covered winter in 2021–22 dinoflagellates dominated over diatoms, a trend also detected by Klais et al. (2011) from 1995 onwards in the northern Baltic Sea. Wintertime mixing and resuspension of cysts in the shallow, coastal Sjöviken combined with the low temperatures required by the dominating dinoflagellate, favours dinoflagellate dominance over diatom dominance during presence of ice-cover (Kremp & Anderson, 2000; Klais et al., 2011). Diatoms are, however, of course still occurring in the pelagic during the dinoflagellate-dominated winters and dominate over dinoflagellates in cell abundance during spring blooms. However, a difference in diatom species was observed between winters. *Chaetoceros* spp. seemed to be more dominant during spring 2022 after the ice-covered winter, while centric diatoms *S. marinoi* and *Thalassiosira* spp. were the more dominant ones during spring 2020 after an ice-free winter, supporting previous findings by for example Haecky et al. (1998) and Piiparinen et al. (2010). Piiparinen et al. (2010) noted that this might be a result of different light-adaptation in diatoms, as low light conditions under the ice influence the community composition.

The dinoflagellate community matured differently during 2019–20 and 2021–22. The 2019–20 community had a low number of dinoflagellate species, some months none at all, while the presence of the ice-cover sowed the seed for a completely distinct community composition in 2021–22. One species in particular, *S. hangoei*, contributed to the compositional difference between the two communities. *S. hangoei* used to be considered a single species, but thanks to high-resolution scanning electron microscopy and rDNA analyses it has been revealed that

there are, in fact, three different species, *Biecheleria baltica* (*Woloszynskia halophila*), *S. hangoei* (*Apocalathium malmogiense*), and *Gymnodinium corollarium.* They are morphologically identical and thus indistinguishable by light microscopy (Spilling et al., 2018; Kremp et al., 2018). In this study, it was not feasible to identify these to species level and thus the collective name *S. hangoei* was used. However, the three species have different spatial distribution in the Baltic Sea basins, and they succeed differently in germination of cysts during winter and encystment during late spring (Spilling et al., 2018). In laboratory experiments on species obtained from the southwest coast of Finland, *S. hangoei* and *B. balthica* formed cysts after being subjected to high temperatures, while *G. corollarium* encystment was triggered by nitrogen limitation. None of the three species increased encystment as a response to phosphorus limitation (Kremp et al., 2009). The low germination success of *B. balthica* and *G. corollarium* are promising, and at least *B. balthica* biomass has increased in the Baltic Sea during the past decades (Spilling et al., 2018). A qualitative field study of these species could reveal which Baltic Sea basin has the potential to act as a carbon sink when encystment is high and germination rates are low. This is yet to be studied in the northern Baltic Sea.

This comparative study was carried out by collecting plankton samples and observing taxonomic differences using light microscopy. Light microscopy has its limitations, one being the overrepresentation of bigger species and subsequent underrepresentation of smaller species. Light microscopy was in this case carried out by several people, though all using the same protocol. Therefore, there may be slight variations in cell enumerations and biomasses, though these should be minimal. In future studies, a complementary DNA analysis of the phytoplankton community would also be beneficial to present a complementary depiction of the community. Especially identifying cryptic species, such as the three different *S. hangoei* species, using DNA analysis would be useful.

Klais et al. (2011) and Spilling et al. (2018) both hypothesised the possible alleviating effects on eutrophication of a dinoflagellate dominance during spring blooms. However, these effects are dependent on the type of dinoflagellate, namely cyst-forming dinoflagellates. The most dominant dinoflagellate during the ice-covered winter 2021–22, both in the sympagic and the pelagic community, was *S. hangoei.* During spring blooms this species creates cysts, which are germinated during the following winter after a period of mandatory dormancy. It has been noted that *S. hangoei* has an advantage over other phytoplankton species in the Baltic Sea, as their resuspended cysts can be germinated within the ice and then re-released into the pelagic

community. This typically occur as temperature drops below 9 ºC during winter (Kremp & Anderson, 2000). So, to maximise carbon capture by this species, it would be effective if an ice-covered, cold winter is followed by a hotter winter, to ensure that the captured carbon is not released again the following winter in cold waters.

According to long-term data, changes in temperature, ice cover, solar irradiation, and wind condition among other patterns lead to later spring blooms of mainly dinoflagellates and the ciliate *M. rubrum*, as opposed to early blooming, rapidly sinking diatoms (Hjerne et al., 2019; Viitasalo & Bonsdorff, 2022). This contradicts my findings, as both dinoflagellates and *M. rubrum* seemed to increase with ice cover and lower temperatures. However, the high nutrient levels may at least partly be the cause of this increase. As a result of increased abundances of dinoflagellates and *M. rubrum*, the benthic system would receive less input in the form of sedimenting diatoms. The decreased benthic production could potentially alleviate anoxic zones. The pelagic secondary production, on the other hand, is favoured by the increases in energy allocation, as high temperatures increase the grazing pressure from zooplankton (Spilling et al., 2018; Hjerne et al., 2019). Camarena-Gómez et al. (2018) noted that even small changes in phytoplankton community composition during spring affect the heterotrophic bacterial community structure and response in bacterial production during summer, with potential cascade effects on cyanobacterial blooms (Spilling et al., 2018).

4.2 Nutrients

Many studies have found that during spring in the Baltic Sea, phosphorus can act as a limiting resource for ice algal growth (Thomas & Dieckmann, 2010). However, Granskog & Kaartokallio (2004) found that atmospheric depositions of nitrogen and phosphorus can play a role in available nutrients, as snow and ice melt during spring. This seems to be the case during the spring of 2022, or at least that the ice cover delays both the tot-N and tot-P peaks compared to the spring of 2020. Studies have shown that a warm spring with low nutrients and salinity will generally lead to an increase in dinoflagellates, whereas a cold spring with high salinity and inorganic nutrients leads to diatom-dominated blooms (Wasmund et al., 2011; Thomas et al., 2017; Enberg et al., 2018). However, this trend could not be found in this particular study, as salinity was low after the ice-covered winter (see supplement 1). The phytoplankton community is tightly connected to the biogeochemical fluxes, as spring blooms in the Baltic Sea are often nitrogen limited (Enberg et al., 2018). The nutrient requirements of diatoms and

dinoflagellates are essentially identical, apart from the need for silica in diatoms (Klais et al., 2011). It is therefore peculiar that r-strategist diatoms dominated over K-strategist dinoflagellates in 2019–20, when nutrients were lower. Generally, dinoflagellates are considered affinity specialists that compete better in low nutrients. In this study, however, they survived better during the high nutrient conditions of 2021–22, especially in regard to tot-P.

Tot-N and tot-P are, however, measurements of the total nitrogen and phosphorus in the pelagic, meaning all dissolved and particulate nitrogen and phosphorus. With this in mind, the reason for both nutrients peaking in February–March 2022 seems reasonable. During this time, both the ice thickness and biomass reached their peaks. Logically, tot-N and tot-P would also be high during this time. This also explains the size difference in peaks of the nutrients, as organisms contain more nitrogen than phosphorus. The significant difference in biomass between 2019–20 and 2021–22 could similarly explain the enormous difference in tot-P between the two cold-water periods. In the Baltic Sea, diatoms generally compete better in nutrient-rich conditions compared to dinoflagellates during spring blooms (Klais et al., 2011), which explains why diatoms increased during late spring in 2022. The unusally high levels of tot-P combined with the presence of ice cover the months prior could explain why dinoflagellates dominated during the early spring of 2022.

The difference in tot-N between the winters 2019–20 and 2021–22 was insignificant, even though they seem to vary in dynamics, with opposite trends during the ice-covered months December to March. The insignificance is probably due to the fact that both years varied within the same range and peaked at similar concentrations, 2021–22 was just slightly delayed. The confidence intervals are also very large, indicating low precision and fit of the GAM. It is also important to keep in mind that Husö biological station received a new nutrient analyser in 2023, before the 2021–22 samples were processed. Nutrient samples from 2019–20 and 2021–22 were thus analysed using different machines and slightly different methodology, and results between years may vary somewhat.

Even though the contrasts between the two cold-water periods are very evident and this study contributes valuable insights, it is important to recognize its limitations and possible improvements for similar studies in the future. For instance, the 0m and 1m data was averaged into pelagic data for 2019–20. To keep these data comparable to the 2021–22 data, 2021–22 were averaged as well. In hindsight, it might have been clearer to keep 0m and 1m data separate. This would have been preferrable especially for the 2021–22 data, since the presence of ice

cover has a strong effect on even a 1m difference in the water column, and especially in a shallow bay like Sjöviken.

In future studies, to truly understand the influence of nutrient levels on phytoplankton, it would be more logical to measure the dissolved nutrients in the pelagic. Measuring the dissolved nutrients gives a better indication of the potential shifts in dominance of phytoplankton taxa due to fluctuations in available nutrients. Especially dissolved Si levels are of interest when studying diatoms versus dinoflagellates during spring blooms. In an extreme case in the Gulf of Riga, dissolved Si limited diatoms during multiple consecutive springs as highly silicified diatoms had bloomed intensely a few years prior, leading to significant dinoflagellate dominance during multiple spring blooms (Klais et al., 2011). Perhaps the levels of dissolved Si were higher during the cold-water period 2019–20, and this is why dinoflagellates were outcompeted. Another case might be that exceptionally low levels of dissolved Si combined with high levels of nitrogen and phosphorus promoted dinoflagellates over diatoms in 2021–22. Kuparinen et al. (2007) noted that dissolved inorganic phosphate concentrations are generally lower in the ice than in the under-ice water, whereas nitrogenous inorganic nutrients are higher in the ice. This would've been an interesting contrast to investigate in this study, as tot-P was very high in under-ice water. Therefore, to comprehend the influence of the ice cover on nutrients both in the sympagic and pelagic, dissolved nutrients should also be measured in the ice in future studies.

4.3 Chlorophyll α, cell abundance, and diversity index

Interestingly, the GAM-analysis did not capture a statistically significant difference in chlorophyll α nor in cell abundance. The seasonal peaks of chlorophyll α indicate a spring bloom in March–April, in accordance with other studies in the northern Baltic Sea (Enberg et al., 2018; Hjerne et al., 2019). During the early ice season of 2022, chlorophyll α was much higher than during the same time in 2020, most likely due to the number of organisms leaving the ice as it matured. However, chlorophyll α reached similarly high levels in March 2020, as during late March 2022, shortly before ice melt. This may be a response to the high tot-N and tot-P during the earlier month of February 2020.

The Shannon diversity index represents the diversity within the community on a monthly basis. A low Shannon index indicates low species diversity or species evenness, that is only one or

few dominating species, within the community. A high Shannon index indicates a diverse or evenly distributed community. The stability of the 2021–22 community compared to the 2019–20 community implies that 2021–22 consisted of a higher number of species, and that these had more evenly distributed cell abundances. This is especially apparent during February and March 2020, when the Shannon index was lowest. During this time one species in particular was more dominant than the rest in the 2019–20 community. The Shannon index fluctuated quite drastically from month to month throughout 2019–20. In contrast, the community consisted of a bigger variation in species, and their call abundances were quite evenly distributed in the community during 2021–22. The pelagic communities should show similar patterns in cell abundance as in Shannon index, since they are closely connected (Tian et al. 2017), however this could not be observed in this study. Unfortunately, as no sampling could take place in April 2020 there are big gaps in our knowledge of the spring community after the ice-free winter 2019–20. For instance, we cannot observe the peaks in chlorophyll, cell abundance, and biomass that likely appeared during this time. Even if the spring communities could not be entirely compared, there are nonetheless clear differences between the years, and not only in nutrient levels.

4.4 The sympagic community and its seeding effect

Generally, the diversity is lower in Baltic Sea ice, since it is considered an extreme environment for many species. A few species from each taxa dominated the ice completely. The biggest contributor to the sympagic community in this study was the dinoflagellate *S. hangoei*. As the ice formed in December, *S. hangoei* made its appearance in the ice and the following month also in the pelagic community. During December no sampling could take place at 8 meters as the ice was too thin to allow passage. Even so, this was the first appearance of *S. hangoei* during the whole cold-water period. Aside from *S. hangoei*, not a single species nor taxa were ever observed in the sympagic community before thriving in the pelagic community. Thus, no widespread seeding effect from the sympagic to the pelagic community could be detected, apart from the contribution of *S. hangoei* to the pelagic community.

The biomass of *S. hangoei* increased in the pelagic community as the ice matured and later as it melted, indicating a seeding effect of this dinoflagellate to the pelagic community from the sympagic community. In mid-April as the ice had melted completely, *S. hangoei* was the most abundant dinoflagellate in the pelagic community. However, as shown by Piiparinen et al.

(2010), it is important to remember the possible contribution of cysts from the sediments during spring, especially in a shallow sampling site such as Sjöviken. During December 2021, the pelagic community experienced optimal temperature for cyst germination. As December 8 m samples were not taken, it is therefore impossible to rule out the germination of sediment cysts as the main contributor of *S. hangoei* to the pelagic community, as opposed to the sympagic community being the main source of cells. However, during severe winters, cysts are resuspended and integrated into newly formed ice (Kremp & Anderson, 2000). There, the light conditions are favourable and *S. hangoei* cysts effectively germinate in the cold ice, which seems to be the case during 2021–22.

It is very likely that *S. hangoei* was seeded to the pelagic community from the ice, as the species was not observed in the water column before ice formation. As expected in Baltic Sea ice, the sympagic cell abundance was not as high as in the underlying pelagic water, nor was it as diverse in species. However, the sympagic biomass measured highest of any recorded biomass in this whole study, including both 2019–20 and 2021–22, just after ice formation. As the ice forms, most microorganisms in the water column are incorporated into the ice (Enberg et al., 2018). It is therefore expected that the sympagic biomass immediately after ice formation is high. Which species dominate the pelagic water is therefore influenced by the presence of ice cover during the cold-water period, as the coupling from the sympagic to the pelagic increases as the ice melts (Leu et al., 2015). To be able to fully understand the seeding effects of the sympagic community to the pelagic spring bloom, using sediment traps under the ice like Haecky et al. (1998) is recommended for future studies. The ice algal production itself is incredibly low in the Baltic Sea ice, around 0.4% of the total annual primary production (Haecky & Andersson, 1999). However, the results in this thesis show that the ice cover plays an important role in the composition of the phytoplankton community, while also affecting the nutrient dynamics.

5 Conclusions

The dinoflagellate community was much more pronounced during the ice-covered cold-water period 2021–22, with great dominance of *S. hangoei*. During spring after an ice-covered winter 2021–22, *Chaetoceros* spp. dominated over centric diatoms *S. marinoi* and *Thalassiosira* spp. that thrived during the spring 2020, after an ice-free winter. Pelagic tot-P was significantly

higher in 2021–22 compared to 2019–20, which seems to have affected the phytoplankton biomass. Future studies should measure dissolved nutrient levels, rather than total nutrient levels, as these are what phytoplankton utilise. Ice cover, among other factors, drives the phytoplankton composition during the cold-water period.

The dinoflagellate *S. hangoei* seems to be the only phytoplankton in Sjöviken to successfully seed to the pelagic spring bloom after ice melt in 2021–22, this is probably due to the protective sympagic habitat and the favourable temperature. The exact effect of the phytoplankton community composition during the cold-water period on specific higher-level organism groups is still unknown. However, the shift to dinoflagellate-dominated spring blooms could potentially alleviate eutrophication in the Baltic Sea. Secondary production in the pelagic could increase, as benthic production would diminish.

6 Summary in Swedish – Svensk sammanfattning

Växtplanktonsamhällets sammansättning under motsatta vinterförhållanden i en grund havsvik i norra Östersjön

6.1 Inledning

Temperaturen i Östersjön stiger stadigt och de största temperaturökningarna iakttas under vintermånaderna. Vintern 2019–20 observerades den starkaste värmeböljan i Östersjön på 20 år, vilket ledde till att praktiskt taget all havsis var frånvarande. Baserat på historiska data var det rentav den minsta isutbredningen sedan 1720!

De vanligaste fototrofiska grupperna är kiselalger, dinoflagellater, klorofyter och kryptofyter, medan ciliater, cercozoa, dinoflagellater och choanoflagellater utgör de vanligaste heterotrofiska grupperna. Dessa växtplanktontaxa fluktuerar med miljövariabler, såsom olika näringshalter. Förekomsten av ett istäcke under vintern leder till lägre primärproduktion och biomassa i pelagialen, vilket resulterar i koncentrerade och kortlivade pelagiska blomningar då isen smälter. Växtplanktonsuccessionen i Östersjön karaktäriseras av alternerande cykler av kiselalger och dinoflagellater. Den allt varmare yttemperaturen leder till tidigare och förlängda vårblomningar. Utöver detta kan dinoflagellater bli mer dominerande än kiselalger efter varmare vintrar, vilket påverkar pelagialens kopplingen till bentos. Under en vårblomning dominerad av dinoflagellater transporteras mindre kol i form av sedimenterande kiselalger till bentos.

Havsisen påverkar starkt det pelagiska växtplanktonsamhället, också efter att den smultit. Växtplanktonsamhällets sammansättning i pelagialen under vårblomningen är annorlunda efter en isfri vinter jämfört med efter en istäckt vinter. Kiselalger är generalister och trivs i de flesta livsmiljöer, medan dinoflagellater är mer specialiserade och mottagliga för nischförändringar. Besåningsteorin (eng. seeding theory) medför att isalger "besår" pelagialen under vårblomningen. Denna teori är mycket omdiskuterad och oftast tillämpbar endast i en del studier.

6.2 Syfte och forskningsfrågor

Syftet med studien var att jämföra variationen hos växtplanktonsamhället under en istäckt kallvattenperiod med en isfri kallvattenperiod. Samhällets koppling till miljövariablerna undersöktes.

Forskningsfrågorna var:

1) Finns det skillnader i växtplanktonsamhällets sammansättning under en isfri vinter 2019–20 respektive en istäckt vinter 2021–22?

2) Kan arter från det sympagiska samhället "beså" pelagialen och därigenom påverka växtplanktonsamhällets sammansättning under våren?

Data under den istäckta vintern jämfördes med data från en tidigare studie av växtplanktonsamhället under en isfri vinter vid samma provtagningsstation. Dessa data erhölls från Ronja Lanndérs pro gradu-avhandling *"Ett växtplanktonsamhälles succession och cellstorleksstruktur under en kallvattenperiod i en grund havsvik på nordvästra Åland"*.

6.3 Material och metoder

Vattenprover och isprover togs månatligen i Sjöviken, på nordvästra Åland, under kallvattenperioden oktober till maj 2021–22. Prover togs för växtplankton, klorofyll a, syrehalt, pH, salinitet och näringshalter. Planktonproverna sedimenterades i Utermöhlkammare innan mikroskopering. Därefter användes ett inverterat fas-kontrastmikroskop för att identifiera växtplankton till artnivå, då detta var möjligt. Planktonen räknades och deras celler mättes. Cellernas storlek och abundanser omvandlades till kolbiomassa genom att utnyttja kolinnehållet för specifika arter eller taxa.

6.4 Resultat

Den största skillnaden mellan växtplanktonsamhällena under kallvattenperioderna 2019–20 och 2021–22 sågs i förekomsten av kiselalger och dinoflagellater. Under 2019–20 dominerade kiselalgerna markant i pelagialen under vårblomningen jämfört med under 2021–22, då dinoflagellaterna framträdde mycket starkt.

Både total fosforhalten och samhällets biomassa i pelagialen var signifikant högre under 2021–22. Tot-N och tot-P nådde sin kulmen i februari–mars 2022, i samband med den tjockaste isen och största biomassan i pelagialen. Den signifikanta skillnaden i biomassa under de två kallvattenperioderna kan möjligen förklaras av den stora skillnaden i tot-P mellan 2019–20 och 2021–22. De förhöjda halterna av tot-P i kombination med förekomsten av istäcket orsakade troligen dominansen av dinoflagellater under vårblomningen 2022.

Det sympagiska samhället dominerades till och med ännu kraftigare av dinoflagellater, i synnerhet av arten *S. hangoei*. Detta var den enda art som uppvisade en besåningsffekt till pelagialen från det sympagiska samhället. *S. hangoei* kan fungera som kolsänka efter sina starka blomningar genom att sedimentera till bentos i form av vilocystor.

6.5 Diskussion

Resultaten motsätter sig tidigare studier, i vilka dinoflagellater i allmänhet ökat efter en varmare vinter. Kiselalgsdominansen under 2019–20, trots lägre näringshalter, skiljer sig från den allmänna trenden att dinoflagellater dominerar bättre i näringsfattiga förhållanden. Under 2021–22 frodades dinoflagellater i höga näringshalter, särskilt vad gäller tot-P. Det är oklart vilken miljövariabel som bidragit mest till den stora förekomsten av dinoflagellater under 2021–22. Omväxlingen till dominans av dinoflagellater under vårblomningen kan eventuellt minska den bentiska kopplingen, då mängden sjunkande kiselalger minskar och sekundärproduktionen ökar i det pelagialen.

Dinoflagellaternas dominans under 2021–22 kan möjligen förklaras av det skyddande habitatet i isen, och de högre fosforhalterna. Vilocystor av dinoflagellaten *S. hangoei* utnyttjade den låga temperaturen i isen för att effektivt gro. Vilocystor av *S. hangoei* som inte lyckas gro följande vår fungerar som kolsänka. Dessa kunde potentiellt minska effekten av övergödningen i Östersjön.

De totala kväve- och fosforhalterna kan vara missvisande eftersom de är mått på allt kväve och fosfor i pelagialen. I framtida studier borde hellre koncentrationerna av lösta näringsämnen mätas. Speciellt halterna löst kisel vore av intresse, eftersom detta är den främsta begränsande faktorn hos kiselalger och kan vara avgörande för dynamiken mellan kiselalger och dinoflagellater. Att undersöka variationer i olika lösta näringsämnen både i de sympagiska och pelagiska habitaten är avgörande för en heltäckande förståelse av näringsämnenas påverkan under vårblomningen.

References

Camarena-Gómez, M. T., Lipsewers, T., Piiparinen, J., Eronen-Rasimus, E., Perez-Quemaliños, D., Hoikkala, L., et al. (2018). Shifts in phytoplankton community structure modify bacterial production, abundance and community composition. *Aquat. Microb. Ecol.* 81, 149–170.

Cederberg, T. (2018). Långtidsserier från Husö biologiska station—Provtagningar 1987—2020. https://www.doria.fi/handle/10024/167044?show=full

Cederberg, T. (2020). Långtidsserier från Husö biologiska station—Provtagningar 1987—2020. https://www.doria.fi/handle/10024/167044?show=full

Enberg, S., Majaneva, M., Autio, R., Blomster, J. & Rintala, J-M. (2018). Phases of microalgal succession in sea ice and the water column in the Baltic Sea from autumn to spring. *Mar. Ecol. Prog. Ser.* 599: 19–34.

Granskog, M.A. & Kaartokallio, H. (2004). An Estimation of the Potential Fluxes of Nitrogen Phosphorus, Cadmium and Lead from Sea Ice and Snow in the Northern Baltic Sea. *Wat., Air, & Soil Poll.* 154, 331–347

Granskog, MA., Kaartokallio, H., Kuosa, H. & Thomas DN., Vainio J (2006). Sea ice in the Baltic Sea: A review. Estuarine Coastal and Shelf. *Science* 70: 145-160.

Grasshoff, K., Ehrhardt, M. and Kremling, K. (1983). Methods of Seawater Analysis. (*2nd edition*) Verlag Chemie Weinhein, New York, 419 p.

Grasshoff, K., Ehrhardt, M., Kremling, K., Anderson, L.G. (Ed.). (1999). Methods of seawater analysis (*3d edition)*. Wiley-VCH.

Grover, J.P. (1991). Resource Competition in a Variable Environment: Phytoplankton Growing According to the Variable-Internal-Stores Model. *Am. Nat.* 138: 811– 835.

Haecky P., Jonsson S. & Andersson A. (1998). Influence of sea ice on the composition of the spring phytoplankton bloom in the northern Baltic Sea. *Polar Biol.* 20: 1–8.

Haecky, P., & Andersson, A. (1999). Primary and bacterial production in sea ice in the northern Baltic Sea. *Aquat. Microb. Ecol.* 20:107−118.

Hjerne, O., Hajdu, S., Larsson, U., Downing, A. S., & Winder, M. Climate driven changes in timing, composition and magnitude of the Baltic Sea phytoplankton spring bloom. *Front. Mar. Sci*. 6: 482.

Järvinen, M., Forsström, L., Huttunen, M., Hällfors, S., Jokipii, R., Niemelä, M. & Palomäki, A. (2011). *Kasviplanktonin laskentamenetelmät.* https://www.ymparisto.fi/fi-fi/vesi/pintavesien_tila/pintavesien_tilan_seuranta/Biologisten_seurantamenetelmien_ohjeet/Kasviplanktonin_tutkimusmenetelmat

Klais, R., Tamminen, T., Kremp, A., Spilling, K., & Olli, K. (2011). Decadal-scale changes of dinoflagellates and diatoms in the anomalous Baltic Sea spring bloom. *PLoS ONE* 6:e21567. doi: 10.1371/journal.pone.0021567

Kremp, A. & Heiskanen, A.-S. (1999). Sexuality and cyst formation of the spring-bloom dinoflagellate Scrippsiella hangoei in the coastal northern Baltic Sea. *Mar. Biol.* 134: 771–777.

Kremp, A. & Anderson, D.M. (2000). Factors regulating germination of resting cysts of the spring bloom dinoflagellate *Scrippsiella hangoei* from the northern Baltic Sea. *J. Plankton Res.* 22 (7): 1311–1327.

Kremp, A., Rengefors, K. & Montresor, M. (2009). Species-specific encystment patterns in three Baltic cold-water dinoflagellates: The role of multiple cues in resting cyst formation. *Limnol. Oceanogr.* 54(4), 1125–1138.

Kremp, A., Hinners, J., Klais, R., Leppänen, A-P & Kallio A. (2018). Patterns of vertical cyst distribution and survival in 100-year-old sediment archives of three spring dinoflagellate species from the Northern Baltic Sea. *Eur. J. Phycol.* 53:2, 135–145.

Kuparinen, J., Kuosa, H., Andersson, A., Autio, R. & Granskog, M.A., (2007). Role of sea-ice biota in nutrient and organic material cycles in the northern Baltic Sea. *Ambio.* 36:149-154

Lanndér, R. (2022). Ett växtplanktonsamhälles succession och cellstorleksstruktur under en kallvattenperiod i en grund havsvik på nordvästra Åland. [Master's thesis, Åbo Akademi University] Doria: https://urn.fi/URN:NBN:fi-fe2022061045585

Leu, E., Mundy, C.J. Assmy, P., Campbell, K., Gabrielsen, T.M., Gosselin, M., Juul-Pedersen, T. & Gradinger, R. 2015. Arctic spring awakening – Steering principles behind the phenology of vernal ice algal blooms. *Prog. Oceanogr.* 139, 151–170.

Majaneva, M., Rintala, J-M., Piisilä, M., Fewer, DP. & Blomster, J. (2012). Comparison of wintertime eukaryotic community from sea ice and open water in the Baltic Sea, based on sequencing of the 18S rRNA gene. *Polar Biol.* 35: 875-889.

Meier, H. E. M., Kniebusch, M., Dieterich, C., Gröger, M., Zorita, E., Elmgren, R., Myrberg, K., Ahola, M. P., Bartosova, A., Bonsdorff, E., Börgel, F., Capell, R., Carlén, I., Carlund, T., Carstensen, J., Christensen, O. B., Dierschke, V., Frauen, C., Frederiksen, M., Gaget, E., Galatius, A., Haapala, J. J., Halkka, A., Hugelius, G., Hünicke, B., Jaagus, J., Jüssi, M., Käyhkö, J., Kirchner, N., Kjellström, E., Kulinski, K., Lehmann, A., Lindström, G., May, W., Miller, P. A., Mohrholz, V., Müller-Karulis, B., Pavón-Jordán, D., Quante, M., Reckermann, M., Rutgersson, A., Savchuk, O. P., Stendel, M., Tuomi, L., Viitasalo, M., Weisse, R., and Zhang, W. (2022). Climate change in the Baltic Sea region: a summary. *Earth Syst. Dynam.* 13, 457–593.

Mikkelsen, D.M, Rysgaard, S. & Glud, R. N. (2008). Microalgal composition and primary production in Arctic sea ice: a seasonal study from Kobbefjord (Kangerluarsunnguaq), West Greenland. *Mar. Ecol. Prog. Ser.* 368: 65–74.

Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I. & Niemkiewicz, E. (2006). *Biovolumes and size-classes of phytoplankton in the Baltic Sea.* HELCOM Balt. Sea Environ. Proc. No. 106, 144pp.

Piiparinen, J., Kuosa, H. & Rintala, J-M. (2010). Winter-time ecology in the Bothnian Bay, Baltic Sea: nutrients and algae in fast ice. *Polar Biol.* 33: 1445–1461.

Sommer, U. (1985). Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton, *Limnol. Oceanogr.* 2.

Sommer, U., Aberle, N., Lengfellner, K., and Lewandowska, A. (2012). The Baltic Sea spring phytoplankton bloom in a changing cli- mate: an experimental approach. *Mar. Biol.* 159, 2479–2490.

Spilling, K., Olli, K., Lehtoranta, J., Kremp, A., Tedesco, L., Tamelander, T., Klais, R., Peltonen, H., & Tamminen, T. (2018). Shifting Diatom—Dinoflagellate Dominance During Spring Bloom in the Baltic Sea and its Potential Effects on Biogeochemical Cycling. *Front. Mar. Sci. 5*, 327.

Thomas, D. N., Kaartokallio, H., Tedesco, L., Majaneva, M., Piiparinen, J. P., Eronen-Rasimus, E., Rintala, J-M., Blomster, J., Vainio, J., & Granskog, M. A. (2017). Life associated with Baltic Sea ice. In P. Snoeijs-Leijonmalm, H. Schubert, & T. Radziejewska (Eds.), *Biological Oceanography of the Baltic Sea* (s. 333- 357). Springer-Verlag GmbH.

Thomas, D.N. & Dieckmann, G.S. (2010). *Sea ice (2nd edition).* Wiley.

Tian, W., Zhang, H., Zhao, L., Zhang, F., & Huang, H. (2017). Phytoplankton Diversity Effects on Community Biomass and Stability along Nutrient Gradients in a Eutrophic Lake. *Int. J. Environ. Res. Public Health*, *14*(1), 95.

Viitasalo, M. & Bonsdorff, E. (2022). Global climate change and the Baltic Sea ecosystem: direct and indirect effects on species, communities and ecosystem functioning. *Earth Syst. Dynam.,* 13: 711–747.

von Schuckmann, K., P.-Y. Le Traon, N. Smith, A. Pascual, S. Djavidnia, P. Brasseur, M. Grégoire (Eds.) (2022). Copernicus Ocean State Report, Issue 6. *J. Oper. Oceanogr.* 15:sup1, s1–s220.

Wasmund, N., Kownacka, J., Göbel, J., Jaanus, A., Johansen, M., Jurgensone, I., Lehtinen, S. & Powilleit, M. (2017). The Diatom/Dinoflagellate Index as an Indicator of Ecosystem Changes in the Baltic Sea 1. Principle and Handling Instruction. *Front. Mar. Sci.* 4: 22.

Wasmund, N., Tuimala, J., Suikkanen, S., Vandepitte, L. & Kraberg, A. (2011). Long-term trends in phytoplankton composition in the western and central Baltic Sea. *J. Mar. Syst.* 87: 145–159.

Winder, M., Berger, S. A., Lewandowska, A., Aberle, N., Lengfellner, K., Sommer, U., et al. (2012). Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. *Mar. Biol.* 159, 2491– 2501.

Winkler, L.W. (1888). Die Bestimmung des im Wasser gelösten Sauerstoffes. *Berichte der deutschen chemischen Gesellschaft 21*, 2843–2854.

Yan D., Yoshida K., Nishioka J., Ito M., Toyota T. & Suzuki K. (2020). Response to Sea Ice Melt Indicates High Seeding Potential of the Ice Diatom Thalassiosira to Spring Phytoplankton Blooms: A Laboratory Study on an Ice Algal Community From the Sea of Okhotsk. *Front. Mar. Sci.* 7: 613.

Supplements
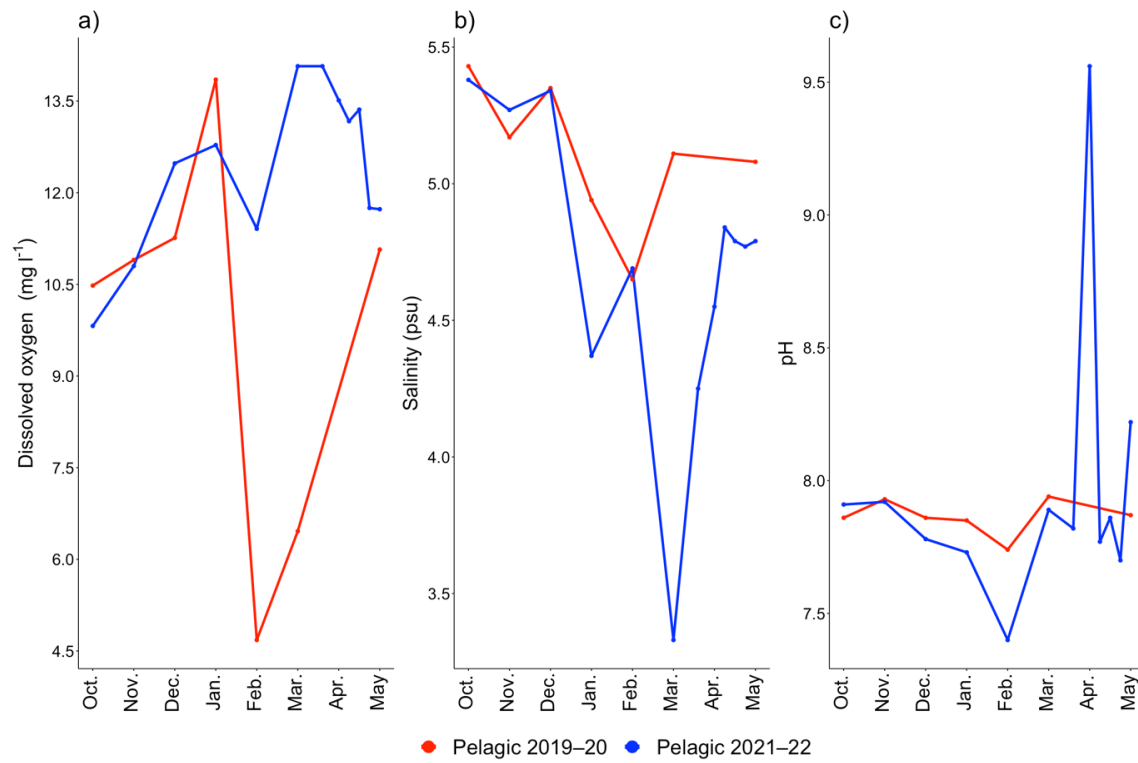
Supplement 1. Environmental variables a) dissolved oxygen, b) salinity, and c) pH in the pelagic during 2019–20 (red) and 2021–22 (blue).