Seasonality of Coastal Picophytoplankton Growth, Nutrient Limitation and Biomass Contribution

Javier Alegria Zufia

Linnaeus University

Hanna Farnelid

Linnaeus University

Catherine Legrand (✉ catherine.legrand@lnu.se)

Linnaeus University

Research Article

Keywords: Picophytoplankton, Baltic Sea, photosynthetic picoeukaryotes (PPE), bioassays, coastal Baltic Sea, Kalmar Sound (K-Station)

Posted Date: April 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-404758/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Picophytoplankton in the Baltic Sea includes picocyanobacteria (*Synechococcus/Cyanobium*) and photosynthetic picoeukaryotes (PPE). Picophytoplankton are thought to be a key component of the phytoplankton community but their seasonal dynamics and relationships with nutrients and temperature are largely unknown. We monitored pico- and larger phytoplankton at a coastal site in Kalmar Sound (K-Station) weekly during 2018. Among the picocyanobacteria, phycoerythrin-rich *Synechococcus* (PE-rich) dominated in spring and summer while phycocyanin-rich *Synechococcus* (PC-rich) dominated during autumn. PE-rich and PC-rich abundances peaked during summer (1.1×10^5 and 2.0×10^5 cells mL⁻¹) while PPE reached highest abundances in spring (1.1×10^5 cells mL⁻¹). PPE was the main contributor to the total phytoplankton biomass (3–73%). To assess nutrient limitation, bioassays with combinations of nitrogen (NO₃ or NH₄) and phosphorus additions were performed. PE-rich and PC-rich growth was mainly limited by nitrogen, with a preference for NH₄ at 15–19°C. The three groups had distinct seasonal dynamics and optimal temperatures for growth were 10°C and 17–19°C for PE-rich, 13–16°C for PC-rich and 11–15°C for PPE. We conclude that picophytoplankton contribute significantly to the carbon cycle in the coastal Baltic Sea and underscore the importance of investigating functional groups to assess the consequences of the combination of high temperature and NH₄ in a future climate.

Introduction

Marine picophytoplankton (here defined as autotrophic cells < 2 µm in diameter) is a diverse group consisting of picocyanobacteria (*Prochlorococcus*, *Synechococcus* and *Cyanobium*), and photosynthetic picoeukaryotes (PPE). In oligotrophic systems, they account for more than 50% of the total chlorophyll *a* (Chl *a*)^{1–3} and can be responsible for most of the primary production^{4–6}. Their success in low nutrient environments is mainly explained by their small size, which provides them with a high surface to volume ratio, resulting in a competitive advantage for nutrient uptake compared to larger phytoplankton cells^{7,8}. Picophytoplankton are similarly abundant also in eutrophic waters⁹, both in coastal^{10,11} and estuarine systems^{12,13}, suggesting that they have a significant role in carbon cycling and ecosystem functions. Understanding which factors control picophytoplankton biomass is important for determining their role in the marine food web.

In the Baltic Sea, an estuary-like semi enclosed large brackish water body, picophytoplankton is a key component of the phytoplankton community and its contribution to Chl *a*, as a proxy for biomass, ranges from 15 to 85%^{14–18}. Baltic Sea picophytoplankton consists of the picocyanobacteria *Synechococcus* and *Cyanobium* and PPE. *Cyanobium* is a genera closely related to *Synechococcus* that has been typically associated with freshwater^{19,20}. For clarity, hereafter we will refer to Baltic Sea picocyanobacteria as *Synechococcus*. The large contribution and diversity of *Synechococcus* in the Baltic Sea has been recently recognized^{15,21–24} but currently there is no systematic monitoring of *Synechococcus* in the Baltic Sea Proper. Coastal areas in the Baltic Sea are very dynamic and nutrient runoff has been related to increases of *Synechococcus* abundances²⁵ but abundance measurements in

coastal areas remain scarce. Likewise, the PPE community is thought to be diverse and important in coastal ecosystems but the distribution and abundance of PPE in the Baltic Sea is largely unknown^{17,26}. Although knowledge of the picophytoplankton in the Baltic Sea is increasing, information about the ecological role, community composition and distribution of picophytoplankton especially in coastal areas is lacking.

Synechococcus can be divided into two functional groups based on the presence of the two phycobiliprotein pigments phycoerythrin (PE) and phycocyanin (PC)^{17,27,28}. PE-rich *Synechococcus* (PE-rich) strains are adapted to blue and green light absorption, and PC-rich *Synechococcus* (PC-rich) strains adapted to red light absorption. Consequently, PE-rich are better adapted to low turbidity waters (generally open waters); meanwhile PC-rich are better adapted to turbid waters (generally coastal waters)^{27,29,30}. The Baltic Sea *Synechococcus* community is dominated by a unique PE pigment cluster^{17,31,32}. However, PC-rich has been observed to have similar contributions as PE-rich in areas with higher turbidity such as the Gulf of Finland, and some coastal areas^{27,33}. PE-rich and PC-rich *Synechococcus* frequently co-occur in estuarine environments and recent studies from diverse coastal areas suggest that physio-ecological adaptations to nutrients and temperature affects their distribution and seasonality^{13,29,34}.

Picophytoplankton seasonal dynamics is determined by temperature, light, nutrient concentration^{35–37} and biotic factors^{14,38}. The few studies focusing on picophytoplankton seasonality in the Baltic Sea suggest that their community composition and abundance have a strong seasonal variation^{21,26}. Maximum *Synechococcus* cell concentrations of 10^5 – 10^6 cells mL⁻¹ have been observed during summer, coinciding with high temperature and low nutrient conditions^{17,26,39,40}. Peak cell abundances for PPE of up to 10^3 cells mL⁻¹ have been reported during the autumn in a non-stratified water column²⁶. Thus, the dynamics and relative importance of *Synechococcus* and PPE along different seasons is expected to vary. In a global perspective, PPE appears to be better adapted to low temperature and NO₃ availability while *Synechococcus* is generally favored by high temperatures and NH₄ availability^{36,41}. *Synechococcus* preference of NH₄ over NO₃ has been observed in laboratory experiments on isolates^{42–44}. Similar observations from natural populations have also been reported from bulk populations in nutrient addition bioassays during summer phytoplankton blooms^{16,25,45}. This was also confirmed by recent studies at the single cell level^{46,47}. In this context, *Synechococcus* occurrence during late summer has been linked to the presence of dinitrogen (N₂)-fixing cyanobacteria blooms^{14,38,48} suggesting that interactions with larger phytoplankton may be an important factor driving seasonal dynamics of picophytoplankton. However, disentangling the nutrient effect from other factors such as temperature is necessary to understand the seasonal changes in picophytoplankton community composition.

This study aims to explore the abundance, net growth rate and nutrient limitation of three functional groups of picophytoplankton (PE-rich, PC-rich and PPE) over an annual cycle at a coastal sampling station located in the Baltic Sea Proper. Weekly field sampling was conducted in March to December in 2018 and a series of nutrient addition bioassays were performed to assess how nutrient limitation drives

picophytoplankton growth. This study provides high resolution data on the dynamics of picophytoplankton and nutrient controls during different seasons. The results show that the response and contribution to total biomass of the different functional groups varies greatly with nutrients and season and highlights the importance of picophytoplankton to the total phytoplankton community throughout the year.

Results

Field observations

The sampling was carried out at the K-station (56°39'25.4"N 16°21'36.6"E, 3m deep), a station located in the southeast coast of Sweden, in the Kalmar Sound, in the city of Kalmar. The Kalmar sound waters are eutrophic and are highly influenced by coastal anthropogenic activities, particularly agriculture. This has affected the yearly primary production, which has roughly doubled over the last century ⁴⁹.

At the K-station, seawater temperature (1 m depth) ranged from 0–24°C from early spring to mid-summer and 18 – 3°C from autumn to winter (Fig. 1a). Temperature was above 20°C from July to September. Salinity varied from 6.5 PSU during spring-summer after the ice melting period up to 7.5 PSU in October after a dry summer (Fig. 1b). The highest concentrations of dissolved inorganic nitrate ($\text{NO}_2 + \text{NO}_3$) were recorded in mid-March (4.3 μM ; Fig. 1c). Nitrate concentrations decreased gradually to the end of the spring and remained low throughout the summer and autumn (< 0.06–0.7 μM) followed by a small rise in the winter. Concentrations of NH_4 were not recorded for 2018 but typically ranged between 1–3 μM at the K-station during 2019 and 2020 (data not shown). The concentrations of dissolved inorganic phosphorus (PO_4) decreased in spring (1 to 0.2 μM) with occasional peaks of up to 1 μM between March and September (Fig. 1d). Silicate (SiO_4) concentrations ranged 4 to 25 μM with strong seasonal peaks in summer and early autumn (Fig. 1e). Total nitrogen (TN) levels ranged 3–10 μM during spring-summer and decreased to below detection concentrations during autumn-winter (Fig. 1f). Total phosphorus (TP) levels increased constantly from spring (5.8 μM) to winter (14.1 μM ; Fig. 1g).

Phytoplankton dynamics

Chl *a*, as a proxy for phytoplankton biomass, showed seasonal variation with a spring bloom maximum in April (up to 15 $\mu\text{g L}^{-1}$), a relatively constant summer bloom (4–8 $\mu\text{g L}^{-1}$) and a gradual decline after late autumn (< 4 $\mu\text{g L}^{-1}$; Fig. 2a). In the spring, the phytoplankton community was dominated by diatoms reaching a maximum biomass of 52 $\mu\text{g C L}^{-1}$ (Fig. 2b and c). After the spring bloom, biomass dropped to 25 – 15 $\mu\text{g C L}^{-1}$ consisting of a diverse community of dinoflagellates, haptophytes, ciliates and large cyanobacteria (Fig. 2c). During June until mid-August, as temperatures increased above 20°C, filamentous nitrogen (N_2)-fixing cyanobacteria, dominated by *Aphanizomenon* (87% relative contribution in June), *Dolichospermum* (69% relative contribution in July), and *Nodularia spumigena* (91% relative contribution in August), bloomed with peaks up to 74.4 $\mu\text{g C L}^{-1}$. In late summer, the larger phytoplankton community composition fluctuated, recording a maximum biomass of 148 $\mu\text{g C L}^{-1}$ due to a bloom of

Euglenophyta (75% relative contribution). During autumn the phytoplankton community biomass decreased to minimum of $6 \mu\text{g C L}^{-1}$ and was dominated by ciliates, dinoflagellates and diatoms (Fig. 2b and c).

Cell abundances of picophytoplankton showed a strong seasonality that differed for the three groups (Fig. 2d). PPE had highest abundance during May and PE-rich and PC-rich reached maximum cell abundances in July. PE and PC-rich maximum cell abundances (PE-rich: $2.6 \times 10^5 \text{ cells mL}^{-1}$, PC-rich: $2.1 \times 10^5 \text{ cells mL}^{-1}$) were more than the double of that of PPE ($1.1 \times 10^5 \text{ cells mL}^{-1}$). *In situ* net growth rates (calculated from *in situ* cell abundances) were low for the three functional groups ($< 0.31 \text{ d}^{-1}$; Fig. 2e). The time span at which *in situ* net growth rates were positive was highly variable during the different seasons for all picophytoplankton groups (Fig. 2e). In spring, the picocyanobacterial community was strongly dominated by PE-rich (Fig. 2f). During summer PC-rich increased its contribution to 50% the picocyanobacterial community. During autumn PC-rich dominated the picocyanobacterial community with a maximum contribution of 65% but decreased down to 10% by the end of November (Fig. 2f).

The reported literature values of picophytoplankton carbon biomass conversion show a large variation depending on the methods used for the determination, the season, region, or taxon in the case of PPE (Supplementary Table S1). In this study we estimated the median and minimum and maximum possible contributions of picophytoplankton to the total phytoplankton community (Fig. 3). Picophytoplankton showed maximum contributions from May to early July (max. May 14th, median: 89% relative contribution). The highest contribution of PE-rich and PC-rich occurred during summer (Fig. 3a, PE-rich June 26th, median: 27% relative contribution, Fig. 3b, PC-rich July 3rd, median: 18% relative contribution). PPE median contribution to the total carbon biomass was close to 40% for most of the year, with maximum contribution of 73% relative contribution during May. Minimum contributions took place in the period between mid-July to early August (median 3–19%, relative contribution; Fig. 3c).

Spearman's rank correlation test showed significant correlations between picophytoplankton cell abundance and abiotic and biotic variables (Table 1). PE-rich abundance correlated negatively with salinity and positively with temperature, total phytoplankton biomass, total cyanobacterial biomass and N_2 -fixing cyanobacterial biomass. PC-rich correlated positively with temperature, total cyanobacterial biomass and N_2 -fixing cyanobacterial biomass. PPE correlated negatively with salinity and salinity.

Table 1

Spearman correlations of PE-rich, PC-rich and PPE (cells mL⁻¹) against abiotic and biotic parameters. Robust correlations ($\rho > 0.25$, adjusted P after Bonferroni correction $P^* < 0.05$) are indicated by values in bold and marked with *.

	PE-rich		PC-rich		PPE	
variables	ρ	P	ρ	P	ρ	P
Temperature (°C)	0.65	2.95 10⁻⁵*	0.82	5.17 10⁻⁹*	0.41	0.01
Salinity (PSU)	-0.78	6.18 10⁻⁸*	0.02	0.88	-0.72	1.63 10⁻⁶*
NO ₂ + NO ₃ (μM)	0.23	0.14	-0.26	0.11	0.13	0.41
PO ₄ (μM)	0.07	0.65	-0.07	0.67	-0.01	0.95
SiO ₄ (μM)	-0.23	0.15	-0.27	0.09	-0.43	6.2 10 ⁻³
Chl <i>a</i> (μg L ⁻¹)	0.43	6.8 10 ⁻³	0.11	0.49	0.24	0.13
Total Phytoplankton Biomass (mg C mL ⁻¹)	0.62	4.91 10⁻⁵*	0.16	0.34	0.42	0.01
Diatoms (mg C mL ⁻¹)	-2.8 10 ⁻³	0.98	-0.34	0.04	-0.10	0.53
Dinoflagellates (mg C mL ⁻¹)	0.36	0.03	0.06	0.69	0.15	0.37
Cyanobacteria (mg C mL ⁻¹)	0.625	2.2 10⁻⁴*	0.61	3.5 10⁻⁴*	0.39	0.03
N ₂ -fixing cyanobacteria (mg C mL ⁻¹)	0.82	7.2 10⁻⁶*	0.73	2.2 10⁻⁴*	0.63	2.51 10 ⁻³

Nutrient limitation of picophytoplankton

A series of 11 bioassays were performed to study nutrient limitation on picophytoplankton along different seasons. The effect of nutrient limitation was analysed by comparing the net growth rates under different nutrient conditions. Net growth rates for PE-rich (-0.07 to 0.82 d⁻¹), PC-rich (-1.10 to 0.78 d⁻¹) and PPE (-0.01 to 0.91 d⁻¹) were in line with previous observations in the Baltic Sea, but generally lower than rates reported from tropical and sub-tropical environments (Fig. 4 and Table 2).

Table 2
Compilation of reported net growth rates (d⁻¹) of *Synechococcus* sp. and PPE from incubation experiments.

<i>Synechococcus</i> sp.				
Area	Temperature (°C)	Dates	Net growth rate (d ⁻¹)	Reference
Baltic Sea (K-station)	6.8–19.2	May–December (2018)	-1.10–0.78 (PC-rich) -0.07–0.82 (PE-rich)	This study
Baltic Sea (Tvärminne Långskär station)	0.7–23.3	All seasons (1988)	-0.07–0.47	26
North West Atlantic (Martha's Vineyard Observatory)	-2–22	All seasons (2003–2019)	0.1–1	35
Mediterranean Sea (Thau Lagoon)	6.5–23.3	Feb – Jan (1999–2000)	0.42–1.64	71
Mediterranean Sea (Bay of Blanes)	11–24	Feb–Jan (1996–1997)	0.2–1.5	59
North Atlantic Ocean (Cape Hatteras, stations P1 and P2)	-	Jul–Aug (1984)	0.54–0.86	72
North Atlantic Ocean (Skidaway Institute of Oceanography)	28.5–31.2	Jun–Aug (2017)	-0.5–2.86	73
North Pacific Subtropical Gyre (Hawaii)	24–25	Aug (1992)	0.65	74
North Pacific Subtropical Gyre (Kaneohe Bay, Hawaii)	-	Sep (1982)	1.42–1.98	75
North West Indian Ocean (R.R.S. Charles Darwin, Gulf of Oman and South east of the Arabian peninsula)	-	Sep–Oct (1986)	0.69–1.02	76
South East Pacific (RV Southern Surveyor, Australia)	14.3–22.5	Oct (2010)	-0.45–0.32	77
PPE				

<i>Synechococcus</i> sp.				
Area	Temperature (°C)	Dates	Net growth rate (d ⁻¹)	reference
Baltic Sea (K-station)	6.8–19.2	May-Dec (2018)	-0.01–0.91	This study
North west Pacific (Martha's Vineyard Observatory)	-2–22	All seasons (2003–2018)	0.1–2.1 (average)	37
North Atlantic Ocean (Skidaway Institute of Oceanography)	28.5–31.2	Jun-Aug (2017)	-0.5–2.86	73
Mediterranean Sea (Thau Lagoon)	6.5–23.3	Feb – Jan (1999–2000)	0.31–2.44	71
North Pacific subtropical gyre (Hawaii)	24–25	Aug (1992)	1.10	74

PE-rich and PC-rich growth patterns and their response to nutrients can be divided into two periods: from May to July and from end-August to October/December. During the first period, PE-rich net growth rates ranged from -0.01 to 0.82 d^{-1} while in the second period they were near-zero (Fig. 4a). During the first period, PE-rich growth was limited exclusively by nitrogen with a preference for NH_4 over NO_3 except on May 9th and June 19th (May 22nd : Control- NH_4 , $P = 2.09 \cdot 10^{-6}$ / June 4th ; Control- NH_4 , $P = 3.52 \cdot 10^{-4}$ / July 3rd, Control- NH_4 , $P = 7.51 \cdot 10^{-5}$). On May 9th net growth rates were co-limited by nitrogen (with a preference for NO_3 over NH_4) and phosphorus (control- $\text{NH}_4 + \text{PO}_4$, $P = 1.61 \cdot 10^{-5}$; control- NO_3 , $P = 1.61 \cdot 10^{-5}$; control- PO_4 , $P = 1.09 \cdot 10^{-3}$; control- $\text{NO}_3 + \text{PO}_4$, $P = 5.16 \cdot 10^{-6}$). On June 19th the NO_3 treatment showed significantly lower rates than the control (control- NO_3 , $P = 0.015$). During the second period, the growth was limited by nitrogen at the end of September (preference for NH_4 , control- NH_4 , $P = 0.01$) and the beginning of October (preference for NO_3 , control- NO_3 , $P = 0.03$). At $< 15^\circ\text{C}$ NO_3 was the preferred form of nitrogen, while at $> 15^\circ\text{C}$ NH_4 was preferred (Fig. 5a). PC-rich growth showed opposite dynamics to PE-rich. In the first period, PC-rich had poor net growth rates while in the second period net growth rates ranged from 0.34 to 0.78 d^{-1} (Fig. 4b). During the first period, PC-rich growth was co-limited by nitrogen (with preference for NH_4) and PO_4 on June 4th, (control- $\text{NH}_4 + \text{PO}_4$, $P = 0.01$) and limited by nitrogen (with preference for NH_4) on July 3rd (control- NH_4 , $P = 7.76 \cdot 10^{-4}$). During the second period, growth was co-limited by nitrogen (both NO_3 and NH_4) and PO_4 during October (October 9th, control- NH_4 , $P = 6.55 \cdot 10^{-4}$ / October 23rd, control- NH_4 , $P = 0.02$). At $< 15^\circ\text{C}$ both NO_3 and NH_4 yielded similar increase in net growth rates, while at $> 15^\circ\text{C}$ NH_4 was the preferred form of nitrogen for PC-rich (Fig. 5b).

PPE net growth rates were low in summer and high in spring and autumn (Fig. 4c). During the two experiments in May, PPE net growth rates ranged from 0.40–0.52 d⁻¹ and increased significantly with PO₄ addition and NH₄ addition on the 6th and 20th of May respectively (May 6th : control-PO₄, P = 0.014 / May 20th, control-NH₄, P = 0.005). In the period from June to August, net growth rates decreased from 0.33 to -0.01 d⁻¹ and the addition of NO₃, NH₄ and PO₄ reduced net growth rates significantly compared to the control (June 4th, control-NO₃, P = 0.001 / June 19th, control-NO₃, P = 9.50 10⁻⁵; control-PO₄, P = 0.02 / August 29th, control-NH₄, P = 0.02). During autumn, net growth rates increased again to 0.56–0.91 d⁻¹. During this period PO₄ limitation was observed during September (September 25th, control-PO₄, P = 1.91 10⁻⁴). The highest net growth rates for PPE rates were at temperatures < 15°C. Nutrient addition at higher temperatures generally caused a significant decrease of the net growth rates compared to the controls (Fig. 5c).

Discussion

Picophytoplankton have a competitive advantage for nutrient uptake in oligotrophic environments where they contribute significantly to the total Chl *a*^{1–3}. However, several observations have pointed out the ecological relevance of picophytoplankton also in coastal and eutrophic environments^{9,11,13,50}. Information about the seasonal abundance of picophytoplankton in the Baltic Sea is limited. As a consequence, picophytoplankton biomass contribution is frequently estimated using Chl *a* fractionation^{14–18} or not included in the calculations^{51,52}. At the K-station during 2018, *Synechococcus* was present throughout the year, with maximum abundances during summer (4.7 x 10⁵ cells mL⁻¹). These numbers were comparable to other observations in the Baltic Sea Proper during summer^{32,39}, suggesting that *Synechococcus* abundances at the coast are as high as in offshore locations. PPE abundances reached 1.1 x 10⁵ cells mL⁻¹ during spring, around two orders of magnitude higher than the maximum abundances reported from the Gulf of Finland²⁶ and one order of magnitude higher than the maximum observed in other estuaries^{29,53}. The current study (K-station) highlights the significant contribution of *Synechococcus* and PPE to the total phytoplankton biomass in the estuarine and eutrophic coastal Baltic Sea over an annual cycle.

Picophytoplankton is composed of multiple functional groups spanning diverse physiological adaptations and niches^{17,29}. Recent studies, in other estuarine and coastal areas, have separated *Synechococcus* into pigment based functional groups (PE-rich and PC-rich) suggesting significant differences in distribution patterns between the groups^{13,54,55}. In this study, the three functional groups, PE-rich, PC-rich and PPE and had significant differences in regard to, 1) seasonal dynamics, 2) biomass contribution, 3) temperature regimes, and 4) nutrient limitation. These results emphasize the importance of high-resolution studies of ecological relevant functional groups in order to understand the dynamics of the genetic and physiologically diverse picophytoplankton^{21,56}.

Seasonal variations in the PE-rich and PC-rich contributions to the *Synechococcus* community has previously been observed in tropical and subtropical estuaries^{28,29,34,55}. At the K-station, PE-rich and PC-rich abundance increased during spring and peaked during early summer while this period PE-rich dominated the *Synechococcus* community. This was consistent with previous observations of PE-rich dominance in the Baltic Sea during the spring-summer period^{17,31,32}. During the autumn, PE-rich abundance declined resulting in PC-rich dominance. These novel findings are in line with different temperature adaptations between PE-rich and PC-rich groups²⁹. These field observations also support the experimental evidence that PC-rich isolates are better adapted to lower irradiance than PE-rich⁵⁷. PPE increase in abundance is usually related to low temperatures and high nutrient concentration^{26,36}. Our observations showed that PPE abundances peaked during spring, at 11–15°C. The low PPE abundance during autumn contrast with the peak abundances observed by Kuosa et al.²⁶ during the same period. These patterns could be explained by the long lasting summer of 2018⁵⁸ which could have shifted PPE favorable temperatures to later in the autumn while concurrent light limitation may have restricted PPE growth³⁷.

The biomass estimates at the K-station, confirmed that the pico-fraction is a major contributor to the total phytoplankton biomass^{17,18,59} and can dominate the phytoplankton community during spring, early summer and autumn. Resolving the biomass estimates into the three functional groups reveal that on an annual basis, PPE was the main contributor except during the bloom of N₂-fixing cyanobacteria at the end of July (> 18°C). This highlights the importance of PPE in coastal environments⁶⁰. High contribution of both *Synechococcus* groups concur with high temperatures, in line with previous studies¹⁵. A community composition shift from PPE to *Synechococcus* can have a large impact for the microbial food web and should be systematically included in future phytoplankton biomass studies and carbon flux models⁶¹.

Niche adaptation to temperature show different patterns among the picophytoplankton groups. Bioassays at the K-station showed the highest net growth rates during spring and beginning of the summer at 10°C and 17–19°C for PE-rich and 11–15°C for PPE. The temperature niche for PC-rich was 13–16°C leading to the highest net growth rates during autumn. The net growth rates of *Synechococcus* were in line with previous observations in temperate ecosystems (Table 2). Similarly to the high-resolution growth dynamic study by Hunter-Cevera et al.⁶², in this Baltic Sea study, net growth showed no increase at > 16–17°C. However, higher net growth rates of *Synechococcus* (up to 2.86 d⁻¹) have been reported in warmer climate (Table 2). Thus, an increase in temperature due to climate change might result in an increase of *Synechococcus* growth at higher latitudes⁶³. The calculated net growth rates in the bioassays did not follow the same seasonality as the *in situ* net growth rates due to the high weekly variation in picophytoplankton cell abundance. These results underscore the importance of high resolution sampling when studying picophytoplankton dynamics.

The bioassays showed that NH_4 was the preferred form of nitrogen for picophytoplankton. The preference of NH_4 over NO_3 for *Synechococcus* has been extensively documented^{42–44,46,47}. This study shows that both PE-rich and PC-rich can use NO_3 and NH_4 at low temperature but prefer NH_4 at high temperature ($> 15–17^\circ\text{C}$). This is in line with the effect of temperature on nitrogen assimilation enzymatic pathways⁴¹. The assimilation of NH_4 generally occurs through the GS-GOGAT pathway, while NO_3 uptake depends on the enzyme nitrate reductase (NR). GS-GOGAT is positively correlated with temperature meanwhile NR is negatively correlated⁴¹. As a result, NH_4 assimilation will be higher than NO_3 assimilation at high temperatures. Thus, the uptake of NH_4 is an advantageous adaptation for *Synechococcus* to compete with other NO_3 specialists such as diatoms under nitrogen limitation during the warm periods⁴¹. It should be noted, that our results shows that PE-rich is better adapted to high temperatures than PC-rich, and as a consequence PE-rich may benefit more from NH_4 uptake. In coastal areas and shallow water ecosystems (< 50 m depth) NH_4 from benthic or riverine origin can be the main nitrogen source for the phytoplankton community^{46,64}, which could benefit PE-rich at high temperatures. The correlation between PE-rich and N_2 -fixing cyanobacteria supports that newly fixed nitrogen in the form of NH_4 may have a key role in controlling PE-rich abundance^{14,38,48}. In line with the observations by Berthelot et al.⁴⁷, PPE growth increased in NH_4 addition treatments during nitrogen limitation. However, nutrient additions at suboptimal temperatures (outside of the temperature niche) resulted in significant reductions of the net growth rates of PPE. This was likely because nutrient addition in a nutrient limited system can favor competitors better adapted for high temperatures such as PE-rich.

This study provides the first annual high resolution description of picophytoplankton abundance and dynamics in the coastal Baltic Sea. It also investigates net growth rates and nutrient limitation of three functional picophytoplankton groups. In this study, PE-rich, PC-rich and PPE showed different seasonal dynamics defined by different temperature niches and nutrient limitation. PPE dynamics in the Baltic Sea are severely understudied. This study shows, for the first time, the importance of picophytoplankton over a full annual cycle and situates PPE as one of the most important components of the phytoplankton community in terms of biomass especially during spring and early summer. The results further suggest that in eutrophic coastal systems where NH_4 is the main nitrogen-species (agricultural landscape), PE-rich will be favored over PPE. This effect could be further magnified during earlier and more extensive blooms of N_2 -fixing cyanobacteria that are projected as a consequence of global warming^{65,66}. Such events could favor PE-rich over PC-rich and PPE, leading to picophytoplankton community shifts having profound consequences on the contribution to the total carbon biomass in coastal areas.

Material And Methods

Field Sampling

Surface water (1 m depth) was sampled weekly from March until December 2018 using a 10L polycarbonate carboy. The temperature and salinity were measured using a

conductivity/temperature/depth sensor CTD® Castaway. Water from the carboy bottle was filtered through a 200 µm mesh gauze to remove large particles. Samples were collected for nutrients, Chl *a*, pico- and larger phytoplankton abundance. Water for the nutrient addition bioassays was collected in 10L acid washed polycarbonate carboys at 11 occasions and experiments were initiated within 1 h of sample collection and incubated in the laboratory under controlled conditions for 48 h.

Abiotic and biotic parameters

Samples for dissolved inorganic nutrients (NO₂ + NO₃ and PO₄, SiO₄, TN and TP) were filtered (400 ml) through a GF/F filter and frozen at -20°C until analysis using standard protocols (UV-Spectrophotometer, Valderrama 1995). Chl *a* was extracted and measured following Jespersen & Christoffersen (1987). Briefly, 50–200 mL seawater was filtered on (A/E) glass fiber filters in duplicates (~1 µm pore size, Pall life Sciences, Ann Arbor, MI, USA) under low vacuum and extracted in ethanol (96%) in darkness. Chl *a* concentrations were measured using a Turner fluorometer (Turner design Model #040, Tucson, USA). Samples for larger phytoplankton (> 5 µm) community composition were collected and counted microscopically (Nikon TMS, Tokyo, Japan) after preservation with acidic Lugol's solution (1% final concentration) following ^{49,67}. Phytoplankton carbon biomass concentration was derived from the cell abundance and carbon biomass ⁶⁸.

Samples for picophytoplankton abundance were fixed with glutaraldehyde solution Grade I 25% in H₂O (Sigma-Aldrich, Missouri, USA; 1% final concentration) and stored at -80°C until flow-cytometry analysis. Cells of PE-rich and PC-rich and PPE were identified and counted using a CyFlow® Cube8 flow cytometer (Partec®, Germany) equipped with a blue pumped solid-state Laser (20 mW) at 488 nm and a red laser diode (25mW) at 638 nm. For each sample, 50 µL were analyzed at an average flow rate of (10 µL s⁻¹). For the cell characterization, five optical parameters were used at a logarithmic scale: Forward scatter (FSC) as a proxy for cell diameter, FL2 (590/50 nm) as a proxy for PE content, FL3 (675/50 nm) as a proxy for Chl *a* and FL4 (675/50 nm) as a proxy for PC content.

Synechococcus was identified and separated into two groups depending on their specific pigment characteristics: PE-rich with a high FL2 signal and PC-rich with a high FL4 signal. PPE was identified due to its large diameter and high FL3 signal. The diameters were estimated in the FSC with the help of 1 µm and 3 µm beads. For a more detailed description of the picophytoplankton identification see Supplementary information and Supplementary Fig. S1. Gating and visualization of the flow cytometric data were carried out using the R (version 3.6.1) packages flowcore, flowWorkspace, openCyto and CytoRsuite ⁶⁹. From picophytoplankton cell abundance data, the *in situ* growth was calculated according to (1).

$$(1) \text{ In situ net growth rate (day}^{-1}\text{)} = \frac{\ln(\epsilon_1/\epsilon_0)}{\text{days between sampling}}$$

The carbon biomass concentration of picophytoplankton was estimated by calculating possible min-max range of relative contribution of PE-rich, PC-rich and PPE to total phytoplankton carbon biomass using a combination of conversion factors estimated for carbon content collected from the literature (Supplementary Table S1).

Nutrient addition bioassays at in situ temperature

To examine the seasonal variation of nutrient (nitrogen and phosphorus) limitation of picophytoplankton at the K-station, a total of 11 short-term (48h) nutrient addition bioassays were conducted during different seasons (see Supplementary Table S2): in spring (7th of May and 22nd of May), summer (4th and 21st of June, 3rd of July and 29th of August), autumn (12th and 25th of September and 9th and 23 of October) and winter (11th of December). The bioassay treatments were addition of NH_4 (200 μM), NO_3 (200 μM), PO_4 (10 μM), NO_3 (200 μM) + PO_4 (10 μM), NH_4 (200 μM) + PO_4 (10 μM) and controls without nutrient addition. Triplicates were done for each treatment in 650 mL acid-washed polycarbonate bottles. The bioassays were incubated in the laboratory at a light intensity of $90 \mu\text{E m}^{-2} \text{s}^{-1}$, a photoperiod of 12L:12D, and at *in situ* temperature provided with a constant inflow and outflow of seawater. Bottles were shaken manually every 24 h to avoid sedimentation. The abundance of PE-rich, PC-rich and PPE were measured at the beginning and the end of each experiment. Net growth rate was calculated according to (2).

$$(2) \text{ Net growth rate (day}^{-1}\text{)} = \frac{\ln(t_{48h}/t_{0h})}{2 \text{ days}}$$

Statistical analysis

The relationship between the picophytoplankton cell abundance and measured biotic and abiotic variables was analyzed using Spearman's rank correlation test ($n = 101$ after omitting NAs). Correlations were considered significant when $p > 0.25$ and P after Bonferroni correction was $P < 0.05$. The bioassays ($n = 18$) were analyzed separately using one-way ANOVA ($P < 0.05$) followed by Tukey's range test ($p < 0.05$) to test the statistical differences between treatments. Normality and heteroscedasticity were assessed via quantile-quantile plots and Cochran's C test ($P = 0.05$) respectively. All statistical analyses were performed using R version 3.6.1⁷⁰.

Declarations

Competing interests

The authors declare no competing interests.

Author contributions

C.L, H.F. and J.A.Z conceived the study, J.A.Z and H.F. conducted the field sampling and bioassay experiments. J.A.Z performed the laboratory and data analysis and plotted the figures. J.A.Z, C.L, and H.F. wrote and edited the manuscript.

Acknowledgments

We are grateful to Anders Månsson, Soran Mahmoudi and Maurice Hirwa for assistance with the K-station sampling and set-up of bioassay experiments, Justyna Kobos and Lidia Nawrocka from University of Gdansk for the microscopic phytoplankton counts and Sonia Bergin from Umeå University for useful conversations on picophytoplankton identification with flow cytometry. This work was funded by a FORMAS future research leader grant (2017 – 00468) to H.F and the Swedish governmental strong research programme EcoChange.

References

1. Agawin, N. S. R., Duarte, C. M. & Agustí, S. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnol. Oceanogr.* **45**, 591–600 (2000).
2. Durand, M. D., Olson, R. J. & Chisholm, S. W. Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep. Res. Part II Top. Stud. Oceanogr.* **48**, 1983–2003 (2001).
3. Pena, M. A., Lewis, M. R. & Harrison, W. G. Primary productivity and size structure of phytoplankton biomass on a transect of the equator at 135°W in the Pacific Ocean. *Deep. Res.* **19**, (1990).
4. Rii, Y. M., Karl, D. M. & Church, M. J. Temporal and vertical variability in picophytoplankton primary productivity in the North Pacific Subtropical Gyre. *Mar. Ecol. Prog. Ser.* **562**, 1–18 (2016).
5. Magazzu, G. & Decembrini, F. Primary production, biomass and abundance of phototrophic picoplankton in the Mediterranean Sea: A review. *Aquat. Microb. Ecol.* **9**, 97–104 (1995).
6. Uitz, J., Claustre, H., Gentili, B. & Stramski, D. Phytoplankton class-specific primary production in the world's oceans: Seasonal and interannual variability from satellite observations. *Global Biogeochem. Cycles.* **24**, GB3016 (2010).
7. Partensky, F., Hess, W. R. & Vaulot, D. Prochlorococcus, a marine photosynthetic prokaryote of global significance. *Microbiol Mol Biol Rev* **63**, 106–127(1999).
8. Pittera, J. *et al.* Connecting thermal physiology and latitudinal niche partitioning in marine Synechococcus. *ISME J.* **8**, 1221–1236 (2014).
9. Caroppo, C. Ecology and biodiversity of picoplanktonic cyanobacteria in coastal and brackish environments. *Biodivers. Conserv.* **24**, 949–971 (2015).
10. Calvo-Díaz, A., Morán, X. A. G., Nogueira, E., Bode, A. & Varela, M. Picoplankton community structure along the northern Iberian continental margin in late winter-early spring. *J. Plankton Res.* **26**, 1069–1081 (2004).

11. Morán, X. A. G. Annual cycle of picophytoplankton photosynthesis and growth rates in a temperate coastal ecosystem: A major contribution to carbon fluxes. *Aquat. Microb. Ecol.* **49**, 267–279 (2007).
12. Sathicq, M. B., Unrein, F. & Gómez, N. Recurrent pattern of picophytoplankton dynamics in estuaries around the world: The case of Río de la Plata. *Mar. Environ. Res.* **161**, 105136 (2020).
13. Paerl, R. W., Venezia, R. E., Sanchez, J. J. & Paerl, H. W. Picophytoplankton dynamics in a large temperate estuary and impacts of extreme storm events. *Sci. Rep.* **10**, 22026 (2020).
14. Ohlendieck, U., Stühr, A. & Siegmund, H. Nitrogen fixation by diazotrophic cyanobacteria in the Baltic Sea and transfer of the newly fixed nitrogen to picoplankton organisms. *J. Mar. Syst.* **25**, 213–219 (2000).
15. Stal, L. J. *et al.* 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 (2003).
16. Stal, L. J., Staal, M. & Villbrandt, M. Nutrient control of cyanobacterial blooms in the Baltic Sea. *Aquat. Microb. Ecol.* **18**, 165–173 (1999).
17. Tamm, M., Laas, P., Freiberg, R., Nõges, P. & Nõges, T. Parallel assessment of marine autotrophic picoplankton using flow cytometry and chemotaxonomy. *Sci. Total Environ.* **625**, 185–193 (2018).
18. Sondergaard, M., Jensen, L. M. & Aertebjerg, G. Picoalgae in Danish coastal waters during summer stratification. *Mar. Ecol. Prog. Ser.* **79**, 139–149 (1991).
19. Komárek, J., Kopecký, J. & Cepák, V. Generic characters of the simplest cyanoprokaryotes Cyanobium, Cyanobacterium and Synechococcus. *Cryptogam. Algal.* **20**, 209–222 (1999).
20. Sánchez-Baracaldo, P., Hayes, P. K. & Blank, C. E. Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. *Geobiology.* **3**, 145–165 (2005).
21. Bertos-Fortis, M. *et al.* Unscrambling cyanobacteria community dynamics related to environmental factors. *Front. Microbiol.* **7**, 625 (2016).
22. Celepli, N. *et al.* Meta-omic analyses of Baltic Sea cyanobacteria: diversity, community structure and salt acclimation. *Environ. Microbiol.* **19**, 673–686 (2017).
23. Herlemann, D. P. *et al.* Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* **5**, 1571–1579 (2011).
24. Hu, Y. O. O., Karlson, B., Charvet, S. & Andersson, A. F. Diversity of pico- to mesoplankton along the 2000 km salinity gradient of the baltic sea. *Front. Microbiol.* **7**, 679 (2016).
25. Lagus, A., Suomela, J., Helminen, H. & Sipura, J. Impacts of nutrient enrichment and sediment on phytoplankton community structure in the northern Baltic Sea. *Hydrobiologia.* **579**, 351–368 (2007).
26. Kuosa, H. Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. *Mar. Ecol. Prog. Ser.* **73**, 269–276 (1991).
27. Stomp, M. *et al.* Colourful coexistence of red and green picocyanobacteria in lakes and seas. *Ecol. Lett.* **10**, 290–298 (2007).

28. Liu, H., Jing, H., Wong, T. H. C. & Chen, B. Co-occurrence of phycocyanin- and phycoerythrin-rich *Synechococcus* in subtropical estuarine and coastal waters of Hong Kong. *Environ. Microbiol. Rep.* **6**, 90–99 (2014).
29. Rajaneesh, K. M., Mitbavkar, S., Anil, A. C. & Sawant, S. S. *Synechococcus* as an indicator of trophic status in the Cochin backwaters, west coast of India. *Ecol. Indic.* **55**, 118–130 (2015).
30. Campbell, L. & Carpenter, E. J. Characterization of phycoerythrin-containing *Synechococcus* spp. populations by immunofluorescence. *J. Plankton Res.* **9**, 1167–1181 (1987).
31. Larsson, J. *et al.* Picocyanobacteria containing a novel pigment gene cluster dominate the brackish water Baltic Sea. *ISME J.* **8**, 1892–1903 (2014).
32. Mazur-Marzec, H. *et al.* Occurrence of cyanobacteria and cyanotoxin in the Southern Baltic Proper. Filamentous cyanobacteria versus single-celled picocyanobacteria. *Hydrobiologia.* **701**, 235–252 (2013).
33. Haverkamp, T. *et al.* Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons. *Environ. Microbiol.* **10**, 174–188 (2008).
34. Jiang, T. *et al.* Temporal and spatial variations of abundance of phycocyanin- and phycoerythrin-rich *Synechococcus* in Pearl River Estuary and adjacent coastal area. *J. Ocean Univ. China.* **15**, 897–904 (2016).
35. Hunter-Cevera, K. R. *et al.* Seasons of Syn. *Limnol. Oceanogr.* **65**, 1–18 (2019).
36. Otero-Ferrer, J. L. *et al.* Factors controlling the community structure of picoplankton in contrasting marine environments. *Biogeosciences.* **15**, 6199–6220 (2018).
37. Fowler, B. L. *et al.* Dynamics and functional diversity of the smallest phytoplankton on the Northeast US Shelf. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 12221(2020).
38. Ploug, H. *et al.* Carbon, nitrogen and O₂ fluxes associated with the cyanobacterium *Nodularia spumigena* in the Baltic Sea. *ISME J.* **5**, 1549–1558 (2011).
39. Albertano, P., Di Somma, D. & Capucci, E. Cyanobacterial picoplankton from the central Baltic Sea: cell size classification by image analyzed fluorescence microscopy. *J. Plankton Res.* **19**, 1405–1416 (1997).
40. Hajdu, S., Höglander, H. & Larsson, U. Phytoplankton vertical distributions and composition in Baltic Sea cyanobacterial blooms. *Harmful Algae.* **6**, 189–205 (2007).
41. Glibert, P. M. *et al.* Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnol. Oceanogr.* **61**, 165–197 (2016).
42. Hood, R. D., Higgins, S. A., Flamholz, A., Nichols, R. J. & Savage, D. F. The stringent response regulates adaptation to darkness in the cyanobacterium *Synechococcus elongatus*. *Proc. Natl. Acad. Sci.* **113**, E4867–E4876(2016).
43. Tan, X. *et al.* Effects of nitrogen on interspecific competition between two cell-size cyanobacteria: *Microcystis aeruginosa* and *Synechococcus* sp. *Harmful Algae.* **89**, 101661 (2019).

44. Moore, L. R., Post, A. F., Rocap, G. & Chisholm, S. W. Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnol. Oceanogr.* **47**, 989–996(2002).
45. Kuuppo, P. *et al.* Fate of increased production in late-summer plankton communities due to nutrient enrichment of the Baltic Proper. *Aquat. Microb. Ecol.* **32**, 47–60 (2003).
46. Klawonn, I. *et al.* Untangling hidden nutrient dynamics: rapid ammonium cycling and single-cell ammonium assimilation in marine plankton communities. *ISME J.* **13**, 1960–1974 (2019).
47. Berthelot, H. *et al.* NanoSIMS single cell analyses reveal the contrasting nitrogen sources for small phytoplankton. *ISME J.* **13**, 651–662 (2018).
48. Stal, L. & Walsby, A. Photosynthesis and nitrogen fixation in a cyanobacterial bloom in the baltic sea. *Eur. J. Phycol.* **35**, 97–108 (2000).
49. Legrand, C. *et al.* Interannual variability of phyto-bacterioplankton biomass and production in coastal and offshore waters of the Baltic Sea. *Ambio.* **44**, 427–438 (2015).
50. Pulina, S. *et al.* Picophytoplankton Seasonal Dynamics and Interactions with Environmental Variables in Three Mediterranean Coastal Lagoons. *Estuaries and Coasts.* **40**, 469–478 (2017).
51. Uusitalo, L., Fleming-lehtinen, V., Ha, H., Jaanus, A. & Ha, S. *Marine Science.* **70**, 408–417 (2013).
52. Olofsson, M., Hagan, J. G., Karlson, B. & Gamfeldt, L. Large seasonal and spatial variation in nano- and microphytoplankton diversity along a Baltic Sea–North Sea salinity gradient. *Sci. Rep.* **10**, 17666 (2020).
53. Mohan, A. P., Jyothibabu, R., Jagadeesan, L., Lallu, K. R. & Karnan, C. Summer monsoon onset-induced changes of autotrophic pico- and nanoplankton in the largest monsoonal estuary along the west coast of India. *Environ. Monit. Assess.* **188**, 93 (2016).
54. Mitbavkar, S., Patil, J. S. & Rajaneesh, K. M. Picophytoplankton as Tracers of Environmental Forcing in a Tropical Monsoonal Bay. *Microb. Ecol.* **70**, 659–676 (2015).
55. Li, J. *et al.* *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 (2019).
56. Not, F., del Campo, J., Balagué, V., de Vargas, C. & Massana, R. New insights into the diversity of marine picoeukaryotes. *PLoS One.* **4**, e7143 (2009).
57. Stomp, M. *et al.* Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature.* **432**, 104–107 (2004).
58. Humborg, C. *et al.* High emissions of carbon dioxide and methane from the coastal Baltic Sea at the end of a summer heat wave. *Front. Mar. Sci.* **6**, 493 (2019).
59. Agawin, N. S. R., Duarte, C. M. & Agustí, S. Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: Seasonality and relationship with temperature. *Mar. Ecol. Prog. Ser.* **170**, 45–53 (1998).
60. Worden, A. Z., Nolan, J. K. & Palenik, B. Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. *Limnol. Oceanogr.* **49**, 168–179 (2004).

61. Schmidt, K. *et al.* Increasing picocyanobacteria success in shelf waters contributes to long-term food web degradation. *Glob. Chang. Biol.* **26**, 5574–5587 (2020).
62. Hunter-Cevera, K. R., Post, A. F., Peacock, E. E. & Sosik, H. M. Diversity of *Synechococcus* at the Martha's Vineyard Coastal Observatory: Insights from Culture Isolations, Clone Libraries, and Flow Cytometry. *Microb. Ecol.* **71**, 276–289 (2016).
63. Stawiarski, B., Buitenhuis, E. T. & Quéré, C. Le. The physiological response of picophytoplankton to temperature and its model representation. *Front. Mar. Sci.* **3**, 164 (2016).
64. Herbert, R. A. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiol. Rev.* **23**, 563–590 (1999).
65. Neumann, T. *et al.* Extremes of temperature, oxygen and blooms in the baltic sea in a changing climate. *Ambio.* **41**, 574–585 (2012).
66. Andersson, A. *et al.* Projected future climate change and Baltic Sea ecosystem management. *Ambio.* **44**, 345–356 (2015).
67. Utermöhl, H. (1958). Zur Vervollkommnung Der Quantitativen Phytoplankton- Methodik. *Mitteilung Int. Vereinigung Fuer Theor. Unde Amgewandte Limnol.* **9**, 1–38 (1958).
68. Edler, L. Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. (Baltic Marine Biologists BMB (Sweden) 1979).
69. Hammill, D. & CytoRSuite. R package, version 0.9.9. <https://dillonhammill.github.io/CytoRSuite>. (2019).
70. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. R version 3.5.1. <https://www.R-project.org/>. (2019).
71. Bec, B., Hussein-Ratrema, J., Collos, Y., Souchu, P. & Vaquer, A. Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: Emphasis on the picoeukaryote community. *J. Plankton Res.* **27**, 881–894 (2005).
72. Campbell, L. & Carpenter, E. Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus* spp. using the sea water dilution and selective inhibitor techniques. *Mar. Ecol. Prog. Ser.* **33**, 121–129 (1986).
73. Anderson, S. R., Diou-Cass, Q. P. & Harvey, E. L. Short-term estimates of phytoplankton growth and mortality in a tidal estuary. *Limnol. Oceanogr.* **63**, 2411–2422 (2018).
74. Landry, M. R., Kirshtein, J. & Constantinou, J. A refined dilution technique for measuring the community grazing impact of microzooplankton. *Mar. Ecol. Prog. Ser.* **120**, 53–63 (1995).
75. Landry, M., Haas, L. & Fagerness, V. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. *Mar. Ecol. Prog. Ser.* **16**, 127–133 (1984).
76. Burkill, P. H., Leakey, R. J. G., Owens, N. J. P. & Mantoura, R. F. C. *Synechococcus* and its importance to the microbial foodweb of the northwestern Indian Ocean. *Deep. Res. Part II.* **40**, 773–782 (1993).
77. Doblin, M. A. *et al.* Nutrient uplift in a cyclonic eddy increases diversity, primary productivity and iron demand of microbial communities relative to a western boundary current. *PeerJ* **4**, e1973(2016).

Figures

Figure 1

K-station weekly measurements during 2018 for (a) temperature (°C), (b) salinity (PSU), (c) NO₂ + NO₃ (μM), (d) PO₄ (μM), (e) SiO₄ (μM), (f) total N (TN; μM) and (g) total P (TP; μM). Vertical dark lines mark the dates when a bioassay was performed.

Figure 2

K-station weekly measurements during for (a) Chl *a* ($\mu\text{g L}^{-1}$), (b) total phytoplankton ($>5 \mu\text{m}$ in diameter) carbon biomass concentration (mg C mL^{-1}) based on microscopy, (c) relative contribution of phytoplankton divisions ($>5 \mu\text{m}$ in diameter) based on microscopy, (d) PE-rich, PC-rich and PPE cell concentration in a logarithmic scale (cells mL^{-1}), (e) PE-rich, PC-rich and PPE in situ net growth rates (d^{-1})

and (f) relative contribution of PE-rich and PC-rich (%) based on flow cytometry cell counts. Vertical dark lines mark the dates when a bioassay was performed.

Figure 3

K-station weekly relative carbon biomass contribution to the total phytoplankton community for (a) PE-rich, (b) PC-rich, (c) PPE. The carbon biomass concentration of picophytoplankton was estimated by calculating the min-max range of relative contribution of PE-rich, PC-rich and PPE to total phytoplankton carbon biomass based on a combination of conversion factors estimated for carbon content collected from the literature.

Figure 4

Bioassay net growth rates (d⁻¹) on each date for (a) PE-rich (day-1), (b) PC-rich (day-1) and (c) PPE (day-1) calculated from cell abundances measured using flow cytometry at T0 and T48. Treatments were control (orange), PO₄ addition (gold), NO₃ addition (green), NO₃ and PO₄ addition (cyan), NH₄ addition (blue) and NH₄ and PO₄ addition (pink). Different letters represent statistically significant differences between treatments. Data is presented as mean $\pm$ sd. Significant nutrient effects are marked with colored shapes; square for NH₄, diamond for NO₃ and circle for PO₄; blue color illustrates a significant increase while red a significant decrease in net growth rates.

Figure 5

Bioassay net growth rates (d-1) as a function of temperature for (a) PE-rich (day-1), (b) PC-rich (day-1) and (c) PPE (day-1) calculated from cell abundances measured using flow cytometry at T0 and T48. Treatments were control (orange), PO₄ addition (gold), NO₃ addition (green), NO₃ and PO₄ addition (cyan), NH₄ addition (blue) and NH₄ and PO₄ addition (pink). Data presented as raw data for each replicate. Dashed lines show the LOESS regression for each treatment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [JavierAZsupplementary1.pdf](#)