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Growth and mortality rates of picophytoplankton in the Baltic Sea Proper

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ABSTRACT: Picophytoplankton (<2 µm diameter), a diverse group of picocyanobacteria and photosynthetic picoeukaryotes, are significant contributors to primary production. Predatory mortality controls picophytoplankton biomass and thereby energy transfer in the marine food web. The 2 major pathways of picophytoplankton mortality are grazing and viral lysis. Grazing passes carbon directly to higher trophic levels, while lysis products are passed into the viral loop. Picophytoplankton are abundant in the Baltic Sea but little is known about their predatory mortality. Using a modification of the dilution approach, we calculated growth and mortality rates of picophytoplankton and studied the effect of predation on community structure during late August and September. The experiments were conducted coinciding with the peak in picophytoplankton abundance (~10^5 cells ml^{-1}) at the Linnaeus Microbial Observatory in the Baltic Sea Proper. The results showed that grazing is an important controller of picocyanobacteria and photosynthetic picoeukaryote populations, while no significant viral lysis effect was detected. Grazing on picocyanobacteria was proportional to growth rates, while grazing on photosynthetic picoeukaryotes exceeded growth. Selective grazing of phylogenetically distinct picocyanobacterial clades had a significant effect on community structure, suggesting that grazing has an impact on the seasonal dynamics of co-occurring clades. Picocyanobacteria had a higher carbon transfer contribution to higher trophic levels than photosynthetic picoeukaryotes at the time of the experiments. The study shows that picophytoplankton are important contributors to carbon cycling in the Baltic Sea microbial food web and should be considered for future ecological models.

KEY WORDS: Picophytoplankton · Picoeukaryotes · *Synechococcus* · Grazing · Viral lysis · Carbon transfer · Baltic Sea

1. INTRODUCTION

Picophytoplankton (<2 µm diameter) are a diverse group of primary producers consisting of picocyanobacteria and photosynthetic picoeukaryotes (PPE), which can dominate phytoplankton biomass in oligotrophic oceans (Pena et al. 1990, Agawin et al. 1998, Durand et al. 2001) as well as coastal, eutrophic, and brackish environments (Søndergaard et al. 1991, Buitenhuis et al. 2012, Caroppo 2015). Due to their small size, picophytoplankton have a high surface to volume ratio which gives them a competitive advantage for nutrient uptake compared to larger phytoplankton cells (Partensky et al. 1999, Pittera et al. 2014). With climate change, picophytoplankton are expected to increase in abundance and replace larger phytoplankton due to increased temperatures, more extended periods of stratification, and de-eutrophication (Bopp et al. 2013, Cabré et al. 2015). Moreover, picocyanobacteria are expected to have a compet-

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itive advantage over PPE in warm, nutrient-deficient environments (Schmidt et al. 2020). Changes in phytoplankton community structure and specifically within the picophytoplankton size fraction could cause alterations in carbon pathways within the microbial food web and have a cascading effect on upper trophic levels (Capuzzo et al. 2017). Thus, quantifying and understanding the mechanisms behind picophytoplankton growth and mortality and its implications for carbon transfer is key to predicting scenarios under climate change conditions.

Picophytoplankton predatory mortality occurs by grazing or viral lysis (Christaki et al. 2002, Wang et al. 2011, Tsai et al. 2012). Carbon pathways through the food web differ depending on whether picophytoplankton cells succumb to lysis or grazing (Fuhrman 1999). When picophytoplankton are grazed, carbon is passed directly to higher trophic levels (Fuhrman 1999, Landry & Calbet 2004). However, when cells are lysed, the lysis products are passed into a semi-enclosed microbial loop and later upcycled through the trophic chain with some energy loss (Calbet & Landry 2004, Suttle 2005). Traditionally, ciliates and flagellates have been considered the main grazers of picophytoplankton (Dolan & Šimek 1998, Hadas et al. 1998, Christaki et al. 1999, Grinienė et al. 2016), but some observations suggest that they can also be grazed by mesozooplankton (Motwani & Gorokhova 2013, Novotny et al. 2021). Grazing has been observed to stabilize but not reduce abundances of the picocyanobacterium *Synechococcus* (Dolan & Šimek 1999, Tsai et al. 2015a, Hunter-Cevera et al. 2020). Conversely, grazers have been observed to effectively control PPE abundances in both marine (Pasulka et al. 2015, Fowler et al. 2020) and brackish environments (Evans et al. 2003). Size is an important factor driving prey selection (Christaki et al. 2002). Most observations suggest that grazing pressure is higher on the larger PPE than the smaller *Synechococcus* (Samuelsson & Andersson 2003, Worden et al. 2004), which could be a consequence of selective pressure due to size or the lower food quality that *Synechococcus* provides grazers (Apple et al. 2011). Prey selection can also be specific to strains within both the *Synechococcus* genera (Stoddard et al. 2007, Apple et al. 2011) and the highly diverse group of PPEs (Blanc-Mathieu et al. 2017) but the mechanisms of selection remain poorly understood. Viral lysis has been reported to exert pressure similar to grazing on picophytoplankton populations (Pasulka et al. 2015, Tsai et al. 2015b). In brackish environments, viral lysis on *Synechococcus* can increase during the cold months, when grazing is reduced (Tsai et al. 2015a), while for PPE, viruses may not cause a population decline (Cottrell & Suttle 1995).

The modified dilution approach (Evans et al. 2003) is a thoroughly replicated experimental design used to calculate mortality rates of phytoplankton (Kimmance et al. 2007, Baudoux et al. 2008, Pasulka et al. 2015, Tsai et al. 2015a, 2018). The setup is based on the dilution technique proposed by Landry & Hassett (1982) to calculate microzooplankton grazing rates, with the additional inclusion of a series of dilutions with virus-free water, which allows for the calculation of viral lysis rates (Evans et al. 2003). This methodological approach has been extensively used in marine environments, although its implementation in brackish waters is still limited (Staniewski & Short 2018). The experimental design operates under 4 assumptions: (1) phytoplankton growth is not affected by the presence or absence of other phytoplankton; (2) mixotrophic phytoplankton are not affected by changes in the concentration of dissolved organic matter, and thus growth is not affected by dilution; (3) grazing is related to prey concentration; and (4) phytoplankton growth is exponential (Landry & Hassett 1982, Cram et al. 2016). Moreover, as heterotrophic bacteria grow faster than phytoplankton in the dilution treatments, short-term experiments are recommended (Cram et al. 2016). In combination with molecular identification, dilution experiments offer insights into mortality dynamics with high taxonomical resolution and have the potential to resolve mechanisms of specific prey selection (Cram et al. 2016).

In this study, we used the modified dilution approach to study grazing and viral mortality as well as carbon transfer rates to upper trophic levels in Baltic Sea Proper picophytoplankton communities, where the picocyanobacteria and PPE communities represent a seasonal contribution of the total phytoplankton biomass up to ~75 and ~15%, respectively (Alegria Zufia et al. 2021). The Baltic Sea picophytoplankton communities are formed by a large array of co-occurring ecotypes with strong seasonality and diverse physiologies (Haverkamp et al. 2009, Bertos-Fortis et al. 2016, Alegria Zufia et al. 2022). The picocyanobacterial population in the Baltic Sea consists of a diverse assemblage of *Synechococcus* sp., *Cyanobium* sp., *Aphanothece* sp., and *Synechocystis* sp. among others, hereafter referred to as SYN. SYN community composition can also be divided into phylogenetic subgroups and clades with distinct physiological characteristics. Baltic Sea Proper SYN communities are dominated by freshwater clades (clade A/B or clade B) from winter to spring, while during summer, low NO_3+NO_2 concentrations and high tempera-

tures promote the dominance of subgroup 5.2 (S5.2), normally coinciding with peak SYN abundances (~10^5 cells ml^{-1}) (Larsson et al. 2014, Alegria Zufia et al. 2022). Conversely, PPE peak abundances (~10^4 cells ml^{-1}) normally take place during early spring and/or in the late-summer to autumn period (Kuosa 1991, Albertano et al. 1997, Tamm et al. 2018, Alegria Zufia et al. 2021). PPE community composition is largely unknown, although some studies indicate the presence of chlorophytes of the Class Mamiellophyceae (Hu et al. 2016).

Picophytoplankton are important contributors to the Baltic Sea carbon cycle (Kuosa 1991, Søndergaard et al. 1991, Stal et al. 2003) and new studies are revealing how environmental conditions control its abundance and community composition (Larsson et al. 2014, Alegria Zufia et al. 2022). However, little is known regarding how mortality controls picophytoplankton abundance and community composition as well as carbon transfer rates to upper trophic levels. Few studies have used the dilution approach to calculate grazing rates for picophytoplankton in the Baltic Sea (Reckermann 1996, Aberle et al. 2007, Grinienė et al. 2016), and there are currently no observations on viral lysis rates. Observations in the Kiel Fjord before and during the spring bloom showed that picophytoplankton are not significantly grazed during this period (Aberle et al. 2007). Reckermann (1996) observed significant grazing rates on the picophytoplankton community during summer in the Gotland Sea, with the daily production of SYN and PPE grazed at 133 and 80%, respectively. Furthermore, Donali et al. (1999) showed significant carbon transfer rates from the picophytoplankton fraction during summer in the Gulf of Riga. Finally, observations during autumn in the Curonian lagoon showed that grazing rates on the picophytoplankton community were higher than their growth (Grinienė et al. 2016). These findings suggest that grazing is an important control for picophytoplankton in the Baltic Sea, but more research is needed to assess the predatory mortality and selectivity on SYN and PPE, respectively.

In this study, we combine the modified dilution approach (Evans et al. 2003) in an *in situ* incubation with a detailed analysis of the community composition to study the effect of grazing and viral lysis on SYN and PPE during and after the phytoplankton summer bloom coinciding with peak SYN abundance. Additionally, we inferred carbon transfer rates to upper trophic levels from the identified mortality rates. This information will provide novel insights into the role of picophytoplankton in the microbial food web in the Baltic Sea Proper.

2. MATERIALS AND METHODS

2.1. Experimental setup

Seawater was collected at 1 m depth from the Linnaeus Microbial Observatory (LMO; 56° 55.8540′ N, 17° 3.6420′ E) approximately 10 km offshore from Kårehamn, Öland, Sweden, in the Baltic Sea Proper on 10 August (Grazing 1) and 14 September (Grazing 2) 2020 using a 5 l Ruttner sampler, and mixed in 70 l acid-washed opaque barrels. Conductivity, temperature, and depth profiles were measured using a CTD probe (AAQ 1186-H, Alec Electronics). The water was transported to the field station in Kårehamn and filtered through a 200 µm mesh filter upon arrival at the shore (~30 min after sampling) to remove large grazers; the water was kept in the shade until the start of the experiments. The experimental setup consisted of a non-filtered seawater control treatment (W), and seawater diluted to 50% (50) and 90% (90) in triplicate-acid-washed 4.7 l Nalgene bottles (Fig. 1). The number of dilutions was limited because of the size of the incubation arrays and the larger volumes needed for molecular work (see below). Grazer-free dilutions were set up with 0.2 µm filtered seawater. In the Grazing 2 experiment, an additional set of dilutions to 50% (50-V) and 90% (90-V) were performed with virus-free seawater, which was produced using a 100 kDa TFF filter (Fig. 1). Three bottles from the W treatment were sampled immediately after they had been filled.

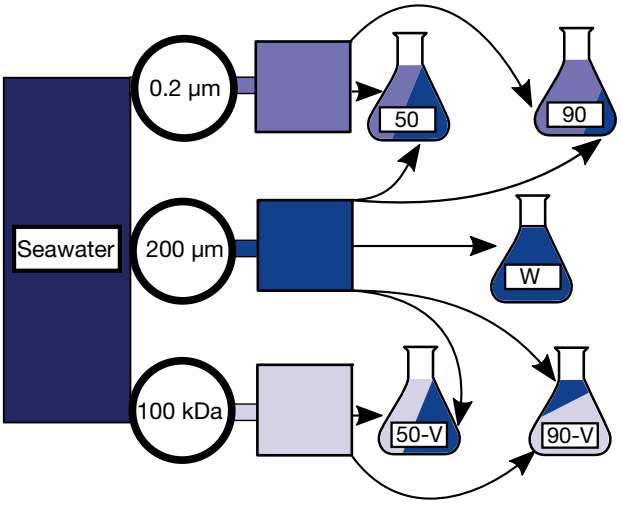

Fig. 1. Schematic illustration of the experimental setup, with the grazer-free 50% dilution (50), the grazer-free 90% dilution (90), the virus- and grazer-free 50% dilution (50-V), the virus- and grazer-free 90% dilution (90-V), and the whole water control treatment (W). Treatments 50-V and 90-V were only included in the Grazing 2 experiment. All treatments were done in triplicate

The remaining bottles were mounted onto incubation arrays positioned outside the Kårehamn harbour at 1 m depth and sampled after a 24 h *in situ* incubation. Water temperature and light intensity were recorded every 5 min for the duration of the experiments using HOBO® loggers.

2.2. Nutrients, chlorophyll *a* and phytoplankton community (>5 µm)

Samples for inorganic nutrients (nitrate-nitrite: NO_3+NO_2; phosphate: PO_4; silicate: SiO_2) were collected in acid-washed polycarbonate bottles (400 ml) and frozen at −20°C until analysis using a 4-channel continuous flow analyzer (San++, Skalar) with standard colorimetric methods (Grasshoff et al. 1983). NO_3 was calculated as the difference between NO_x and NO_2. Samples for chlorophyll *a* (chl *a*) concentration, as a proxy for phytoplankton biomass, were collected (500 ml) and filtered through a 25 mm diameter glass fiber filter (A/E) under low vacuum (>0.2 bar [200 hectopascal]). Chl *a* was extracted in 96% ethanol for 24 h and measured using a fluorometer (Turner Design model #040; Jespersen & Christoffersen 1987).

Samples for larger phytoplankton (>5 µm) community composition were collected and counted microscopically (Nikon TMS) after preservation with acidic Lugol's solution (1% final concentration) following Utermöhl (1958). Phytoplankton carbon biomass concentration was calculated using cell abundances and estimates of carbon content per cell based on taxonomy and size fraction (Edler 1979, HELCOM Phytoplankton Expert Group 2013).

2.3. Picophytoplankton abundance and community composition

Picophytoplankton and nanoflagellate abundance samples were fixed with glutaraldehyde solution Grade I 25% in H_2O (1% final concentration) and stored at −80°C until analysis with a BD FACSverse (BD Biosciences) flow cytometer equipped with a blue and a red laser. Picophytoplankton were counted as 3 populations: PPE, phycoerythrin (PE)-rich SYN cells and phycocyanin (PC)-rich SYN cells based on the gating described in Alegria Zufia et al. (2021). For identifying the populations, 4 optical parameters were used at a logarithmic scale: forward scatter as a proxy for cell diameter, FL2 (590/50 nm, blue laser dependent) as a proxy for PE content, FL3 (675/50 nm, blue laser dependent) as a proxy for chl *a*, and FL4 (675/50 nm, red laser dependent) as a proxy for PC content (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m735p063_supp.pdf).

To count the nanoflagellates, samples were stained with SYBRgreen (Sigma-Aldrich; 1% final concentration), and the population was identified using side scatter (SSC), FL1 (530/30 nm, blue laser dependent), and FL3 as described in Christaki et al. (2011). Briefly, stained cells were identified (FL1 vs. SSC), and then heterotrophic cells were discriminated from autotrophic cells following a 2-step process: first using FL3 vs. SSC and finally, FL3 vs. FL1 (Fig. S2). Gating and visualization of the flow cytometric data was done using FCSalyzer v.0.9.22-alpha (Mostböck 2021).

Picophytoplankton (SYN and PPE) community composition was determined through 16S rRNA gene amplicon sequencing. Samples for DNA extraction were collected by filtering 500 ml of seawater through a 0.22 µm Sterivex membrane filter and were then stored at −80°C until extraction. The DNA was extracted using the FastDNA™ SPIN Kit for Soil from MP Biomedicals according to the manufacturer's instructions with the addition of 1 h incubation with Proteinase K (20 mg ml^{-1}, final concentration) at 55°C. A 2-step PCR amplification was performed, as described in Mattsson et al. (2021), targeting the V3–V4 region of the 16S rRNA gene using the 341F and 805R primers (Herlemann et al. 2011). Samples were pooled at equal concentrations and sequenced using MiSeq Illumina technology (2 × 300 bp) at the National Genomics Infrastructure in Stockholm.

The 16S rRNA gene amplicon data was denoised and screened for chimera removal with ampliseq (v1.1; https://github.com/nf-core/ampliseq), which runs on QIIME2 (2019.10; Caporaso et al. 2010) and DADA2 (1.10.0; Callahan et al. 2016). Taxonomy assignment of the amplicon sequencing variants (ASVs) was done using the SILVA 132 database with a 90% identity threshold. The Shannon diversity index was calculated, and the bacterial community composition was observed to assess whether the dilution treatments in the experiment affected the overall bacterial community (e.g. shifts in community composition or loss of diversity). The utilization of 16S rRNA primers also results in the amplification of chloroplast sequences associated with eukaryotic cells (Hu et al. 2016, Li et al. 2019). Consequently, we filtered the obtained sequences into 2 distinct data sets comprising ASVs from SYN and chloroplasts. Each data set was subsampled to the level of the least deeply sequenced sample (SYN: 269; chloroplasts: 121) using scaling with ranked subsampling (Bec et al. 2005).

Chloroplast ASVs were mapped to the PhytoRef database (Decelle et al. 2015) to identify the corresponding species. ASVs affiliating with species classified with a diameter of <2 µm were identified as PPE. For the phylogenetic analysis for SYN, only ASVs that represented >1% of the total SYN reads were included. All ASVs were first aligned using ClustalW and later included in a maximum likelihood method in MEGAX software (Kumar et al. 2018) following the GTamura-Nei model (bootstrap values inferred from 1000 replicates). The differences in community composition between treatments and experiments were analyzed by applying a permutational multivariate analysis of variance (PERMANOVA) comparing the community composition of dilution treatments with control treatments after 24 h. All statistical analyses were performed using R v.3.6.1 (R Core Team 2019) and the 'vegan' package (Oksanen et al. 2020).

2.4. Calculation of SYN and PPE growth and mortality rates

SYN and PPE net growth rates (μ_{env}, d^{-1}) were calculated according to Eq. (1):

$$\mu_{env} = \frac{\ln(N_{t2}/N_{t1})}{t2-t1} \quad (1)$$

where N_{t1} refers to the starting cell abundance (cells ml^{-1}), N_{t2} refers to the final cell abundance in the experiment (cells ml^{-1}) and t refers to the number of experimental days. Specific growth rates (μ, d^{-1}) were taken as the y-intercept, while the grazing (m_g, d^{-1}) and viral (m_v, d^{-1}) rates were derived from the slope from a simplified linear regression of μ_{env} versus dilution (Liu et al. 1995). The slope from the 0.2 µm dilution series regression equation represented m_g, while the slope of the 100 kDa dilution series represented $m_g + m_v$.

The significance of each regression was determined to check whether the slopes were significantly different from 0 ($p = 0.05$). If the regression of the 0.2 µm dilution series was not significant, $m_g = 0$. Furthermore, an F-test was employed to assess the significance of the differences between the regressions obtained within each experiment ($p = 0.05$). The calculations of m_v were based on the criteria specified in Staniewski & Short (2018):

(1) if both regressions were significant and significantly different from each other: $m_v = (m_g + m_v) - m_g$;
(2) if both regressions were significant but not significantly different from each other: $m_v = 0$;
(3) if both regressions were not significant: $m_v = 0$;
(4) if m_g was significant but $m_g + m_v$ was not: $m_v = -m_g$;
(5) if $m_g + m_v$ was significant but m_g was not: $m_v = m_g + m_v$.

Additionally, a power analysis was performed to test the sensitivity of the modified dilution approach to detect viral mortality (Kimmance et al. 2007), using the R package 'pwr' (Champley 2020). The power analysis was calculated at a significance level of 0.05 and 2 tails while Cohen's D (d) was calculated with Eq. (2):

$$d = \frac{\Delta b}{\sqrt{\frac{(\sigma^2_{m_g} + \sigma^2_{m_v + m_g}}{2}}} \quad (2)$$

where Δb is the difference between the slopes of the regressions of the 2 dilution series and $\sigma_{m_v+m_g}$ and σ_{m_g} are the standard deviation of the 100 kDa and 0.2 µm series, respectively. If the power was <80%, m_v was considered not determined (n.d.) since it was interpreted that the experiment lacked the sensitivity to detect the viral effect.

The carbon biomass (B, µg C l^{-1}) of PPE and SYN at the beginning of the experiment was calculated according to Eq. (3):

$$B = C \times N_{t1} \quad (3)$$

where C is the estimated cell carbon content of SYN and PPE (µg C $cell^{-1}$). There are several C values for SYN and PPE available in literature (Buitenhuis et al. 2012); in this study, we included all C values available for SYN and PPE from a literature compilation of conversion factors (Alegria Zufia et al. 2021), obtaining one B value for each C.

Production rates (P, µg C l^{-1} d^{-1}) for SYN and PPE were calculated according to Eq. (4):

$$P = B - 1 \quad (4)$$

The carbon transfer rate (F, µg C l^{-1} d^{-1}) was calculated according to Eq. (5):

$$F = B^{m_{g/v}} - 1 \quad (5)$$

Since a different value for B was calculated for each C, corresponding P and F values were calculated for each B.

3. RESULTS

3.1. Nutrients, chl *a*, and phytoplankton community (>5 µm)

The temperature during the 24 h *in situ* incubations ranged from 20.3–23.3°C during Grazing 1 and 10.7–16.0°C during Grazing 2 (Table 1, Fig. S3). Salinity

Table 1. Initial conditions of the Grazing 1 and Grazing 2 experiments. Temperature and salinity were measured *in situ*. NO_3+NO_2, PO_4, SiO_2, chl *a*, phytoplankton, photosynthetic picoeukaryotes (PPE), picocyanobacteria (SYN), nanoflagellates, and ciliates were measured from the experimental bottles (data are presented as mean ± SD)

Date	Grazing 1 10 August 2020	Grazing 2 14 September 2020
Temperature (°C)	21.28 ± 0.69	11.48 ± 0.43
Salinity (PSU)	7.15	7.15
NO_3+NO_2 (µM)	0.35 ± 0.04	0.51 ± 0.12
PO_4 (µM)	0.38 ± 0.04	0.38 ± 0.01
SiO_2 (µM)	9.60 ± 0.67	10.2 ± 0.92
Chl *a* (µg l^{-1})	3.3 ± 0.3	1.9 ± 0.0
Phytoplankton (mg C m^{-3})	219.0 ± 109	8.65 ± 9.01
SYN (cells ml^{-1})	1.1×10^5	2.6×10^5
PPE (cells ml^{-1})	3.0×10^4	1.9×10^4
Nanoflagellates (cells l^{-1})	$1.1 \times 10^7 \pm 1.3 \times 10^6$	$5.3 \times 10^6 \pm 8.1 \times 10^5$
Ciliates (cells ml^{-1})	$3.2 \times 10^4 \pm 1.4 \times 10^4$	$4.4 \times 10^3 \pm 4.8 \times 10^4$

was the same in both experiments (7.15 PSU; Table 1). Initial nutrient concentrations were similar for PO_4 (0.38 µM for Grazing 1 and 0.38 µM for Grazing 2) and SiO_2 (9.6 µM for Grazing 1 and 10.2 µM for Grazing 2) but NO_3+NO_2 was higher in Grazing 2 (0.51 µM) than Grazing 1 (0.35 µM; Table 1). During Grazing 1, daylight was ~3 h longer and light intensity was higher than during Grazing 2 (Fig. S3).

The initial concentration of chl *a* in Grazing 1 was higher than in Grazing 2 (3.3 and 1.9 µg l^{-1} respectively; Table 1). After 24 h, the dilution gradient remained in the 50 and 90 treatments, measuring ~50 and ~10% of the chl *a* concentration compared to that of W (Fig. S4). Phytoplankton biomass (>5 µm diameter) had an initial concentration of 219 µg C l^{-1} in Grazing 1, and the phytoplankton community was dominated by cyanobacteria (55%; of which 92% were filamentous cyanobacteria) followed by ciliates (36%; Fig. S4). Over the 24 h incubation, the contribution of ciliates increased slightly in both the W and dilution treatments (50 and 90) and became the larger contributor to phytoplankton biomass. In Grazing 2, the initial phytoplankton biomass was 8.7 µg C l^{-1} and was dominated by ciliates (62%) followed by cyanobacteria (10%; Fig. S4). Dinoflagellates, Euglenophyta, Crysophyta, Chlorophyta, and Cryptophyta were also present in higher proportion than in Grazing 1 and showed various responses in the different treatments over the 24 h incubation. Nanoflagellates had an initial concentration of 1.1×10^4 cells ml^{-1} in Grazing 1 and 5.3×10^4 cells ml^{-1} in Grazing 2 (Table 1). Ciliates had an initial concentration of 3.2×10^4 cells ml^{-1} in Grazing 1 and 4.4×10^4 cells ml^{-1} in Grazing 2 (Table 1).

3.2. Picophytoplankton abundance and community composition

Picophytoplankton were counted as 3 populations: PPE, PE-rich SYN and PC-rich SYN. PC-rich SYN were not observed in either of the experiments. The initial SYN cell abundance was lower in Grazing 1 than in Grazing 2 at 1.1×10^5 and 2.6×10^5 cells ml^{-1}, respectively (Table 1). PPE cell abundances were similar for both experiments at 3.0×10^4 and 2.7×10^4 cells ml^{-1}, respectively (Table 1). In Grazing 1, initial PPE biomass was higher than SYN (SYN median value: 19.7 µg C l^{-1}, PPE median value: 29.2 µg C l^{-1}), while in Grazing 2, initial SYN biomass was higher than PPE (SYN median value: 44.3 µg C l^{-1}, PPE median value: 26.7 µg C l^{-1}; Fig. S5).

The analysis of the 16S rRNA gene libraries composition revealed that the Shannon diversity index remained constant throughout Grazing 1 and Grazing 2, confirming that the dilution did not affect diversity (Fig. S6). At the start of Grazing 1, the bacterial community was dominated by cyanobacteria (42%) and proteobacteria (17%). After 24 h, cyanobacteria decreased while the proteobacteria contribution increased and dominated the community. At the start of Grazing 2, the bacterial community was dominated by actinobacteria (39%) and proteobacteria (23%; Fig. S4). The bacterial community remained stable after 24 h with the exception of 90-V, where actinobacteria contribution decreased and proteobacteria increased.

SYN sequences had an average relative contribution of 3.2 and 4.4% to the total bacterial community at the start of Grazing 1 and Grazing 2, respectively. The phylogenetic analysis showed that the SYN community was formed by S5.1, S5.2/B (S5.2 and clade B could not be separated by the analysis), and clade A (Fig. S7). Observations on the data set filtered for SYN sequences showed that the starting SYN community composition differed between experiments. In Grazing 1, the SYN community was dominated by S5.2/B, followed by clade A (Fig. 2A). In Grazing 2, the starting community was dominated by clade S5.2/B, followed by A and S5.1. In Grazing 1, PERMANOVA showed significant differences in the community composition among treatments. After 24 h in the 90 treatment, S5.2/B had a mean 14% higher contribution, and clade A and S5.1 had a 40 and 27% lower contribution, respectively compared to the W treatment (Table 2, Fig. 2A). No significant differences were observed in Grazing 2.

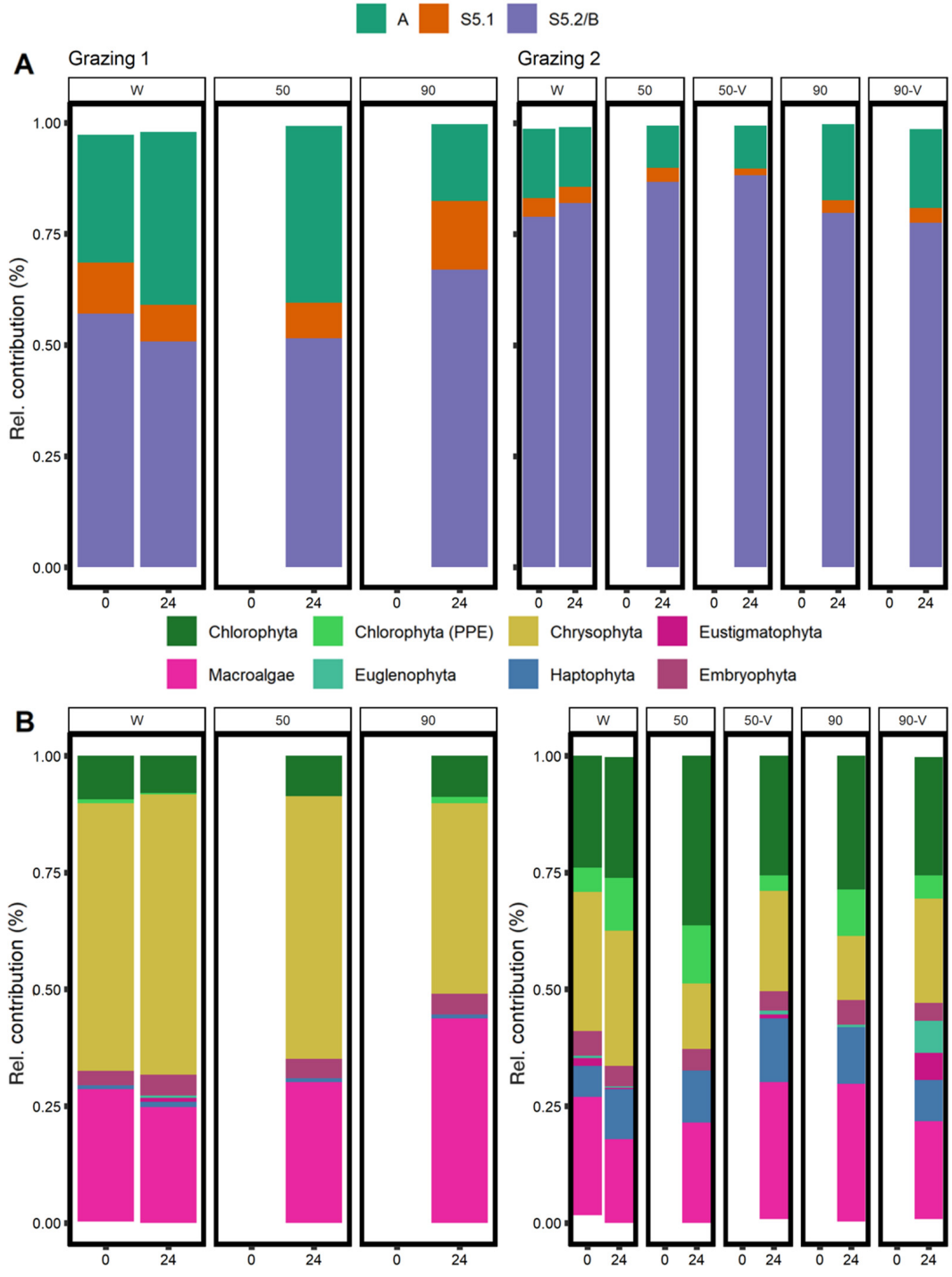

Fig. 2. (A) Picocyanobacterial (SYN) clade composition based on 16S rRNA gene V3–V4 amplicon sequencing in Grazing 1 and Grazing 2 and (B) chloroplast sequences in Grazing 1 and Grazing 2 at the initial time point (0) and after 24 h *in situ* incubation. Treatments consisted of a seawater control treatment (W), seawater diluted to 50% (50) and 90% (90) with grazer-free water in Grazing 1 and Grazing 2. Additionally, Grazing 2 involved an extra set of dilutions to 50% (50-V) and 90% (90-V) with virus-free water

Chloroplast sequences had an average relative contribution of 3.4 and 3.7% to the total 16S rRNA gene amplicon sequencing libraries at the start of Grazing 1 and Grazing 2, respectively. At the beginning of the experiments, Chrysophyta dominated the chloroplast sequences in both Grazing 1 (relative contribution 57%; Fig. 2B) and Grazing 2 (relative contribution 29%; Fig. 2B). A total of 19 ASVs affiliating with PPEs

Table 2. PERMANOVA analysis comparing the 16S rRNA gene libraries of picocyanobacteria (SYN) and chloroplasts within treatments and time points for the Grazing 1 and Grazing 2 experiments. SS: sum of squares; MS: mean squares; *p < 0.05

SYN	df	SS	MS	*F* model	R^2	Pr(>*F*)
Grazing 1						
Treatment	2	0.115	0.057	8.244	0.767	0.018*
Residuals	5	0.034	0.006		0.232	
Total	7	0.149			1	
Grazing 2						
Treatment	4	0.108	0.027	0.807	0.287	0.655
Residuals	8	0.267	0.033		0.712	
Total	12	0.375			1	

were identified as chlorophytes of the Phylum Mamiellophyta (Table 3). The chloroplast identification provides an approximation of the PPE community; however, it does not provide a complete list of the PPE community and thus no statistical analysis was performed on them.

3.2. Mortality, production, and carbon transfer rates

Grazing rates on SYN (Grazing 1: −1.1 d^{-1}, $F_{1,7}$ = 45.48, p < 0.001; Grazing 2: −0.24 d^{-1}, $F_{1,7}$ = 8.05, p < 0.025) were equal to the growth rates in both experiments (Grazing 1: 1.1 d^{-1}; Grazing 2: 0.24 d^{-1}; Table 4, Fig. 3A,C). Grazing rates on PPE (Grazing 1: −0.62 d^{-1}, $F_{1,7}$ = 73.74, p < 0.001; Grazing 2: −0.31 d^{-1}, $F_{1,7}$ = 5.72, p < 0.048) exceeded the growth rates in both experiments (Grazing 1: 0.49 d^{-1}, Grazing 2: 0.025 d^{-1}; Table 4, Fig. 3B,D). The regression lines in the 100 kDa dilution series in Grazing 2 were not significant for either SYN or PPE (SYN: $F_{1,7}$ = 1, p = 0.15; PPE: $F_{1,7}$ = 0.001, p = 0.97) and were not significantly different from the 0.2 µm dilution series (SYN: $F_{1,14}$ = 1.69, p = 0.21; PPE: $F_{1,14}$ = 1.18, p = 0.58); hence, $m_v = -m_g$ in both SYN (m_v = 0.24 d^{-1}; Table 4) and PPE (m_v = 0.31 d^{-1}; Table 4). The power analysis indicated that the experiment had enough sensitivity to determine m_v in SYN ($\sigma_{m_v+m_g}$ = 0.82, σ_{m_g} = 0.09, power = 94%); however, it lacked the sensitivity to determine m_v in PPE ($\sigma_{m_v+m_g}$ = 0.60, σ_{m_g} = 0.13, power = 32%); hence, m_v in PPE was treated as 'not determined' (Table 4).

The production and carbon transfer rates to higher trophic levels were calculated based on the growth and grazing rates. In Grazing 1, SYN had a higher median production than PPE (SYN median value: 25.6 µg C l^{-1} d^{-1}; PPE: 4.2 µg C l^{-1} d^{-1}), while in Grazing 2, SYN had higher production rate than PPE (SYN median value: 1.5 µg C l^{-1} d^{-1}; PPE: 0.1 µg C l^{-1} d^{-1}; Fig. S5). The low primary production rate for PPE was a result of the negative growth rate in Grazing 2. Carbon transfer rates were higher for SYN compared to PPE in Grazing 1 (median values, SYN: 25.6 µg C l^{-1} d^{-1}, PPE: 7.1 µg C l^{-1} d^{-1}), but similar in Grazing 2 (median values, SYN: 1.5 µg C l^{-1} d^{-1}, PPE: 1.8 µg C l^{-1} d^{-1}; Fig. 4).

Table 3. Amplicon sequence variants (ASVs) affiliated with photosynthetic picoeukaryotes (PPE) in the Grazing 1 and Grazing 2 experiments. Accession numbers (PhytoRef and NCBI), species identification, and % identity are also given along with the number of counts in each experiment

ASV ID	Acc. Number (PhytoRef)	Acc. Number (NCBI)	Species	ID (%)	Counts in Grazing 1	Counts in Grazing 2
ASV_01207	4604	FN563099	*Bathycoccus prasinos*	100	0	12
ASV_01799	4604	FN563099	*Bathycoccus prasinos*	100	2	0
ASV_00387	765		*Micromonas pusilla*	100	21	409
ASV_00469	4487	FN563097	*Micromonas pusilla*	100	0	295
ASV_00353	168		*Micromonas* sp.	100	98	443
ASV_01973	6245	NC_012575	*Micromonas* sp.	94.2	1	0
ASV_01374	672		*Ostreococcus lucimarinus*	98.6	0	7
ASV_01916	167	AY702161	*Ostreococcus* sp.	96.7	0	1
ASV_00320	683	AY702141	*Ostreococcus tauri*	100	0	412
ASV_00528	6244	NC_008289	*Ostreococcus tauri*	100	0	219
ASV_01103	6244	NC_008289	*Ostreococcus tauri*	99.8	0	17
ASV_00944	748		*Picochlorum* sp.	98.9	0	30
ASV_00312	32	AY702125	*Rhizochromulina* sp.	95.6	95	485
ASV_00427	32	AY702125	*Rhizochromulina* sp.	96.6	167	0
ASV_00464	32	AY702125	*Rhizochromulina* sp.	96.6	127	0
ASV_00577	32	AY702125	*Rhizochromulina* sp.	95.8	49	87
ASV_00695	32	AY702125	*Rhizochromulina* sp.	95.3	0	6

Table 4. Growth and mortality rates (d^{-1}) from the Grazing 1 and Grazing 2 experiments for picocyanobacteria (SYN) and photosynthetic picoeukaryotes (PPE). If the power was <80%, m_v was considered not determined (n.d.)

	SYN		PPE	
	Grazing 1	Grazing 2	Grazing 1	Grazing 2
Net growth rate (μ_{env})	0.12	0.026	−0.10	−0.24
Specific growth rate (μ)	1.1	0.24	0.49	0.025
Grazing mortality (m_g)	−1.1	−0.24	−0.62	−0.31
% of daily production grazed	100	100	126	1240
Viral mortality (m_v)	–	0.24	–	0.31 (n.d.)

4. DISCUSSION

The combination of the modified dilution approach (Evans et al. 2003) with a detailed analysis of community structure is a method that allows us to study the effect of grazing and viral mortality on specific taxa (Cram et al. 2016). This setup operates under the assumption that growth rates remain constant after the dilutions, allowing fast-growing organisms with high mortality rates to increase in abundance. In

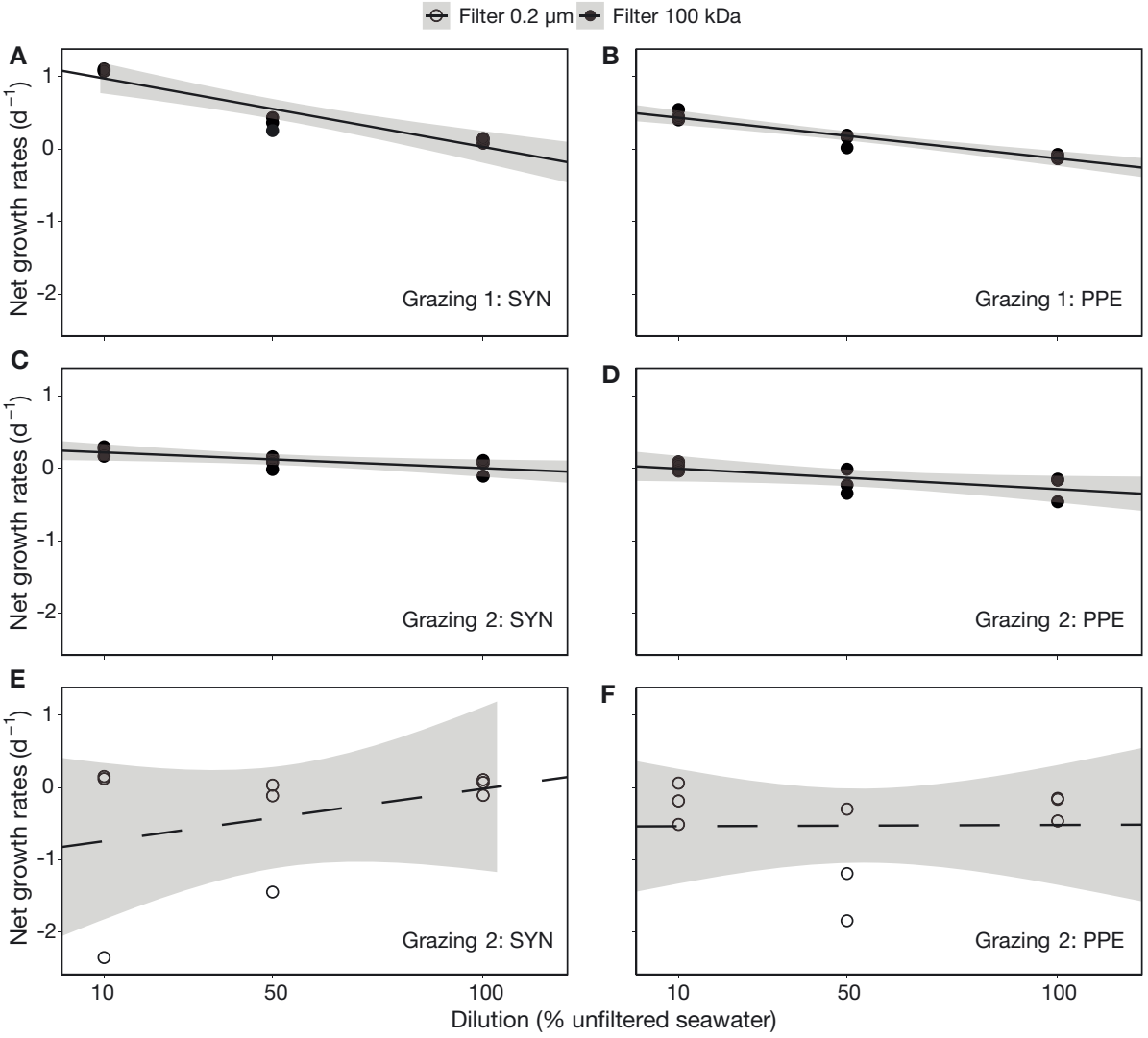

Fig. 3. Net growth rates versus 0.2 µm filter dilution series for (A) Grazing 1 picocyanobacteria (SYN) and (B) photosynthetic picoeukaryotes (PPE), Grazing 2 filter dilution series at 0.2 µm for (C) SYN and (D) PPE, and Grazing 2 filter dilution series at 100 kDa for (E) SYN and (F) PPE. Filled and unfilled circles: net growth rates in the grazer-free (0.2 µm filter) and virus-free (100 kDa filter) treatments, respectively. The linear regressions equations from Grazing 1 were: SYN 1.1 − 1.1x (grazer-free) and PPE 0.49 − 0.62x (grazer-free). The linear regression equations from Grazing 2 were: SYN 0.24 − 0.24x (grazer-free), −0.82 + 0.8x (virus-free) and PPE 0.025 − 0.31x (grazer-free), −0.54 + 0.02x (virus-free)

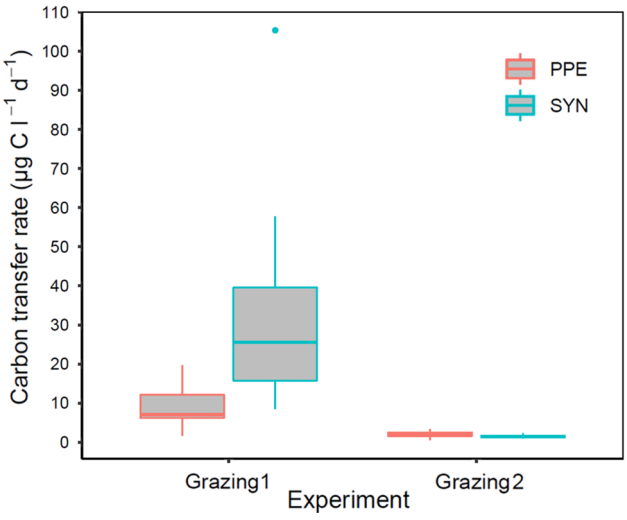

Fig. 4. Carbon transfer rates from each experiment (Grazing 1 and Grazing 2) for picocyanobacteria (SYN) and photosynthetic picoeukaryotes (PPE). Rates were calculated based on all the *C* values available for SYN and PPE from a literature compilation of conversion factors (Alegria Zufia et al. 2021). Boxplots represent median and 25–75% interquartile non-outlier range; points beyond are outliers

this study, 2 experiments (Grazing 1 and Grazing 2) were performed which represented bloom and post-bloom microbial communities in the Baltic Sea Proper. To mimic natural conditions, incubations were done using *in situ* incubation arrays. Grazing 1 exhibited higher temperatures and lower NO_3 concentrations than Grazing 2, displaying typical physiochemical characteristics of the respective seasons (Alegria Zufia et al. 2022, Fridolfsson et al. 2023). The experiments provide novel insights about the mortality pressure of different functional groups of picophytoplankton.

For SYN, equal levels of mortality (grazing and viral lysis) and growth rates were observed, suggesting a tight coupling between the processes. Grazing may stabilize the accumulation of SYN in temperate (Hunter-Cevera et al. 2020) and sub-tropical regions (Tsai et al. 2008). Thus, we hypothesize that grazing is an important factor for keeping SYN abundances at ~10^5 cells ml^{-1} during the summer–autumn period in the Baltic Sea (June–October; Fig. S3) (Kuosa 1991, Mazur-Marzec et al. 2013, Alegria Zufia et al. 2021). Such dynamics are common in other high-latitude locations, where phytoplankton peak abundances are determined by a trade-off between temperature, nutrient availability, and the time required for grazers to reach critical biomass where grazing rates equal growth (Landry et al. 2002, Verity et al. 2002, Schmoker et al. 2013). In the Baltic Sea Proper, time series observations and nutrient bioassay experiments revealed that SYN growth is strongly limited by nitrogen before reaching peak abundances during the summer bloom (Alegria Zufia et al. 2021). Further experiments using a modified dilution approach during the early stages of the summer bloom could offer relevant information about the role of grazers in controlling environmental SYN abundances.

Grazing rates on PPE were higher than growth rates, which indicates that grazing could be a factor determining PPE bloom decline (Kimmance et al. 2007, Fowler et al. 2020). Previous reports in the Baltic Sea during mid-summer showed that SYN was more intensively grazed than PPE (Reckermann 1996). However, during the time of the experiments, a higher proportion of PPE production than SYN production was lost to grazing, in line with most observations in marine environments (e.g. Samuelsson & Andersson 2003, Worden et al. 2004). The results of this study are among the few reports of the largely unknown PPE composition and dynamics in the Baltic Sea, despite its ecological relevance in productive coastal areas (Kuosa 1991, Tamm et al. 2018, Alegria Zufia et al. 2021, 2022).

Median carbon transfer rates of the total picophytoplankton community (SYN and PPE) (Grazing 1: 32.6 µg C l^{-1} d^{-1}; Grazing 2: 3.2 µg C l^{-1} d^{-1}) were in a similar range as a study in the Gulf of Riga based on tritiated thymidine incorporation experiments (summer: 35.1 µg C l^{-1} d^{-1}; autumn: 14.6 µg C l^{-1} d^{-1}; Donali et al. 1999). In that study, picophytoplankton had a large contribution to the total primary production: 65% during summer and 44% during autumn. These results suggest that picophytoplankton carbon transfer rates during late summer are also important in the Baltic Sea Proper. Furthermore, the experimental results showed that carbon transfer rates were higher for SYN despite PPE's dominance in terms of biomass and production in Grazing 1. This indicates that SYN, although it is very small and is considered a low-quality food (Christaki et al. 2002, Apple et al. 2011), is a significant contributor to carbon cycling as previously indicated by (Kuosa 1991).

Observations on viral mortality rates generally range between −0.1 and 0.1 d^{-1} (Staniewski & Short 2018). Moreover, the methodological limitations associated with the modified dilution approach have led authors to conclude that this method lacks the power to consistently detect mortality rates of <0.1 d^{-1} (Kimmance et al. 2007, Staniewski & Short 2018). In this study, $m_v > 0$ was observed for SYN (0.24 d^{-1}), but the experiment lacked the sensitivity to detect the viral effect for PPE (Kimmance et al. 2007). Future

studies might consider increasing the dilution of the treatments to increase the power to detect viral mortality rates (Staniewski & Short 2018). Dilution approaches with higher dilution levels have the advantage that they can accurately detect non-linear responses. However, a recent study indicated that 2-point dilution, as used in this study, and 4- to 5-point dilution methods result in similar rate estimates for growth and mortality (Morison & Menden-Deuer 2017). It should also be taken into account that the present study represents a single measurement that took place during late summer, whereas studies that included experiments across different seasons showed that viral mortality was more prevalent during cold seasons (Wells & Deming 2006, Tijdens et al. 2008). Therefore, it would be necessary to perform repeated studies across different seasons to understand the role of viruses in picophytoplankton mortality.

The Baltic Sea is warming rapidly (Neumann et al. 2012, Meier et al. 2019). Warmer temperatures are expected to decrease overall phytoplankton biomass while increasing picophytoplankton biomass (Bopp et al. 2013, Cabré et al. 2015, Legrand et al. 2015). Time-series observations in other environments indicate that SYN has a competitive advantage over PPE under climate change conditions (Schmidt et al. 2020), thus higher contributions of SYN to the carbon transfer pathways could be expected. Microzooplankton (flagellates and ciliates) are considered the main grazers of SYN, and different SYN strains can significantly affect their growth efficiency (Apple et al. 2011). In Grazing 1, the significantly lower contribution of clade A in the 90% dilution compared to the control could indicate that this clade is selectively grazed, while the higher contribution of clade 5.2/B in the same treatment could indicate higher grazing resistance by this clade. Clades A and B have been observed to dominate SYN communities in the Baltic Sea Proper, particularly during the autumn to spring period in the Baltic Sea (Alegria Zufia et al. 2022). Thus, grazing may play an important role in SYN community structure and dynamics. Recent observations in the Baltic Sea revealed that SYN may also be an important food source for mesozooplankton during summer, and that the preference towards SYN can be different among mesozooplankton species (Motwani & Gorokhova 2013, Novotny et al. 2021). However, SYN lacks elemental biomolecules necessary for mesozooplankton growth (Patil et al. 2007, Jónasdóttir 2019, Ruess & Müller-Navarra 2019). Thus, to make accurate assessments on the fate of the energy transfer through the trophic chain under climate change conditions, we first would need to understand how the growth of different species of grazers is affected by an increase of SYN abundance over PPE.

In summary, this study shows that grazing is an important control for picophytoplankton during the late summer in the Baltic Sea. SYN mortality was tightly coupled with growth while PPE grazing effectively reduced its biomass. In the case of SYN, results indicate that grazing may have an important role in SYN community composition and seasonality. Since SYN abundance and community composition undergo strong seasonal changes, similar studies should be conducted over longer timescales to further to understand the relationship between grazing and viral mortality with SYN seasonal dynamics. Picophytoplankton grazing also showed high contributions to the carbon pool relative to the rest of the phytoplankton community, potentially situating this size fraction as one of the most important components of the phytoplankton community during summer.

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LITERATURE CITED

Aberle N, Lengfellner K, Sommer U (2007) Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. Oecologia 150:668–681

Agawin NSR, Duarte CM, Agustí S (1998) Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: seasonality and relationship with temperature. Mar Ecol Prog Ser 170:45–53

Albertano P, Di Somma D, Capucci E (1997) Cyanobacterial picoplankton from the central Baltic Sea: cell size classification by image analyzed fluorescence microscopy. J Plankton Res 19:1405–1416

Alegria Zufia J, Farnelid H, Legrand C (2021) Seasonality of coastal picophytoplankton growth, nutrient limitation and biomass contribution. Front Microbiol 12:786590

Alegria Zufia J, Legrand C, Farnelid H (2022) Seasonal dynamics in picocyanobacterial abundance and clade composition at coastal and offshore stations in the Baltic Sea. Sci Rep 12:14330

Apple JK, Strom SL, Palenik B, Brahamsha B (2011) Variability in protist grazing and growth on different marine *Synechococcus* isolates. Appl Environ Microbiol 77: 3074–3084

Baudoux AC, Veldhuis MJW, Noordeloos AAM, Van Noort G, Brussaard CPD (2008) Estimates of virus- vs. grazing induced mortality of picophytoplankton in the North Sea during summer. Aquat Microb Ecol 52:69–82

Bec B, Husseini-Ratrema J, Collos Y, Souchu P, Vaquer A (2005) Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: emphasis on the picoeukaryote community. J Plankton Res 27:881–894

Bertos-Fortis M, Farnelid HM, Lindh MV, Casini M, Andersson A, Pinhassi J, Legrand C (2016) Unscrambling *Cyanobacteria* community dynamics related to environmental factors. Front Microbiol 7:625

Blanc-Mathieu R, Krasovec M, Hebrard M, Yau S and others (2017) Population genomics of picophytoplankton unveils novel chromosome hypervariability. Sci Adv 3:e1700239

Bopp L, Resplandy L, Orr JC, Doney SC and others (2013) Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosciences 10: 6225–6245

Buitenhuis ET, Li WKW, Vaulot D, Lomas MW and others (2012) Picophytoplankton biomass distribution in the global ocean. Earth Syst Sci Data 4:37–46

Cabré A, Marinov I, Leung S (2015) Consistent global responses of marine ecosystems to future climate change across the IPCC AR5 earth system models. Clim Dyn 45: 1253–1280

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol Oceanogr 49:51–57

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13: 581–583

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Capuzzo E, Lynam CP, Barry J, Stephens D and others (2017) A decline in primary production in the North Sea over 25 years, associated with reductions in zooplankton abundance and fish stock recruitment. Glob Chang Biol 24: e352–e364

Caroppo C (2015) Ecology and biodiversity of picoplanktonic cyanobacteria in coastal and brackish environments. Biodivers Conserv 24:949–971

Champley S (2020) pwr: basic functions for power analysis. R package version 1.3-0. https://github.com/heliosdrm/pwr

Christaki U, Jacquet S, Dolan JR, Vaulot D, Rassoulzadegan F (1999) Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnol Oceanogr 44: 52–61

Christaki U, Courties C, Karayanni H, Giannakourou A, Maravelias C, Kormas KA, Lebaron P (2002) Dynamic characteristics of *Prochlorococcus* and *Synechococcus* consumption by bacterivorous nanoflagellates. Microb Ecol 43:341–352

Christaki U, Courties C, Massana R, Catala P, Lebaron P, Gasol JM, Zubkov MV (2011) Optimized routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I. Limnol Oceanogr Methods 9: 329–339

Cottrell MT, Suttle CA (1995) Dynamics of lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla*. Limnol Oceanogr 40:730–739

Cram JA, Parada AE, Fuhrman JA (2016) Dilution reveals how viral lysis and grazing shape microbial communities. Limnol Oceanogr 61:889–905

Decelle J, Romac S, Stern RF, Bendif EM and others (2015) PhytoREF: a reference database of the plastidial 16S rRNA gene of photosynthetic eukaryotes with curated taxonomy. Mol Ecol Resour 15:1435–1445

Dolan JR, Šimek K (1998) Ingestion and digestion of an autotrophic picoplankter, *Synechococcus*, by a heterotrophic nanoflagellate, *Bodo saltans*. Limnol Oceanogr 43: 1740–1746

Dolan JR, Šimek K (1999) Diel periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: analysis of food vacuole contents. Limnol Oceanogr 44:1565–1570

Donali E, Olli K, Heiskanen AS, Andersen T (1999) Carbon flow patterns in the planktonic food web of the Gulf of Riga, the Baltic Sea: a reconstruction by the inverse method. J Mar Syst 23:251–268

Durand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic time-series station in the Sargasso Sea. Deep Sea Res II 48: 1983–2003

Edler L (1979) Recommendations on methods for marine biological studies in the Baltic Sea: phytoplankton and chlorophyll. Balt Mar Biol 5:1–38

Evans C, Archer SD, Jacquet S, Wilson WH (2003) Direct estimates of the contribution of viral lysis. Aquat Microb Ecol 30:207–219

Fowler BL, Neubert MG, Hunter-Cevera KR, Olson RJ, Shalapyonok A, Solow AR, Sosik HM (2020) Dynamics and functional diversity of the smallest phytoplankton on the Northeast US Shelf. Proc Natl Acad Sci USA 117:12221

Fridolfsson E, Bunse C, Lindehoff E, Farnelid H and others (2023) Multiyear analysis uncovers coordinated seasonality in stocks and composition of the planktonic food web in the Baltic Sea Proper. Sci Rep 13:11865

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548

Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis, 2nd edn. Wiley Verlag, Berlin

Grinienė E, Šulčius S, Kuosa H (2016) Size-selective microzooplankton grazing on the phytoplankton in the Curonian Lagoon (SE Baltic Sea). Oceanologia 58:292–301

Hadas O, Malinsky-Rushansky N, Pinkas R, Cappenberg TE (1998) Grazing on autotrophic and heterotrophic picoplankton by ciliates isolated from Lake Kinneret, Israel. J Plankton Res 20:1435–1448

Haverkamp THA, Schouten D, Doeleman M, Wollenzien U, Huisman J, Stal LJ (2009) Colorful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea. ISME J 3:397–408

HELCOM Phytoplankton Expert Group (2013) Phytoplankton biovolume and carbon content. Helsinki Commission

Herlemann DPR, Labrenz M, Jürgens K, Bertilsson S, Wan-

iek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5:1571–1579

Hu YOO, Karlson B, Charvet S, Andersson AF (2016) Diversity of pico- to mesoplankton along the 2000 km salinity gradient of the Baltic Sea. Front Microbiol 7:679

Hunter-Cevera KR, Neubert MG, Olson RJ, Shalapyonok A, Solow AR, Sosik HM (2020) Seasons of *Syn*. Limnol Oceanogr 65:1085–1102

Jespersen AM, Christoffersen K (1987) Measurements of chlorophyll-a from phytoplankton using ethanol as extraction solvent. Arch Hydrobiol 109:445–454

Jónasdóttir SH (2019) Fatty acid profiles and production in marine phytoplankton. Mar Drugs 17:151

Kimmance SA, Wilson WH, Archer SD (2007) Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: limitations associated with method sensitivity in natural waters. Aquat Microb Ecol 49: 207–222

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Kuosa H (1991) Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. Mar Ecol Prog Ser 73:269–276

Landry MR, Calbet A (2004) Microzooplankton production in the oceans. ICES J Mar Sci 61:501–507

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. Mar Biol 67:283–288

Landry MR, Selph KE, Brown SL, Abbott MR and others (2002) Seasonal dynamics of phytoplankton in the Antarctic Polar Front region at 170° W. Deep Sea Res II 49: 1843–1865

Larsson J, Celepli N, Ininbergs K, Dupont CL, Yooseph S, Bergman B, Ekman M (2014) Picocyanobacteria containing a novel pigment gene cluster dominate the brackish water Baltic Sea. ISME J 8:1892–1903

Legrand C, Fridolfsson E, Bertos-Fortis M, Lindehoff E, Larsson P, Pinhassi J, Andersson A (2015) Interannual variability of phyto-bacterioplankton biomass and production in coastal and offshore waters of the Baltic Sea. Ambio 44:427–438

Li J, Chen Z, Jing Z, Zhou L and others (2019) *Synechococcus* bloom in the Pearl River Estuary and adjacent coastal area—with special focus on flooding during wet seasons. Sci Total Environ 692:769–783

Liu H, Campbell L, Landry MR (1995) Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. Mar Ecol Prog Ser 116:277–287

Mattsson L, Sörenson E, Capo E, Farnelid HM and others (2021) Functional diversity facilitates stability under environmental changes in an outdoor microalgal cultivation system. Front Bioeng Biotechnol 9:651895

Mazur-Marzec H, Sutryk K, Kobos J, Hebel A and others (2013) Occurrence of cyanobacteria and cyanotoxin in the Southern Baltic Proper. Filamentous cyanobacteria versus single-celled picocyanobacteria. Hydrobiologia 701:235–252

Meier HEM, Dieterich C, Eilola K, Gröger M and others (2019) Future projections of record-breaking sea surface temperature and cyanobacteria bloom events in the Baltic Sea. Ambio 48:1362–1376

Morison F, Menden-Deuer S (2017) Doing more with less? Balancing sampling resolution and effort in measurements of protistan growth and grazing-rates. Limnol Oceanogr Methods 15:794–809

Mostböck S (2021) FCSalyzer. https://sourceforge.net/projects/fcsalyzer

Motwani NH, Gorokhova E (2013) Mesozooplankton grazing on picocyanobacteria in the Baltic Sea as inferred from molecular diet analysis. PLOS ONE 8:e79230

Neumann T, Eilola K, Gustafsson B, Müller-Karulis B, Kuznetsov I, Meier HEM, Savchuk OP (2012) Extremes of temperature, oxygen and blooms in the Baltic Sea in a changing climate. Ambio 41:574–585

Novotny A, Zamora-Terol S, Winder M (2021) DNA metabarcoding reveals trophic niche diversity of micro and mesozooplankton species. Proc R Soc B 288:20210908

Oksanen J, Blanchet FG, Friendly M, Kindt R and others (2020) vegan: community ecology package. R package version 2.5-7. https://CRAN.R-project.org/web/packages/vegan/vegan.pdf

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol Mol Biol Rev 63:106–127

Pasulka AL, Samo TJ, Landry MR (2015) Grazer and viral impacts on microbial growth and mortality in the southern California Current Ecosystem. J Plank Res 37:320–336

Patil V, Källqvist T, Olsen E, Vogt G, Gislerød HR (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacult Int 15:1–9

Pena MA, Lewis MR, Harrison WG (1990) Primary productivity and size structure of phytoplankton biomass on a transect of the equator at 135° W in the Pacific Ocean. Deep-Sea Res A, Oceanogr Res Pap 37:295–315

Pittera J, Humily F, Thorel M, Grulois D, Garczarek L, Six C (2014) Connecting thermal physiology and latitudinal niche partitioning in marine *Synechococcus*. ISME J 8: 1221–1236

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reckermann M (1996) Ultraphytoplankton and protozoan communities and their interactions in different marine pelagic ecosystems (Arabian Sea and Baltic Sea). Mar Sci Rep No. 14. Leibniz-Institut für Ostseeforschung Warnemünde (Leibniz Institute for Baltic Sea Research), Rostock

Ruess L, Müller-Navarra DC (2019) Essential biomolecules in food webs. Front Ecol Evol 7:269

Samuelsson K, Andersson A (2003) Predation limitation in the pelagic microbial food web in an oligotrophic aquatic system. Aquat Microb Ecol 30:239–250

Schmidt K, Birchill AJ, Atkinson A, Brewin RJW and others (2020) Increasing picocyanobacteria success in shelf waters contributes to long-term food web degradation. Glob Change Biol 26:5574–5587

Schmoker C, Hernández-León S, Calbet A (2013) Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions. J Plankton Res 35: 691–706

Søndergaard M, Jensen LM, Ærtebjerg G (1991) Picoalgae in Danish coastal waters during summer stratification. Mar Ecol Prog Ser 79:139–149

Stal L, Albertano P, Bergman B, Von Bröckel K and others (2003) BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea — responses to a changing environment. Cont Shelf Res 23:1695–1714

Staniewski MA, Short SM (2018) Methodological review and meta-analysis of dilution assays for estimates of virus- and grazer-mediated phytoplankton mortality. Limnol Oceanogr Methods 16:649–668

Stoddard LI, Martiny JBH, Marston MF (2007) Selection and characterization of cyanophage resistance in marine *Synechococcus* strains. Appl Environ Microbiol 73:5516–5522

Suttle CA (2005) Viruses in the sea. Nature 437:356–361

Tamm M, Laas P, Freiberg R, Nõges P, Nõges T (2018) Parallel assessment of marine autotrophic picoplankton using flow cytometry and chemotaxonomy. Sci Total Environ 625:185–193

Tijdens M, Van De Waal DB, Slovackova H, Hoogveld HL, Gons HJ (2008) Estimates of bacterial and phytoplankton mortality caused by viral lysis and microzooplankton grazing in a shallow eutrophic lake. Freshw Biol 53: 1126–1141

Tsai AY, Chiang KP, Chang J, Gong GC (2008) Seasonal variations in trophic dynamics of nanoflagellates and picoplankton in coastal waters of the western subtropical Pacific Ocean. Aquat Microb Ecol 51:263–274

Tsai AY, Gong GC, Sanders RW, Chiang KP, Huang JK, Chan YF (2012) Viral lysis and nanoflagellate grazing as factors controlling diel variations of *Synechococcus* spp. summer abundance in coastal waters of Taiwan. Aquat Microb Ecol 66:159–167

Tsai AY, Gong GC, Hu SL, Chao CF (2015a) The effect of grazing and viral lysis on the diel variations of *Synechococcus* spp. abundance in the East China Sea. Estuar Coast Shelf Sci 163:108–115

Tsai AY, Gong GC, Huang YW, Chao CF (2015b) Estimates of bacterioplankton and *Synechococcus* spp. mortality from nanoflagellate grazing and viral lysis in the subtropical Danshui River estuary. Estuar Coast Shelf Sci 153: 54–61

Tsai AY, Gong GC, Chung CC, Huang YT (2018) Different impact of nanoflagellate grazing and viral lysis on *Synechococcus* spp. and picoeukaryotic mortality in coastal waters. Estuar Coast Shelf Sci 209:1–6

Utermöhl H (1958) Zur vervollkommnung der quantitativen phytoplanktonmethodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Verity PG, Wassmann P, Frischer ME, Howard-Jones MH, Allen AE (2002) Grazing of phytoplankton by microzooplankton in the Barents Sea during early summer. J Mar Syst 38:109–123

Wang K, Wommack KE, Chen F (2011) Abundance and distribution of *Synechococcus* spp. and cyanophages in the Chesapeake Bay. Appl Environ Microbiol 77:7459–7468

Wells LE, Deming JW (2006) Significance of bacterivory and viral lysis in bottom waters of Franklin Bay, Canadian Arctic, during winter. Aquat Microb Ecol 43:209–221

Worden AZ, Nolan JK, Palenik B (2004) Assessing the dynamics and ecology of marine picophytoplankton: the importance of the eukaryotic component. Limnol Oceanogr 49:168–179

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