DOCTORAL SCHOOL AT THE FACULTY OF OCEANOGRAPHY AND GEOGRAPHY UNIVERSITY OF GDAŃSK

Allelopathy in different phenotypes of the picoplanktonic cyanobacteria *Synechococcus* sp.: A key feature determining the structure of phytoplankton communities in the Baltic Sea

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Oddziaływanie allelopatyczne różnych fenotypów pikoplanktonowej sinicy *Synechococcus* sp.: mechanizm decydujący o strukturze fitoplanktonu w Morzu Bałtyckim

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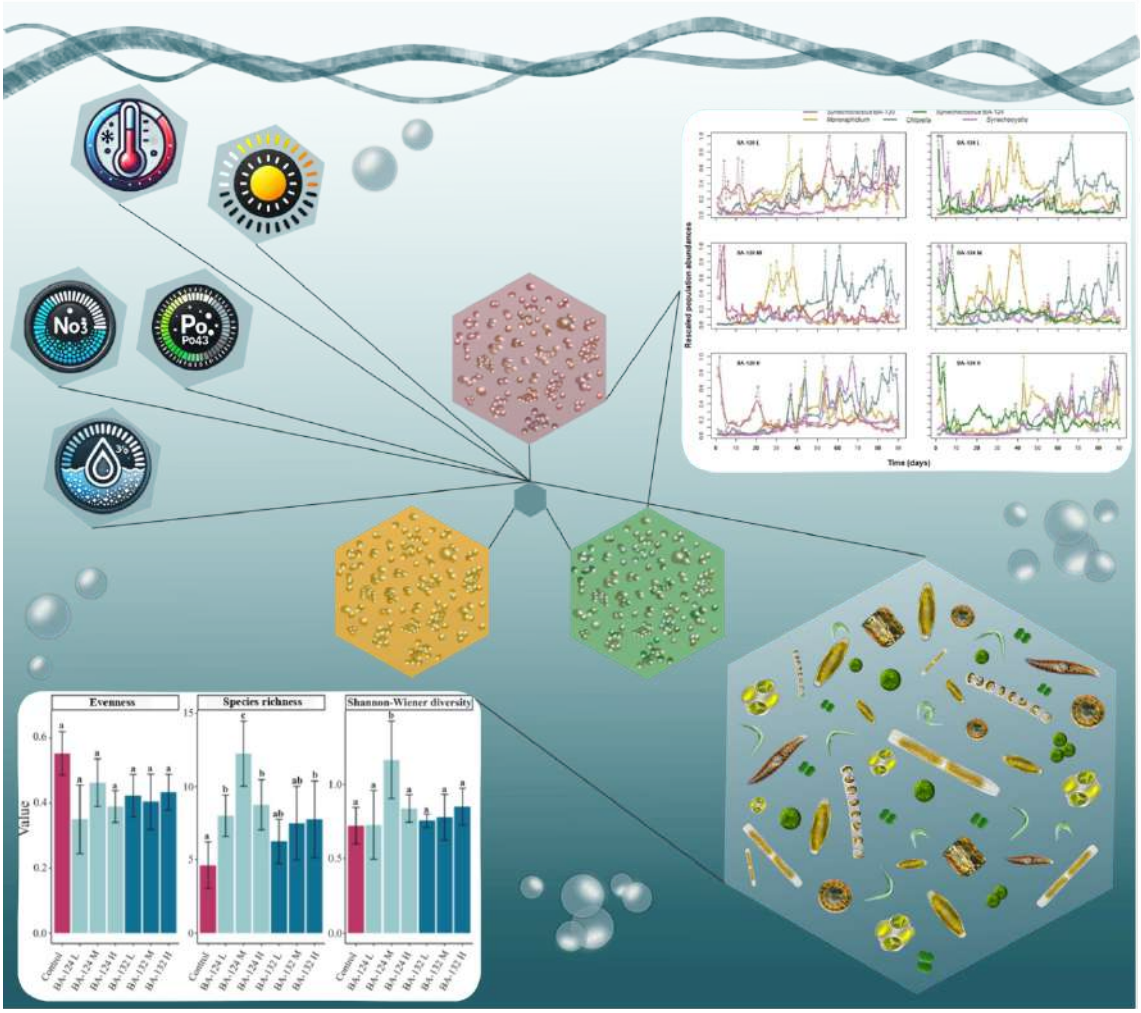

Key words: allelopathy, *Synechococcus*, diversity, phytoplankton, Baltic Sea

Słowa kluczowe: allelopatia, *Synechococcus*, różnorodność, fitoplankton, Morze Bałtyckie

2 ABSTRACT IN ENGLISH

2 STRESZCZNIE PO ANGIELSKU

This doctoral dissertation investigates the role allelopathic properties of three dominant *Synechococcus* sp. phenotypes in shaping the diversity of phytoplankton communities. The study focuses on picocyanobacteria from the genus *Synechococcus* and microalgae isolated from the coastal waters of the Gulf of Gdańsk. The methodological approach combines laboratory experiments and ecological modeling to examine the allelopathic effects of *Synechococcus* sp. phenotypes on phytoplankton community structure. Initially, short-term allelopathic assays were conducted under various abiotic conditions to assess the influence of environmental factors on the allelopathic activity of co-

phytoplankton species was performed. Once these basic grounds were set, we tested the effect of allelopathy in long-term competition experiments that were conducted with *Synechococcus* and a selected group of coexisting phytoplankton species, also developing a mechanistic model to assess whether allelopathy and nutrient competition drive species dynamics. Finally, in a further step to upscale complexity, we tested whether *Synechococcus* sp. allelopathy can act as a key factor in structuring natural phytoplankton communities driven to equilibrium in the laboratory.

To achieve this aim, the following hypotheses were formulated:

H1 Abiotic factors that promote the growth of *Synechococcus* phenotypes enhance their allelopathic activity.

H2 Allelopathy exhibited by different *Synechococcus* phenotypes affect co-occurring phytoplankton species. The allelopathic effect is different depending on the *Synechococcus* phenotype, and target phytoplankton species.

H3 The strength of allelopathy from *Synechococcus* phenotypes influences plankton community diversity: Low allelopathic strength is associated with lower diversity due to dominance by strongest competitors. Intermediate allelopathic intensity increases community diversity due to coexistence between allelopathic weak competitors and sensitive stronger competitors. High allelopathic intensity reduces diversity due to dominance by *Synechococcus* phenotypes.

Based on formulated hypotheses the main conclusions of the thesis include:

- The abiotic factors that enhance the growth of the studied *Synechococcus* phenotypes also increase their allelopathic activity. However, optimal salinity does not always have the same effect, with moderate salinity (8 PSU) intensifying interspecific interactions. This salinity was related to their place of origin.
- The allelopathic effect varied depending on the *Synechococcus* phenotype and the target phytoplankton species. Both positive and negative allelopathic effects were observed. *Synechococcus* Type 3a exhibited the strongest allelopathic activity, while the diatoms showed the most sensitive among the studied species.
- Allelopathy in interplay with competition for a limiting resource (nitrate) promoted oscillatory coexistence of four selected phytoplankton species. However, the initial strength of allelopathy did not influence the outcome of competition experiments.
- The allelopathy of *Synechococcus* was shown to enhance the diversity of natural phytoplankton communities driven to equilibrium in long-term experiments only for the Type 1 (BA-124). This indicates that, given equal inoculum sizes, Type 1 was more allelopathic than Type 3a (BA-132) on a per-cell basis.

3 ABSTRACT IN POLISH

3 STRESZCZNIE PO POLSKU

Niniejsza rozprawa doktorska dotyczy wykazania roli trzech dominujących fenotypów *Synechococcus* sp. w kształtowaniu różnorodności zbiorowisk fitoplanktonu. Praca koncentruje się na pikoplanktonowych siniach z rodzaju *Synechococcus* oraz mikroglonach wyizolowanych z przybrzeżnych wód Zatoki Gdańskiej. Aby wykazać

oddziaływanie allelopatyczne różnych fenotypów *Synechococcus* sp. na strukturę zbiorowisk fitoplanktonu, w pracy zestawiono eksperymenty laboratoryjne z modelowaniem ekologicznym. Celem niniejszej pracy było określenie czy oddziaływanie allelopatyczne może stanowić czynnik kształtujący zbiorowiska fitoplanktonu w Morzu Bałtyckim.

W niniejszej pracy sformułowano następujące hipotezy badawcze:
H1 Czynniki abiotyczne sprzyjające wzrostowi fenotypów *Synechococcus* powodują także zwiększenie ich aktywności allelopatycznej.
H2 Aktywność allelopatyczna różnych fenotypów *Synechococcus* wpływa na współwystępujące gatunki fitoplanktonu. Oddziaływanie allelopatyczne różni się w zależności od fenotypu *Synechococcus* oraz badanego gatunku targetowego.
H3 Siła oddziaływania allelopatycznego fenotypów *Synechococcus* kształtuje różnorodność zbiorowisk fitoplanktonu:

- Niska siła oddziaływania allelopatycznego jest związana z mniejszą różnorodnością fitoplanktonu, ze względu na dominację najsilniejszych konkurentów.
- Średnia siła allelopatii zwiększa różnorodność zbiorowisk, dzięki koegzystencji słabszych konkurentów, które są aktywne allelopatycznie oraz silniejszych konkurentów, które są wrażliwe na allelopatię.
- Wysoka siła oddziaływania allelopatycznego redukuje różnorodność, prowadząc do dominacji fenotypów *Synechococcus*.

Na podstawie przeprowadzonych badań wysunięto następujące wnioski:

- Czynniki abiotyczne sprzyjające wzrostowi badanych fenotypów *Synechococcus* jednocześnie zwiększają ich aktywność allelopatyczną. Jednak warunki optymalnego zasolenie nie zawsze mają ten sam efekt, ponieważ najsilniejszą aktywność allelopatyczną badanych fenotypów zaobserwowano w zasoleniu, z którego pierwotnie zostały one wyizolowane (8 PSU).
- Siła oddziaływań allelopatycznych zależała zarówno od fenotypu *Synechococcus*, jak i od badanego gatunku fitoplanktonu. Zaobserwowano zarówno pozytywne, jak i negatywne oddziaływanie allelopatyczne. *Synechococcus* Typ 3a wykazał najsilniejszą aktywność allelopatyczną, podczas gdy okrzemki były najbardziej wrażliwą grupą spośród badanych organizmów.
- Długoterminowe eksperymenty laboratoryjne oraz sporządzony model nie wykazały, aby limitacja azotu wpływała na siłę oddziaływania allelopatycznego. Niespodziewanie wykazano natomiast oscylacyjną koegzystencję wszystkich badanych sinic i mikroglonów.
- Oddziaływanie allelopatyczne *Synechococcus* przyczyniło się do zwiększenia różnorodności zbiorowisk fitoplanktonu w długoterminowych eksperymentach jedynie dla Typu 1 (BA-124). Oznacza to, że przy identycznej początkowej liczebności, Typ 1wykazuje silniejsze oddziaływanie allelopatyczne niż Typ 3a (BA-132).

4 LIST OF SCIENTIFIC WORKS CONSTITUTING THE DOCTORAL THESIS

4 WYKAZ PRAC NAUKOWYCH STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

1. **Konarzewska, Z.**, Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Latała, A., 2022. Effects of light intensity, temperature, and salinity in allelopathic interactions between coexisting *Synechococcus* sp. phenotypes. *Marine Environmental Research*, *179*, 105671. **IF = 3.0; 100 MNiSW**.

2. **Konarzewska, Z.,** Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Latała, A., 2025. Impacts of Nutrient Dynamics on Three Picocyanobacterial Populations: Laboratory Experiments and Seasonal Surveys in the Baltic Sea Region. *Estuarine, Coastal and Shelf Science*. **IF = 2.6; 100 MNiSW.**

3. **Konarzewska, Z.**, Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Vasconcelos, V., Latała, A., 2020. Assessment of the allelochemical activity and biochemical profile of different phenotypes of picocyanobacteria from the genus *Synechococcus*. *Marine Drugs*, *18*(4), 179. **IF = 4.9; 100 MNiSW.**

4. **Konarzewska, Z.,** Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Vasconcelos, V., Latała, A., 2025. Take this Waltz: allelopathy induces oscillatory coexistence in marine phytoplankton. Aim at: *The American Naturalist.* **IF = 2.4; 100 MNiSW.**

5. **Konarzewska, Z.,** Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Morais, J., Vasconcelos, V., Latała, A., 2025. Allelopathy of *Synechococcus* sp. phenotypes influences the structure of coexisting phytoplankton communities driven to equilibrium. *Marine Ecology Progress Series.* **IF = 2.2; 70 MNiSW.**

5 LIST OF OTHER PUBLICATIONS AND CHAPTERS BY THE DOCTORAL CANDIDATE

5 WYKAZ PUBLIKACJI I ROZDZIAŁÓW MONOGRAFII DOKTORANTA NIEUJĘTYCH W ROZPRAWIE DOKTORSKIEJ

1. Śliwińska-Wilczewska S., Wiśniewska K., **Konarzewska Z.,** Cieszyńska A., Barreiro Felpeto A., Lewandowska A.U., Latała A. 2021. The current state of knowledge on taxonomy, modulating factors, ecological roles, and mode of action of phytoplankton allelochemicals. *Science of the Total Environment, 773*, 145681. **IF = 6.551, 200 MNiSW.**

2. Śliwińska-Wilczewska, S., Wiśniewska, K. A., Budzałek, G., **Konarzewska, Z.,** 2021. Phenomenon of Allelopathy in *Cyanobacteria. In Ecophysiology and Biochemistry of Cyanobacteria* (pp. 225–254). Springer, Singapore, **20 MNiSW.**

3. Śliwińska-Wilczewska S., **Konarzewska Z.,** Wiśniewska K., Konik M. 2020. Photosynthetic pigments changes of three phenotypes of picocyanobacteria *Synechococcus* sp. under different light and temperature conditions. *Cells, 9*, 2030. **IF = 4.366, 140 MNiSW.**

4. Barreiro Felpeto A., Śliwińska-Wilczewska S., Klin M., **Konarzewska Z.**, Vasconcelos V. 2019. Temperature-dependent impacts of allelopathy on growth, pigment and lipid content between a sub-polar strain of *Synechocystis* sp. CCBA MA-01 and coexisting microalgae. *Hydrobiologia, 835*, 117–128. **IF = 2.165, 200 MNiSW.**

6 JUSTIFICATION FOR SCIENTIFIC RESEARCH

6 UZASADNIENIE PODJĘTEJ PROBLEMATYKI BADAWCZEJ

Picocyanobacteria are a polyphyletic group of aquatic microorganisms (<2 μm diameter), with *Synechococcus* sp. representing a key component of marine planktonic communities, in oligotrophic (Hunter-Cevera et al., 2020; Coello-Camba and Agustí, 2021), temperate, and brackish (Kuosa et al., 1991; Zufia et al., 2022) environments. Temperate ecosystems generate rapid community shifts over short timescales, driven by the seasonality of planktonic communities (Hunter-Cevera et al., 2020; Zufia et al., 2022), providing a unique and dynamic habitat for *Synechococcus*. This promotes the high phenotypic diversity observed within the genus, allowing adaptation to changing environmental conditions and dynamic water systems (Jasser et al., 2011; Holtermann et al., 2014; Śliwińska-Wilczewska et al., 2025). It also facilitates the coexistence of freshwater and marine clades (Sanchez-Baracaldo et al., 2008; Aguilera et al., 2023), as well as the presence of clades unique to the Baltic Sea (Haverkamp et al., 2009; Larsson et al., 2014).

Synechococcus from the Baltic Sea belongs to cluster 5 of picocyanobacteria (Castenholz, 2001). Within this cluster, some sub-clusters (5.1, 5.2, or 5.3) are primarily found in freshwater ecosystems, while others are adapted to marine environments (Aquilera et al., 2023). This distribution highlights the versatility and broad ecological range of *Synechococcus* (Callieri et al., 2022). Their further classification is based on differences in the composition of their phycobiliproteins, including phycocyanin (PC) and phycoerythrin (PE) (Six et al., 2007; Haverkamp et al., 2009).

Picocyanobacteria from the genus *Synechococcus* plays a crucial role in carbon flux and primary production across various aquatic environments, including oligotrophic oceans, coastal waters, estuaries, and freshwater systems (Jasser and Callieri, 2017; Wilk-Woźniak, 2019; Visintini et al., 2021; Zufia et al., 2021). *Synechococcus* serves as a key food source for ciliates, flagellates, and larger zooplankton, contributing to the stability of microbial food webs (Zufia et al., 2024). Beyond its role in nutrient cycling, *Synechococcus* can also influence ecosystem dynamics through the formation of massive blooms and the production of toxic compounds, which may disrupt marine food webs and biogeochemical processes (Śliwińska-Wilczewska et al., 2018a). Despite its ecological significance, knowledge of Baltic *Synechococcus* phenotypes remains limited, highlighting the need for further research to better understand its function in marine and freshwater environments.

The diversity of *Synechococcus* phenotypes is a key factor in their ecological adaptability, with environmental factors serving as proven drivers of their diversity and abundance (Callieri, 2017; Park et al., 2024). Various environmental factors, including light availability (Mella-Flores et al., 2012), temperature (Kuosa, 1991; Tamm et al., 2018; Zufia et al., 2021), salinity (Aguilera et al., 2023), and nutrient availability (Stal et al., 1999; Zufia et al., 2021), play critical roles in shaping the distribution and dynamics of *Synechococcus* in natural aquatic habitats. These factors are particularly relevant due to the ongoing shifts in global environmental conditions which particularly amplify variability in coastal and brackish ecosystems (Hunter-Cevera et al., 2020).

Temperature is a particularly important factor influencing *Synechococcus* growth and diversity (Hunter-Cevera et al., 2020). Higher temperatures generally enhance the

growth of certain phenotypes, a trend that becomes especially pronounced during seasonal fluctuations in *Synechococcus* abundance (Zufia et al., 2022; Stevens et al., 2024). Additionally, rising temperatures contribute to increased phenotypic diversity within *Synechococcus* populations, further shaping their ecological distribution (Coello-Camba and Agusti, 2024). The highest abundance of *Synechococcus* in the Baltic Sea (about 10^5 cells mL^{-1}) is observed during the summer season (Zufia et al., 2021), which is associated with higher temperature between 18 and 20°C (Kuosa, 1991; Tamm et al., 2018; Zufia et al., 2021). Limiting factors such as grazing (Celepli et al., 2017; Zufia et al., 2024) can regulate *Synechococcus* populations; however, they also contribute to reinforcing spring and fall blooms in temperate waters (Zufia et al., 2024). In these seasons, *Synechococcus* growth is primarily regulated by temperature in spring and by light availability in fall (Hunter-Cevera et al., 2020). Optimal growth of certain *Synechococcus* phenotypes occurred under lower light conditions (Eigemann et al., 2018). This adaptation to low light intensities provides a competitive advantage in changing aquatic environments, particularly where light availability is projected to decrease (Callieri, 2017). Nitrate and phosphate concentrations can also influence growth of *Synechococcus*. Nutrient concentrations in the Baltic Sea vary seasonally and geographically, with higher levels typically found in northern regions and lower concentrations in southern areas (Granéli et al., 1990; Konik et al., 2023). It is worth mentioning here, that marine *Synechococcus* clades (5.1) in the Baltic Sea are limited by nitrate (Schindler, 2006; Zufia et al., 2021), whereas freshwater clades (5.3) are limited by phosphate (Mills et al., 2004). Salinity can influence the abundance of *Synechococcus* by affecting the spatial distribution and diversity of its phenotypes within different salinity ranges (Bertos-Fortis et al., 2016; Aguilera et al., 2023). These environmental drivers, combined with *Synechococcus* phenotypic diversity, underscore their ecological success and adaptability in various marine environments. The research suggests the sensitivity of *Synechococcus* to changes in temperature, light availability, and nutrient conditions indicates that global warming can significantly alter the timing and magnitude of their blooms (Flombaum et al., 2013; Paerl et al., 2018; Hunter-Cevera et al., 2020). These blooms play a pivotal role in ecosystems, driven by the pressures of coastal eutrophication (Sorokin et al., 2004; Li et al., 2019) and increasing temperature (Flombaum et al., 2013; Dutkiewicz et al., 2015).

In the Baltic Sea, *Synechococcus* blooms have been documented (Zufia et al., 2021), and have been shown to exhibit allelopathic effects, suppressing competitors (Śliwińska-Wilczewska et al., 2017a). Recently, studies have been conducted on the allelopathic influence of Baltic *Synechococcus* phenotype on phytoplankton species occurring in the same environment (Śliwińska-Wilczewska et al., 2017b; 2018b). Given the ecological differences between *Synechococcus* phenotypes, particularly in relation to their photosynthetic pigment profiles and environmental adaptations, a broader investigation into their allelopathic activity is needed to better understand their role in phytoplankton community dynamics.

At the same time, the paradoxical high phytoplankton diversity, considering how low is the number of potential limiting resources (light, nutrients) that they share in an environment of very limited spatial heterogeneity (Hutchinson, 1961). Traditional models suggest that competition for limiting resources should lead to competitive exclusion of most of the species. The number of species coexisting in equilibrium must equal the number of limiting resources, given that a trade-off in the competitive ability between available

resources for the coexisting species (Tilman, 1977). Yet, phytoplankton communities exhibit a remarkable level of species diversity despite this theoretical constrain. Explanations for this phenomenon have included factors such as higher spatial heterogeneity than previously perceived, driven by hydrodynamics (Peters and Marrasé, 2000), avoidance of exclusion due to complex life cycles (McQuoid and Hobson, 1996), periodic fluctuations of resource supply in a species set composed by almost-equal competitors (Grenney et al., 1973), and faster demographic than mixing rates (Richerson et al., 1970). All these hypotheses, in the end, suggest that plankton communities are not really in equilibrium, so there is no time for competitive exclusion to occur. An explanation that still involves equilibrium would be deterministic chaos (Huisman and Weissing, 1999) for which there is very little experimental evidence (Benincà et al., 2008). Nowadays it is clearly viewed that phytoplankton communities are out of equilibrium, and that they develop in a constantly changing spatial-temporal niche. However, the lack of time for exclusion does not resolve everything. It is still unclear how, at a given spatial-temporal niche, so many species seem to coexist in what have to be differentiated niches. Therefore, there must be more mechanisms influencing niche differentiation than the originally assumed, essentially light and nutrient competition (Tilman 1977; Stomp et al., 2004). Some mechanisms already suggested were differential grazing pressure (Sommer and Sommer, 2006), and viral interactions (Flynn et al., 2022), Here, allelopathy presents an alternative, yet relatively understudied, mechanism that may also shape phytoplankton community structure by introducing a new dimension for niche differentiation.

Phytoplankton allelopathy is a biological process in which biochemicals secreted by one algal species influence the growth and photosynthetic activity of another, either positively or negatively (Legrand et al., 2003). Studies on allelopathy have primarily focused on short-term bioassays, using "*cross-culturing*" or "*mixed-culture*" methods (Suikkanen et al., 2004; Barreiro et al., 2019). The "*cross-culturing*" method involves the addition of cell-free filtrates from the donor species to a target species, allowing researchers to investigate allelopathic influence while eliminating direct competition between the two species (Suikkanen et al., 2004). This method has been widely used to assess allelopathic effects on both monocultures and natural phytoplankton assemblages (Suikkannen et al. 2005; Śliwińska-Wilczewska et al., 2017a). While "*cross-culturing*" highlights competitive interactions among studied strains, "*mixed-culture*" experiments make it challenging to isolate the specific effects of allelopathy (Suikkanen et al., 2004). All the above referred experiments are useful to detect the existence of allelopathic interactions. However, they do not capture the effect of more complex environmental factors. Additionally, all these short-term assays do not capture the effect of allelopathy in species dynamics and community structure.

An experimental approach focused on the ecological dynamics involves the development of theoretical models including the effect of allelopathy on interspecific interactions. Simple theoretical models suggest that allelopathy in phytoplankton competition can enable an inferior competitor to exclude a stronger resource competitor or promote species coexistence (Chao and Levin 1981; Durret and Levin 1997; Roy and Chattopadhyay 2007; Roy 2009). Barreiro et al. (2018a) presented both theoretical and experimental evidence suggesting that allelopathy can facilitate the coexistence of two phytoplankton species competing for a single limited resource. However, this framework has

yet to be extended to more complex planktonic communities and did not concern picocyanobacteria from the genus *Synechococcus*.

Despite increasing recognition of allelopathy as an important ecological mechanism, significant knowledge gaps remain regarding its role in shaping natural phytoplankton communities. The objective of this research is through a combination of laboratory experiments and ecological modeling to investigate the potential influence of allelopathy from different *Synechococcus* phenotypes as a driver of phytoplankton community structure.

7 HYPOTHESES AND AIMS

7 HIPOTEZY I CELE

The **primary aim** of this thesis was to investigate the role of *Synechococcus* allelopathy in the structuring of phytoplankton community in the Baltic Sea.

To achieve this aim, the following hypotheses were formulated:

H1 Abiotic factors that promote the growth of *Synechococcus* phenotypes enhance their allelopathic activity.

H2 Allelopathy exhibited by different *Synechococcus* phenotypes affect co-occurring phytoplankton species.

- The allelopathic effect is different depending on the *Synechococcus* phenotype, and target phytoplankton species.

H3 The strength of allelopathy from *Synechococcus* phenotypes influences plankton community diversity:

- Low allelopathic strength is associated with lower diversity due to dominance by strongest competitors.
- Intermediate allelopathic intensity increases community diversity due to coexistence between allelopathic weak competitors and sensitive stronger competitors.
- High allelopathic intensity reduces diversity due to dominance by *Synechococcus* phenotypes.

To test these hypotheses, the following steps were performed:

1. Determination of the effect of abiotic factors (temperature, light intensity, salinity, and nutrient concentrations) on the allelopathic activity of *Synechococcus* phenotypes.
2. Evaluation of the allelopathic effects of *Synechococcus* phenotypes on various cyanobacterial and microalgal strains.
3. Testing the effect of different strengths of allelopathy in an outcome of competition with a group of selected co-occurring phytoplankton species.
4. Demonstratingthe effect of different strengths of allelopathy on the diversity of natural phytoplankton communities co-occurring with *Synechococcus* phenotypes.

8 METHODOLOGY AND RESEARCH DESIGN

8 METODYKA I KONCEPCJA BADAN

8.1 MATERIAL OF THE STUDY

All studied organisms were isolated from the coastal waters of the Gulf of Gdańsk (Baltic Sea) and were maintained in the Culture Collection of Baltic Algae (CCBA) at the University of Gdańsk (Latała et al., 2006).

The phenotypes of *Synechococcus* used in the study consisted of three strains, differentiated based on phycobiliproteins content (Six et al., 2005; 2007; Larson et al., 2014, Table 1). Field surveys have shown that all three phenotypes of picocyanobacteria occur in the Baltic Sea (Aguilera et al., 2023). Previous studies have proven only the allelopathic activity of Type 1 against the co-existing phytoplankton species (Śliwińska-Wilczewska et al., 2016; 2017a; 2018b). However, the allelopathic activity of the remaining phenotypes has not yet been studied, which served as the focus of **Publications 1-3.**

Table 1. Phenotypic characterization of *Synechococcus* used in this thesis.

Synechococcus **phenotype (classification by Six et al., 2007)**	**Dominant phycobiliprotein**	***Synechococcus*** **symbol in CCBA**	**Classification by color**
Type 1	Phycoerythrin (PEI) *	BA-124	Green strain
Type 2	Phycocyanin (PC)	BA-120	Red strain
Type 3a	PC, PEI and PEII*	BA-132	Brown strain

*Phycoerythrin is divided into two forms PEI and PEII

As a result of the initial screening of coexisting microalgae among CCBA strains (**Publication 3**, Table 2), three strains were chosen for further investigation (**Publication 4**). The chosen species are sensitive to allelopathy of *Synechococcus* and are expected to be a better competitor for nitrate, which was later investigated in **Publication 4**.

To effectively test hypothesis 3 and achieve aim 4, further studies on the allelopathic activity of *Synechococcus* phenotypes were conducted using natural phytoplankton communities brought to equilibrium under laboratory conditions (**Publication 5**).

Table 2. Donor and target *Synechococcus* and phytoplankton organisms used in the allelopathy experiments of this thesis.

Synechococcus phenotype	Target organism with symbol in CCBA	Publication
Type 1, Type 2, Type 3a	*Synechococcus* BA-120, BA-124, BA-132	Publication 1 and 2
Type 1, Type 2, Type 3a	**Cyanophyta** *Planktolyngbya* sp. BA-50 *Aphanizomenon* sp. BA-69 *Nostoc* sp. BA-81 *Synechocystis* sp. BA-121 *Phormidium* sp. BA-141 *Pseudanabaena* sp. BA-142 **Chlorophyta** *Monoraphidium convolutum* var. *pseudosabulosum* BA-17 *Chlorella fusca* BA-18 *Kirchneriella obesa* BA-51 *Monoraphidium* sp. BA-165 *Chlorella* sp. BA-167 *Oocystis* cf. *submarina* BA-172 **Heterokontophyta** *Cyclotella meneghiniana* BA-10 *Amphora coffeaeformis* BA-16 *Navicula perminuta* BA-30 *Nitzschia fonticola* BA-34 *Fistulifera saprophila* BA-56 *Skeletonema marinoi* BA-98	Publication 3
Type 1, Type 2	*Synechocystis* sp. BA-121 *Chlorella* sp. BA-167 *Monoraphidium convolutum* var. *pseudosabulosum* BA-17	Publication 4
Type 2, Type 3a	Natural phytoplankton community	Publication 5

8.2 CULTURE CONDITIONS

Cyanobacterial and microalgal strains from CCBA were grown in f/2 medium (Guillard, 1975) (**Publications 1–3**). The culture medium was prepared using Baltic Sea water (salinity 8 ‰), filtered through glass fiber filters (Whatman GF/C), and autoclaved. Experiments were conducted under standard conditions of photosynthetically active radiation (PAR) at 10 µmol photons m^{-2} s^{-1} with a photoperiod of 16:8 (light:dark), at 18°C and salinity 8 ‰. Exceptions were made for experiments testing the influence of abiotic

factors on allelopathy, where the standard conditions described above were selectively manipulated (**Publications 1–2**).

Experiments with the selected CCBA phytoplankton strains (**Publication 4**) and with natural communities (**Publication 5**), were conducted under the same conditions as described above, except that nitrate (NO_3^-) concentration was held constant at 120 µM to induce nitrate limitation. Due to the previous experience of the main supervisor of the dissertation (Barreiro et al., 2018a) the experiments necessary to prepare the model of interspecific competition for nitrate with allelopathy (nitrate competition experiments, and the pair-wise comparison short allelopathic assays), were conducted in CIIMAR, University of Porto, those experiments were conducted at 25 µmol photons m^{-2} s^{-1} PAR, with a photoperiod of 16:8 (light:dark), at 23°C and salinity 8 ‰ (**Publication 4**). In those experiments, medium composition was modified, with nitrate concentration of 320 µM.

8.3 NITRATE ANALYSIS

For the analysis of nitrate (NO_3^-), NitraVer® 5 nitrate reagent pads were used to generate the color reaction. The absorbance was measured with a DR6000 spectrophotometer (Hach-Lange, Loveland, USA). Nitrate was measured to ensure no differences between control and experimental treatments (**Publications 1**, **2**, and **3**), as well as to confirm nitrate limitation and the attainment of equilibrium in nitrate competitive ability experiments (**Publication 4**) and in semi-continuous natural community cultures (**Publication 5**).

8.4. SHORT-TERM ALLELOPATHIC ASSAYS

We conducted the short-term allelopathic assays to study the influence of the abiotic factors on the allelopathic strength of three studied phenotypes (**Publication 1** and **2**), the sensitivity of co-occurring species to allelopathy from three *Synechococcus* phenotypes (**Publication 3**), and the pair-wise comparison of the chosen phytoplanktonic species and two *Synechococcus* phenotypes (**Publication 4**).

The method used in **Publication 1-4** consisted of a cell-free filtrate addition. The methodology followed the previous works by Suikkanen et al., 2004 and Śliwińska-Wilczewska et al., 2016; 2017a; 2018b. The experimental cultures consisted of the target organism being studied, either a different *Synechococcus* phenotype (**Publication 1** and **2**) or a co-existing phytoplankton species (**Publication 3** and **4**). These cultures were treated with a cell-free filtrate, prepared by filtering picoplanktonic cultures through 0.45-µm filters (Macherey-Nagel MN GF-5).

Additionally, in **Publication 1** and **2**, the mixed culture experiments were conducted following the methodology in Barreiro Felpeto et al. (2019). In those experiments, an equal amount of different *Synechococcus* phenotypes were added to the target species. In both methods, the control cultures consisted of adding f/2 medium instead of filtrate or *Synechococcus* culture.

8.5 PIGMENTS ASSAY

To further study the allelopathic effect of *Synechococcus* in **Publication 3** the pigment content of chlorophyll a (Chl *a*) and carotenoids (Car), was measured in target organisms,

after 7 days of exposure. Pigments were extracted using 90% acetone in the dark at 6°C for 1 hour, followed by centrifugation at 13,000 rpm for 1 minute. Absorbance was measured at 480, 665, and 750 nm using a spectrophotometer. Chlorophyll *a* concentration was calculated using formulas specific to cyanobacteria, green algae, and diatoms (Jeffrey and Humphrey, 1975), and carotenoid concentrations were determined using the method of Strickland and Parsons (1972).

8.6. FLUORESCENCE ASSAY

The effect of allelopathy on chlorophyll fluorescence was studied (**Publication 3**) following the methodology in Campbell et al. (1998) and Śliwińska-Wilczewska et al. (2016). The effects of cell-free filtrates from three *Synechococcus* phenotypes on the chlorophyll fluorescence of target species were assessed using the maximum PSII quantum efficiency (F_v/F_m). Measurements were taken with a Pulse Amplitude Modulation (PAM) fluorometer after 7 days of exposure. Samples were filtered through glass fiber filters and kept in the dark for 10 minutes prior to measurement.

8.7 DETERMINATION OF CELL ABUNDANCES

8.7.1 DETERMINATION OF CELL ABUNDANCES BY FLOW CYTOMETRY

In **Publications 1** and **2**, *Synechococcus* abundance was measured using a BD Accuri C6 Plus flow cytometer with blue (480 nm) and red (640 nm) lasers at a flow rate of 14 µL min^{-1}. Events were recorded in list mode, and the instrument was calibrated with SPHERO Rainbow Calibration Particles and Spherotech Validation Beads. Cell counts were recorded on days 0, 1, 3, and 7 of the experiment, following the method of Śliwińska-Wilczewska et al. (2016; 2018a).

In **Publication 3**, cell abundances of cyanobacteria and microalgae were estimated with optical density (OD) calibrated with cell counts (N mL^{-1}). For this calibration, the cell counts of *cyanobacteria,* green algae and diatoms were made with a BD Accuri C6 Plus flow cytometer. Then, linear regression models were fit between OD and cell counts (Table 5 in **Publication 3**).

8.7.2 DETERMINATION OF CELL ABUNDANCES BY MICROSCOPIC COUNTS

In this study, the cell counts were determined using various methods. The abundances of filamentous cyanobacteria were counted from 48 large squares in Bürker chamber (Guilard and Sierocki, 2005), on day 7 of the experiments (**Publication 3**). Daily cell abundances were performed using the Neubauer chamber (in the experiments for independent parameterization of the model) and using Bürker chamber in the long-term experiments with the selected phytoplankton strains (**Publication 4**). Furthermore, daily abundances of cells using either Ütermöhl or Bürker counting chambers, depending on the phytoplankton abundance in natural communities, were performed in **Publication 5.** Cells were counted using a Tomic SFC-18 light microscope at magnification 40x or 20x depending on the cell size.

8.8 MODEL OF INTERSPECIFIC COMPETITION FOR NITRATE WITH ALLELOPATHY

In **Publication 4** it was constructed a model of interspecific competition for a single limiting resource (nitrate) with allelopathic interactions among 4 phytoplankton strains (including one of the three *Synechococcus* phenotypes). This included independent experiments to parameterize the competitive ability for nitrate of each of the species, and short term allelopathic assays between all the pair-wise combination of strains (described in section 8.4).

In order to conduct the nitrate competitive ability experiments, medium-term growth experiments were performed in batch culture, using nitrate as limiting resource. In these experiments were estimated the daily cell abundances and nitrate concentration (see section 8.3).

MODEL FORMULA

$$\frac{dN}{dt} = \partial(N_0 - N) - F_1(N)\frac{1}{\eta_1}P_1 - F_2(N)\frac{1}{\eta_2}P_2 - F_3(N)\frac{1}{\eta_3}P_3 - F_4(N)\frac{1}{\eta_4}P_4$$

$$\frac{dP_1}{dt} = F_1(N)P_1 - \varphi_1(P_2)\varphi_2(P_2)\gamma_{21}P_1 - \varphi_1(P_3)\varphi_2(P_3)\gamma_{31}P_1 - \varphi_1(P_4)\varphi_2(P_4)\gamma_{41}P_1 - \partial P_1$$

$$\frac{dP_2}{dt} = F_2(N)P_2 - \varphi_1(P_1)\varphi_2(P_1)\gamma_{12}P_2 - \varphi_1(P_3)\varphi_2(P_3)\gamma_{32}P_2 - \varphi_1(P_4)\varphi_2(P_4)\gamma_{42}P_2 - \partial P_2$$

$$\frac{dP_3}{dt} = F_3(N)P_3 - \varphi_1(P_1)\varphi_2(P_1)\gamma_{13}P_3 - \varphi_1(P_2)\varphi_2(P_2)\gamma_{23}P_3 - \varphi_1(P_4)\varphi_2(P_4)\gamma_{43}P_3 - \partial P_3$$

$$\frac{dP_4}{dt} = F_4(N)P_4 - \varphi_1(P_1)\varphi_2(P_1)\gamma_{14}P_4 - \varphi_1(P_2)\varphi_2(P_2)\gamma_{24}P_4 - \varphi_1(P_3)\varphi_2(P_3)\gamma_{34}P_4 - \partial P_4$$

Where:

$$F_i(N) = \frac{\mu_{max_i}N}{K_i + N}$$

$$\varphi_1(P_i) = e^{-aP_i} + b$$

$$\varphi_2(P_i) = max\left[\frac{P_i - n_0}{P_i}, 0\right]$$

For i = 1, 2, 3, 4

Where: μ_{max} - the maximum growth rate, K_i - the half saturation constants for growth with the limiting nutrient, N - the concentration of limiting nutrient in the culture, N_0 - the inflowing concentration of limiting nutrient, ∂ - the dilution rate of the system, P_i (being i = 1, 2, 3, 4) are the population abundances of the species in competition (species 1, 2, 3 and 4), η_i - the yield coefficients of species i, γ - the "killing parameter" denoting the allelopathic effect, , φ - function for allelochemical production. a - the rate of the process, b - a lower threshold. n_0 is the minimum abundance of the allelopathic species required to produce the allelopatic effects.

The parameters obtained from these nitrate competitive ability experiments and the pair-wise allelopathy assays (section 8.4) were further optimized to fit the actual data obtained from the long-term experiments described below (section 8.9). The optimized

models served as the basis to study the behavior of this system by model simulations extrapolating to different scenarios and equilibrium analysis.

8.9 LONG-TERM COMPETITION EXPERIMENTS

The aim of these experiments was to observe the outcome of ecological interspecific competition in experimental systems driven to equilibrium. In order to drive these experimental systems to equilibrium, we performed both continuous culture (**Publication 4**), and in semi-continuous culture conditions (**Publication 5**). The experimental design followed the previous works from the supervisor from CIIMAR (Barreiro et al., 2018a). Briefly, the growth in the system in equilibrium was controlled by a single limiting resource (nitrate). The species were inoculated and their dynamics (and those of the limiting resource) followed in the long-term. In **Publication 4**, these experiments were performed with four strains of Baltic phytoplankton species, including one of the two *Synechococcus* phenotypes, and lasted for 90 days. In **Publication 5**, these experiments were run with natural phytoplankton communities plus one of the three *Synechococcus* phenotypes and lasted for 79 days.

8.10 STATISTICAL ANALYSIS

Statistical analyses were conducted to evaluate the effects of abiotic factors, allelopathic interactions, and experimental treatments on *Synechococcus* phenotypes, target species, and plankton community dynamics. Two-factor ANOVA was employed to examine the effects of temperature, irradiance, and salinity on mixed cultures and cell-free filtrates, with post-hoc Dunnett tests used to compare experimental conditions with controls (**Publication 1**). Repeated measures ANOVA was used to assess the effects of *Synechococcus* filtrates on the growth and fluorescence of target species, while one-way ANOVA evaluated changes in chlorophyll *a* and carotenoid concentrations on the seventh day of exposure (**Publication 3**).

For experiments investigating nutrient concentrations, general linear models were applied, followed by ANOVA and post-hoc Tukey and Dunnett tests. The normality of residuals was verified using the Shapiro-Wilk test, and non-normal variables were transformed with an optimization method. Outliers were identified with the Bonferroni test (**Publication 2**).

To analyze long-term community dynamics, hierarchical cluster analysis with Bray-Curtis distance was performed to determine community homogeneity over time, with significance assessed using 1000 bootstrap repetitions. Correspondence analysis (CA) was conducted to explore relationships between taxa and treatments. ANOVA with Tukey post-hoc tests were applied to evaluate the effects of treatments on species richness, evenness, and Shannon-Wiener diversity indices. A Monte-Carlo approach was used to ensure the robustness of the results, simulating increased sample size by data randomizations (**Publication 5**).

Statistical analyses were performed using Statistica® 13.1 (**Publications 1** and **3**) and R (R Core Team, 2023; with the stats, car, DescTools, vegan, and factoextra packages; **Publications 2, 4,** and **5**).

9 THE SCIENTIFIC WORKS CONSTITUTING THE DOCTORAL THESIS

9 WYKAZ PRAC NAUKOWYCH STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

9.1 PUBLICATION 1

9.1 PUBLIKACJA 1

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Effects of light intensity, temperature, and salinity in allelopathic interactions between coexisting *Synechococcus* sp. phenotypes

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ABSTRACT

Organisms from the *Synechococcus* genus constitute one of the major contributors to oceanic primary production, broadly distributed in waters with wide range of environmental conditions. This work investigated the influence of abiotic factors (temperature, irradiance, and salinity) on the strength of allelopathic interactions between different phenotypes of picoplanktonic cyanobacteria of the genus *Synechococcus* sp. (Type 1, Type 2, and Type 3a) employing mixed cultures and cell-free filtrate assays. The response variables studied were population growth and content of photosynthetic pigments: chlorophyll *a* (Chl *a*), carotenoids (Car), phycocyanin (PC), phycoerythrin (PE), and allophycocyanin (APC). Temperature was shown to be the most significant abiotic factor impacting the allelopathy of *Synechococcus* sp. phenotypes, with the Type 2 most significantly impacted. Irradiance also had a significant effect, having the largest effect on allelopathy of Type 3a phenotype. Changes in salinity had the greatest effect on allelopathy of Type 1. Our study has shown the significant influence of temperature, irradiance, and salinity on the strength of allelopathic compounds secreted by *Synechococcus* sp. phenotypes, with temperature the most significantly affecting allelopathic properties. Moreover, we discovered that the allelopathic response to changing environmental factors is highly phenotype-specific. This differential response of allelopathy could help different phenotypes of *Synechococcus* sp. to coexist in the water column.

1. Introduction

The genus *Synechococcus* is a polyphyletic group of non-diazotrophic picocyanobacteria with high ecological importance both in freshwater and marine environments. Organisms from the *Synechococcus* genus constitute one of the major contributors to oceanic primary production, broadly distributed in waters with a wide range of temperature, irradiance, and salinity (Flombaum et al., 2013; Kim et al., 2018).

In the frame of global change, it has been forecasted increased dominance of diazotrophic cyanobacteria and *Synechococcus* sp. in ocean planktonic communities (Flombaum et al., 2013; Dutkiewicz et al., 2015; Li et al., 2019). Bloom forming phytoplankton species are favored by eutrophication of coastal areas, ocean acidification, and rising water temperature (Suikkanen et al., 2013; Paerl, 2018; Zhu et al., 2020; Bao and Gao, 2021; Śliwińska-Wilczewska et al., 2021). An increase in the frequency and geographic spread of *Synechococcus* sp. blooms has already been detected (Oziel et al., 2017; Li et al., 2019). Undesirable effects of these blooms could be anoxia and light deprivation in the water column (Allen and Gillooly, 2006). Moreover, these changes are especially prominent in coastal and estuarial areas, with the Baltic Sea, as a semi-enclosed sea with low biodiversity, being more prone to environmental changes (Philippart et al., 2011). Studies showed that a decrease in salinity caused by a an increase in water temperature (up to 5 °C (HELCOM, 2013)) amplified by increased river runoff (Neumann et al., 2012) favors picoplanktonic cyanobacteria species in the phytoplankton community (Berner et al., 2018).

Synechococcus sp. phenotypes occurring in the marine habitats differ in their dominant phycobiliproteins, which enabled them to occupy different ecological niches (Scanlan et al., 2009; Scanlan, 2012; Callieri et al., 2022). Phenotypes with dominance of PC (highest absorption at 600–650 nm) are adapted to the high irradiance levels of surface waters (Six et al., 2007; Haverkamp et al., 2009; Larsson et al., 2014). Other phenotypes are characterized by the dominance of PE, (highest absorption at 450–600 nm) providing adaptation to greater depths and

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lower light intensities. Śliwińska-Wilczewska et al. (2018a) differentiated three main Baltic *Synechococcus* phenotypes. The green strain with the dominance of PC (Type 1), the red strain with a high amount of the first type of phycoerythrin (PEI) (Type 2), and the brown strain with PC, PEI, and PEII (Type 3). Furthermore, Type 3 can be divided into types (a–d) with low to high phycoerythrobilin (PEB) and orange-colored phycourobilin (PUB) ratio (Six et al., 2007; Haverkamp et al., 2009). The Baltic Sea is characterized by high water dynamics (Holtermann et al., 2014) which forces picocyanobacteria to share similar spatial or temporal distribution in the water column. Therefore, it is difficult to talk about clear different niches occupied by these organisms. Regarding light, conditions are as variable as in other systems due to the depth gradient and seasonal changes. Pigment specialization allows for the coexistence of *Synechococcus* species in the water column (Stomp et al., 2004). The difference in composition of the photosynthetic pigments, especially phycobiliproteins, expands the light spectrum available for each phenotype, increasing biodiversity of phototrophic organisms (Ernst et al., 2003).

However, apart from pigment composition, these picocyanobacteria have other mechanisms that allow them to coexist. We hypothesize that allelopathy is one of these mechanisms and can be a significant factor in the occurrence of Baltic *Synechococcus* sp. phenotypes at various depths. Nevertheless, allelopathic interactions between different phenotypes has not been previously studied.

Allelopathy is spread among primary producers from all aquatic environments (Gross, 2003; Śliwińska-Wilczewska et al., 2021). The production of allelochemical compounds is a strategy by which they can compete with the co-occurring phytoplankton species, affecting interspecies competition and influencing the diversity of phytoplankton communities (Fistarol et al., 2003; Suikkanen et al., 2004; Barreiro Felpeto et al., 2018a). Allelopathic compounds released by some species may also contribute to the formation of monospecific blooms (Figueredo et al., 2007; Suikkanen et al., 2011).

Recent studies demonstrated allelopathic effects of the three *Synechococcus* phenotypes on co-existing phytoplankton species (Śliwińska-Wilczewska et al., 2018b; Konarzewska et al., 2020). Conducted studied has shown that the secretion and the strength of allelopathic substances produced by *Synechococcus* sp. phenotypes varied depending on the studied phenotype, with brown phenotype proven the strongest (Konarzewska et al., 2020).

The present work aims to investigate the influence of environmental factors (temperature, light, and salinity) on the strength of allelopathic interactions between three different phenotypes of *Synechococcus* sp. The experimental approach consists in the study of growth in mixed cultures, as well as effects in growth and content of photosynthetic pigments (Chl *a*, Car, PC, PE, and APC) after the addition of cell-free filtrate under different temperatures (13, 18, and 23 °C) and irradiances of 50 and 100 μmol photons $m^{-2}s^{-1}$ representing conditions at the surface waters and below it. this represents the hypothetical distribution of the phenotypes in the water column. As well as the salinity values of 3 and 13‰, which influence the special distribution of studied phenotypes. Moreover, as previously mentioned, the strength of allelopathic interaction depends heavily on the studied phenotype. That is why the study aims to determine intra-species allelopathic interaction, which may be important in explaining the variety of *Synechococcus* phenotypes in the brackish ecosystem.

2. Materials and methods

2.1. Species and standard culture conditions

The three phenotypes of picoplanktonic cyanobacteria *Synechococcus* Type 1 (green strain), Type 2 (red strain), and Type 3a (brown strain), were isolated from the coastal zone of Gulf of Gdańsk, Baltic Sea and obtained from the Culture Collection of Baltic Algae (CCBA), located in the Institute of Oceanography at the University of Gdansk (Latała et al., 2006), where they are maintained as strains *Synechococcus* sp. (BA-124) *Synechococcus* sp. (BA-120), and *Synechococcus* sp. (BA-132), respectively. Cyanobacterial cultures were grown in an f/2 medium (Guillard, 1975). Culture media was prepared using Baltic Sea water (salinity 8‰) measured with salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany) filtered through glass fiber filters (Whatman GF/C) and autoclaved. The light was provided by an artificial light source (Cool White 40W, Sylvania, USA). Measurements of PAR (Photosynthetically Active Radiation) were made using the Li-Cor meter, model LI-189 with a cosine collector. The standard culturing conditions of the picocyanobacterial strains were 18 °C of temperature and photosynthetically active radiation (PAR) of 10 μmol photons $m^{-2}s^{-1}$ with photoperiod L:D 16:8.

2.2. Culture conditions for the study of abiotic factors

In order to test the effect of irradiance in mixed cultures and in cell-free filtrate assays, the organisms were cultured modifying the standard conditions detailed above (i.e., 10 μmol photons $m^{-2}s^{-1}$, 18 °C, 8‰) to irradiances of 50 and 100 μmol photons $m^{-2}s^{-1}$. For the evaluation of the effect of temperature, the standard conditions were modified to 13 and 23 °C. These experiments were conducted in thermostat chambers (Biogenet, fitotron chamber, Józefów, Poland). The test of different salinities was carried put modifying the standard conditions to salinities of 3 and 13‰, which were chosen based on salinity in the Baltic Sea region. All cultures were acclimated to the specific conditions for one week. These conditions were then set for the mixed cultures and for the cultures of the donor phenotype in the case of the cell-free filtrate assays.

2.3. Allelopathy assays on growth of Synechococcus sp. phenotypes using mixed cultures

The mixed cultures experiments were prepared according to the method described by Barreiro Felpeto et al. (2018a). In these experiments, the initial ratio of donor picocyanobacteria to target phenotypes was adjusted to 1:1 in the same way as detailed above for the cell-free filtrate experiments. Mixed cultures were set in 25-mL Erlenmeyer flasks, with 10 mL of the tested phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3a) in the logarithmic growth phase and adding 10 mL of another phenotype. In all experiments, the initial Chl *a* concentration was 0.4 μg Chl *a* mL^{-1}. For this purpose, all picocyanobacterial cultures were first diluted with the fresh f/2 medium to obtain the initial Chl *a* concentration (0.4 μg Chl *a* mL^{-1}) and then proper donor culture was added to target phenotype (V = 10 mL), which contained the same initial Chl *a* concentration.

The co-cultures of Type 2 and Type 3a,were undistinguishable due to pigments changes in various abiotic conditions (Fig. S1 in Supplementary material). The control cultures were prepared by adding 10 mL of f/2 medium to 10 mL of culture of each phenotype. As the cultures were in logarithmic growth phase, adding 10 mL of f/2 media to the control did not caused difference to the treatment groups. Moreover, lack of difference in the amount of nutrients was confirmed in a similar experiment carried out by Barreiro Felpeto et al. (2018a). The experiments were conducted in triplicates.

Population growth of each *Synechococcus* sp. phenotypes was recorded on 1st, 3rd, and 7th days of the experiment, in order to detect initial changes in phenotypes growth, as well as the response and trend of growth in studied conditions. We also selected day 1, 3, and 7 for recorded the growth so that we could compare the data with other studies on *Synechococcus* sp. allelopathy (Śliwińska-Wilczewska et al., 2016; 2017; Śliwińska-Wilczewska and Latała, 2018; Barreiro Barreiro Felpeto et al., 2019; Konarzewska et al., 2020). The results of growth experiments in mixed cultures were presented as the percent of the control culture.

2.4. Allelopathy assays on growth of Synechococcus sp. phenotypes and pigment content with cell-free filtrate

The cell-free filtrates of *Synechococcus* strains were prepared by filtering picoplanktonic culture through a 0.45-μm filter (Macherey-Nagel MN GF-5).The experiments were conducted by adding 10 mL of this cell-free filtrate from cultures of each *Synechococcus* sp. phenotype to 25-mL Erlenmeyer flasks, containing 10 mL of the target phenotype of *Synechococcus* sp. (Type 1, Type 2, and Type 3a). Control cultures were carried out by the addition of 10 mL of f/2 medium instead of cell-free filtrate. In all experiments, the initial Chl *a* concentration was 0.4 μg Chl *a* mL^{-1}. Thus, the abundances of the initial cultures were set from 10^5 to 10^6 cells mL^{-1}. The experiments were conducted in triplicates.

Population growth was recorded on 1st, 3rd, and 7th days of the experiment and the results of growth experiments after the addition of cell-free filtrate were presented as the percent of the control culture. The photosynthetic pigments content of chlorophyll *a* (Chl *a*), carotenoids (Car), phycocyanin (PC), phycoerythrin (PE), and allophycocyanin (APC) was analyzed in the last day of the experiments.

Chl *a* and Car were measured in 8 mL samples. Extraction was performed in 2 mL of 90% acetone, which was then kept in low temperature and light for about a day. This extract was centrifuged for 1 min at 13,000 rpm min^{-1}. Measurements were performed in a Thermoscientific spectrophotometer (Multiskan GO UV-VIS, Thermo Scientific, Massachusetts, USA), at wavelengths of 480, 665, and 750 nm. Chl *a* was estimated with the formula from Jeffrey and Humphrey (1975). Car pigment concentration was estimated using the formula from Strickland and Parsons (1972).

In order to measure the phycobiliproteins content, 8 mL of culture were filtered using 0.45 μm Macherey-Nagel MN GF-5 filter and refrigerated for about a day. Afterward, pigment extraction continued in a reagent consisting of 2 mL of 0.25 M Trizma Base, 10 mM binary EDTA, 2 mg mL^{-1} lysozyme, and the addition of HCl. This extract was centrifuged for 1 min at 13,000 rpm and measured using Thermoscientific spectrophotometer at 565, 620, 650, and 750 nm. Concentrations of PC, PE, and APC were estimated with the formulas from Bennett and Bogorad (1973) and Bryant et al. (1979).

2.5. Estimation of cell abundances

In all the experiments, picocyanobacterial abundance was measured with flow cytometer (BD Accuri C6 Plus; BD Biosciences, San Jose, CA, USA), using the method by Śliwińska-Wilczewska et al. (2018c). The measurements were carried out using the combination of the blue laser (480 nm) and the red laser (640 nm), with the flow rate of 100 μl min^{-1} (measured with the FL3 and FL4 detectors). Each event was recorded in a list mode. The flow cytometer was calibrated with SPHERO Rainbow Calibration Particles and Spherotech 6- and 8- Peak Validation Beads (BD Biosciences). The number of cells were recorded at the beginning of the experiment (day 0) and then on the 1st, 3rd, and 7th day of the experiment.

2.6. Statistical analyses

Data from the experiments with mixed cultures and cell-free filtrates under varying temperatures, irradiances, and salinities were analyzed with two-factor analysis of variance and a post-hoc Dunnett test. The values were presented as mean ± SD (n = 3). Asterisks indicated significant difference with post hoc Dunnett's test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The software employed was Statistica® 13.1.

3. Results

3.1. Influence of abiotic factors on growth of Synechococcus sp. phenotypes in mixed cultures experiments

The results of the growth of the combination of the three phenotypes in the different abiotic conditions are shown in Fig. 1. Individually, each Type responded strongly to studied abiotic factors, resulting in different growth depending on the environmental condition. In most cases, a significant inhibition of growth was detected. Furthermore, the factor that most significantly influenced the interaction between phenotypes was shown to be the temperature (ANOVA, $p < 0.001$, for Type 1, Type 2, and Type 3a; Fig. 1 (A-C)). The strongest detrimental effect was observed between the Type 1 and Type 3a at 13 °C (1A and 3A) and 23 °C (1C and 3C), with the highest decrease in cell abundance of Type 3a at 23 °C, with a decline of 6.87×10^7 cell mL^{-1} relative to control, on the 7th day. In addition, strong effect on cell abundance occurred in Type 2 at 13 °C and 18 °C (2A-B) and Type 3a at 18 °C (3B) cultured with Type 1, with stronger negative effect at higher temperature. The same trend could be seen in Type 1 phenotype, with highest decrease of abundance after the addition of Type 3a (1C), with a decrease of 3.6×10^7 cell mL^{-1} compared to control.

There was significant growth inhibition in mixed cultures at irradiances of 50 μmol photons $m^{-2}s^{-1}$ and 100 μmol photons $m^{-2}s^{-1}$ (ANOVA, $p < 0.001$, for Type 1, Type 2, and Type 3a, respectively; Fig. 1 (D-E)) stronger at higher irradiance. These experiments showed that Type 1 growth was most stunted in the presence of both phenotypes in 100 μmol photons $m^{-2}s^{-1}$, with the highest growth decline (3.29×10^7 cell mL^{-1} compered to control) caused by Type 2, on the last day of the study (1E). Whereas, in 50 μmol photons $m^{-2}s^{-1}$ Type 1 stunt the growth of Type 2 and more notably Type 3a (with the decline of 1.09×10^7 and 2.46×10^7 cell mL^{-1} on day 7th, respectively; 2D and 3D). In both irradiance conditions was observed strong competition between Type 1and Type 3a.

Salinity also showed to significantly affect allelopathic interactions of *Synechococcus* sp. phenotypes (ANOVA, $p < 0.001$, for Type 1, Type 2, and Type 3a; Fig. 1 (F-G)). Stronger inhibitory effect of Type 1 was observed in lower salinity, with a decline of Type 2 and Type 3a abundances, relative to the control (2F and 3F), of 2.69×10^7 cell mL^{-1} and 2.96×10^7 cell mL^{-1} respectively. On the other hand, the presence of both Type 2 and Type 3a phenotypes caused stimulation of growth of Type 1 at higher salinity (1G). The most significant increase was observed on day 7th and constituted 1.98×10^7 and 1.58×10^7 cell mL^{-1} compared to control.

3.2. Influence of abiotic factors on growth of Synechococcus sp. phenotypes after the addition of cell-free filtrate

Our experiments showed significant effect of filtrate obtained from the *Synechococcus* sp. phenotypes on the growth of each respective phenotype (ANOVA, $p < 0.001$, for Type 1, Type 2, and Type 3a, Fig. 2).

Our results have shown that the higher the temperature, the stronger the allelopathic effect. The Type 2 phenotype was the most sensitive to cell-free filtrate at the highest temperature (2C), with the Type 1 and the Type 3a as a donor, inducing a growth inhibition of 65% and 88%, respectively. Type 2 was also affected by Type 1, and Type 3a filtrates at temperatures of 18 °C (2B) and 13 °C (2A).The effect of the filtrate obtained from the Type 2 significantly affected the growth of Type 3a in all studied temperatures (3A-C), while filtrate from Type 1 affected growth at 18 °C (3B) and 23 °C (3C). Growth of Type 1 was inhibited only at the highest temperature (1C).

The different levels of irradiance significantly affected the allelopathic effect (D-E). Type 2 was the most significantly inhibited by filtrate from other phenotypes, with the highest decrease of growth observed at lower irradiance (2D), which constituted 60% after the addition of Type 3a and 46% after the addition of Type 1. Type 1 showed

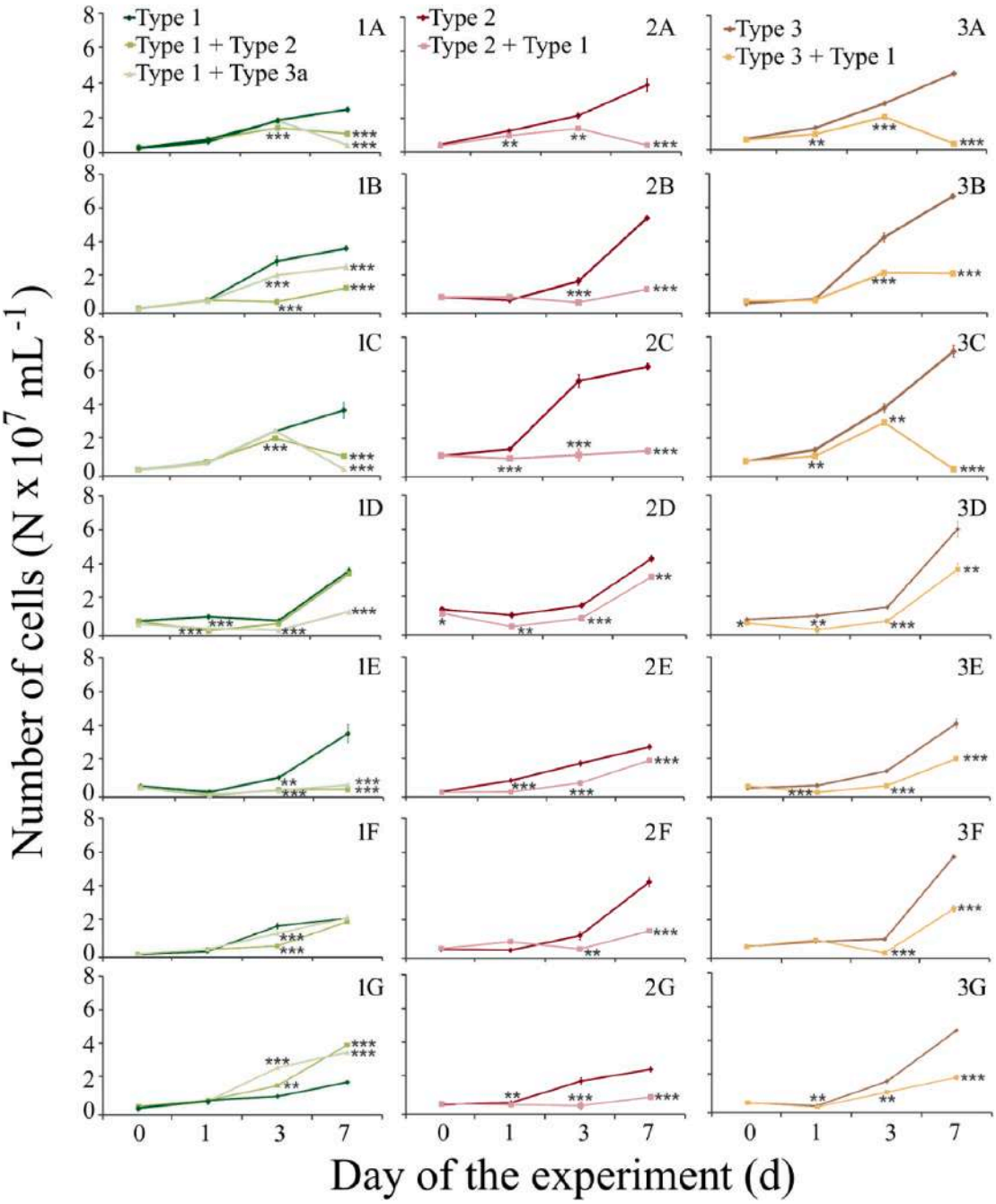

Fig. 1. Number of cells (N × 10^7 cell mL^{-1}) of phenotypes of *Synechococcus* sp.: Type 1 (1st column), Type 2 (2nd column), and Type 3a (3rd column)), after 0, 1st 3rd, and 7th day of the experiment with co-cultivation of phenotypes. At different temperatures: 13 °C (A), 18 °C (B), and 23 °C (C); irradiances: 50 μmol photons $m^{-2}s^{-1}$ (D), 100 μmol photons $m^{-2}s^{-1}$ (E) and salinity: 3‰ (F) and 13‰ (G). The values are mean ± SD (n = 3). Asterisks indicates significant difference with post hoc Dunnett's test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

inhibition of growth by 37% and 22% at lower irradiance (1D), and increase in number of cells by 25%, after the addition of Type 2 filtrate in higher irradiance (1E). Nonetheless, Type 3a showed a higher sensitivity to cell-free filtrate produced by both phenotypes at higher irradiance (3E), with a decrease in the number of cells of 50% and 58% after the addition of filtrate from Type 1 and Type 2 phenotype, respectively.

These results showed that the allelopathic effect of *Synechococcus* sp. phenotypes decreases with increasing salinity. The Type 2 showed the strongest inhibition of growth under the effect of Type 1, being inhibited by 61% and 36% relative to the control, respectively, at both salinities (2F-G). Cell-free filtrate obtained from Type 2 stimulated the growth of Type 1 by 25% and 94% respectively for both salinities (1F-G). Type 3a was only affected by the addition of Type 2 at the higher salinity level (3G).

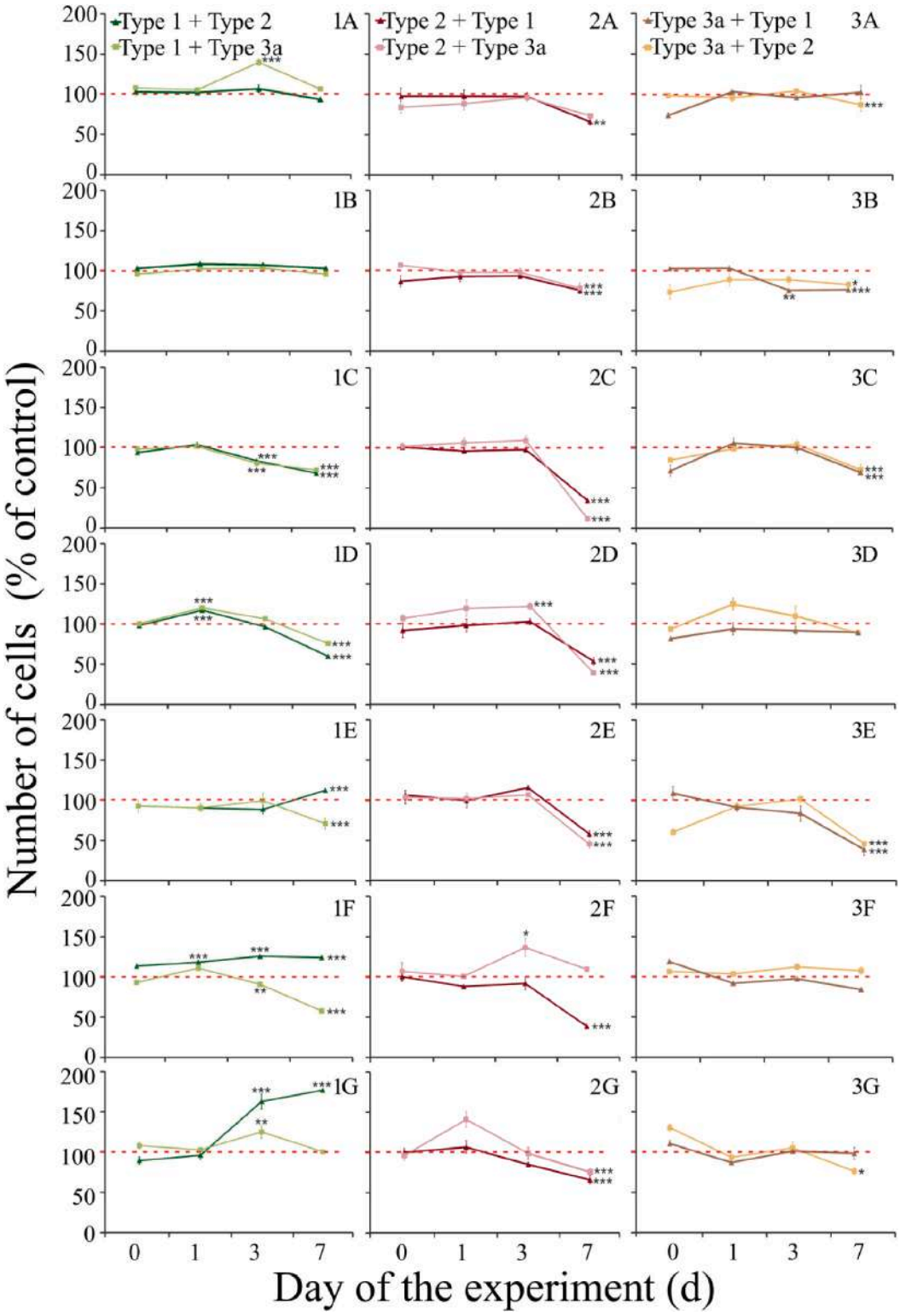

Fig. 2. Number of cells (% of control) of phenotypes of *Synechococcus* sp.: Type 1 (1st column), Type 2 (2nd column), and Type 3a (3rd column), after 0, 1st 3rd, and 7th day of the cell-free filtrate experiment. At different temperatures: 13 °C (A), 18 °C (B), and 23 °C (C); irradiances: 50 μmol photons $m^{-2}s^{-1}$ (D), 100 μmol photons $m^{-2}s^{-1}$ (E) and salinity: 3‰ (F) and 13‰ (G). The values are mean ± SD (n = 3). Asterisks indicates significant difference with post hoc Dunnett's test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3.3. Influence of abiotic factors on chlorophyll a and carotenoids content in Synechococcus sp. phenotypes after the addition of cell-free filtrate

The photosynthetic pigment concentrations were then presented in pg × cell (Figs. 3 and 4), as well as pg × mL^{-1} (Figs. S2 and S3 in Supplementary material).

The cell-free filtrate significantly affected the content of Chl *a* (ANOVA, $p < 0.001$, for Type 1, Type 2, and Type 3a, Fig. 3) and Car (ANOVA, $p < 0.001$, for Type 1, Type 2, and Type 3a) concentration. Depending on the phenotype there could be a decrease or an increase of pigments. The most significant decrease in Chl *a* was for Type 1 after the addition of filtrate from both phenotypes at the highest salinity level (1F-G), with a decrease of 0.70 pg and 0.50 pg × $cell^{-1}$, compared to the control, with filtrate from Type 2 also decreasing the Chl *a* concentration of Type 1 in low salinity (1G). However, the Type 1 was the only phenotype for which salinity was decreasing Chl *a* concentration. For Type 2 and Type 3a temperature was the factor having more influence in the decreases of Chl *a* content (2 and 3 A-C). The largest decrease in Chl *a* was caused by Type 3a in both other phenotypes in 23 °C (1C and 2C). Type 2 also reduced the Chl *a* concentration of both other phenotypes at the highest temperature (1C and 3C). However, in some instances the filtrate increased the Chl *a* content. The largest stimulation was caused by Type 1 filtrate, both at 18 °C and 23 °C (2 and 3B–C). Moreover, at 100 μmol photons $m^{-2}s^{-1}$ irradiance, Type 2 and Type 3a also increased Chl *a* content (1E).

The Car content was also significantly affected by filtrate obtained from the three phenotypes of *Synechococcus* sp. in different temperatures and irradiance conditions. However, salinity showed no significant impact in Car content (F-G). In most cases, the addition of cell-free filtrate obtained from *Synechococcus* sp. resulted in increased values of Car content compared to the control. A significant increase in Car was recorded in 18 °C (B), with the greatest increase in Car observed for the Type 2 (2B) after the addition of Type 3a filtrate (0.82 pg × $cell^{-1}$) and Type 1 (0.16 pg × $cell^{-1}$), as well as Type 3a (3B) after the addition of filtrate from both other phenotypes, with an increase of 0.06 pg × $cell^{-1}$. Moreover, Type 1 was susceptible to both phenotypes at 13 °C (1A), with

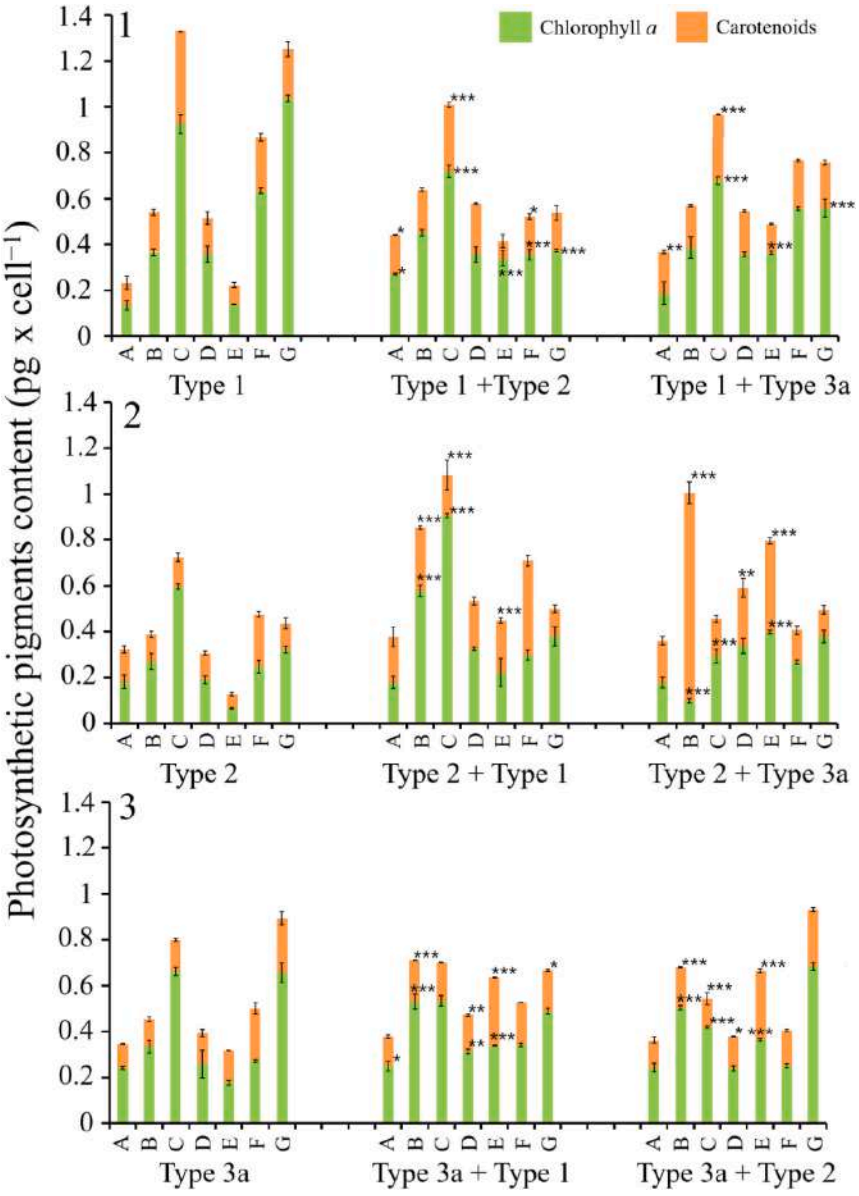

Fig. 3. Chl *a* and Car content (pg × $cell^{-1}$) of phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3a), after 7th day of the experiment with the addition of cell-free filtrate. At different temperatures: 13 °C (A), 18 °C (B), and 23 °C (C); irradiances: 50 μmol photons $m^{-2}s^{-1}$ (D), 100 μmol photons $m^{-2}s^{-1}$ (E) and salinities: 3‰ (F) and 13‰ (G). The values refer to means (n = 3, mean ± SD). Asterisk indicates significant difference from post hoc Dunnett's test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

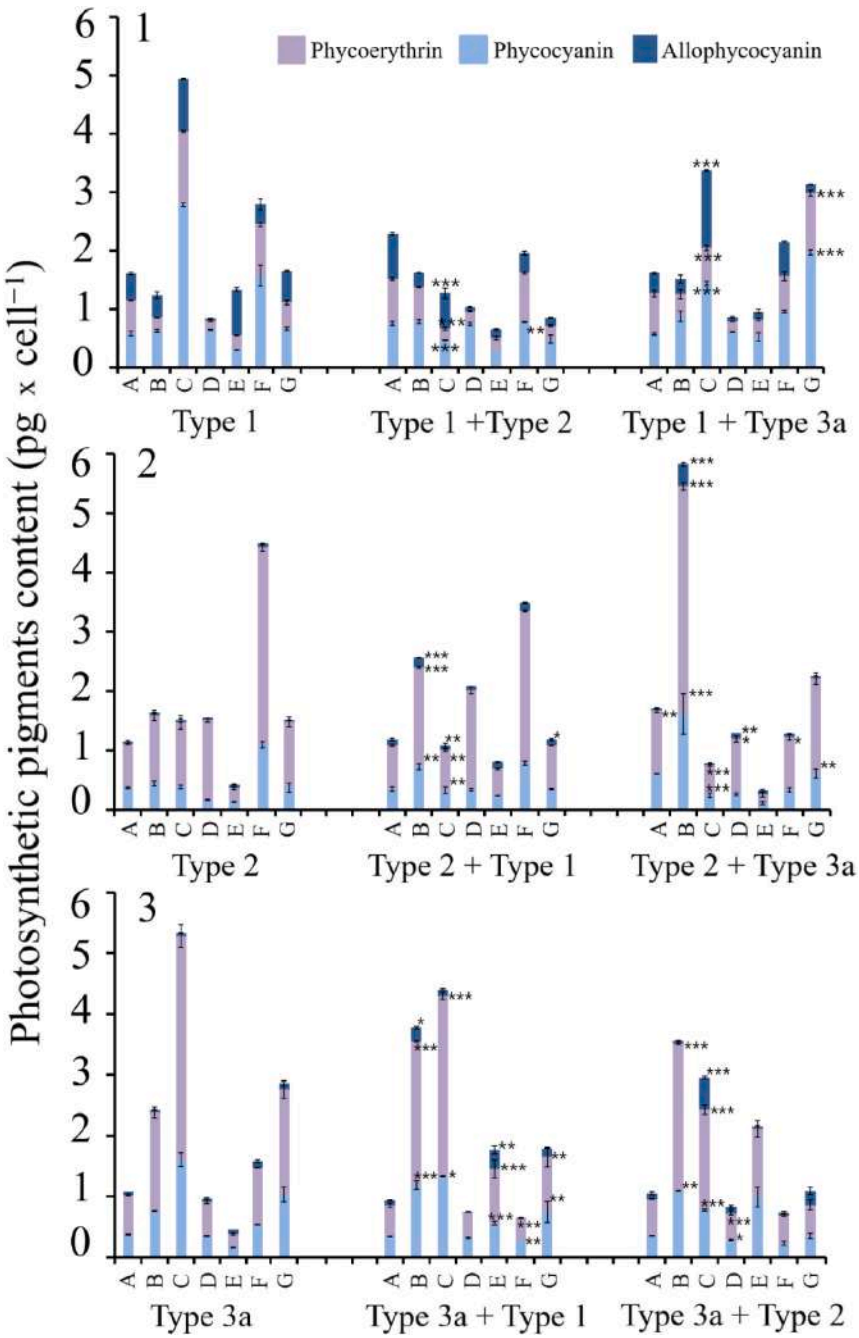

Fig. 4. Photosynthetic pigments content (pg × cell^{-1}) of phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3a), after 7th day of the experiment with the addition of cell-free filtrates. At different temperatures: 13 °C (A), 18 °C (B), and 23 °C (C); irradiances: 50 μmol photons m^{-2}s^{-1} (D), 100 μmol photons m^{-2}s^{-1} (E) and salinities: 3‰ (F) and 13‰ (G) conditions. The values refer to means (n = 3, mean ± SD). Asterisk indicates significant difference from post hoc Dunnett's test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

an increase of 0.08 and 0.09 pg × cell^{-1} after the addition of Type 2 and Type 3a filtrates, respectively. Furthermore, at 100 μmol photons m^{-2}s^{-1} irradiance there was significant stimulation of the Car content of Type 2 (2E) phenotype with the highest increase constituting 0.35 pg × cell^{-1} after the addition of cell-free filtrate from Type 3a. At irradiance of 50 μmol photons m^{-2}s^{-1} there was a significant increase for the Type 2 (2D) of *Synechococcus* sp. after the addition of Type 3a (0.14 pg × cell^{-1}) and for Type 3a (3D) after the addition of Type 1 (0.02 pg × cell^{-1}). However, the filtrate obtained from Type 2 and Type 3a resulted in decrease of Car content of Type 1 by 0.14 pg × cell^{-1} at 23 °C (1C).

3.4. Influence of abiotic factors on phycobiliproteins content in Synechococcus sp. phenotypes after the addition of cell-free filtrate

The PC (ANOVA, $p < 0.001$ for Type 1, Type 2, and Type 3a, Fig. 4) and PE (ANOVA, $p < 0.001$ for Type 1, Type 2, and Type 3a, Fig. 4) content was always significantly affected by all the cell-free filtrates, in all experimental conditions. A significant increase in PC content was observed in the filtrate from 18 °C, with the highest increase in PC observed for the *Synechococcus* sp. Type 3a (3B), and more significantly Type 2 (2B), after the addition of cell-free filtrate from both strains. At the same temperature, there was stimulation of PC content of Type 2 equal to 1.18 pg × cell^{-1} and 0.29 pg × cell^{-1} after the addition of each

Type 1 and 3a, respectively (2B). However, a significant increase in PC content of Type 1 was detected only at the highest salinity caused by filtrate from Type 3a and Type 2 and constituted 1.31 pg × cell^{-1} and 20.24 pg × cell^{-1} respectively (1G). In the highest irradiance conditions, filtrate from Type 1 caused an increase of the PC content for Type 3a by 0.4 pg × cell^{-1} (3E). On the other hand, cell-free filtrates also showed reduction of the PC content. At the highest temperature tested, there was always an effect of decreasing PC content, with the highest decrease found for Type 1 (1C) after the addition of Type 2 (2.32 pg × cell^{-1}) and Type 3a (1.35 pg × cell^{-1}) compared to the control. Furthermore, a significant decrease was noted after the addition of the Type 1, with the highest inhibition of PC content found for Type 3a (0.27 and 0.29 pg × cell^{-1}), in the salinities of 3‰ (3F) and 13‰ (3G), respectively.

An increase in PE content was shown at 18 °C, for Type 3a (3B) and Type 2 (2B), with the highest PE value observed for the *Synechococcus* sp. Type 2 after the addition of Type 3a (by 2.68 pg × cell^{-1}) and Type 1 (by 0.52 pg × cell^{-1}). Furthermore, at the highest irradiance level, Type 1 stimulated PE content of Type 3a (by 0.67 pg × cell^{-1}; 3E), whereas at the lowest salinity Type 3a increased the PE content in Type 1 (1F). There was also an inhibitory effect in PE content, for Type 2 (2C) and Type 3a, with the highest decrease found for Type 3a (3C) after the addition of Type 2 (2.02 pg × cell^{-1}) and Type 1 (0.72 pg × cell^{-1}), at the highest studied temperature. At the irradiance of 50 μmol photons m^{-2}s^{-1} there was a significant inhibitory effect between Type 2 (2D) and Type 3a (3D), with the highest decrease in PE of Type 2 constituting 0.41 pg × cell^{-1} compared to control. In the lowest salinity level, the addition of Type 3a caused a decrease of PE content in Type 2 of 2.44 pg × cell^{-1} (2F). Type 3 was sensitive to filtrate from Type 1 in salinities of 3‰ and 13‰ (3F-G).

Cell-free filtrate stimulated APC content of all phenotypes (ANOVA, $p < 0.001$ for Type 1, Type 2, and Type 3a), with temperature causing the strongest effect. There was an increase of APC content recorded in 18 °C for Type 1 (1B) and Type 2 (2B), with the highest increase for Type 2 after the addition of Type 3a (0.34 pg × cell^{-1}) and Type 1 (0.11 pg × cell^{-1}). At the highest temperature level tested, APC contents of Type 2 (2C) and Type 3a (3C) were increased by filtrate from Type 1 and Type 2 respectively. In the lowest irradiance level tested, the APC content of Type 2 (2D) was increased by the effect of Type 3a (by 0.07 pg × cell^{-1}), whereas in the highest irradiance level, APC content of Type 3a (3E) was increased (0.23 pg × cell^{-1}) by filtrate from Type 1. The only significant effect of cell-free filtrate from cyanobacteria in salinity 13‰ was recorded after the addition of filtrate obtained from Type 1 and resulted in increased the APC content of Type 2, by 0.07 pg × cell^{-1} (2G).

4. Discussion

4.1. Influence of abiotic factors on allelopathic interaction of Synechococcus sp. phenotypes

Studies regarding allelopathic interactions of *Synechococcus* sp. phenotypes are very scarce. However, conducted studies have shown negative effect of co-cultured strains (Paz-Yepes et al., 2013; Kovács et al., 2018). Moreover Paz-Yepes et al. (2013) showed an inhibition of growth of *Synechococcus* strains, and dominance of one strain when cultured with two different strains. Nevertheless, allelopathic interaction of *Synechococcus* sp. phenotypes in different environmental conditions were never studied.

Our data show that temperature is the most significant environmental factor in which both the co-cultivation of phenotypes and the addition of cell-free filtrates, negatively affected the other phenotypes. With an increase of temperature, the strength of the negative interaction of all three phenotypes was increasing. Literature data confirmed that cell-free filtrate from picoplanktonic cyanobacteria increase their potency with increased water temperature (Noaman et al., 2004). Śliwińska-Wilczewska et al. (2016) showed stronger negative effects of filtrate from *Synechococcus* sp. (Type 1) on growth of diatom *Navicula perminuta* at higher temperatures. Also, Barreiro Felpeto et al. (2019) showed that the inhibition of growth of *Porphyridium purpureum*, *Fistulifera* sp., and *Chlorella vulgaris* after the addition of filtrate from *Synechocystis* sp. was stronger in the highest temperature. According to laboratory works, higher temperatures may benefit species like *Synechococcus* relative to other common marine phytoplankton like diatoms and cocclithophores (Dutkiewicz et al., 2015; Li et al., 2019). Previous studies showed that an increase in temperature increased production and toxicity of allelochemicals secreted by cyanobacteria, promoting their bloom formation (Ame et al., 2003; Flombaum et al., 2013). Moreover, these species are also favored by other human-driven global environmental changes, such as eutrophication of coastal areas, ocean acidification (Paerl, 2018). An increase in frequency and geographic occurrence of their blooms has already been recorded (Oziel et al., 2017; Li et al., 2019).

Our work demonstrated that irradiance is also a significant environmental factor for the allelopathic properties of these strains. Previous works showed stronger allelopathic effect at the higher irradiance levels, which can indicate stronger competition in surface waters (Eigemann et al., 2018). However, there is no necessarily an exclusion of either strain, co-existence of three strains is also possible to be due to the different pigment composition (Stomp et al., 2004).

There are very few studies about the effect of irradiance in allelopathic properties. Few studies demonstrated increase in inhibitory effect with the increase of irradiation (Dyble et al., 2006; Antunes et al., 2012). Śliwińska-Wilczewska et al. (2016) demonstrated the inhibitory effect of *Synechococcus* sp. phenotypes only in 190 μmol photons m^{-2}s^{-1}, while Barreiro Felpeto et al. (2018a) showed cytolysis in high light condition in experiment studying interaction between *Synechococcus* sp. and *Nodularia spumigena.* Other literature data demonstrated that with the rise of irradiance, growth of *Synechococcus* sp. Type 1, Type 2, and Type 3a decreased. Śliwińska-Wilczewska et al. (2020) showed that the *Synechococcus* sp. Type 2 was the most susceptible to high irradiance. However, Bao and Gao (2021), showed a decline in growth rate at higher irradiance in the PE-rich strain. Phenotypes with dominance of PE, are adapted to greater depths, and thus lower light intensity (Larsson et al., 2014). This is why higher irradiance negatively affected growth rate. *Synechococcus* sp. were shown to occur dominantly in the lower part of the euphotic zone, with lower light conditions (Piazena et al., 2002; Flombaum et al., 2013). The wide range of irradiance conditions in which *Synechococcus* sp. phenotypes can occur, could by the result of periodic mixing, which can led to high light exposition (Flombaum et al., 2013).

In our experiments we demonstrated that salinity is the least significant environmental factor affecting allelopathic properties. Previous studies have shown that salinity plays an important role in the secretion of allelopathic compounds (Brutemark et al., 2015). Śliwińska-Wilczewska et al. (2016) showed increased allelopathic effect in the lowest salinity, similarly to the present work. Cyanobacteria from genus *Synechococcus* show high adaptation to changes in salinity conditions, finding high abundances of these organisms in open ocean, coastal waters and estuaries (Moisander et al., 2002; Kim et al., 2018). Previous studies have shown that salinity can be a factor in differentiation of *Synechococcus* phenotypes. Studies conducted by Kim et al. (2018) showed that in the salinity ranging from 2.5 in the river mouth to 35 PSU salinity of an open ocean waters, different *Synechococcus* sp. strains dominated. However, since *Synechococcus* sp. is exposed to significant and frequent salinity changes, its plasticity suggests that this one is less significant than other environmental factors (Ludwig and Bryant, 2012).

4.2. Influence of abiotic factors on chlorophyll a and carotenoids content in Synechococcus sp. phenotypes after the addition of cell-free filtrate

Our data show that temperature is the most significant environmental factor on the allelopathic effect altering the Chl *a* and Car content

in *Synechococcus* sp. phenotypes. There were both negative and positive effect depending on the phenotype. In the highest temperature cell-free filtrate showed more inhibitory effect. Salinity significantly decreased Chl *a* value of only Type 1 with the highest inhibition in salinity 13‰. The Car pigments content of Type 2 and Type 3a were most significantly affected by temperature and irradiance, causing the increase of their value.

The decrease in the content of pigments was directly related to the decrease in the number of cells of these organisms, with the exception of Type 1 in salinity 13. Previously conducted studies (e.g., Sukenik et al., 2002; Śliwińska-Wilczewska et al., 2017; Barreiro-Felpeto et al., 2018b) have shown the decrease of Chl *a* and increase in Car content, after the addition of cell-free filtrates. Śliwińska-Wilczewska et al. (2017) and Barreiro-Felpeto et al. (2018a), showed a significant negative effect of filtrate from *Synechococcus* sp. phenotype on Chl *a* content and a stimulation of Car content. Konarzewska et al. (2020) demonstrated a significant decrease of Chl *a* and an increase of Car content of co-existing phytoplankton species, after the addition of cell-free filtrate obtained from all three *Synechococcus* sp. phenotypes. Literature data showed that the decrease of Chl *a* content, indicative of damage to the thylakoid membranes and inhibition of Chl *a* biosynthesis, and the increase of Car, are consequences of cell exposition to stressors (Machado et al., 2015). However, in this study was also recorded an increase of Chl *a* content mainly at 18 °C for the *Synechococcus* sp. Type 3a. Furthermore, in irradiance conditions of 100 μmol photons $m^{-2}s^{-1}$ the cell-free filtrate most significantly increased the Chl *a* content. The stimulation of Chl *a* content may be caused by inhibition of cell division, without destroying thylakoid membranes or inhibiting photosynthesis, which could be considered a mild effect of allelopathy. Gleason and Paulson (1984) described similar results after adding 1 μgmL^{-1} of cyanobacterin obtained from *Scytonema hofman*nii to Synechococcus sp. These authors reported that small amounts of cyanobacterin caused an inhibition of cell division but did not affect the thylakoid membranes of this picocyanobacterium. A similar mechanism may occur for as-yet unidentified allelopathic compounds produced by *Synechococcus* sp. to other phenotypes of this genus.

4.3. Influence of abiotic factors on phycobiliproteins content in *Synechococcus sp. phenotypes after the addition of cell-free filtrate*

In most cases temperature, irradiance and salinity significantly altered the effect of cell-free filtrate from phenotypes of *Synechococcus* sp. on content of phycobiliproteins in cyanobacterial cells. Temperature was the most significant environmental factor influencing the phycobiliproteins content. Śliwińska-Wilczewska et al. (2020) showed that the content of phycobiliproteins increased with temperature. Kana and Glibert (1987) showed that irradiance intensity significantly affects the phycobiliproteins in cyanobacteria cells, with lower concentrations of pigments at higher temperatures. Moisander et al. (2002) showed that in conditions of high salinity the concentration of phycobiliproteins shifts during acclimation. Existing works indicate that a decrease of phycobiliproteins with increase in light may be associated with photo acclimation. With the decrease of Chl *a* content the phycobiliproteins assume the function of main photosynthetic pigment in the cell (Fernández--Rojas et al., 2014).

5. Conclusions

This work shows the significant influence of the environmental conditions (temperature, irradiance, and salinity) on the allelopathic compounds secreted by *Synechococcus* sp. phenotypes, with temperature the most significantly affecting allelopathic properties and salinity the least. There were negative interactions of the three phenotypes. The response to changing environmental factors is highly phenotype-specific. This differential responses of allelopathy could allow these phenotypes of *Synechococcus* sp. to coexist across different niches in the water column. Our study showed a remarkable importance of temperature and light in the allelopathy of *Synechococcus* strains, suggesting that their role in phytoplankton communities could be reinforced in future scenarios, as a result of global change.

Author contributions

Z.K.: Conceptualization, Investigation, Writing - Original draft preparation, S.Ś.W.: Methodology, Supervision, Writing - Review & Editing, A.B.F.: Supervision, Writing - Review & Editing, A.L.: Supervision, Writing - Review & Editing. All authors approved the submitted version.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2022.105671.

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Supplementary material

Effects of light intensity, temperature, and salinity in allelopathic interactions between coexisting *Synechococcus* sp. phenotypes

Zofia Konarzewska[a,*], Sylwia Śliwińska-Wilczewska[a,b], Aldo Barreiro Felpeto[c], Adam Latała[a]

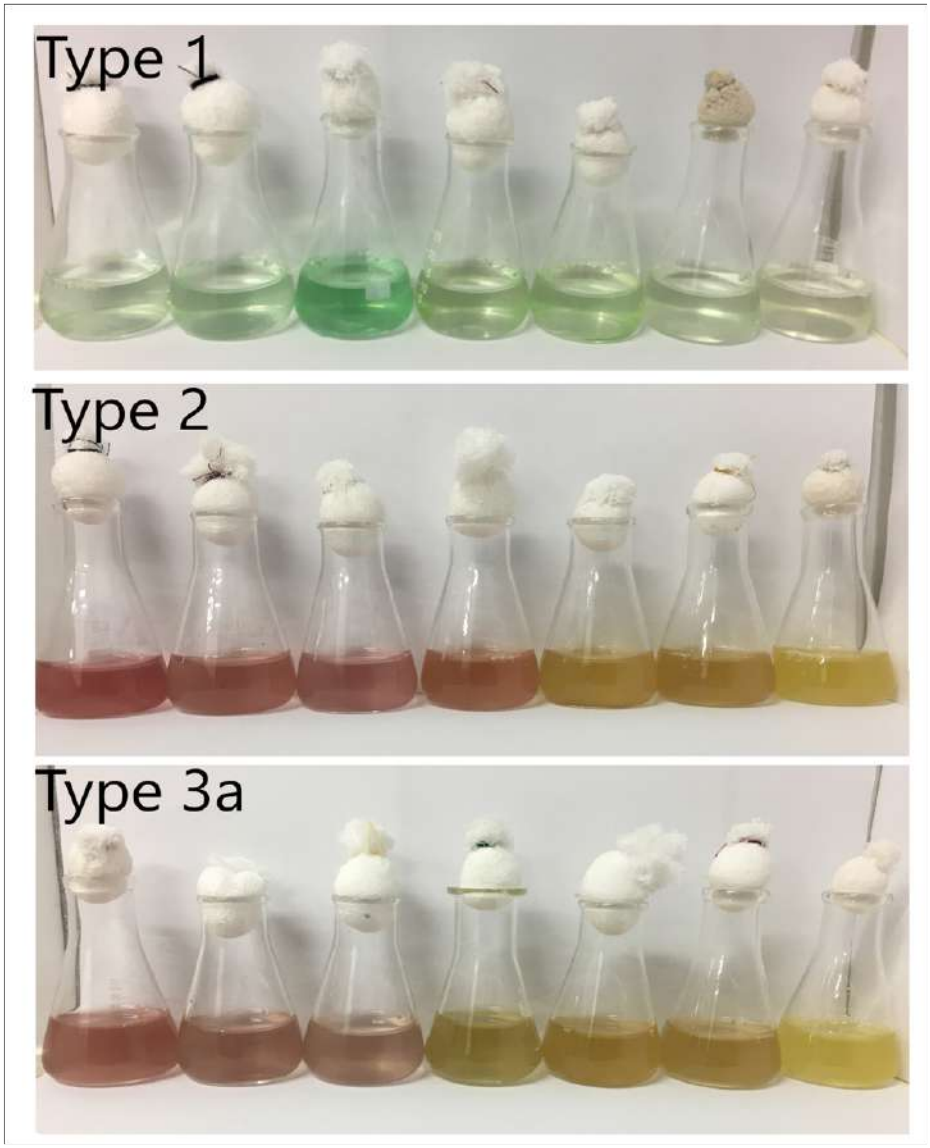

FIGURE S1. Phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3a) in varied temperature (13˚C, 18˚C and 23˚C), salinity (3 and 13‰), and irradiance (50 and 100 µmol photons $m^{-2}s^{-1}$) conditions.

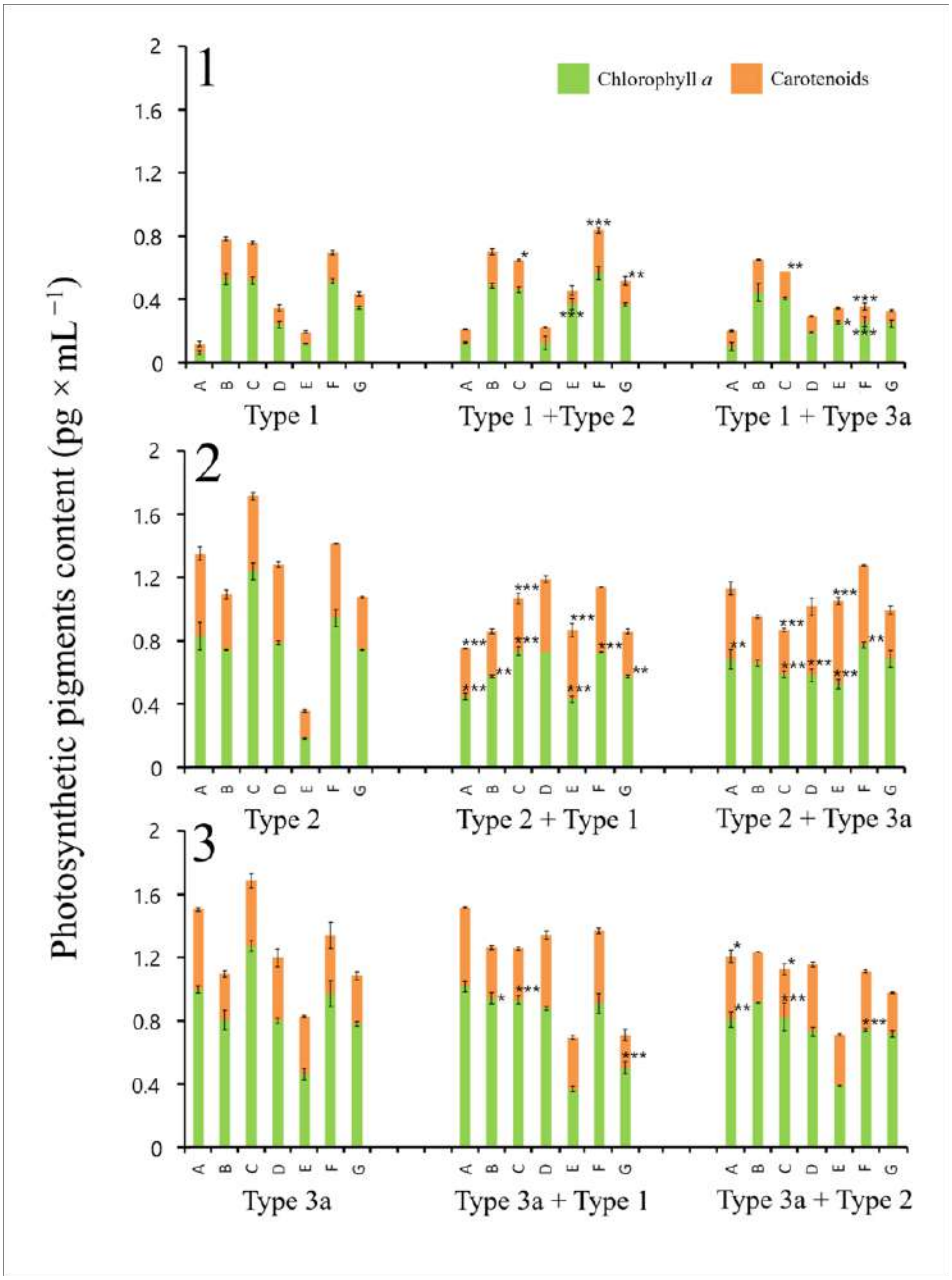

FIGURE S2. Chl *a* and Car content (pg × mL^{-1}) of phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3a), after 7th day of the experiment with the addition of cell-free filtrate. At different temperatures: 13˚C (A), 18˚C (B), and 23˚C (C); irradiances: 50 µmol photons $m^{-2}s^{-1}$ (D), 100 µmol photons $m^{-2}s^{-1}$ (E) and salinities: 3‰ (F) and 13‰ (G). The values refer to means (n = 3, mean ± SD). Asterisk indicates significant difference from post hoc Dunnett's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

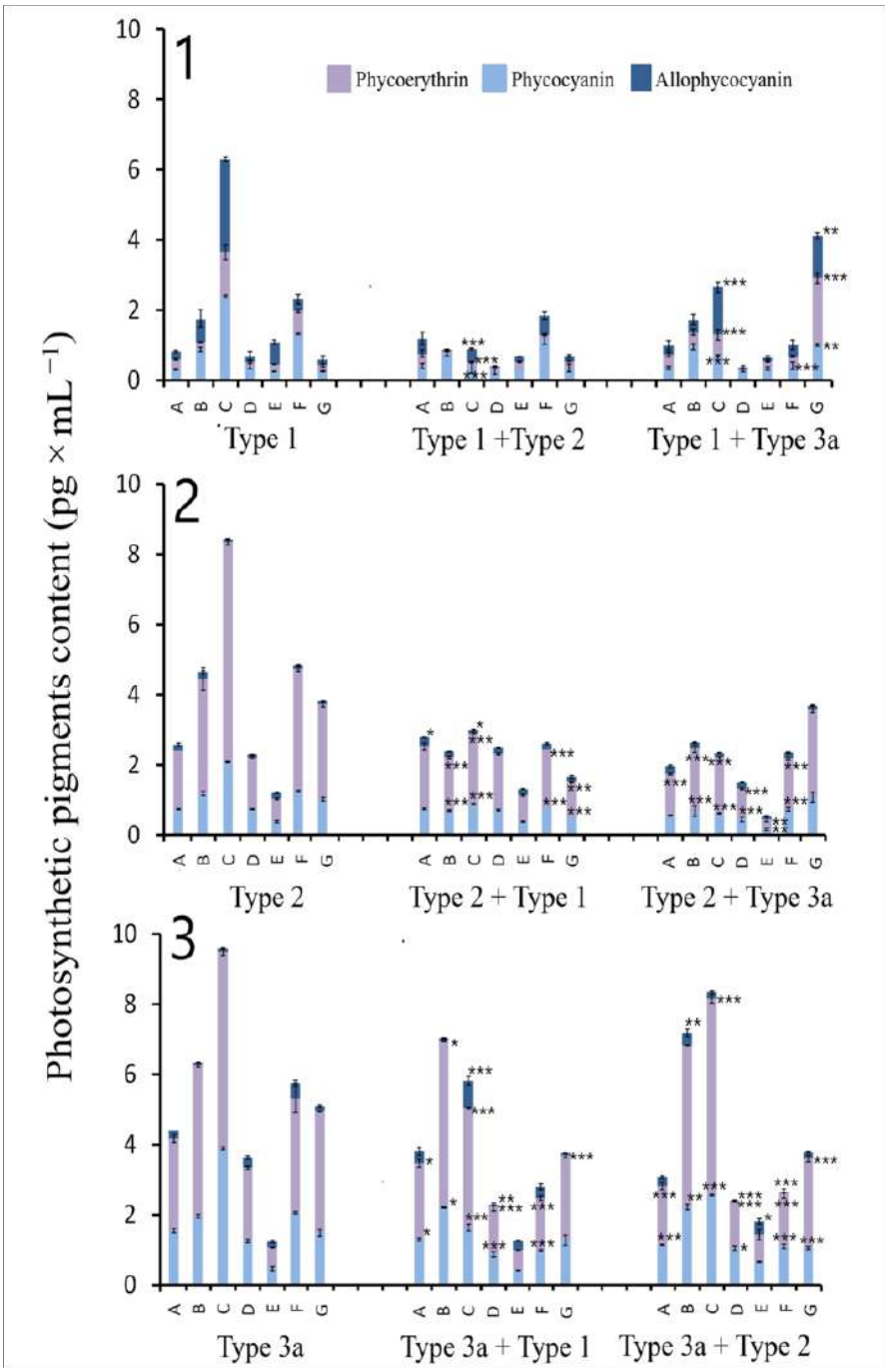

FIGURE S3 Photosynthetic pigments content (pg × mL^{-1}) of phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3a), after 7th day of the experiment with the addition of cell-free filtrates. At different temperatures: 13˚C (A), 18˚C (B), and 23˚C (C); irradiances: 50 µmol photons $m^{-2}s^{-1}$ (D), 100 µmol photons $m^{-2}s^{-1}$ (E) and salinities: 3‰ (F) and 13‰ (G) conditions. The values refer to means (n = 3, mean ± SD). Asterisk indicates significant difference from post hoc Dunnett's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

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were as follows:

Konarzewska Zofia 70%:

Conceptualization, Investigation, Writing - Original draft preparation

Śliwińska-Wilczewska Sylwia 15%:

Methodology, Supervision, Writing - Review & Editing

Barreiro Felpeto Aldo 10%:

Supervision, Writing - Review & Editing

Latała Adam 5%:

Supervision, Writing - Review & Editing

Konarzewska Zofia

Śliwińska-Wilczewska Sylwia

Barreiro Felpeto Aldo

Latała Adam

9.2 PUBLICATION 2

10.2 PUBLIKACJA 2

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Impacts of nutrient dynamics on three picocyanobacterial populations: laboratory experiments and seasonal surveys in the Baltic Sea region

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ABSTRACT

Picocyanobacteria, characterized by their small size (<2 μm) and high adaptability, are widespread in aquatic ecosystems, yet their seasonal dynamics and responses to environmental parameters remain poorly understood, particularly in brackish waters. Therefore, it is critical to understand how various environmental factors (including nutrient concentrations) and inter-specific interactions between coexisting phenotypes influence the distribution and dynamics of *Synechococcus*. Especially given their potential contribution to primary production and the formation of cyanobacterial blooms in vulnerable ecosystems like the Baltic Sea, where forecasts and modeling data predict a significant increase in nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentration in this coming decade. We used laboratory tests and environmental data to examine how a disturbed balance of NO_3^- and PO_4^{3-} will affect the occurrence and interactions between three main (Type 1, Type2, and Type 3a) populations of *Synechococcus* sp. in the Baltic Sea. This study demonstrates differential growth inhibition among *Synechococcus* sp. phenotypes (Type 1, Type 2, and Type 3a) when exposed to filtrate from Type 2 and Type 3a at higher NO_3^- concentrations and increased inhibition by Type 1 and Type 3a under low PO_4^{3-} conditions, highlighting differences between nutrient adaptation within phenotypes. All phenotypes tested produced MC-LR (pg $cell^{-1}$) and their concentration depended on the availability of NO_3^- and PO_4^{3-} in the medium, particularly with PE-rich *Synechococcus* Type 2 showing the highest microcystin content under low NO_3^- conditions, highlighting potential adaptive responses to environmental stress. We found that the abundance of all tested *Synechococcus* sp. phenotypes in the Baltic Sea was high in spring and fall, with phycoerythrin (PE)-rich Type 2 picocyanobacteria dominating. The PCA analysis has shown that the main factors correlated with the abundance of *Synechococcus* sp. phenotypes are the abundances of all three phenotypes, nitrate concentration and temperature, which align with previous studies. The study highlights the

potential dominance and ecological implications of PE-rich *Synechococcus* phenotypes, emphasizing the complex intrapopulation dynamics and environmental-microbial interactions within brackish ecosystems.

Keywords: nutrient availability; allelopathic interactions; eutrophication; *Synechococcus*; MC-LR, toxicity

2. INTRODUCTION

The unicellular picocyanobacteria of the genus *Synechococcus* exhibit high adaptability and are widely distributed across aquatic ecosystems (Scanlan et al., 2009). This genus, characterized as polyphyletic, displays considerable diversity in pigment content, taxonomy, and physiology (Partensky et al., 1999; Six et al., 2007; Mazard et al., 2012). While previous studies have emphasized *Synechococcus* sp. dominance in oligotrophic environments (Partensky et al., 1999; Agawin et al., 2000), recent observations underscore the ecological significance of picophytoplankton also in eutrophic environments (Morán, 2007; Mazur-Marzec et al., 2013; Paerl et al., 2020).

Various environmental factors, including light (Moore et al., 1995; Mella-Flores et al., 2012), water column stability (Eigemann, et al., 2018), temperature (Eigemann, et al., 2018; Konarzewska et al., 2022), salinity (Calepli et al., 2017), grazing pressure (Kuosa, 1991; Calepli et al., 2017), and nutrient availability (Stal et al., 1999), influence the distribution and dynamics of *Synechococcu*s cells. In our previous publications (Konarzewska et al., 2020, 2022), we described three predominant picocyanobacteria phenotypes: Type 1 (rich in phycocyanin; PC), Type 2 (with organisms dominated mainly by phycoerythrin I; PEI), and Type 3a (with PC, PEI and PEII). Recent evidence has further confirmed the existence and classification of these three types in the Baltic Sea (Aguilera et

al., 2023). A previous study reported the distribution of *Synechococcu*s sp. in the upper water layer (Stal et al., 2003) and its ability to coexist within this niche alongside *Dolichospermum* sp. and *Nodularia spumigena* (Eigemann, et al., 2018). Unlike filamentous cyanobacteria, Baltic Sea *Synechococcus* sp. do not fix nitrogen in heterocysts and instead rely on ammonium from nitrogen-fixers or regeneration (ammonification) as their primary nitrogen source, supplemented by dissolved organic nitrogen (Ohlendieck et al., 2000). Hence, *Synechococcus* sp. is potentially more sensitive to fluctuations in nutrient concentrations than filamentous cyanobacteria. Despite the notable influence of nutrient concentrations, information regarding the impact of NO_3^- and PO_4^{3-} on the abundance and allelopathic interactions of picophytoplankton in the Baltic Sea remains limited.

The natural characteristics of the Baltic Sea, including its coastal geography, relatively shallow average depth, and limited connection to the North Sea, render the Baltic Sea region particularly susceptible to anthropogenic influences (Wulff et al., 1990). Consequently, nutrient pollution of the Baltic Sea has markedly increased over the past century, resulting in clear indications of widespread eutrophication (Wulff et al., 1990) and a corresponding rise in the occurrence of cyanobacterial blooms (Paerl and Huisman, 2008, 2009). Despite their small size (< 2 µm), *Synechococcus* sp. can constitute up to 80% of the total cyanobacterial biomass and significantly contribute to primary production during cyanobacterial blooms (Stal et al., 2003). These blooms pose a significant threat to the Baltic Sea ecosystem due to toxin production and the formation of a large biomass that often leads to oxygen depletion deeper in the water column (Konik et al., 2023).

The nutrient concentration in surface waters of the Baltic Sea exhibits seasonal and regional fluctuations. Absolute nutrient loading can influence interspecies competition and occasionally lead to phytoplankton blooms (Downing et al., 2001; McCarthy et al., 2009). Higher NO_3^- concentrations are typically observed in northern regions, whereas lower values are more common in the Baltic Sea proper and southern regions. In these areas, NO_3^-

deficiency, limited PO_4^{3-} access, and cyanobacterial blooms occur more frequently during summer (Granéli et al., 1990; Konik et al., 2023). It is crucial to recognize that cyanobacterial blooms significantly suppress biological diversity and play a pivotal role in ecosystem services by contributing to the regulation of nutrient stoichiometry and homeostasis in marine systems (Deutsch et al., 2007).

Nutrient concentration can also significantly influence the production of secondary metabolites in cyanobacteria and microalgae (Yaakob et al., 2021). Under phosphorus-limiting conditions, certain green algae (DellaGreca et al., 2010) and golden algae (Granéli and Johansson, 2003a, b) exhibit increased allelochemical production. Conversely, Allen et al. (2017) and Śliwińska-Wilczewska and Latała (2018) observed a positive correlation between the allelochemical production of cyanobacteria and NO_3^- and PO_4^{3-} concentrations.

Microcystin (MC) is classified as a known toxin and is recognized as an allelopathic compound produced by cyanobacteria (Yang et al., 2014). MCs comprise a family of over 50 structurally similar hepatotoxins produced by different species of cyanobacteria. The most widespread member of this family is MC-LR (where L stands for leucine and R for arginine, indicating the variable amino acids). This toxin primarily damages the liver by disrupting the cytoskeleton through the inhibition of protein phosphatase 1 (PP1) and 2A (PP2A). In addition, microcystins have been associated with human diseases such as gastroenteritis and allergic/irritant reactions (Dawson, 1998). Recent studies have shown that *Synechococcus* sp. is also capable of producing this known toxic substance. The presence of microcystin in *Synechococcus* sp. cells or cultures has been confirmed in various locations, including the Salton Sea and Florida Bay (Carmichael and Li, 2006; Gantar et al., 2009), the Mediterranean Sea (Vareli et al., 2012), the Portuguese coast (Martins et al., 2005), Belgian waters (Van Hassel et al., 2022) and Brazilian sewage ponds (Furtado et al., 2009). In addition, Wiśniewska et al. (2022) detected MC-LR in *Synechococcus* sp. isolated from the air over the Baltic Sea. It is noteworthy that this toxin can bioaccumulate, allowing its

harmful effects to spread through the trophic chain and potentially affect terrestrial animals and humans (Orr et al., 2003; Paldavičienė et al., 2015; Puschner, 2018).

Other potential allelopathic substances produced by *Synechococcus* sp. are thionsulfolipid (Kunimitsu et al., 1993), β-N-methylamino-L-alanine (BMAA) (Cox et al., 2005), geosmin (GSM) (Graham et al., 2008), 2-methylisoborneol (MIB) (Jakubowska and Szeląg-Wasielewska, 2015), hemolysin (Puschner, 2018), 7-deoxysedoheptulose (Brilisauer et al., 2019), eicosan, 10-methyl-, oxime-, methoxy-phenyl-silanediol, dimethyl (Konarzewska et al., 2020). However, the influence of nutrient availability on the production of these compounds remains unknown. Moreover, the response to nutrient availability is phenotype-specific, suggesting potentially different ecological mechanisms to cope with resource limitations (McLean et al., 2021).

In recent decades, anthropogenic impacts have led to recognizable changes in NO_3^- and PO_4^{3-} levels in coastal areas, significantly affecting cyanobacterial diversity and interactions, as discussed in the work of Malone and Newton (2020). Nevertheless, our current understanding of how NO_3^- and PO_4^{3-} concentrations specifically affect the ecophysiology of different *Synechococcus* sp. is scarce. Our study was designed to investigate the potential influence of different NO_3^- and PO_4^{3-} concentrations on abundance, growth rates, allelopathic interactions between phenotypes, and MC-LR production in three primary picocyanobacteria populations (Type 1, Type 2, Type 3a). The aim of this research is to determine whether the expected changes in nutrient dynamics favor the prevalence of certain picocyanobacterial phenotypes in coastal areas and whether this prevalence is associated with an escalation of microcystin production.

2. MATERIALS AND METHODS

2.1. Effect of NO_3^- and PO_4^{3-} concentration on Synechococcus sp. phenotypes in laboratory conditions

2.1.1. Culture conditions

The laboratory experiments were carried out with three phenotypes of picoplanktonic cyanobacteria isolated from the southern Baltic Sea: *Synechococcus* Type 1 (CCBA124; PC-rich strain), Type 2 (CCBA120; PE-rich strain) and Type 3a (CCBA132; and Type 3 with PC, PEI and PEII strain), which were stored in the Culture Collection of Baltic Algae (CCBA) at the University of Gdansk (Latała et al., 2006). All tested phenotypes of picocyanobacteria were cultivated at 18ºC and photosynthetically active radiation (PAR) of 10 µmol photons $m^{-2}s^{-1}$ with photoperiod L:D 16:8. *Synechococcus* sp. phenotypes were cultured in f/2 medium (Guillard, 1975) with altered concentrations of nitrate (NO_3^-) and phosphate (PO_4^{3-}) as shown in Table 1. Before inoculation of the cultures, the nutrient concentration was measured according to the method of Śliwińska-Wilczewska and Latała (2018) to ensure equal initial nutrient concentrations for the control and experimental cultures. Nutrients were measured using NitraVer® 5 nitrate and PhosVer® 3 phosphate reagent pads and the DR6000 spectrophotometer (Hach, Loveland, USA).

Different nutrient conditions were investigated: standard f/2 medium (f/2), high NO_3^- (HN), low NO_3^- (LN), high PO_4^{3-} (HP) and low PO_4^{3-} (LP). The composition of the standard medium and the modified f/2 medium at different nutrient concentrations is shown in Table 1. Experiments were also carried out with low NO_3^- and PO_4^{3-} (LN LP) concentrations, however, due to the insufficient nutrient concentration, the organisms did not grow sufficiently for further analysis.

Table 1. The composition of medium in different nutrient concentrations.

Nutrients					
	f/2	HN	LN	HP	LP

$NaNO_3$	883 µM	4415 µM	176.6 µM	883 µM	883 µM
$NaH_2PO_4 \cdot H_2O$	36.3 µM	36.3 µM	36.3 µM	181.5 µM	7.26 µM
$NaSiO_3 \cdot 9H_2O$			107 µM		
TraceMetals					
Na_2EDTA			11.7 µM		
$FeCl_3 \cdot 6H_2O$			11.7 µM		
$CuSO_4 \cdot 5H_2O$			0.0393 µM		
$ZnSO_4 \cdot 7H_2O$			0.0765 µM		
$CoCl_2 \cdot 6H_2O$			0.042 µM		
$MnCl_2 \cdot 4H_2O$			0.91 µM		
$Na_2MoO_4 \cdot 2H_2O$			0.026 µM		
Vitamins					
Thiamine-HCl (vitamin B1)			0.296 µM		
Biotin (vitamin H)			2.05 µM		
Cyanocobalamin (vitamin B12)			0.369 µM		

2.1.2. Growth rate of Synechococcus sp. phenotypes

We determined the specific growth rate (µ) of *Synechococcus* sp. Type 1, Type 2 and Type 3a under the influence of different NO_3^- and PO_4^{3-} concentrations (described in section 2.2.1). The µ of the three *Synechococcus* sp. phenotypes was calculated using the equation $\mu = \ln(N_2/N_1)/t$, where N_1 and N_2 represent the number of cells on the first and last day of measurements at time point (t = 7 days), respectively (Guillard, 2005).

2.1.3. The effect of NO_3^- and PO_4^{3-} concentrations on the inter-phenotype allelopathic interactions

To investigate the effects of NO_3^- and PO_4^{3-} concentration on allelopathic interactions between phenotypes, experiments were carried out with the addition of cell-free filtrate and

in mixed cultures, in both cases with a selected donor phenotype and two target phenotypes (Fig. 1.).

In the experiment to study the allelopathy effect in cell-free filtrate, 10 mL of the target *Synechococcus* sp. phenotype was added to 10 mL of the donor filtrate. To obtain the filtrate, the cultures of *Synechococcus* sp. were filtered through a 0.45 µm filter (Macherey-Nagel MN GF-5 GmbH, Germany). The control contained 10 mL of the target *Synechococcus* sp. phenotype and 10 mL of modified medium.

In the mixed culture test, 10 mL of another phenotype was added to the 10 mL of the *Synechococcus* phenotype, with control cultures as described above. The mixed culture of Type 2 and Type 3a was not tested because the pigment changes in the different treatments made them indistinguishable.

The initial Chl *a* concentration was 0.4 µg Chl *a* mL^{-1} in all experiments, with initial cell numbers ranging from 1 - $4x10^7$ mL^{-1} depending on phenotype and experimental conditions. Chl *a* concentration was estimated based on the extraction in 90% acetone and absorbance measurements using a spectrophotometer at specific wavelengths and using established formulas for cyanobacteria according to Jeffrey and Humphrey (1975).

The growth of each *Synechococcus* sp. phenotype studied in monocultures and mixed cultures was determined based on the initial and 1st, 3rd and 7th day cell counts. The cell counts of the organisms studied were measured using a flow cytometer as described above (Section 2.1). The use of the blue laser (480 nm) and the red laser (640 nm) made it possible to distinguish between the phenotypes of *Synechococcus* sp. in both monocultures and mixed cultures. The results of the growth experiments were expressed as a percentage of the control. All experiments were performed in three replicates.

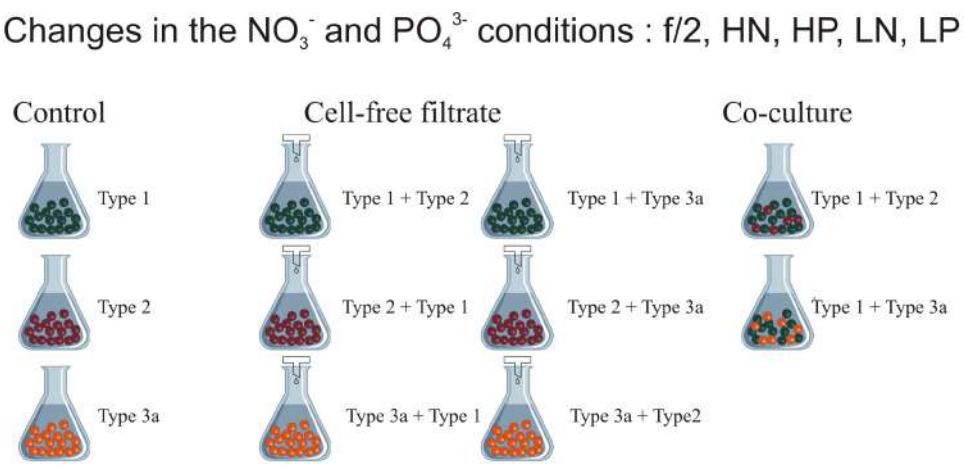

Fig.1. Scheme of the conducted experiments.

2.2. The influence of NO_3^- and PO_4^{3-} concentration on the picocyanobacterial cell-specific MC-LR content

We determined the cell-specific MC-LR (pg cell^{-1}) content of *Synechococcus* sp. Type 1, Type 2 and Type 3a under the influence of different NO_3^- and PO_4^{3-} concentrations (described in section 2.2.1) on day 7 of the experiment, and the MC-LR content was analyzed using the Microcystin-LR ELISA kit (Abnova, Taipei, Taiwan) according to the kit instructions (Parez and Chu, 2020). Absorbance was measured at a wavelength of 450 nm using the Thermo Scientific Multiscan Go microplate reader (Thermo Scientific, Waltham, MA, USA).

2.3. Determination of the occurrence of picocyanobacteria populations from the field samples

The environmental samples from the southern Baltic Sea were taken during three expeditions with the R/V Oceanograf in March, July and September 2022 at 14 sampling stations (Fig. 2, Table 1S). The values of temperature and salinity in the surface layer were measured using CTD sonde The concentrations of NO_3^- and PO_4^{3-}, were determined by the SatBaltic Remote Sensing System (Satellite Monitoring of the Baltic Sea Environment; https://www.satbaltyk.pl/).

Fig.2. Location of the sampling stations.

To determine the presence of picocyanobacteria populations, water was collected from the surface layer using a 5L container. Samples were stored in 1L plastic bottles at 4ºC in the dark until they were transported to the laboratory, where 5ml of each sample was filtered through 2µm filters (Whatman GF/C). The abundance of picocyanobacteria was then estimated measuring the 100µL of sample using a flow cytometer (BD Accuri C6 Plus; BD Biosciences, San Jose, CA, USA) with a flow rate of 100 µl min^{-1} according to the method described by Śliwińska-Wilczewska et al. (2018a). Three main populations of picocyanobacteria (PC-rich Type 1, PE-rich Type 2 and Type 3 with PC, PEI and PEII) were distinguished using blue laser (480 nm) and red laser (640 nm). Type 3 can be categorized

into various classes (from a to d) based on the PEB (phycoerythrobilin), and PUB (phycourobilin) ratio.

2.4. Statistical analyses

Statistical analyzes were performed using R version 2.15.2 (R core Team, 2023).

To analyze the effect of nutrient concentrations on the allelopathic interactions between the three Synechococcus sp. phenotypes, phenotypes, in their growth rates and in their MC-LR production, specific general linear models were performed, with lm() function implemented in package stats, and the analysis of variance (ANOVA) with the Anova() function from package car (Fox and Weisberg, 2019). When ANOVA showed significant main effects for a categorical factor, post-hoc pairwise Tukey comparisons between factor levels were performed with the emmeans() function from the homonym package (Lenth, 2023), for experiments with growth rate and MC-LR concentrations and Dunnett test for comparing several treatments with a control, using DunnettTest function from the DescTools package (Signorell, 2023). Normal distribution of model residuals was tested with Shapiro-Wilks test, implemented in shapiro.test() function from stats R package. Outliers were removed based on the Bonferroni outliers test, implemented with the outlierTest() function from car R package. In those cases when residuals did not follow a normal distribution, the variable was normalized with the optimal transformation, using the bestNormalize() function of the homonym package (Peterson, 2021).

The PCA analysis was performed using the prcomp() function and fviz_pca_biplot() function from factoextra R package (Kassambara and Mundt, 2020) in order to visualize the obtained results in a biplot. Spearman correlation coefficient was used to test the relationships between the abundance of picocyanobacteria populations and nitrate and

phosphate concentrations at the sampling stations during each month, with cor.test() function from package stats (R core Team, 2023).

3. RESULTS

3.1. Influence of NO_3^- and PO_4^{3-} concentrations on Synechococcus phenotypes in laboratory conditions

3.1.1. Effect of NO_3^- and PO_4^{3-} concentrations on picocyanobacterial growth rate

Significant differences were found in the growth rate (μ) when performing an ANOVA with μ as the dependent variable and considering nutrient condition and *Synechococcus* phenotype as factors (Fig. 3, Tables 2-4S).

The fastest growth rates ($\mu;d^{-1}$) were observed under moderate NO_3^- and moderate PO_4^{3-} (f/2) conditions for all *Synechococcus* sp. phenotypes studied, with values of 0.17, 0.43 and 0.30 per day for Type 1, Type 2, and Type 3a, respectively. Above all, the different NO_3^- conditions had the greatest influence on the growth rate of Type 3a, which showed higher rates at low NO_3^- levels ($\mu = 0.08$ in HN and 0.23 in LN). Conversely, Types 1 and 2 showed higher growth rates under elevated NO_3^- concentrations ($\mu = 0.12$ and 0.16, respectively) than under NO_3^- deficient conditions ($\mu = 0.10$ and 0.13, respectively). In addition, the results showed that changes in PO_4^{3-} conditions had the greatest effect on Type 2 (with $\mu = 0.15$ in HP and 0.39 in LP). Similarly, the growth rate of Type 1 ($\mu = 0.14$) under low PO_4^{3-} concentration exceeded that under PO_4^{3-}-rich conditions ($\mu = 0.05$). However, for Type 3a, increased PO_4^{3-} conditions resulted in an increased growth rate ($\mu = 0.14$) compared to low PO_4^{3-} conditions ($\mu = 0.11$).

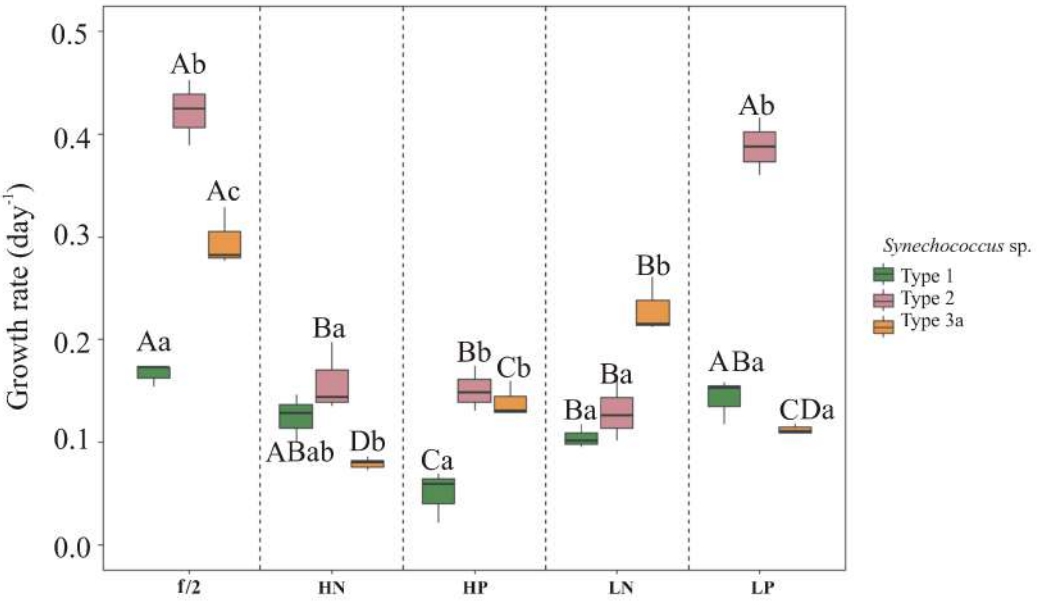

Fig.3. Growth rate (d^{-1}) of *Synechococcus* sp. phenotypes at different NO_3^- and PO_4^{3-} conditions (where: f/2 – standard f/2 media; HN– high NO_3^-; HP – high PO_4^{3-}; LN– low NO_3^-; LP– low PO_4^{3-}). The values are shown as a median ± minimal and maximal values (n = 3). Big letters (A-D) indicate significant difference in the Tukey post-hoc pairwise comparisons for Phenotype, and small letters (a-c) indicate post-hoc pairwise comparisons for nutrient conditions as factor.

3.2. Effect of NO_3^- and PO_4^{3-} concentration on inter-phenotype allelopathic interactions in monocultures and mixed cultures

The effects of nutrient conditions on the strength of allelopathic interactions between phenotypes are shown in Fig. 4. An ANOVA and a post-hoc Dunnet test were performed using % of growth relative to the control, as dependent variable and nutrient condition and day of the experiment as factors, finding significant differences, for all of the studied *Synechococcus* phenotypes. The statistical significance of the coefficients from the general

linear model for the influence of different nitrate (NO_3^-) and phosphate ($PO4^{3-}$), concentrations on inter-phenotype allelopathic interactions are shown in Table 5S.

NO_3^- concentration significantly affected the allelopathic activity of Type 2 and Type 3a. Elevated NO_3^- levels led to an inhibitory effect of both phenotypes on the target organisms. Under HN conditions, the cell-free filtrate of Type 2 significantly inhibited the growth of two other phenotypes, with a reduction of 56% for Type 1 and 19% for Type 3a. In addition, the filtrate of Type 3a inhibited the growth of Type 1 in HN medium by 62% compared to the control. Conversely, both phenotypes stimulated the growth of the target organisms under low NO_3^- conditions. The addition of cell-free filtrate of Type 3a stimulated the growth of two other phenotypes under LN conditions, resulting in a 49% increase for Type 1 and a 90% increase for Type 2 compared to the control. Similarly, the addition of filtrate of Type 2 stimulated the growth of Type 3a by 28% compared to the control.

In addition, a significant effect of PO_4^{3-} concentration on the allelopathic activity of Type 1 and Type 3a was observed. LP conditions resulted in an inhibitory effect of both phenotypes on the target organisms. The filtrate obtained from Type 1 inhibited the growth of Type 2 (by 77%) and Type 3a (by 16%) compared to the control. In addition, the filtrate of Type 3a under LP medium conditions inhibited the growth of Type 2 by 50% compared to the control. Conversely, excess PO_4^{3-} stimulated the growth of the target organisms. In high PO_4^{3-} concentration (HP), the filtrate of Type 1 and Type 3a increased the number of cells of phenotype Type 2 by 25% and 5%, respectively.

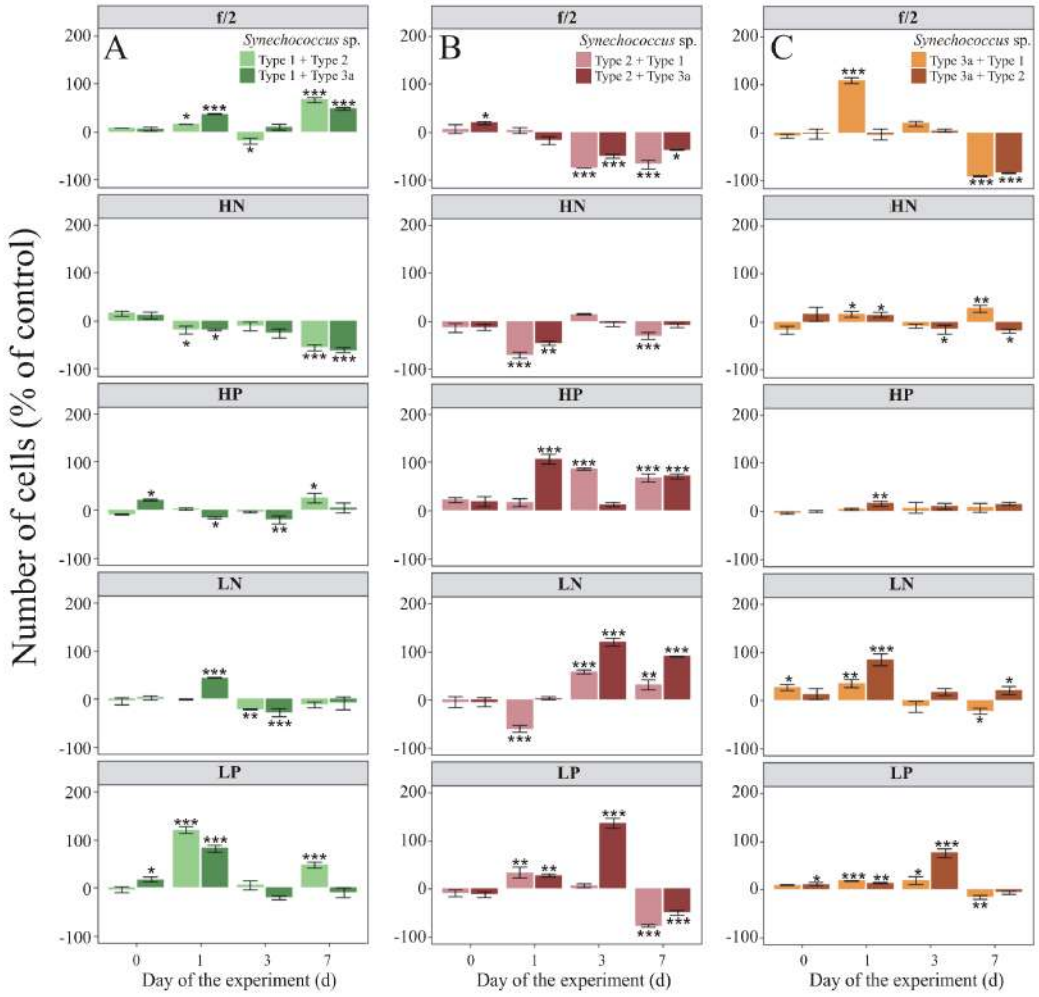

Fig.4. Number of cells (% of control, where control equal 0 calculated for each day of the experiment and for each tested phenotype) of *Synechococcus* sp.: Type 1 (A), Type 2 (B), and Type 3a (C) grown in monocultures with the addition of cell-free filtrate obtained from different *Synechococcus* sp. phenotypes at different NO_3^- and PO_4^{3-} conditions (where: f/2 – standard f/2 media; HN– high NO_3^-; HP – high PO_4^{3-}; LN– low NO_3^-; LP– low PO_4^{3-}). The values are mean ± SD (n = 3). Asterisks indicates significant differences in the post-hoc Dunnet test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

An analysis of variance (ANOVA) and subsequent post-hoc Dunnett test were conducted, employing the percentage of growth relative to the control as the dependent

variable. Nutrient condition and the day of the experiment were considered as factors, revealing significant differences in the interactions among *Synechococcus* phenotypes (Fig.5).

The interaction between Type 2 and Type 1 showed differences depending on the nutrient concentration. In the experiments we performed, we observed a mutual inhibition of both phenotypes under f/2, HN, and LP conditions, with the highest inhibition of Type 1 (by 76%) occurring under HN conditions. In contrast, under LN and HP conditions, we observed a pattern in which the Type 2 phenotype was stimulated (by 72% and 59%, respectively), while the Type 1 phenotype was inhibited (by 63% and 62%, respectively). Overall, co-cultivation of Type 1 and Type 2 resulted in a dominance of Type 2, except under LP conditions, where low PO_4^{3-} conditions clearly favored Type 1 and resulted in a lower growth reduction (32%) compared to Type 2 (87%).

Furthermore, the experiments performed showed that different nutrient concentrations influenced the co-culture of Type 1 and Type 3a. At all NO_3^- concentrations tested, co-culture of Type 1 with Type 3a resulted in significant inhibition (by 67% in f/2, 87% in HN and 63% in LN), with Type 3a showing less pronounced inhibition (by 19% in HN and 19% in LN, compared to the control). However, when Type 1 was cultured with Type 3a under low PO_4^{3-} conditions, a stimulation of 23% was observed.

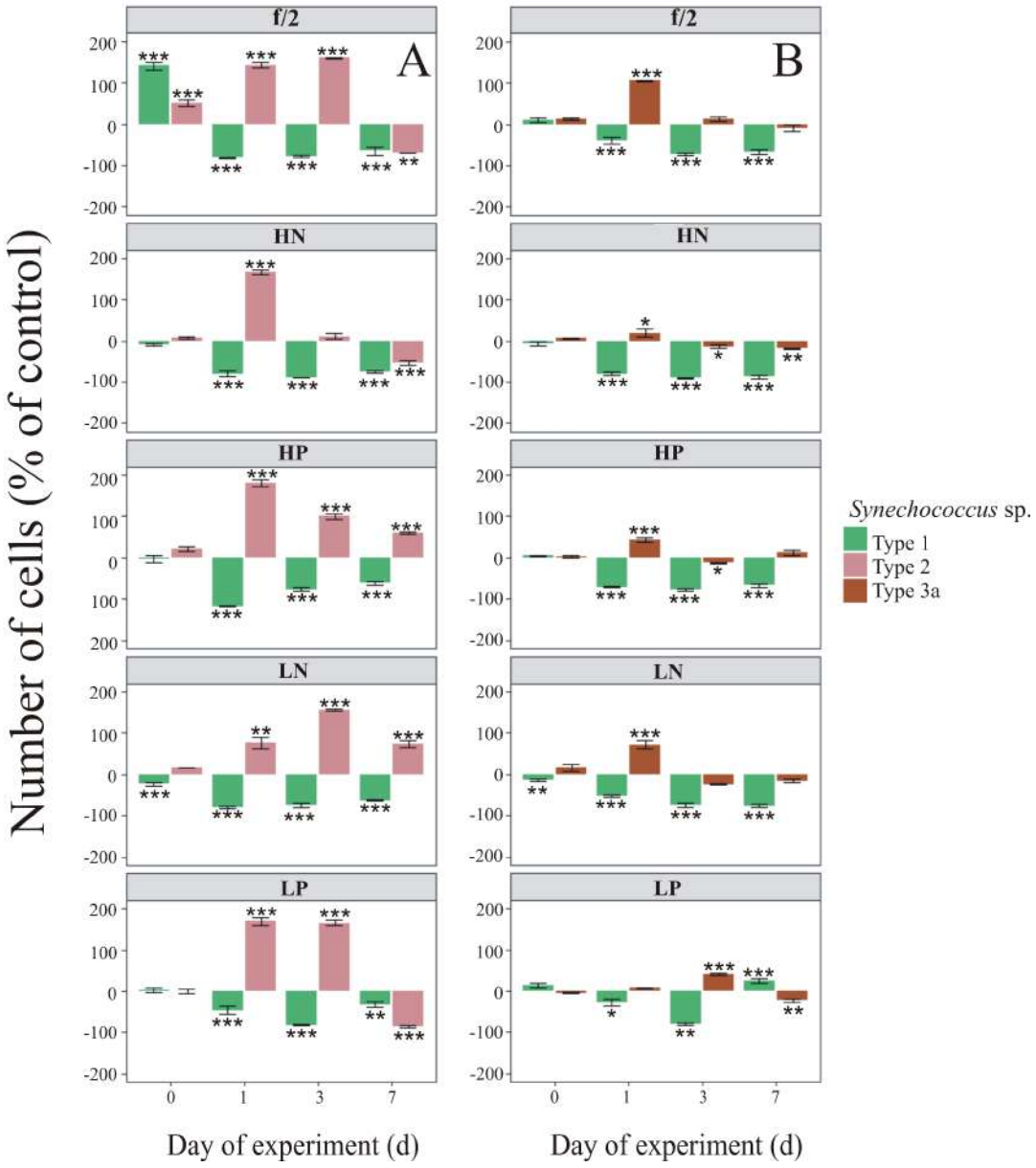

Fig.5. Cell abundance (% of control, where control equal 0 calculated for each day of the experiment and for each tested phenotype) of *Synechococcus* sp.: Type 1 and Type 2 (A) and Type 1 and Type 3a (B). Each plot represents two phenotypes grown in mixed cultures at different NO_3^- and PO_4^{3-} conditions (where: f/2 – standard f/2 media; HN– high NO_3^-; HP– high PO_4^{3-}; LN– low NO_3^-; LP– low PO_4^{3-}). The values are mean ± SD (n = 3). Asterisks indicates significant difference in post-hoc Dunnett test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3.3. Influence of nutrient concentrations on the cell-specific microcystin content

The concentrations of NO_3^- and PO_4^{3-} had a significant effect on the cell-specific microcystin-LR (MC-LR) content in the picocyanobacteria examined, as determined by ANOVA using MC content as dependent variable and nutrient concentrations as factors (see Tables 7-9S). The MC-LR content in the medium (µg mL^{-1}) was also quantified (see Table 6S in the supplementary materials).

This study highlights that all phenotypes tested showed the ability to produce MC-LR and that their production varied depending on the availability of NO_3^- and PO_4^{3-} in the medium (see Fig. 6). It was observed that MC-LR content was higher at LN concentrations than at HN conditions in all phenotypes studied. In particular, the highest cell-specific MC-LR content was observed in *Synechococcus* Type 2 (0.073 pg $cell^{-1}$) under low NO_3^- concentrations. In addition, increased levels of cell-specific MC-LR were observed in Type 1 with 0.053 and 0.056 pg $cell^{-1}$ under f/2 and LP conditions, respectively. In general, among the phenotypes tested, the lowest cell-specific MC-LR content was observed for *Synechococcus* sp. Type 3a, ranging from 0.012 pg $cell^{-1}$ under HN to 0.032 pg $cell^{-1}$ under HP conditions.

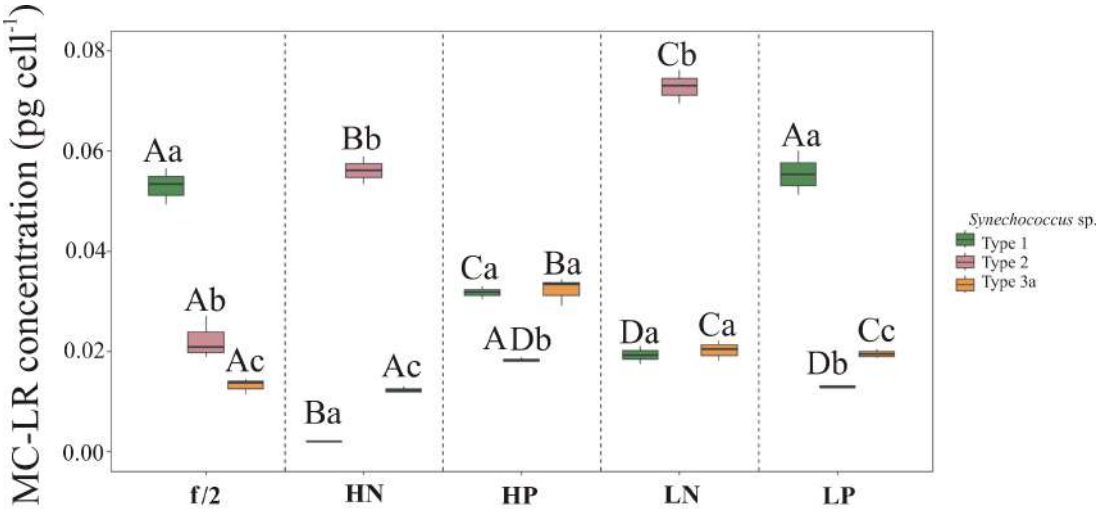

Fig.6. Cell-specific MC-LR content (pg cell^{-1}) in *Synechococcus* sp. (Type 1, Type 2, and Type 3a) at different NO_3^- and PO_4^{3-} conditions (where: f/2 – standard f/2 medium; HN – high NO_3^-; HP– high PO_4^{3-}; LN– low NO_3^-; LP– low PO_4^{3-}). The values are shown as a median ± minimal and maximal values (n = 3). Big letters (A-D) indicate significant difference in the post-hoc pairwise comparisons with different phenotype as factors and nutrient conditions as covariate, small letters (a-c) indicate Tukey post-hoc pairwise comparisons with nutrient conditions as factors and different phenotypes as covariate.

3.4. Influence of NO_3^- and PO_4^{3-} concentration on the occurrence and abundance of picocyanobacteria phenotypes in the southern Baltic Sea region

Our study showed that the number of cells of all picocyanobacterial phenotypes had no significant correlation with the concentrations of NO_3^- and PO_4^{3-} in the studied area (Spearman test $p > 0.05$, see Table 10S in the supplementary materials), with the exception of a positive correlation between NO_3^- concentration and the occurrence of phenotype Type 1 in March (Spearman test $p < 0.01$). At all of the sampled stations, *Synechococcus* sp. Type 2 was the most abundant phenotype, with a higher representation in July. Type 1 was the least abundant phenotype, with a higher representation in March and September, particularly at Sw2 station.

The lowest abundance picocyanobacteria phenotype was observed in July, with a peak of 2460 cells mL^{-1} at station KS1. The highest abundance of picocyanobacteria phenotypes occurred in SO3 station in March and reached 22860 cells mL^{-1}. However, across stations picocyanobacteria were most abundant in September, ranging from 3360 cells mL^{-1} to 14533 cells mL^{-1} at stations KS3 and LIVIA, respectfully. While all three cyanobacterial phenotypes were present in the sampled stations, Type 1 picocyanobacteria had the lowest abundance, peaking at 1723 cells mL^{-1} in March at station SO3. In all months

and at all stations, Type 2 picocyanobacteria consistently dominated (Fig. 3), with abundance ranging from 93cells mL^{-1} in July to 14277 cells mL^{-1} in March at station SO3. Picocyanobacteria of Type 3a were most abundant in March and reached a peak value of 6860 cells mL^{-1} at station SO3.

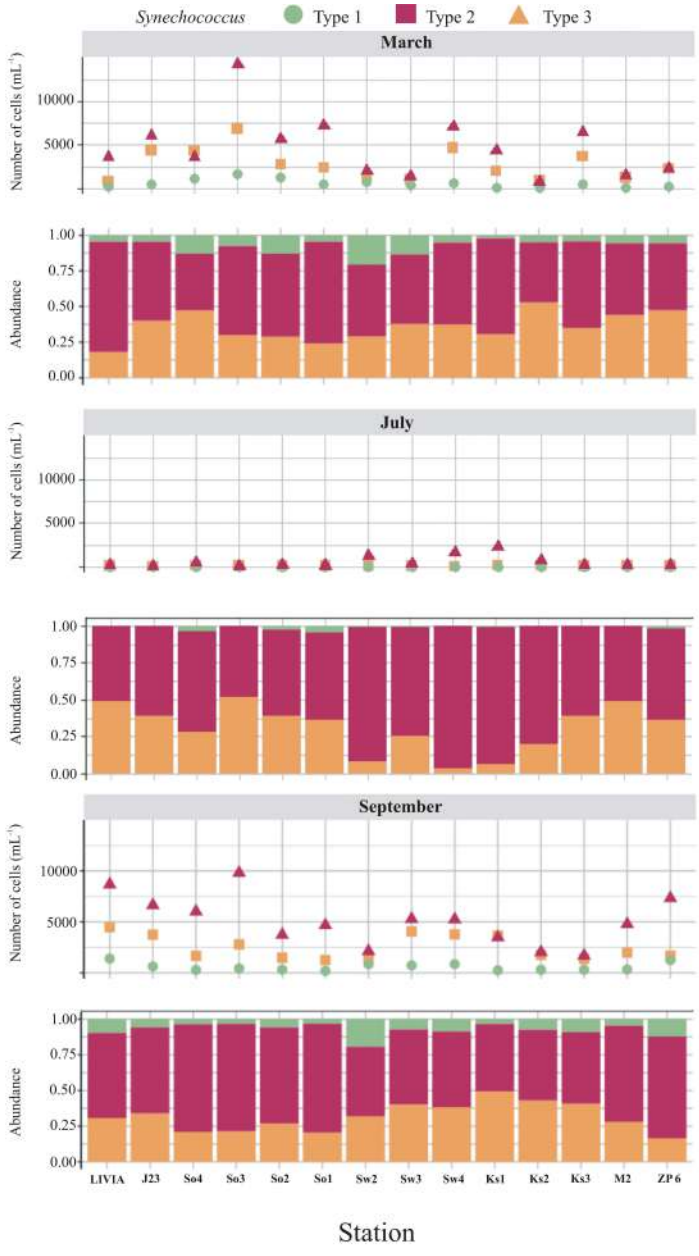

Fig.7. Cell abundances (mL^{-1}) and abundance of picocyanobacteria Type 1, Type 2, and Type 3a based on flow cytometry cell counts at different sample locations detected in March, July, and September of 2022.

This study shows a spatial variability of NO_3^- and PO_4^{3-} concentrations at the different sampling stations in the southern Baltic Sea (Fig. 8). The highest NO_3^- concentration occurred in March and ranged from 1.6µML^{-1} (station KS1) to 7.52 µML^{-1} (station SW3). The lowest NO_3^- concentration was observed in July, with values between 0.07 µML^{-1} and 1.7 µML^{-1} in SW2 and KS3 respectively. Regarding PO_4^{3-}, this nutrient peaked in July, observed mainly at stations M2 and ZP6 with values of 0.57 µML^{-1} and 0.52 µML^{-1} respectively, whereas the lowest PO_4^{3-} concentration was recorded in March, with values ranging from 0.1 µML^{-1} in the east of the Gulf of Gdansk to 0.35 µML^{-1} in the western region.

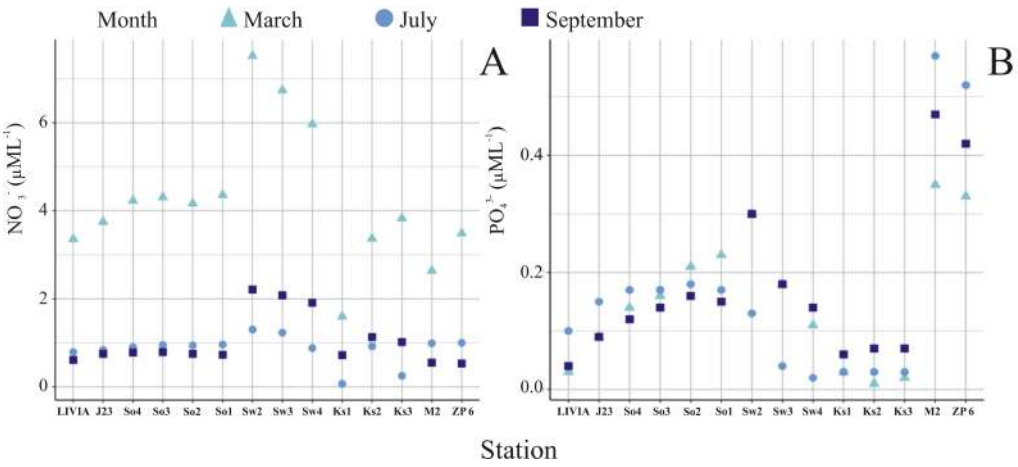

Fig.8.Nitrate (NO_3^-) (A) and phosphate (PO_4^{3-}) (B) concentrations at different sample locations detected in March, July, and September of 2022.

The principal components analysis revealed significant association between the distribution of *Synechococcus* sp. phenotypes, NO_3^- and temperature. The first two principal components explained an accumulated 60.8% of the total variance, (Figure 9). First principal component (PC1) represented 48.3% of the variance, and was mainly correlated with

temperature, NO_3^- and the abundances of the three *Synechococcus* sp. phenotypes. Second principal component (PC2) explained 19.5% of the total variation and was characterized mostly by the correlation with salinity.

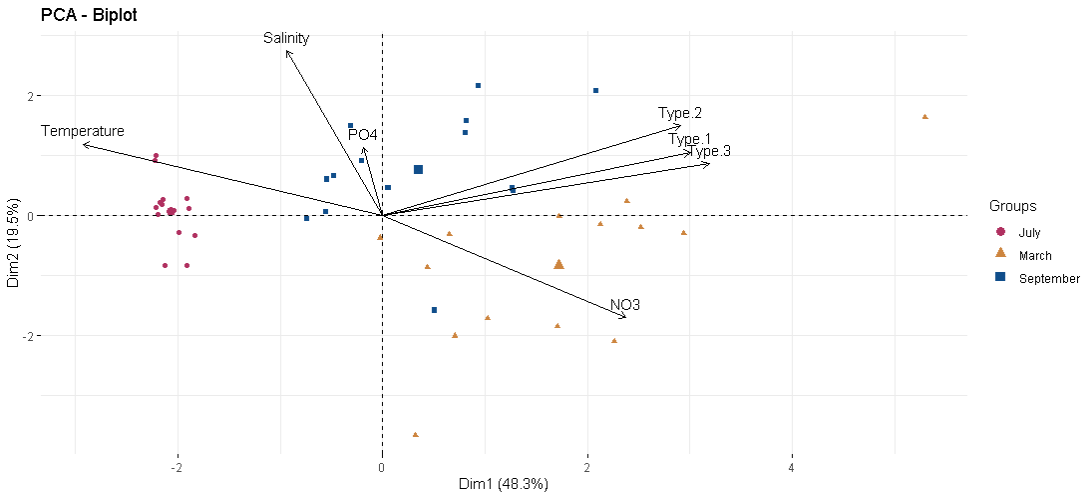

Fig.9. Principal component analysis ordination plot of *Synechococcus* Type 1, Type 2 and Type 3a, temperature (ºC), salinity, nitrate (NO_3^-) and phosphate (PO_4^{3-}) divided by groups of March, July, and September of 2022.

4. DISCUSION

4.1. Influence of NO_3^- and PO_4^{3-} concentrations on inter-phenotype allelopathic interactions

Despite the great ecological importance of *Synechococcus* sp. there is little knowledge about their allelopathic effects on other phenotypes within the same genus, as well as intrapopulation dynamics in varied nutrient conditions.

This study indicates that after the addition of filtrate of *Synechococcus* sp. Type 2 and Type 3a, the inhibition of the growth of the target phenotypes was detected at higher NO_3^- concentrations. On the other hand, we have shown that Type 1 and Type 3a

demonstrated increased negative effect on growth of other phenotype in low PO_4^{3-} conditions. Similarly, in co-cultures *Synechococcus* sp. Type 1, dominated over other phenotypes only in low PO_4^{3-} conditions, which can be due to adaptive responses to environmental stress of PC-rich strain to limited PO_4^{3-} conditions, rarely found in turbid, coastal waters.

Previous studies have emphasized the crucial role of NO_3^- and PO_4^{3-} concentrations in shaping the composition of phytoplankton and influencing their interactions based on resource competition theory (Tilman, 1982; Edwards et al., 2011; Grosse et al., 2017). Studies by Granéli and Johansson (2003a, b) have shown that, both PO_4^{3-} and NO_3^- limitation led to higher toxicity of the allelochemicals produced by *Prymnesium parvum*. In addition, higher production of allelochemicals stimulated by PO_4^{3-} decrease was observed in green alga *Chlorella vulgaris* (DellaGreca et al., 2010), *Trichormus doliolum* (Von Elert and Juettner, 1997), *Nostoc* (Bloor and England, 1991; Kurmayer, 2011), *Cylindrospermopsis raciborskii* (Antunes et al., 2012), *Microcystis* (Krueger et al., 2012), as well as in *Synechococcus* sp. (Sim et al., 2023).

4.2. Influence of nutrient concentrations on the microcystin content of Synechococcus phenotypes

Microcystins are cyclic heptapeptides that have a general structure that includes γ-linked d-glutamic acid (Lawton and Edwards, 2001). Moreover, some modifications, including esterification of glutamic acid, lead to changes in the toxicity of microcystins (Harada et al., 1990; Rinehart et al., 1994). *Synechococcus* sp. can reduce nitrates to ammonium and convert it to glutamine via type III glutamine synthetases, an enzyme crucial for nitrogen metabolism and protein synthesis, which can subsequently be used to produce microcystin (Kumada et al., 1993; Karp et al., 2019). Therefore, it appears that NO_3^-

concentration may influence the production of microcystin. Microcystin is produced by many different genera of cyanobacteria (Lawton and Edwards, 2001; Paldavičienė et al., 2015; Puschner, 2018), including *Synechococcus*, which is described in detail in a review by Śliwińska-Wilczewska et al. (2018). In our study, we showed that all tested *Synechococcus* sp. phenotypes (Type 1, Type 2, and Type 3a) were able to produce MC-LR and that their concentration depends on the availability of NO_3^- and PO_4^{3-} in the medium. We observed that *Synechococcus* sp. Type 2 produced the highest microcystin content under low NO_3^- conditions. Previous studies have shown that *Synechococcus* sp. can utilize the abundant phycoerythrin (PE) as an internal NO_3^- source under low NO_3^- conditions (Wyman et al., 1985). Therefore, it is possible that in our experiments, PE-rich *Synechococcus* sp. Type 2 was using this additional NO_3^- source for its microcystin production.

The production of microcystins can increase with increasing biomass of cyanobacteria during their growth (Paerl et al., 2016; Glibert, 2017; Harke et al., 2017), although it does not always correlate directly with cell abundance (Briand et al., 2005). In our study, the highest amount of microcystin produced by *Synechococcus* Type 2 correlated with the lowest growth rate of this phenotype. On the other hand, Van de Waal et al. (2014) demonstrated an increase in the production of nitrogenous toxins at high NO_3^- concentrations, which is consistent with the C:nutrient hypothesis (Hamilton et al., 2001). This hypothesis states that organisms prioritize metabolic needs, such as growth, over the production of secondary metabolites. Nonetheless, it appears that for *Synechococcus* sp. Type 2, the highest production of microcystins correlates with the lowest growth rates of the studied phenotype, which may indicate a potential association between the increased synthesis of microcystin and potential protective mechanisms of picocyanobacterial cells in response to stress conditions (Ginn et al., 2010; Pimentel and Giani, 2014).

4.3. Effect of NO_3^- and PO_4^{3-} concentration on the occurrence and abundance of picocyanobacterial phenotypes in the southern Baltic Sea regions

Given the detailed knowledge of the Baltic Sea as documented by e.g. Heckwolf et al. (2021), this aquatic system is ideally suited for the formulation and validation of hypotheses on the central role of nutrient availability in controlling the occurrence and abundance of major picocyanobacterial phenotypes.

The PCA analysis has shown that the main environmental factors influencing the abundance of *Synechococcus* sp. phenotypes are NO_3^- concentration and temperature, which aligned with previous studies (Eigmann et al., 2018; Otero-Ferrer et al., 2018; Alegria Zufia, et al., 2021). Studies conducted by Otero-Ferrer et al., (2018) had also shown that sea surface temperature and NO_3^- supply are the primary factors influencing the biomass variability of different picoplankton subgroups. In contrast, although surface light levels have a great impact on diversity and niche differentiation of *Synechococcus* phenotypes (Haverkamp et al., 2009), generally had a minor impact on picoplankton special variability (Otero-Ferrer et al., (2018). Additionally, out PCA shows that the three phenotype's abundance are strongly related to each other, suggesting a high degree of niche overlap. There was a minimum differentiation of the three phenotypes in the second axis, mainly related with salinity and PO_4^{3-}.

We have shown that three phenotypes of picocyanobacteria occur in the southern Baltic Sea, with a clear predominance of picocyanobacteria rich in phycoerythrin (Type 2), which agrees with previous studies (Mazur-Marzec et al., 2013; Alegria Zufia, et al., 2021). The present study provides evidence for the high abundance of *Synechococcus* sp. phenotypes in the southern Baltic Sea during the spring and fall seasons. In turn, the lowest abundance of picocyanobacteria was found in July. Although this conclusion seems to be contradictory with the previous results, showing the peak of abundance of picoplanktonic

cells in the summer season (Hajdu et al., 2007; Tamm et al., 2018; Alegria Zufia, et al., 2021). Recent studies highlighted distinct autecological preferences of *Synechococcus* sp. related to temperature and irradiance, which influence its vertical distribution. Unlike *N. spumigena* that thrives at the surface and *Dolichospermum* sp. found in the subsurface layer above the thermocline, *Synechococcus* sp. also occupies this subsurface zone (Hajdu et al., 2007; Eigmann et al., 2018). Moreover, the studies conducted by Eigmann et al., (2018) has shown that in terms of growth responses, *Synechococcus* sp. demonstrates optimal growth in low-light and low-temperature settings, consistent with previous research highlighting its adaptation to these conditions (Stal et al., 2003). Notably, *Synechococcus* sp. is sensitive to increased light intensity and variations in temperature, showing significant growth suppression under high-light conditions (approximately 60% inhibition) and to a lesser extent with temperature changes (around 20% inhibition). Also, studies have shown that *Synechococcus* sp. dominated in cooler marine environments (below 20°C), which typically exhibit intermediate to high levels of NO_3^-supply (Otero-Ferrer et al., 2018). This corresponds to our study in which the fastest growth rate of all tested phenotypes was observed under intermediate NO_3^- and PO_4^{3-}conditions. These findings emphasize the species' preference for specific environmental parameters, potentially influencing its vertical distribution within the water column during bloom events and may explain lower abundances of *Synechococcus* sp. cells in July in surface waters.

Grazing pressure by zooplankton has also been shown as an important factor influencing the seasonal abundance of picoplankton in the Baltic sea. Some studies showed high grazing rates by flagellates (Kuosa, 1991), but other zooplankton organisms like cladocerans, rotifers and copepod naupli also showed high grazing on picocyanobacteria (Motwani and Gorokhova, 2013). Grazing pressure during summer blooms was shown to be higher on picoplanktonic species over filamentous cyanobacteria (Motwani et al., 2018),

which may contribute to lower amount of picoplankton relative to filamentous cyanobacteria in summer season.

It is noteworthy that the abundance of picocyanobacteria cells showed significant variation between sample sites and surprisingly was not dependent on NO_3^- and PO_4^{3-} concentrations at these sites, with the exaptation of Type 1 phenotype in March. Type 1 is a species rich in PC that thrives in turbid coastal waters (Haverkamp et al., 2009). This is supported by the high abundance of this phenotype observed closest to the shore such as at the SW2 station, where the lowest salinity observed in September was noted. Previous studies have indicated salinity largely influences the special composition of picoplanktonic cyanobacteria (Bertos-Fortis et al., 2016). Recent studies shas shown a higher diversity of picoplanktonic cyanobacteria at brackish waters, as well as a notable shift with a distinctive shift of composition dependent on salinity ranges, in the Baltic Sea (Celepli, et al., 2017).

In July, *Synechococcus* sp. Type 1 (with the dominance of PC) was the least abundant phenotype. Previous studies have shown poorer adaptation to higher temperatures in PC-rich picocyanobacteria (Aguilera et al., 2021). Moreover, the same study had proven a preference of NH_4 over NO_3^- of PE-rich *Synechococcus* as a main source of NO_3^-, predicting higher abundance of PE-rich over other picoplanktonic organisms in eutrophic coastal waters. Furthermore, PE-rich *Synechococcus* sp. phenotype is adapted to lower light conditions and can occur lower in the water column (Haverkamp et al., 2009).

Based on our results and previous works, we can affirm that the structure of picophytoplankton communities in the Baltic Sea will be subject to changes driven by the predicted temperature increase (Suikkanen et al., 2013; Paerl, 2018), earlier and increased filamentous cyanobacteria blooms (Andersson et al., 2015), as well as the shift in nutrient regimes (Paerl, 2018). These alterations may increase dominance of PE-rich *Synechococcus* sp. phenotypes, shifting the composition and carbon biomass contribution in these communities along coastal areas of the Baltic Sea.

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AUTHOR CONTRIBUTIONS

Z.K.: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - Original draft preparation, S.Ś-W.: Conceptualization, Supervision, Writing - Review & Editing, A.B.F.: Supervision, Writing - Review & Editing, A.L.: Supervision, Writing - Review & Editing. All authors approved the submitted version.

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Supplementary material

Impacts of nutrient dynamics on three picocyanobacterial populations: laboratory experiments and seasonal surveys in the Baltic Sea region

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Table 1S. Values of *Synechococcus* Type 1, Type 2 and Type 3a, temperature (ºC), salinity, nitrate (NO_3^-) and phosphate (PO_4^{3-}) on sampling stations in March, July, and September of 2022.

Station	Type 1 (cells/mL)	Type 2 (cells/mL)	Type 3(cells/mL)	Salinity	Temperature (ºC)	NO_3^- (µmol/L)	PO_4^{3-} (µmol/L)
				March			
LIVIA	207	3550	833	7.24	4.45	3.36	0.03
J23	500	6073	4380	6.66	5.77	3.75	0.09
So4	1180	3610	4307	6.25	6.69	4.23	0.14
So3	1723	14277	6860	6.25	6.69	4.31	0.16
So2	1263	5653	2797	6.66	6.78	4.17	0.21
So1	450	7153	2413	6.97	5.75	4.36	0.23
Sw2	803	1963	1130	5.33	6.62	7.52	0.3
Sw3	390	1413	1097	6.47	5.66	6.74	0.18
Sw4	637	7123	4650	6.88	5.24	5.97	0.11
KS1	140	4340	1977	3.33	6.43	1.6	0.03
KS2	90	767	960	1.84	7.06	3.37	0.01
KS3	450	6390	3663	2.92	6.56	3.83	0.02
M2	163	1447	1270	6.94	5.4	2.64	0.35
ZP6	270	2237	2260	6.97	5.34	3.49	0.33
				July			
LIVIA	0	133	130	7.42	20.95	0.79	0.1
J23	0	100	63	7.02	20.95	0.84	0.15
So4	27	500	203	6.98	20.6	0.9	0.17
So3	0	93	100	7.45	20.4	0.95	0.17
So2	10	197	133	7.47	20.41	0.94	0.18
So1	10	127	77	7.39	20.42	0.96	0.17
Sw2	13	1203	100	6.32	20.72	1.3	0.13
Sw3	3	310	107	7.04	20.05	1.23	0.04
Sw4	7	1647	57	7.55	19.55	0.88	0.02
KS1	33	2270	157	7.25	19.92	0.07	0.03
KS2	0	727	180	5.51	21.34	0.92	0.03
KS3	0	193	123	5.35	21.43	0.25	0.03
M2	0	167	163	7.47	20.32	0.99	0.57
ZP6	7	197	117	7.47	20.52	1	0.52
				September			
LIVIA	1400	8663	4470	7.47	14.72	0.61	0.04
J23	633	6640	3743	7.45	14.92	0.75	0.09
So4	283	6007	1673	7.47	14.56	0.78	0.12
So3	440	9797	2793	7.46	14.29	0.79	0.14
So2	320	3727	1487	7.41	13.64	0.75	0.16
So1	203	4673	1253	7.26	14.02	0.73	0.15
Sw2	843	2130	1390	2.39	14.6	2.21	0.3

Sw3	737	5277	4057	5.81	14.27	2.08	0.18
Sw4	857	5243	3770	5.91	14.36	1.91	0.14
KS1	253	3480	3650	6.9	14.33	0.72	0.06
KS2	307	2027	1760	6.99	14.25	1.13	0.07
KS3	307	1683	1370	6.85	14.51	1.02	0.07
M2	327	4777	1983	7.37	13.98	0.55	0.47
ZP6	1250	7323	1690	7.35	13.8	0.53	0.42

Table 2S. ANOVA of the growth rate (day^{-1}) of *Synechococcus* sp. phenotypes with nutrient conditions and phenotypes as factors; Sum Sq, sum of squares; Df, degrees of freedom; F value, Fisher's F-test statistic.

Response: Growthrate	Sum Sq	Df	F value	*Pr(>F)*
Synechococcus sp.	0.133	2	119.971	< 0.001
Condition	0.207	4	93.243	< 0.001
Synechococcus sp.: Condition	0.157	8	15.251	< 0.001
Residuals	0.017	30		

Table 3S. Post-hoc Tukey pairwise comparisons test of the growth rate (day^{-1}) of *Synechococcus* sp. with phenotype as factor: SE, standard error; df, degrees of freedom; t. ratio, ratio of the difference between the mean of the two sample sets and the variation that exists within the sample sets.

Condition =f/2					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.256	0.020	30	-13.311	< 0.001
Type 1 – Type 3a	-0.130	0.020	30	-6.736	< 0.001
Type 2 – Type 3a	0.127	0.020	30	6.575	< 0.001
Condition = HN					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.034	0.020	30	-1.770	0.1969
Type 1 – Type 3a	0.045	0.020	30	2.332	0.0665
Type 2 – Type 3a	0.079	0.020	30	4.102	< 0.01
Condition = HP					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.101	0.020	30	-5.249	< 0.001
Type 1 – Type 3a	-0.090	0.020	30	-4.659	< 0.001
Type 2 – Type 3a	0.011	0.020	30	0.590	0.8266
Condition = LN					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.026	0.020	30	-1.352	0.3786
Type 1 – Type 3a	-0.125	0.020	30	-6.522	< 0.001
Type 2 – Type 3a	-0.100	0.020	30	-5.170	< 0.001
Condition = LP					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.245	0.020	30	-12.755	< 0.001
Type 1 – Type 3a	0.031	0.020	30	1.591	0.2649
Type 2 – Type 3a	0.276	0.020	30	14.346	< 0.001

Table 4S. Post-hoc Tukey pairwise comparisons of the growth rate(day^{-1}) of *Synechococcus* sp. phenotypes with nutrient condition as factor: SE, standard error; df, degrees of freedom; t. ratio, ratio of the difference between the mean of the two sample sets and the variation that exists within the sample sets.

Synechococcus sp. = Type 1:					
contrast	estimate	SE	df	t.ratio	p.value
(f/2) – HN	0.042	0.020	30	0.019	0.2109
(f/2) – HP	0.117	0.020	30	6.062	< 0.001
(f/2) – LN	0.062	0.020	30	0.019	0.0228
(f/2) – LP	0.024	0.020	30	1.229	0.7348
HN – HP	0.075	0.020	30	3.872	< 0.01
HN – LN	0.020	0.020	30	1.046	0.8319
HN – LP	-0.019	0.020	30	-0.961	0.8702
HP – LN	-0.054	0.020	30	-2.826	0.0590
HP – LP	-0.093	0.020	30	-4.833	< 0.001
LN – LP	-0.039	0.020	30	-2.007	0.2872
Synechococcus sp. = Type 2:					
contrast	estimate	SE	df	t.ratio	p.value
(f/2) – HN	0.264	0.020	30	13.731	< 0.001
(f/2) – HP	0.272	0.020	30	14.125	< 0.001
(f/2) – LN	0.293	0.020	30	15.196	< 0.001
(f/2) – LP	0.034	0.020	30	1.785	0.4006
HN – HP	0.008	0.020	30	0.393	0.9947
HN – LN	0.028	0.020	30	1.464	0.5927
HN – LP	-0.230	0.020	30	-11.946	< 0.001
HP – LN	0.021	0.020	30	1.071	0.8198
HP – LP	-0.237	0.020	30	-12.340	< 0.001
LN – LP	-0.258	0.020	30	-13.411	< 0.001
Synechococcus sp. = Type 3a:					
contrast	estimate	SE	df	t.ratio	p.value
(f/2) – HN	0.217	0.020	30	11.258	< 0.001
(f/2) – HP	0.157	0.020	30	8.139	< 0.001
(f/2) – LN	0.066	0.020	30	3.450	0.0135
(f/2) – LP	0.184	0.020	30	9.556	0.0305
HN – HP	-0.060	0.020	30	-3.119	< 0.001
HN – LN	-0.150	0.020	30	-7.808	< 0.01
HN – LP	-0.032	0.020	30	-1.702	< 0.01
HP – LN	-0.090	0.020	30	-4.689	< 0.001
HP – LP	0.027	0.020	30	1.417	< 0.001
LN – LP	0.118	0.020	30	6.106	0.9950

Table 5S. Linear model coefficient estimates and their significance for the influence of different nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations on inter-phenotype allelopathic interactions. SE, standard error; df, degrees of freedom.

Synechococcus sp. Type 1					
Nutrients	estimate	SE	df	t value	p value
NO_3^-	3.352	5.762	28	0.582	0.565
PO_4^{3-}	0.730	0.194	28	3.755	< 0.01
Synechococcus sp. Type 2					
Nutrients	estimate	SE	df	t value	p value
NO_3^-	-14.234	5.965	28	-2.387	< 0.05
PO_4^{3-}	0.240	0.265	28	0.904	0.374
Synechococcus sp. Type 3a					
Nutrients	estimate	SE	df	t value	p value

NO_3^-	-13.734	5.241	28	-2.620	< 0.05
PO_4^{3-}	0.502	0.221	28	2.274	< 0.05

Table 6S. MC-LR concentration (µg mL^{-1}) in *Synechococcus* sp. (Type 1, Type 2, and Type 3a) at different nitrate and phosphates conditions (where: f/2 – standard f/2 media; HN – high NO_3; HP – high PO_4; LN – low NO_3; LP – low PO_4).

Synechococcus sp.	MC-LR concentration (µgmL^{-1})				
	f/2	HN	HP	LN	LP
Type 1	1.473	0.155	1.482	1.163	1.485
Type 2	0.473	1.035	0.283	1.362	1.454
Type 3a	1.113	0.947	1.652	1.108	1.762

Table 7S. ANOVA of the *Synechococcus* sp. phenotypes MC-LR content (pg $cell^{-1}$) with nutrient conditions as factors and different phenotypes as covariate; Sum, sum of squares; Df, degrees of freedom.

Response: MC-LR content	Sum	Df	F value	*p*
Synechococcus sp.	0.002	2	194.41	< 0.001
Condition	0.001	4	38.41	< 0.001
Synechococcus sp.: Condition	0.014	8	296.43	< 0.001

Table 8S. Tukey post-hoc pairwise comparisons of *Synechococcus* sp. phenotypes of MC-LR content (pg $cell^{-1}$) with phenotypes as factor: SE, standard error; df, degrees of freedom; t. ratio, ratio of the difference between the mean of the two sample sets and the variation that exists within the sample sets.

Condition = f/2					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	0.031	0.002	30	15.316	< 0.001
Type 1 – Type 3a	0.040	0.002	30	19.805	< 0.001
Type 2 – Type 3a	0.009	0.002	30	4.489	< 0.01
Condition = HN					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.054	0.002	30	-26.878	< 0.001
Type 1 – Type 3a	0.010	0.002	30	-5.068	< 0.001
Type 2 – Type 3a	0.044	0.002	30	21.810	< 0.001
Condition = HN					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	0.014	0.002	30	6.702	< 0.001
Type 1 – Type 3a	-0.001	0.002	30	-0.234	0.9702
Type 2 – Type 3a	-0.014	0.002	30	-6.936	< 0.001
Condition = LN					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	-0.054	0.002	30	-26.671	< 0.001
Type 1 – Type 3a	-0.001	0.002	30	0.497	0.8735
Type 2 – Type 3a	0.053	0.002	30	26.174	< 0.001
Condition = LP					
contrast	estimate	SE	df	t ratio	p value
Type 1 – Type 2	0.043	0.002	30	21.168	< 0.001
Type 1 – Type 3a	0.036	0.002	30	17.927	< 0.001

Type 2 – Type 3a	-0.007	0.002	30	3.241	< 0.01

Table 9S. Post-hoc pairwise comparison of the *Synechococcus* sp. phenotypes obtained from MC-LR content (pg cell^{-1}) with nutrient conditions as factor: SE, standard error; df, degrees of freedom; t. ratio, ratio of the difference between the mean of the two sample sets and the variation that exists within the sample sets.

Synechococcus sp. = Type 1:					
contrast	estimate	SE	df	t.ratio	p.value
(f/2) – HN	0.051	0.002	30	25.345	< 0.001
(f/2) – HP	0.021	0.002	30	10.602	< 0.001
(f/2) – LN	0.034	0.002	30	16.801	< 0.001
(f/2) – LP	-0.003	0.002	30	-1.237	< 0.001
HN – HP	-0.030	0.002	30	-14.742	< 0.001
HN – LN	-0.017	0.002	30	-8.544	< 0.001
HN – LP	-0.054	0.002	30	-26.582	< 0.001
HP – LN	0.013	0.002	30	6.198	< 0.001
HP – LP	-0.024	0.002	30	-11.839	< 0.001
LN – LP	-0.036	0.002	30	-18.038	< 0.001
Synechococcus sp. = Type 2:					
contrast	estimate	SE	df	t.ratio	p.value
(f/2) – HN	-0.034	0.002	30	-16.849	< 0.001
(f/2) – HP	0.004	0.002	30	1.989	0.2957
(f/2) – LN	-0.051	0.002	30	-25.186	< 0.001
(f/2) – LP	0.009	0.002	30	4.615	< 0.001
HN – HP	0.038	0.002	30	18.837	< 0.001
HN – LN	-0.017	0.002	30	-8.337	< 0.001
HN – LP	0.043	0.002	30	21.464	< 0.001
HP – LN	-0.055	0.002	30	-27.174	< 0.001
HP – LP	0.005	0.002	30	2.627	0.0907
LN – LP	0.060	0.002	30	29.801	< 0.001
Synechococcus sp. = Type 3a:					
contrast	estimate	SE	df	t.ratio	p.value
(f/2) – HN	0.001	0.002	30	0.472	0.9893
(f/2) – HP	-0.019	0.002	30	-9.436	< 0.001
(f/2) – LN	-0.007	0.002	30	-3.500	0.0119
(f/2) – LP	-0.006	0.002	30	-3.114	< 0.05
HN – HP	-0.020	0.002	30	-9.908	< 0.001
HN – LN	-0.008	0.002	30	-3.973	< 0.01
HN – LP	-0.007	0.002	30	-3.586	<0.01
HP – LN	0.012	0.002	30	5.936	< 0.001
HP – LP	0.013	0.002	30	6.322	< 0.001
LN – LP	0.001	0.002	30	0.386	0.9950

Table 10S. Spearman's rank correlation test ($p < 0.05$) of relations between abundance of picocyanobacterial populations and nitrate (NO_3^-) and phosphate (PO_4^{3-}), concentration at sampling stations during individual months: rho: Spearman's rank correlation.

March		
Synechococcus sp.Type 1		
Nutrients	p value	rho
NO_3^-	0.0075	0.6799
PO_4^{3-}	0.3936	0.2475
Synechococcus sp.Type 2		
Nutrients	p value	rho

NO_3^-	0.4086	0.2396
PO_4^{3-}	0.6032	-0.1523
Synechococcus sp.Type 3a		
Nutrients	p value	rho
NO_3^-	0.3099	0.2923
PO_4^{3-}	0.9224	-0.0287
July		
Synechococcus sp.Type 1		
Nutrients	p value	rho
NO_3^-	0.7197	0.1055
PO_4^{3-}	0.9062	0.0347
Synechococcus sp. Type 2		
Nutrients	p value	rho
NO_3^-	0.8754	-0.0462
PO_4^{3-}	0.06211	-0.5106
Synechococcus sp. Type 3a		
Nutrients	p value	rho
NO_3^-	0.5677	-0.1672
PO_4^{3-}	0.5958	0.1554
September		
Synechococcus sp.Type 1		
Nutrients	p value	rho
NO_3^-	0.8752	0.0463
PO_4^{3-}	0.4218	0.2335
Synechococcus sp. Type 2		
Nutrients	p value	rho
NO_3^-	0.2201	-0.3498
PO_4^{3-}	0.8052	0.0726
Synechococcus sp. Type 3a		
Nutrients	p value	rho
NO_3^-	0.5677	-0.1672
PO_4^{3-}	0.3293	-0.2816

AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

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were as follows:

Konarzewska Zofia 60%:

Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing-Original draft preparation

Śliwińska-Wilczewska Sylwia15%:

Conceptualization, Supervision, Writing - Review & Editing

Barreiro Felpeto Aldo 15%:

Supervision, Writing - Review & Editing

Latała Adam 10%:

Supervision, Writing - Review & Editing

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Śliwińska-Wilczewska Sylwia

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Article

Assessment of the Allelochemical Activity and Biochemical Profile of Different Phenotypes of Picocyanobacteria from the Genus *Synechococcus*

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Abstract: Organisms belonging to *Synechococcus* sp. genera are observed in all freshwater, brackish, and marine waters of the world. They play a relevant role in these ecosystems, since they are one of the main primary producers, especially in open ocean. Eventually, they form mass blooms in coastal areas, which are potentially dangerous for the functioning of marine ecosystems. Allelopathy could be an important factor promoting the proliferation of these organisms. According to the authors' best knowledge, there is no information on the allelopathic activity and allelopathic compounds exhibited by different *Synechococcus* sp. phenotypes. Therefore, the research conducted here aimed to study the bioactivity of compounds produced by three phenotypes of *Synechococcus* sp. by studying their influence on the growth, chlorophyll fluorescence, and photosynthetic pigments of eighteen cyanobacteria and microalgae species. We demonstrated that three different *Synechococcus* sp. phenotypes, including a phycocyanin (PC)-rich strain (Type 1; green strain) and phycoerythrin (PE)-rich strains containing phycoerythrobilin (PEB) and phycocyanobilin (PCB) (Type 2; red strain and Type 3a; brown strain), had a significant allelopathic effect on the selected species of cyanobacteria, diatoms, and green algae. For all green algae, a decrease in cell abundance under the influence of phenotypes of donor cyanobacteria was shown, whereas, among some target cyanobacteria and diatom species, the cell-free filtrate was observed to have a stimulatory effect. Our estimates of the stress on photosystem II (F_v/F_m) showed a similar pattern, although for some diatoms, there was an effect of stress on photosynthesis, while a stimulatory effect on growth was also displayed. The pigment content was affected by allelopathy in most cases, particularly for chlorophyll *a*, whilst it was a bit less significant for carotenoids. Our results showed that *Synechococcus* sp. Type 3a had the strongest effect on target species, while *Synechococcus* sp. Type 1 had the weakest allelopathic effect. Furthermore, GC-MS analysis produced different biochemical profiles for the *Synechococcus* strains. For every phenotype, the most abundant compound was different, with oxime-, methoxy-phenyl- being the most abundant substance for *Synechococcus* Type 1, eicosane for *Synechococcus* Type 2, and silanediol for *Synechococcus* Type 3a.

Keywords: allelochemicals; phytochemicals; picocyanobacteria; *Synechococcus* sp.

1. Introduction

Picoplanktonic cyanobacteria of the genus *Synechococcus* are of great importance for the functioning of marine ecosystems, due to their significance in the composition of phytoplankton communities [1]. Moreover, they are capable of producing harmful secondary metabolites [2], as well as creating blooms that play a significant role in the environment, which are enhanced as a result of the increasing eutrophication of coastal ecosystems [1,3] and global warming [4,5]. It is also possible that picocyanobacteria have an advantage over other organisms due to their ability to adapt to changing environmental conditions [2] and their allelopathic activity [6].

The dominant photosynthetic pigments (phycobiliproteins) in phycobilisomes constitute the basis of the classification of organisms from the genus *Synechococcus*, which is the most abundant picoplanktonic genus in the Baltic Sea [7]. Phycobilisomes of these picoplanktonic cyanobacteria can contain four different phycobiliproteins: phycocyanin (PC), two forms of phycoerythrin (PE) known as PEI and PEII, and allophycocyanin (APC). PC can tie phycoerythrobilin (PEB) and phycocyanobilin (PCB). PEI binds either only PEB or both PEB and PUB, whereas PEII always binds both PEB and PUB [8–10]. Furthermore, research conducted by Six et al. [9] created a classification of marine *Synechococcus* consisting of three large groups: Type 1 consists of phenotypes with PC; Type 2 consists of phenotypes with a dominance of PEI; and Type 3 incorporates phenotypes with PC and two types of PE, which can be subdivided into different types (from a to d) based on the PEB and PUB ratio, with Type 3a having a low PEB:PUB ratio [9]. All three phenotypes of picocyanobacteria occur in the Baltic Sea [11,12].

One of the reasons why allelopathy is a subject of interest for researchers is because it can favor the dominance of a species in the ecosystem [13]. However, the effect of allelochemicals depends on the nature of the interaction between donor and target organisms and the activity of the chemical compounds responsible for this interaction. Nevertheless, knowledge on the substances excreted by picoplanktonic cyanobacteria of the genus *Synechococcus* is still scarce. Recently, studies have been conducted on the allelopathic activity of a green strain of *Synechococcus*, with a higher amount of PC (Type 1) on phytoplankton species occurring in the same environment [2]. This indicates that these picocyanobacteria could constitute a source of allelochemical substances not previously identified. The aim of our work was to determine the bioactivity of compounds produced by a strain from each of the three phenotypes of *Synechococcus* sp. (Type 1, Type 2, and Type 3); the latter two of which have were been explored. The bioactivity was assessed by studying their influence on the growth, photosynthetic parameters, and pigment composition of coexisting phytoplanktonic species. In addition, a biochemical profile of each strain was obtained by GS-MS analysis.

2. Results and Discussion

2.1. Allelopathic Effect of Different Synechococcus sp. Phenotypes on the Growth of Targeted Species of Phytoplankton

Our results showed a significant effect of all three phenotypes of *Synechococcus* sp. on the growth of all target species of cyanobacteria and microalgae, except the green algae *Chlorella fusca* (Figure 1, Table S1 in Supplementary Materials). Experiments revealed that the filtrate obtained from donor picocyanobacteria, in the majority of cases, had a negative effect on the growth of target species, with the highest inhibition caused by *Synechococcus* Type 3a (brown strain) (Figure 1). The most significant allelopathic effect was observed for the diatom *Skeletonema marinoi* (ANOVA, $F_{9,32} = 99.0$; $p < 0.001$) and constituted 77% (Dunnett HSD, $p < 0.001$), 35% (Dunnett HSD, $p < 0.001$), and 20% (Dunnett HSD, $p < 0.001$), respectively, of growth observed in the control. Furthermore, the cyanobacterium for which the highest inhibition of growth was observed was *Nostoc* sp. (ANOVA, $F_{9,32} = 283.1$; $p < 0.001$) and constituted 31% (Dunnett HSD, $p < 0.001$), 34% (Dunnett HSD, $p < 0.001$), and 33% (Dunnett HSD, $p < 0.001$), respectively, compared to the control. A positive effect on growth was observed in one cyanobacterium, *Aphanizomenon* sp., by *Synechococcus* Type 1 (green strain) and Type 3a (brown strain), and diatoms species *Navicula perminuta* under the influence of *Synechococcus* Type 2 (red strain). The

highest growth was noted for *Nitzschia fonticola* after the addition of cell-free filtrate of the green strain, which resulted in more than twice the number of cells of the control.

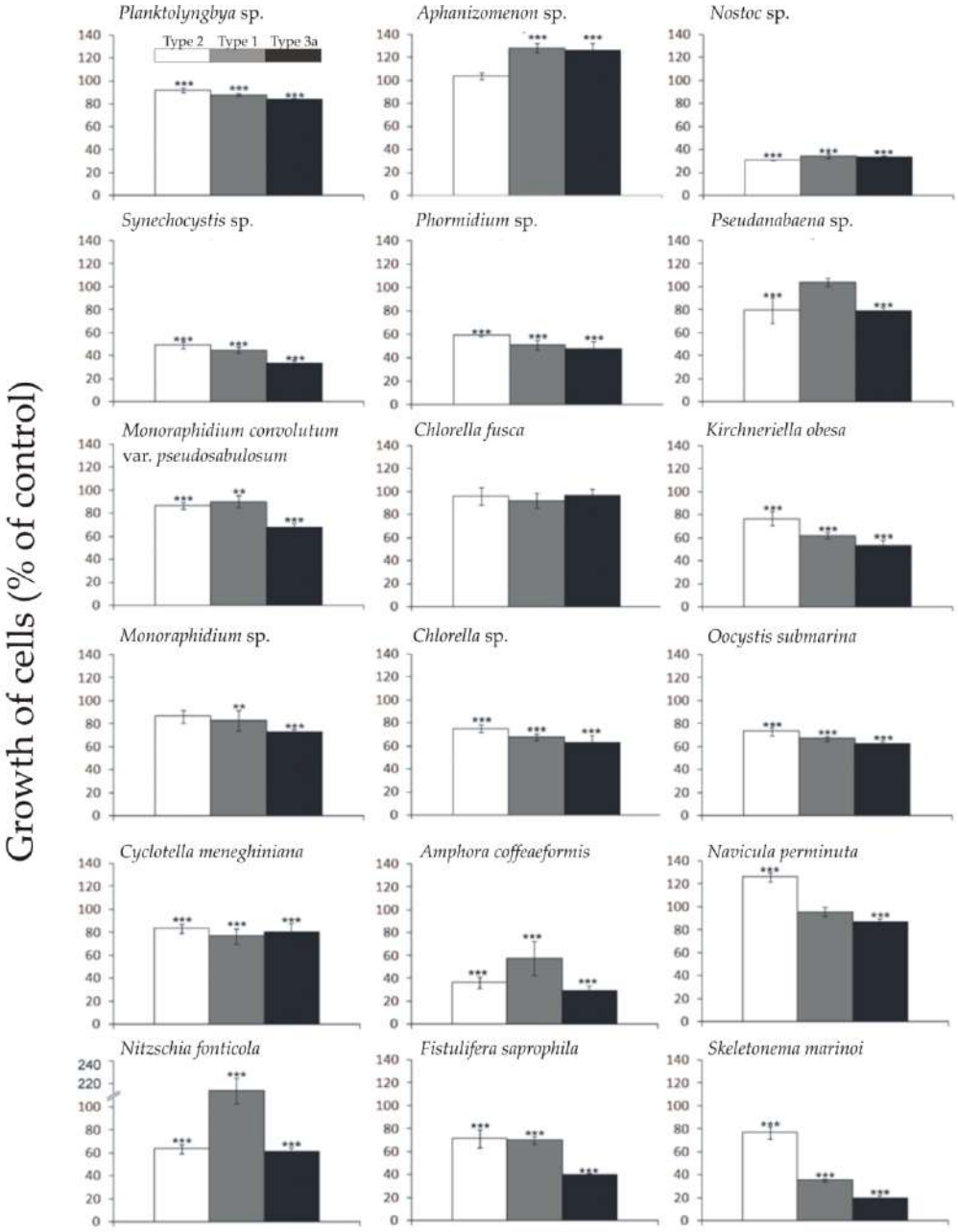

Figure 1. Growth of cells (% of control) of targeted cyanobacteria and microalgae, after the seventh day of the experiment with the addition of filtrate obtained from cultures of different *Synechococcus* sp. phenotypes. The values refer to means (n = 3, mean ± SD). Asterisk indicates significant difference identified by a post hoc Dunnett test, compared with the control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

In most cases, the highest allelopathic activity was demonstrated for *Synechococcus* Type 3a (brown strain). This phenotype belongs to cluster 5.1, which includes organisms that do not require an increased amount of nutrients [11]. Moreover, organisms in this clade prefer transparent ocean

waters [14], and are thus not abundant in relatively nutrient-rich waters of the Baltic Sea. The strong growth-inhibiting response of the target phytoplankton strains to allelopathic compounds produced by the brown *Synechococcus* strain may have resulted from the geographical avoidance of both producer and target organisms and the consequent lack of adaptation.

Several studies have shown a negative effect of the *Synechococcus* sp. Type 1 filtrates on different phytoplankton species, such as diatom *Navicula perminuta* [15], filamentous cyanobacteria *Nostoc* sp. and *Phormidium* sp. [16], bloom-forming cyanobacteria *Nodularia spumigena* [17], and *Porphyridium purpureum* and *Prymnesium parvum* [18]. Dominance by certain phytoplankton species in the community can be explained by the inhibition caused in the growth of co-existing species. If other environmental conditions are also favorable, this can lead to blooms of those species. This results in a change of the natural structure of the phytoplankton complex, for example, during the summer in the Baltic Sea [19,20].

It is believed that the plankton community maintains its diversity due to temporal (seasonal) heterogeneity driving them to a permanent non-equilibrium state, preventing species exclusion by a succession of seasonal communities [21]. Some factors contributing to the structuring of these seasonal communities have already been experimentally demonstrated, such as the light gradient [22]. However, allelopathy can also be considered as a driver of the plankton community structure [23]. Nevertheless, the effect of allelochemicals depends on the nature of the interaction between donor and target organisms and the activity of the chemical compounds responsible for this interaction.

In previous studies, it was demonstrated that allelopathic compounds may have self-stimulating properties [24]. Stimulation of the growth of the cyanobacteria *Aphanizomenon flos-aquae* under the influence of the filtrate from *Synechococcus* sp. was observed in studies conducted by Śliwińska-Wilczewska et al. [16]. Studies conducted by Bar-Yosef et al. [25] may be helpful in understanding the stimulating properties of allelopathic substances. Research has shown that *Aphanizomenon* can induce the alkaline phosphatase secretion of co-existing phytoplankton species, as a succession strategy. That is why an increase of target organisms is beneficial for bloom-forming *Aphanizomenon*.

2.2. Allelopathic Effect of Different Synechococcus sp. Phenotypes on the Chlorophyll Fluorescence of Studied Species of Phytoplankton

Our results showed significant effects of all three *Synechococcus* sp. phenotypes on the maximum PSII quantum efficiency (F_v/F_m) of the target species (Table 1, Table S2 in Supplementary Materials). The allelopathic effects on F_v/F_m were species-specific. Cell-free filtrate inhibited the F_v/F_m of all studied diatoms and green algae showed the strongest inhibition of the F_v/F_m, except *Oocystis submarina*, for which a positive effect was observed. However, cyanobacteria, in most cases, showed an increase in the F_v/F_m, suggesting a stimulatory effect of allelopathy. Among these, the highest increase was shown for *Phormidium* sp. (ANOVA, $F_{6.24} = 15.9$; $p < 0.001$) in response to *Synechococcus* Type 2 and constituted 158% of the control (Dunnett HSD, $p < 0.001$). However, the highest decrease in F_v/F_m was also observed for cyanobacterium. In *Nostoc* sp. (ANOVA, $F_{6.24} = 11.7$; $p < 0.001$), F_v/F_m dropped to only 29% of the control (Dunnett HSD, $p < 0.001$) after being exposed to *Synechococcus* Type 3a.

Prince et al. [26] showed a significant decrease in the F_v/F_m fluorescence parameter of the species *Akashiwo* cf. *sanguinea*, *Amphora* sp., *Asterionellopsis glacialis*, *Prorocentrum minimum*, and *Skeletonema costatum*in response to *Karenia brevis* (dinoflagellate). However, the studies conducted by Śliwińska-Wilczewska et al. [15] did not show a significant influence of diatom *Navicula perminuta* on the F_v/F_m value after the addition of filtrate obtained from *Synechococcus* sp. The filtrate from *Synechococcus* sp. also decreased the fluorescence parameter of *P. purpureum* and *Stichococcus bacillaris*, but increased the F_v/F_m of *P. parvum* [18]. Experiments conducted on freshwater species by Kovacs et al. [27] showed a significant decrease in the F_v/F_m of green alga *Scenedesmus quadricauda* after the addition of coexisting freshwater picocyanobacteria *Cyanobium gracile* and *Cylindrospermopsis raciborskii*. In addition, it was shown that the cell-free filtrate of picocyanobacteria *Synechocystis* sp. inhibits this same fluorescence parameter of the coexisting microalgae species *P. purpureum* and *Fistulifera* sp. [6].Our

work and the mentioned literature show a predominantly inhibitory effect on this parameter. A low index of photosynthesis parameters is indicative of irregularities in the process of photosynthesis, low physiological conditions, and the impact of potential stress factors on the studied plant organisms [27]. Studies show that moderations in photosynthesis or the influence of chemicals alters fluorescence kinetics and may be an indicator of environmental changes [28]. A decrease of the main physiological process of co-occurring algae species is a competitor strategy, which can determine the domination of this species in the environment. The results showed that the influence of allelochemicals varied, depending on the strain, which may indicate that the composition of the phytoplankton community can heavily influence the nature of allelopathic interactions, as well as the seasonal succession [27].

Table 1. Effect of filtrate from different *Synechococcus* sp. phenotypes on the chlorophyll fluorescence parameter F_v/F_m obtained after the seventh day of the experiment. Asterisk indicates significant difference identified by a post hoc Dunnett test, compared with the control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Target Species	Effect on Phenotypes of *Synechococcus* sp.		
	Type 1	**Type 2**	**Type 3a**
Cyanobacteria			
Planktolyngbya sp.	+ *	0	0
Aphanizomenon sp.	+ **	+ ***	+ ***
Nostoc sp.	– ***	– ***	– ***
Synechocystis sp.	0	0	0
Phormidium sp.	+ ***	+ ***	+ ***
Pseudanabaena sp.	– ***	– ***	0
Green algae			
Monoraphidium convolutum var. *pseudosabulosum*	– ***	– ***	– ***
Chlorella fusca	– ***	– ***	– ***
Kirchneriella obesa	0	+ **	– **
Monoraphidium sp.	– ***	– ***	– ***
Chlorella sp.	– ***	– ***	– ***
Oocystis cf. *submarina*	+ ***	+ ***	+ ***
Diatoms			
Cyclotella meneghiniana	– *	– **	– *
Amphora coffeaeformis	– **	0	– ***
Navicula perminuta	– ***	0	0
Nitzschia fonticola	– ***	0	– ***
Fistulifera saprophila	– **	0	– ***
Skeletonema marinoi	0	0	– ***

2.3. Allelopathic Effect of Different Synechococcus sp. Phenotypes on the Photosynthetic Pigments of Studied Species of Phytoplankton

This study has shown that the filtrate from all phenotypes of *Synechococcus* sp. negatively affected the content of chlorophyll *a* and carotenoid pigments of the cyanobacteria *Phormidium* sp. and *Nostoc* sp., green algae *M. convolutum* var. *pseudosabulosum*, *K. obesa*, *Monoraphidium* sp., and *O. submarina*, as well as thediatoms *A. coffeaeformis*, *N. fonticola*, and *F. saprophila* (Table 2, Table S3 in Supplementary Materials). The largest decrease of chlorophyll *a* was found for the species *Monoraphidium* sp. with filtrate from phenotype 3a (6% relative to the control, *t*-test, $p < 0.001$) and the largest decrease of carotenoids was found for the species *M. convolutum var. pseudosabulosum* (16% relative to the control,

t-test, $p < 0.001$). The green algae were shown to be the group most susceptible to allelopathic effects. The decrease in the content of pigments also appeared to be associated with the decrease in the number of cells of these organisms, with the exception of *N. fonticola*, which showed an increase in the number of cells under the influence of *Synechococcus* Type 1. Among the positive effects detected for the chlorophyll *a* content, the highest increase was demonstrated for cyanobacterium from the genus *Planktolyngbya*. There were no positive effects on carotenoid pigments observed. In general, carotenoids were much less affected than chlorophyll *a*.

In line with our results, Suikkanen et al. [20] observed a significant decrease in the chlorophyll *a* content of *Rhodomonas*sp. after being exposed to filtrate from cyanobacteria *A. flos-aquae* and *N. spumigena*. Additionally, a significant reduction of chlorophyll *a* in *Phormidium* sp., *Rivularia* sp., and *N. spumigena* was demonstrated in response to the filtrate from *Synechococcus* sp. [16,17]. Inhibition of the chlorophyll *a* value shows a drop in the efficiency of photosynthesis, which demonstrates the activity of the cell's defense mechanism and response to stress factors [28,29].

Table 2. Effect of filtrate from different *Synechococcus* sp. phenotypes on photosynthetic pigments (chlorophyll *a* and carotenoids) obtained after the seventh day of the experiment. Asterisk indicates significant difference, compared with the control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Target Species	Effect on Phenotypes of *Synechococcus* sp.					
	Type 1		Type 2		Type 3a	
	Photosynthetic Pigments					
	Chl *a*	Car	Chl *a*	Car	Chl *a*	Car
Cyanobacteria						
Planktolyngbya sp.	+ **	0	+ *	– *	+ **	0
Aphanizomenon sp.	+ *	0	0	0	+ **	0
Nostoc sp.	– *	– **	– *	– *	– *	– **
Synechocystis sp.	– *	0	– *	0	– *	0
Phormidium sp.	– ***	– ***	– ***	– ***	– ***	– *
Pseudanabaena sp.	– *	– ***	– *	0	– ***	– ***
Green algae						
Monoraphidium convolutum var. *pseudosabulosum*	– *	– **	– *	– **	– **	– ***
Chlorella fusca	– *	– *	0	0	– *	0
Kirchneriella obesa	– ***	– **	– ***	– **	– ***	– **
Monoraphidium sp.	– ***	– **	– ***	– **	– ***	– ***
Chlorella sp.	– ***	0	– ***	0	– ***	0
Oocystis cf. *submarina*	– ***	– **	– **	– **	– **	– **
Diatoms						
Cyclotella meneghiniana	– *	0	0	0	– ***	– **
Amphora coffeaeformis	– ***	– ***	– ***	– ***	– **	– ***
Navicula perminuta	– *	– *	0	0	– *	– *
Nitzschia fonticola	– ***	– **	– ***	– **	– ***	– ***
Fistulifera saprophila	– ***	– ***	– ***	– ***	– ***	– ***
Skeletonema marinoi	0	– **	0	0	– ***	– ***

2.4. GC-MS Analysis

GC-MS analysis identified the most abundant chemical compounds from every cell-free filtrate from *Synechococcus* sp. Type 1, Type 2, and Type 3a (Table 3) that did not occur in f/2 medium and thus may potentially be allelopathic substances. All substances present in the filtrates are shown in

Table S4 in the Supplementary Materials, as well as the GC-MS chromatogram profiles of f/2 medium and three phenotypes of *Synechococcus* sp. in Figures S1–S4. Twenty-two chemical compounds not detected in the f/2 medium were found in *Synechococcus* Type 1 (green strain). The most abundant one was eicosane, 10-methyl- (Figure 2), with the peak area of 64.98%. *Synechococcus* Type 2 (red strain) showed 10 compounds that were not present in the f/2 medium. In this strain, the most abundant was oxime-, methoxy-phenyl-, with a peak area of 12.91%. GC-MS analysis revealed only five compounds produced by *Synechococcus* Type 3a (brown strain), with the most abundant of them being silanediol, dimethyl, with a 13.03% peak area. In previous studies, compounds detected in algae having antimicrobial properties included inter alia fatty acids, hydrocarbons, phenols, terpenes, and indoles [30–32], and some of these substances also occurred in the analysis carried out in this study. Chemicals from these classes were proven to have an antimicrobial and antialgal role, and can also be ranked among phytochemicals.

Table 3. The most abundant phytochemicals identified in different phenotypes of picocyanobacteria from the genus *Synechococcus* Type 1 (green strain), Type 2 (red strain), and Type 3a (brown strain) by GC-MS.

Name of Compound	RT	Molecular	MW	Type 1		Type 2		Type 3a	
				Peak Area %	SI	Peak Area %	SI	Peak Area %	SI
Silanediol, dimethyl-	4.301	$C_2H_8O_2Si$	92	ND	ND	ND	ND	13.03	97
Oxime-, methoxy-phenyl-	8.444	$C_8H_9NO_2$	151	0.79	83	12.91	83	4.51	83
Eicosane, 10-methyl-	15.060	$C_{21}H_{44}$	296	64.98	96	6.73	94	ND	ND

RT = retention time, MW = molecular weight, ND = not detected, and SI = NIST (mass spectral libraries) Match Factors.

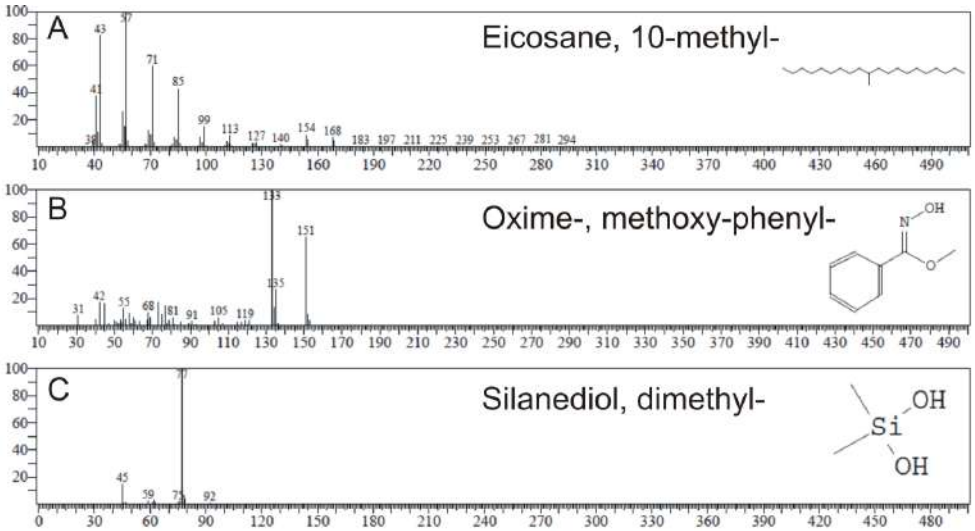

Figure 2. GC-MS spectrum of identified compounds which dominated in the cultures of different *Synechococcus* sp. phenotypes, including Type 2 (**A**), Type 1 (**B**), and Type 3a (**C**), and were not present in the f/2 medium.

Previous studies carried out using Gas Chromatography-Mass Spectrometry (GC-MS) analysis detected eicosane in species of red algae *Laurencia obtusa* var. *pyramidata* [33], brown algae *Turbinaria ornata* [34], and *Cystoseira barbata*, which was proven to show antimicrobial activity [35]. In addition, oxime-, methoxy-phenyl- found in *Synechococcus* Type 2 was identified in the bacteria *Sorangium*

cellulosum [36] and *Pseudomonas aeruginosa* [37], fungi species *Aspergillus terreus* [38], and the leaf extract of *Alstonia scholaris* [39], and was proven to have antimicrobial, antibacterial, and antifungal properties. Furthermore, the most abundant substance in *Synechococcus* Type 3a was proven to be silanediol, which has protease inhibitor properties [40]. Moreover, studies conducted by Madsen et al. [41] have proven that silanediols represent a novel zinc binding group (ZBG) with properties that can be used for the development of histone deacetylase inhibitors, which have an important role inter alia in apoptosis [42].

Picocyanobacteria have also been rarely studied with respect to their potential as producers of novel bioactive allelopathic compounds. Our results indicate that different phenotypes of *Synechococcus* sp. may serve as a potential source of interesting bioactive compounds, whose characterization requires detailed investigation.

3. Materials and Methods

3.1. Studied Species

In this study, the experiments were conducted using *Synechococcus* strains from Type 1 (green strain), Type 2 (red strain), and Type 3a (brown strain), obtained from the Culture Collection of Baltic Algae (CCBA) as strains *Synechococcus* sp. (BA-120), *Synechococcus* sp. (BA-124), and *Synechococcus* sp. (BA-132), respectively. Eighteen species of cyanobacteria, green algae, and diatoms (Table 4) were cultivated in the CCBA at the Institute of Oceanography, University of Gdańsk, Poland [43], to be tested as target strains for investigation of the allelopathic activity of *Synechococcus*.

Table 4. Microalgae and cyanobacteria species tested for allelopathic activity.

Target Species	Identification in CCBA Collection
Cyanobacteria	
Planktolyngbya sp.	BA-50
Aphanizomenon sp.	BA-69
Nostoc sp.	BA-81
Synechocystis sp.	BA-121
Phormidium sp.	BA-141
Pseudanabaena sp.	BA-142
Chlorophyta	
Monoraphidium convolutum var. *pseudosabulosum*	BA-17
Chlorella fusca	BA-18
Kirchneriella obesa	BA-51
Monoraphidium sp.	BA-165
Chlorella sp.	BA-167
Oocystis cf. *submarina*	BA-172
Bacilariophyta	
Cyclotella meneghiniana	BA-10
Amphora coffeaeformis	BA-16
Navicula perminuta	BA-30
Nitzschia fonticola	BA-34
Fistulifera saprophila	BA-56
Skeletonema marinoi	BA-98

3.2. Culture Condition

Cyanobacterial and microalgal cultures were grown in f/2 medium [44]. Culture media was prepared with Baltic Sea water (salinity 8‰) filtered through glass fiber filters (Whatman GF/C) and autoclaved. All organisms were grown under the conditions of photosynthetic active light (PAR) of 10 µmol photons $m^{-2}s^{-1}$ with the photoperiod L:D 16:8, at 18 °C and 8‰. All cultures were acclimated to experimental conditions for 7 days. Light was provided by an artificial light source (Cool White 40W, Sylvania, Wilmington, MA, USA). Measurements of the PAR radiation intensity were made using the Li-Cor meter, model LI-189, with a cosine collector.

3.3. Determination of the Allelopathic Effect of Cell-Free Filtrates

Allelopathic effects were tested according to a method proposed by Suikkanen et al. [19], with modifications. Experimental treatments were prepared by adding 10 mL of the cell-free filtrate (f/2 medium in controls) to 25-mL Erlenmeyer flasks containing 10 mL of cell suspensions of the targeted species. In all experiments, the initial Chl *a* concentration in the cultures was 0.4 µg Chl *a* mL^{-1}. *Synechococcus* sp. strains were filtered through a 0.45-µm filter (Macherey-Nagel MN GF-5). Tests were conducted in triplicate and the results of the experiments are presented as the mean value of three independent measurements.

3.4. Cell Density Assays

Cell abundances of cyanobacteria and microalgae were estimated with previously determined linear regression models on the basis of the optical density (OD) and number of cells (NmL^{-1}) in the cultures. Estimates of the cell abundance of picocyanobacteria, green algae, and diatoms were carried out using the BD Accuri™ C6 Plus flow cytometer. Filamentous cyanobacteria cells were counted using a Tomic SFC-18 light microscope and the Bürker chamber, and were counted from 48 large squares [45]. Obtained data were used to fit a linear regression model of the number of cells and optical density (Table 5). Cell abundances were calculated on the seventh day of the exposure of target cells in experimental and control cultures.

Table 5. Linear regression and correlation coefficients (*r*) used to calculate the number (*N*) of studied picocyanobacteria, cyanobacteria, green algae, and diatom cells in cultures based on optical density (OD) measurements.

Studied Strain	Linear Regression	Correlation Coefficient (*r*)
BA-120	$N = 4242096 \cdot OD - 35834$	0.97
BA-124	$N = 93029379 \cdot OD - 98415$	0.99
BA-132	$N = 139120177 \cdot OD - 44353$	0.99
BA-50	$N = 74916153 \cdot OD + 46981$	0.92
BA-69	$N = 6716526 \cdot OD - 86633$	0.96
BA-81	$N = 39891877 \cdot OD - 11899$	0.95
BA-121	$N = 163917381 \cdot OD - 246275$	0.98
BA-141	$N = 86779699 \cdot OD - 44781$	0.98
BA-142	$N = 126415680 \cdot OD + 100972$	0.98
BA-17	$N = 24943668 \cdot OD - 263873$	0.99
BA-18	$N = 14395782 \cdot OD + 100101$	0.97
BA-51	$N = 12365968 \cdot OD - 246229$	0.99
BA-165	$N = 13120468 \cdot OD + 10489$	0.99
BA-167	$N = 3678299 \cdot OD + 274144$	0.93

Table 5. *Cont.*

Studied Strain	Linear Regression	Correlation Coefficient (*r*)
BA-172	$N = 3363550 \cdot OD + 91273$	0.98
BA-10	$N = 8775538 \cdot OD - 1251$	0.98
BA-16	$N = 4385135 \cdot OD + 15527$	0.98
BA-30	$N = 6412449 \cdot OD - 8836$	0.97
BA-34	$N = 8050792 \cdot OD + 17824$	0.98
BA-56	$N = 7326981 \cdot OD - 57789$	0.99
BA-98	$N = 38103552 \cdot OD + 75013$	0.97

where *N*—cells in 1 mL of medium and OD—optical density of the culture.

3.5. Fluorescence Assay

The effects of the filtrate of three different *Synechococcus* sp. phenotypes on the chlorophyll fluorescence of target species were determined using the maximum PSII quantum efficiency—F_v/F_m (where F_v—the difference between the maximum and minimum fluorescence and F_m—the maximum fluorescence) [46]. The measurements were conducted using a Pulse Amplitude Modulation (PAM) fluorometer (FMS1, Hansatech, King's Lynn, United Kingdom) after 7 days of the experiment. Samples were filtered through glass fiber filters (Whatman GF/C, Saint Louis, MO, USA). Before measurement, the filtered sample was kept in the dark for approximately 10 min [15].

3.6. Pigments Assay

Photosynthetic pigments of target species were examined for the control and experiments with additions of the cell-free filtrate obtained from three different *Synechococcus* sp. phenotypes after 7 days of exposure. In the experiment, pigment extraction (4 mL of the material) was carried out in experimental flasks in 2 mL of 90% acetone in the dark and at a low temperature of −60 °C for about 1 hour. After this time, the extract was centrifuged for 1 min at 13,000 rpm min^{-1}. Absorbance measurements were then carried out in 1 cm glass cuvettes on a Becker spectrophotometer model DU 530 at wavelengths of 480, 665, and 750 nm. For the determination of chlorophyll *a*, different formulas of cyanobacteria, green algae, and diatoms was used, as described by Jeffrey and Humphrey [47]. The concentration of carotenoid pigments was calculated using the formula employed by Strickland and Parsons [48].

3.7. GC–MS Analysis

GC-MS analysis was carried out on a Shimadzu QP 2017 SE LOG 149 system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Zebron 5MSi (30 m, I.D 0.25 mm × 0.25 µm, Phenomenex, Part no.: 7HG-G018-11, Serial nr. 357092), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.5 mL min^{-1} and an injection volume of 0.5 EI was employed (split ratio of 10:1) with an injector temperature of 300 °C; ion-source temperature 260 °C. The oven temperature was programmed from 30 °C (isothermal for 5 min), with an increase of 20 °C min^{-1}, to 300 °C, ending with a 3 min isothermal at 300 °C. Mass spectra were taken at 70 eV, a scan-interval of 0.5 s, and fragments from 40 to 550 Da [49]. Compounds were identified by fitting coefficients (SI) using the NIST library ver. 14.

3.8. Statistical Analyses

Repeated measures ANOVA was used to test the effect of *Synechococcus* strains on the growth and fluorescence of the targeted species onday0, 1, 3 (data not shown), and 7 of the experiment. A post hoc Dunnett test was used to determine significant differences between the control and the experimental treatments. One-way ANOVA was used to test the effect of picocyanobacterial filtrates on

the chlorophyll *a* and carotenoid pigments in control and experimental cultures on the seventh day of the experiment. Data are reported as the mean ± standard deviation (SD). Levels of significance were * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. The statistical analyses were performed using the Statistica® 13.1 software.

4. Conclusions

This work demonstrated the significant allelopathic effect of all three phenotypes of picoplanktonic cyanobacteria *Synechococcus* sp. on the growth and photosynthetic activity of selected species of Baltic cyanobacteria, green algae, and diatoms. GC-MS analysis of the cell-free filtrate showed the presence of chemical compounds that may potentially be allelopathic substances, of which the most dominant ones all had either antimicrobial or cell-damaging properties. Phenotypes of *Synechococcus* sp. displayed interesting and valuable allelopathic activities and further analysis of the substances they produce is essential, particularly due to the potential role of allelochemicals in structuring the plankton community.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-3397/18/4/179/s1, Figure S1: Chromatogram that shows the results of the analysis of sample of f/2 medium prepared using HS-SPME, Figure S2: Chromatogram that shows the results of the analysis of sample of phenotype Type 1 of *Synechococcus* sp. prepared using HS-SPME, Figure S3: Chromatogram that shows the results of the analysis of sample of phenotype Type 2 of *Synechococcus* sp. prepared using HS-SPME, Figure S4: Chromatogram that shows the results of the analysis of sample of phenotype Type 3a of *Synechococcus* sp. prepared using HS-SPME, Table S1:Number of cells of studied cyanobacteria and microalgae obtained after 7th day of the experiment for control and culture with the addition of filtrate obtained from cultures of cyanobacteria *Synechococcus* sp. (BA-120, BA-124 and BA-132), Table S2: Value of fluorescence of chlorophyll *a* (F_v/F_m parameter) of studied cyanobacteria and microalgae obtained after 7th day of the experiment for control and culture with the addition of filtrate obtained from cultures of cyanobacteria *Synechococcus* sp. (BA-120, BA-124 and BA-132), Table S3: Value of photosynthetic pigments (chlorophyll *a* and carotenoid pigments) of studied cyanobacteria and microalgae obtained after 7th day of the experiment for control and culture with the addition of filtrate obtained from cultures of cyanobacteria *Synechococcus* sp. (BA-120, BA-124 and BA-132), Table S4: Phytochemicals identified in different phenotypes of picocyanobacteria from the genus *Synechococcus* Type 1 (green strain), Type 2 (red strain), and Type 3a (brown strain) by GC-MS. The compounds found in both the f/2 medium and picocyanobacterial cultures are highlighted in italics.

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Supplementary material

Assessment of the Allelochemical Activity and Biochemical Profile of Different Phenotypes of Picocyanobacteria from the Genus *Synechococcus*

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Table S1. Number of cells of studied cyanobacteria and microalgae obtained after 7th day of the experiment for control and culture with the addition of filtrate obtained from cultures of cyanobacteria *Synechococcus* sp. (BA-120, BA-124 and BA-132).

***Planktolyngbya* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.22	0.232	0.238	0.23	0.009165
OD (750 nm)	0.141	0.143	0.149	0.144333	0.004163
Number of cells (cell/ml)	10610159	10759991	11209488	10859879	311900.8
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.177	0.176	0.178	0.177	0.001
OD (750 nm)	0.133	0.131	0.134	0.132667	0.001528
Number of cells (cell/ml)	10010829	9860997	10085746	9985857	114436.3
% of control	94.35136	91.64503	89.97508	91.99049	2.208501
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.167	0.17	0.172	0.169667	0.002517
OD (750 nm)	0.125	0.127	0.129	0.127	0.002
Number of cells (cell/ml)	9411500	9561332	9711165	9561332	149832.3
% of control	88.70273	88.86004	86.63344	88.0654	1.242609
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.159	0.162	0.165	0.162	0.003
OD (750 nm)	0.119	0.12	0.124	0.121	0.002646
Number of cells (cell/ml)	8962003	9036919	9336584	9111836	198209.5
% of control	84.46625	83.98631	83.2918	83.91479	0.590484
Aphanizomenon sp.					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.094	0.099	0.1	0.097667	0.003215
OD (750 nm)	0.065	0.068	0.069	0.067333	0.002082
Number of cells (cell/ml)	349941.2	370090.8	376807.3	365613.1	13981.56
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.096	0.1	0.107	0.101	0.005568
OD (750 nm)	0.065	0.071	0.072	0.069333	0.003786
Number of cells (cell/ml)	349941.2	390240.3	396956.9	379046.1	25428.36
% of control	100	105.4445	105.3474	103.5973	3.115744
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.12	0.126	0.131	0.125667	0.005508
OD (750 nm)	0.079	0.086	0.083	0.082667	0.003512
Number of cells (cell/ml)	443972.6	490988.2	470838.7	468599.8	23587.66
% of control	126.8706	132.667	124.9548	128.1641	4.015523
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.113	0.124	0.122	0.119667	0.005859
OD (750 nm)	0.076	0.086	0.083	0.081667	0.005132

Number of cells (cell/ml)	423823	490988.2	470838.7	461883.3	34466.53
% of control	121.1126	132.667	124.9548	126.2448	5.884205

***Nostoc* sp.**

	$Control_1$	$Control_2$	$Control_3$	Mean	SD
OD (665 nm)	0.245	0.241	0.243	0.243	0.002
OD (750 nm)	0.17	0.165	0.166	0.167	0.002646
Number of cells (cell/ml)	6769720	6570261	6610153	6650044	105544
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
OD (665 nm)	0.068	0.068	0.066	0.067333	0.001155
OD (750 nm)	0.052	0.052	0.051	0.051667	0.000577
Number of cells (cell/ml)	2062479	2062479	2022587	2049181	23031.59
% of control	30.46623	31.39112	30.59819	30.81851	0.500264
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
OD (665 nm)	0.067	0.069	0.069	0.068333	0.001155
OD (750 nm)	0.055	0.059	0.059	0.057667	0.002309
Number of cells (cell/ml)	2182154	2341722	2341722	2288533	92126.34
% of control	32.23404	35.64123	35.42614	34.4338	1.908084
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
OD (665 nm)	0.069	0.065	0.067	0.067	0.002
OD (750 nm)	0.058	0.053	0.056	0.055667	0.002517
Number of cells (cell/ml)	2301830	2102370	2222046	2208749	100392.4
% of control	34.00185	31.99828	33.61566	33.20526	1.062961

***Synechocystis* sp.**

	$Control_1$	$Control_2$	$Control_3$	Mean	SD
OD (665 nm)	0.105	0.112	0.116	0.111	0.005568
OD (750 nm)	0.08	0.082	0.087	0.083	0.003606
Number of cells (cell/ml)	12867115	13194950	14014537	13358868	591012.5
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
OD (665 nm)	0.06	0.057	0.059	0.058667	0.001528
OD (750 nm)	0.043	0.04	0.042	0.041667	0.001528
Number of cells (cell/ml)	6802172	6310420	6638255	6583616	250387.9
% of control	52.86478	47.82451	47.36692	49.35207	3.050687
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
OD (665 nm)	0.055	0.053	0.056	0.054667	0.001528
OD (750 nm)	0.039	0.037	0.038	0.038	0.001
Number of cells (cell/ml)	6146503	5818668	5982585	5982585	163917.4
% of control	47.76908	44.09769	42.68843	44.85173	2.622918
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
OD (665 nm)	0.043	0.04	0.042	0.041667	0.001528
OD (750 nm)	0.03	0.028	0.029	0.029	0.001
Number of cells (cell/ml)	4671246	4343412	4507329	4507329	163917.4
% of control	36.30376	32.91723	32.16181	33.79427	2.205862

***Phormidium* sp.**

	$Control_1$	$Control_2$	$Control_3$	Mean	SD
OD (665 nm)	0.149	0.156	0.139	0.148	0.008544
OD (750 nm)	0.108	0.115	0.098	0.107	0.008544
Number of cells (cell/ml)	9327426	9934884	8459630	9240647	741446.1
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
OD (665 nm)	0.071	0.083	0.065	0.073	0.009165
OD (750 nm)	0.063	0.071	0.057	0.063667	0.007024
Number of cells (cell/ml)	5422340	6116578	4901662	5480193	609520.6
% of control	58.13329	61.56667	57.9418	59.21392	2.039789

	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.067	0.057	0.065	0.063	0.005292
OD (750 nm)	0.056	0.054	0.054	0.054667	0.001155
Number of cells (cell/ml)	4814882	4641323	4641323	4699176	100204.6
% of control	51.62069	46.71743	54.86437	51.0675	4.101547
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.066	0.059	0.054	0.059667	0.006028
OD (750 nm)	0.059	0.05	0.045	0.051333	0.007095
Number of cells (cell/ml)	5075221	4294204	3860305	4409910	615667.2
% of control	54.41181	43.22349	45.63209	47.75579	5.888735

***Pseudanabaena* sp.**

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.247	0.254	0.255	0.252	0.004359
OD (750 nm)	0.161	0.167	0.167	0.165	0.003464
Number of cells (cell/ml)	20453896	21212391	21212391	20959559	437916.8
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.212	0.206	0.2	0.206	0.006
OD (750 nm)	0.143	0.138	0.112	0.131	0.016643
Number of cells (cell/ml)	18178414	17546336	14259528	16661426	2103976
% of control	88.87507	82.71739	67.22264	79.60503	11.1567
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.255	0.252	0.25	0.252333	0.002517
OD (750 nm)	0.174	0.171	0.169	0.171333	0.002517
Number of cells (cell/ml)	22097300	21718053	21465222	21760192	318139.2
% of control	108.0347	102.3838	101.1919	103.8701	3.655508
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.168	0.161	0.175	0.168	0.007
OD (750 nm)	0.13	0.125	0.134	0.129667	0.004509
Number of cells (cell/ml)	16535010	15902932	17040673	16492872	570039.9
% of control	80.84039	74.97001	80.33358	78.71466	3.252847

Monoraphidium convolutum* var. *pseudosabulosum

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.423	0.469	0.476	0.456	0.028792
OD (750 nm)	0.335	0.373	0.38	0.362667	0.024214
Number of cells (cell/ml)	8092256	9040115	9214721	8782364	603994
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.362	0.379	0.391	0.377333	0.014572
OD (750 nm)	0.302	0.317	0.325	0.314667	0.011676
Number of cells (cell/ml)	7269115	7643270	7842819	7585068	291246.9
% of control	89.82804	84.54837	85.11185	86.49608	2.899279
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.379	0.38	0.405	0.388	0.014731
OD (750 nm)	0.322	0.32	0.342	0.328	0.012166
Number of cells (cell/ml)	7767988	7718101	8266861	7917650	303452.8
% of control	95.99286	85.37613	89.71364	90.36088	5.337877
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.274	0.278	0.287	0.279667	0.006658
OD (750 nm)	0.244	0.246	0.254	0.248	0.005292
Number of cells (cell/ml)	5822382	5872269	6071819	5922157	131989.5

% of control	71.95005	64.9579	65.89259	67.60018	3.795973
Chl*orella fusca*					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.27	0.302	0.281	0.284333	0.016258
OD (750 nm)	0.22	0.245	0.23	0.231667	0.012583
Number of cells (cell/ml)	3267173	3627068	3411131	3435124	181143
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.275	0.26	0.269	0.268	0.00755
OD (750 nm)	0.226	0.214	0.223	0.221	0.006245
Number of cells (cell/ml)	3353548	3180798	3310360	3281569	89901.63
% of control	102.6437	87.69614	97.04583	95.79523	7.551853
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.254	0.251	0.269	0.258	0.009644
OD (750 nm)	0.209	0.207	0.222	0.212667	0.008145
Number of cells (cell/ml)	3108819	3080028	3295965	3161604	117246.8
% of control	95.15319	84.91785	96.62381	92.23162	6.376446
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.271	0.281	0.253	0.268333	0.014189
OD (750 nm)	0.226	0.233	0.211	0.223333	0.01124
Number of cells (cell/ml)	3353548	3454318	3137611	3315159	161805.9
% of control	102.6437	95.23722	91.98155	96.62083	5.464085
Kirchneriella obesa					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.496	0.527	0.542	0.521667	0.023459
OD (750 nm)	0.404	0.433	0.451	0.429333	0.023714
Number of cells (cell/ml)	4749622	5108235	5330823	5062893	293241.2
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.381	0.356	0.366	0.367667	0.012583
OD (750 nm)	0.341	0.326	0.33	0.332333	0.007767
Number of cells (cell/ml)	3970566	3785077	3834540	3863394	96052.08
% of control	83.59752	74.09754	71.9315	76.54218	6.205338
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.294	0.294	0.308	0.298667	0.008083
OD (750 nm)	0.269	0.269	0.283	0.273667	0.008083
Number of cells (cell/ml)	3080216	3080216	3253340	3137924	99952.93
% of control	64.85182	60.29903	61.02885	62.0599	2.445253
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.234	0.248	0.297	0.259667	0.033081
OD (750 nm)	0.214	0.226	0.272	0.237333	0.030616
Number of cells (cell/ml)	2400088	2548480	3117314	2688627	378595.2
% of control	50.53219	49.88963	58.47717	52.96633	4.783329
***Monoraphidium* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.416	0.464	0.436	0.438667	0.024111
OD (750 nm)	0.33	0.374	0.349	0.351	0.022068
Number of cells (cell/ml)	4340243	4917544	4589532	4615773	289543.5
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.355	0.361	0.38	0.365333	0.013051
OD (750 nm)	0.294	0.299	0.315	0.302667	0.01097
Number of cells (cell/ml)	3867907	3933509	4143436	3981617	143927
% of control	89.11727	79.9893	90.28014	86.46224	5.635804
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD

OD (665 nm)	0.36	0.329	0.358	0.349	0.017349
OD (750 nm)	0.3	0.273	0.297	0.29	0.014799
Number of cells (cell/ml)	3946629	3592377	3907268	3815425	194165.2
% of control	90.93106	73.05225	85.13434	83.03922	9.121682
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.29	0.306	0.292	0.296	0.008718
OD (750 nm)	0.25	0.262	0.252	0.254667	0.006429
Number of cells (cell/ml)	3290606	3448052	3316847	3351835	84352.81
% of control	75.81616	70.11735	72.26982	72.73445	2.877676

Chl*orella* sp.

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.457	0.529	0.507	0.497667	0.036896
OD (750 nm)	0.389	0.451	0.431	0.423667	0.031644
Number of cells (cell/ml)	1705002	1933057	1859491	1832517	116395.5
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.312	0.328	0.324	0.321333	0.008327
OD (750 nm)	0.289	0.304	0.3	0.297667	0.007767
Number of cells (cell/ml)	1337172	1392347	1377634	1369051	28571.02
% of control	78.42643	72.02824	74.08661	74.84709	3.266182
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.268	0.285	0.282	0.278333	0.009074
OD (750 nm)	0.252	0.267	0.265	0.261333	0.008145
Number of cells (cell/ml)	1201075	1256250	1248893	1235406	29958.01
% of control	70.44421	64.98773	67.16318	67.53171	2.74684
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.263	0.242	0.256	0.253667	0.010693
OD (750 nm)	0.247	0.227	0.241	0.238333	0.010263
Number of cells (cell/ml)	1182684	1109118	1160614	1150805	37751.13
% of control	69.36553	57.37637	62.41569	63.05253	6.019896

Oocystis* cf. *submarina

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.327	0.328	0.344	0.333	0.009539
OD (750 nm)	0.27	0.27	0.286	0.275333	0.009238
Number of cells (cell/ml)	999431.5	999431.5	1053248	1017370	31071.14
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.218	0.229	0.214	0.220333	0.007767
OD (750 nm)	0.192	0.201	0.19	0.194333	0.005859
Number of cells (cell/ml)	737074.6	767346.6	730347.5	744922.9	19708.6
% of control	73.74939	76.7783	69.34239	73.29003	3.739181
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.187	0.201	0.2	0.196	0.00781
OD (750 nm)	0.168	0.179	0.18	0.175667	0.006658
Number of cells (cell/ml)	656349.4	693348.5	696712	682136.6	22395.62
% of control	65.67227	69.37428	66.14888	67.06515	2.01392
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.188	0.179	0.181	0.182667	0.004726
OD (750 nm)	0.167	0.158	0.162	0.162333	0.004509
Number of cells (cell/ml)	652985.9	622713.9	636168.1	637289.3	15167.09
% of control	65.33573	62.30681	60.40058	62.68104	2.488765

Cyclotella meneghiniana

	Control$_1$	Contro$_{l2}$	Control$_3$	Mean	SD
OD (665 nm)	0.096	0.111	0.11	0.105667	0.008386

OD (750 nm)	0.062	0.071	0.07	0.067667	0.004933
Number of cells (cell/ml)	542832.4	621812.2	613036.7	592560.4	43288.7
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.078	0.081	0.084	0.081	0.003
OD (750 nm)	0.052	0.056	0.061	0.056333	0.004509
Number of cells (cell/ml)	455077	490179.1	534056.8	493104.3	39571.09
% of control	83.8338	78.83074	87.11662	83.26038	4.172598
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.074	0.074	0.072	0.073333	0.001155
OD (750 nm)	0.052	0.051	0.052	0.051667	0.000577
Number of cells (cell/ml)	455077	446301.4	455077	452151.8	5066.559
% of control	83.8338	71.77431	74.23324	76.61378	6.372443
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.076	0.074	0.074	0.074667	0.001155
OD (750 nm)	0.055	0.053	0.054	0.054	0.001
Number of cells (cell/ml)	481403.6	463852.5	472628.1	472628.1	8775.538
% of control	88.68366	74.59688	77.09621	80.12558	7.516125
Amphora coffeaeformis					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.037	0.033	0.038	0.036	0.002646
OD (750 nm)	0.024	0.02	0.024	0.022667	0.002309
Number of cells (cell/ml)	120770.2	103229.7	120770.2	114923.4	10127.04
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.014	0.013	0.012	0.013	0.001
OD (750 nm)	0.007	0.006	0.005	0.006	0.001
Number of cells (cell/ml)	46222.95	41837.81	37452.68	41837.81	4385.135
% of control	38.27346	40.52885	31.01151	36.60461	4.973302
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.018	0.022	0.018	0.019333	0.002309
OD (750 nm)	0.01	0.014	0.01	0.011333	0.002309
Number of cells (cell/ml)	59378.35	76918.89	59378.35	65225.2	10127.04
% of control	49.16638	74.51236	49.16638	57.61504	14.63351
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.01	0.011	0.012	0.011	0.001
OD (750 nm)	0.003	0.004	0.005	0.004	0.001
Number of cells (cell/ml)	28682.41	33067.54	37452.68	33067.54	4385.135
% of control	23.74956	32.03297	31.01151	28.93135	4.516527
Navicula perminuta					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.053	0.057	0.054	0.054667	0.002082
OD (750 nm)	0.045	0.048	0.046	0.046333	0.001528
Number of cells (cell/ml)	279724.2	298961.6	286136.7	288274.1	9795.178
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.068	0.068	0.069	0.068333	0.000577
OD (750 nm)	0.057	0.058	0.059	0.058	0.001
Number of cells (cell/ml)	356673.6	363086	369498.5	363086	6412.449
% of control	127.509	121.4491	129.1336	126.0306	4.049972
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.051	0.056	0.058	0.055	0.003606
OD (750 nm)	0.042	0.045	0.046	0.044333	0.002082
Number of cells (cell/ml)	260486.9	279724.2	286136.7	275449.2	13348.58

% of control	93.12274	93.56528	100	95.56267	3.849202
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.049	0.049	0.05	0.049333	0.000577
OD (750 nm)	0.04	0.04	0.041	0.040333	0.000577
Number of cells (cell/ml)	247662	247662	254074.4	249799.4	3702.229
% of control	88.53791	82.84074	88.79478	86.72448	3.365865
Nitzschia fonticola					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.047	0.048	0.051	0.048667	0.002082
OD (750 nm)	0.037	0.038	0.04	0.038333	0.001528
Number of cells (cell/ml)	315703.3	323754.1	339855.7	326437.7	12297.79
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.027	0.031	0.033	0.030333	0.003055
OD (750 nm)	0.021	0.024	0.026	0.023667	0.002517
Number of cells (cell/ml)	186890.6	211043	227144.6	208359.4	20260.72
% of control	59.19819	65.1862	66.8356	63.74	4.018851
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.07	0.074	0.074	0.072667	0.002309
OD (750 nm)	0.05	0.054	0.055	0.053	0.002646
Number of cells (cell/ml)	420363.6	452566.8	460617.6	444516	21300.39
% of control	224.9249	214.4429	202.7861	214.0513	11.07462
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.029	0.029	0.028	0.028667	0.000577
OD (750 nm)	0.023	0.023	0.022	0.022667	0.000577
Number of cells (cell/ml)	202992.2	202992.2	194941.4	200308.6	4648.127
% of control	64.29841	62.69951	57.36006	61.45266	3.633339
Fistulifera saprophila					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.075	0.078	0.078	0.077	0.001732
OD (750 nm)	0.062	0.065	0.064	0.063667	0.001528
Number of cells (cell/ml)	396483.8	418464.8	411137.8	408695.5	11192.15
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
OD (665 nm)	0.059	0.058	0.053	0.056667	0.003215
OD (750 nm)	0.051	0.048	0.044	0.047667	0.003512
Number of cells (cell/ml)	315887	293906.1	264598.2	291463.8	25731.51
% of control	79.67211	70.23437	64.35754	71.42134	7.725975
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
OD (665 nm)	0.057	0.056	0.058	0.057	0.001
OD (750 nm)	0.047	0.046	0.048	0.047	0.001
Number of cells (cell/ml)	286579.1	279252.1	293906.1	286579.1	7326.981
% of control	72.28015	66.73253	71.48603	70.16624	3.00007
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
OD (665 nm)	0.034	0.036	0.035	0.035	0.001
OD (750 nm)	0.03	0.031	0.03	0.030333	0.000577
Number of cells (cell/ml)	162020.4	169347.4	162020.4	164462.8	4230.234
% of control	40.86432	40.46874	39.40782	40.24696	0.753154
Skeletonema marinoi					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
OD (665 nm)	0.14	0.155	0.144	0.146333	0.007767
OD (750 nm)	0.1	0.11	0.099	0.103	0.006083
Number of cells (cell/ml)	3885368	4266404	3847265	3999679	231774.9
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD

OD (665 nm)	0.113	0.13	0.12	0.121	0.008544
OD (750 nm)	0.076	0.09	0.07	0.078667	0.010263
Number of cells (cell/ml)	2970883	3504333	2742262	3072492	391064.5
% of control	76.46336	82.13786	71.27822	76.62648	5.431658
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
OD (665 nm)	0.047	0.053	0.052	0.050667	0.003215
OD (750 nm)	0.033	0.037	0.035	0.035	0.002
Number of cells (cell/ml)	1332430	1484844	1408637	1408637	76207.1
% of control	34.29354	34.80319	36.614	35.23691	1.219515
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
OD (665 nm)	0.026	0.028	0.022	0.025333	0.003055
OD (750 nm)	0.02	0.021	0.016	0.019	0.002646
Number of cells (cell/ml)	837084	875187.6	684669.8	798980.5	100812.5
% of control	21.54452	20.51347	17.79628	19.95142	1.936301

Table S2. Value of fluorescence of chlorophyll *a* (F_v/F_m parameter) of studied cyanobacteria and microalgae obtained after 7th day of the experiment for control and culture with the addition of filtrate obtained from cultures of cyanobacteria *Synechococcus* sp. (BA-120, BA-124 and BA-132).

***Planktolyngbya* sp.**					
	$Control_1$	$Control_2$	$Control_3$	Mean	SD
F_v/F_m	0.371	0.386	0.374	0.377	0.007937
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
F_v/F_m	0.422	0.406	0.384	0.404	0.019079
% of control	113.7466	105.1813	102.6738	107.2006	5.806025
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
F_v/F_m	0.465	0.464	0.445	0.458	0.011269
% of control	125.3369	120.2073	118.984	121.5094	3.370713
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
F_v/F_m	0.456	0.424	0.399	0.426333	0.028572
% of control	122.9111	109.8446	106.6845	113.1467	8.602523
Aphanizomenon sp.					
	$Control_1$	$Control_2$	$Control_3$	Mean	SD
F_v/F_m	0.298	0.294	0.3	0.297333	0.003055
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
F_v/F_m	0.338	0.333	0.334	0.335	0.002646
% of control	113.4228	113.2653	111.3333	112.6738	1.163563
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
F_v/F_m	0.334	0.321	0.318	0.324333	0.008505
% of control	112.0805	109.1837	106	109.0881	3.041396
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
F_v/F_m	0.356	0.344	0.327	0.342333	0.014572
% of control	119.4631	117.0068	109	115.1566	5.471417
***Nostoc* sp.**					
	$Control_1$	$Control_2$	$Control_3$	Mean	SD
F_v/F_m	0.502	0.497	0.498	0.499	0.002646
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD

F_v/F_m	0.208	0.222	0.22	0.216667	0.007572
% of control	41.43426	44.66801	44.17671	43.42633	1.742579
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.115	0.188	0.097	0.133333	0.048191
% of control	22.90837	37.82696	19.47791	26.73775	9.755513
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.143	0.1	0.19	0.144333	0.045015
% of control	28.48606	20.12072	38.15261	28.9198	9.023765
***Synechocystis* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.449	0.417	0.43	0.432	0.016093
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.393	0.346	0.36	0.366333	0.024132
% of control	87.52784	82.97362	83.72093	84.7408	2.442401
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.524	0.412	0.438	0.458	0.058617
% of control	116.7038	98.80096	101.8605	105.7884	9.575977
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.444	0.25	0.375	0.356333	0.098338
% of control	98.88641	59.95204	87.2093	82.01592	19.97999
***Phormidium* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.439	0.439	0.435	0.437667	0.002309
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.716	0.69	0.672	0.692667	0.022121
% of control	163.0979	157.1754	154.4828	158.252	4.407351
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.692	0.632	0.672	0.665333	0.030551
% of control	157.631	143.9636	154.4828	152.0258	7.157321
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.646	0.62	0.642	0.636	0.014
% of control	147.1526	141.2301	147.5862	145.323	3.551176
***Pseudanabaena* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.441	0.418	0.424	0.427667	0.01193
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.374	0.367	0.361	0.367333	0.006506
% of control	84.80726	87.79904	85.14151	85.91594	1.63936
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.411	0.398	0.383	0.397333	0.014012
% of control	93.19728	95.21531	90.33019	92.91426	2.454828
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.35	0.334	0.331	0.338333	0.010214
% of control	85.15815	83.9196	86.42298	85.16691	1.251712
Monoraphidium convolutum* var. *pseudosabulosum					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.772	0.771	0.772	0.771667	0.000577
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.691	0.691	0.688	0.69	0.001732
% of control	89.50777	89.62387	89.11917	89.41694	0.264324

	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.716	0.718	0.718	0.717333	0.001155
% of control	92.74611	93.12581	93.00518	92.95904	0.194009
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.645	0.642	0.637	0.641333	0.004041
% of control	83.54922	83.26848	82.51295	83.11022	0.535956
Chl*orella fusca*					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.255	0.248	0.249	0.250667	0.003786
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.195	0.193	0.19	0.192667	0.002517
% of control	76.47059	77.82258	76.30522	76.86613	0.832427
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.157	0.15	0.15	0.152333	0.004041
% of control	61.56863	60.48387	60.24096	60.76449	0.706917
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.118	0.106	0.108	0.110667	0.006429
% of control	46.27451	42.74194	43.37349	44.12998	1.883872
Kirchneriella obesa					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.748	0.747	0.745	0.746667	0.001528
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.781	0.778	0.777	0.778667	0.002082
% of control	104.4118	104.1499	104.2953	104.2857	0.131181
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.735	0.74	0.739	0.738	0.002646
% of control	98.26203	99.06292	99.19463	98.83986	0.504729
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.724	0.724	0.723	0.723667	0.000577
% of control	96.79144	96.92102	97.04698	96.91981	0.127772
***Monoraphidium* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.584	0.578	0.583	0.581667	0.003215
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.347	0.339	0.343	0.343	0.004
% of control	59.41781	58.65052	58.83362	58.96732	0.400736
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.388	0.383	0.388	0.386333	0.002887
% of control	66.43836	66.26298	66.55232	66.41788	0.145752
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.24	0.244	0.237	0.240333	0.003512
% of control	41.09589	42.21453	40.6518	41.32074	0.805265
Chl*orella* sp.					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.719	0.719	0.718	0.718667	0.000577
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.679	0.681	0.681	0.680333	0.001155
% of control	94.43672	94.71488	94.8468	94.66613	0.209341
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.629	0.623	0.623	0.625	0.003464
% of control	87.48261	86.64812	86.7688	86.96651	0.451012

	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.606	0.608	0.605	0.606333	0.001528
% of control	84.28373	84.56189	84.26184	84.36915	0.167275
Oocystis* cf. *submarina					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.688	0.671	0.672	0.677	0.009539
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.87	0.848	0.854	0.857333	0.011372
% of control	126.4535	126.3785	127.0833	126.6385	0.387095
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.82	0.817	0.811	0.816	0.004583
% of control	119.186	121.7586	120.6845	120.543	1.292084
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.841	0.845	0.825	0.837	0.010583
% of control	122.2384	125.9314	122.7679	123.6459	1.996974
Cyclotella meneghiniana					
	Control$_1$	Contro$_{12}$	Control$_3$	Mean	SD
F_v/F_m	0.828	0.826	0.824	0.826	0.002
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.817	0.805	0.805	0.809	0.006928
% of control	98.6715	97.45763	97.69417	97.9411	0.643506
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.814	0.81	0.811	0.811667	0.002082
% of control	98.30918	98.06295	98.42233	98.26482	0.183748
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.815	0.809	0.807	0.810333	0.004163
% of control	98.42995	97.94189	97.93689	98.10291	0.283236
Amphora coffeaeformis					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.783	0.804	0.804	0.797	0.012124
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.667	0.625	0.667	0.653	0.024249
% of control	85.18519	77.73632	82.9602	81.96057	3.823722
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.556	0.556	0.5	0.537333	0.032332
% of control	71.00894	69.15423	62.18905	67.45074	4.650161
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.4	0.571	0.571	0.514	0.098727
% of control	51.08557	71.0199	71.0199	64.37512	11.50909
Navicula perminuta					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.632	0.588	0.579	0.599667	0.028361
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.613	0.608	0.614	0.611667	0.003215
% of control	96.99367	103.4014	106.0449	102.1466	4.654239
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.493	0.487	0.489	0.489667	0.003055
% of control	78.00633	82.82313	84.45596	81.76181	3.353243
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.582	0.595	0.605	0.594	0.011533

% of control	92.08861	101.1905	104.4905	99.25653	6.42315
Nitzschia fonticola					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.789	0.787	0.787	0.787667	0.001155
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.714	0.74	0.729	0.727667	0.013051
% of control	90.4943	94.02795	92.63024	92.38416	1.779635
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.427	0.434	0.431	0.430667	0.003512
% of control	54.11914	55.14612	54.76493	54.67673	0.519143
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.4	0.438	0.429	0.422333	0.019858
% of control	50.69708	55.65438	54.5108	53.62076	2.595734
Fistulifera saprophila					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.795	0.802	0.798	0.798333	0.003512
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.79	0.794	0.806	0.796667	0.008327
% of control	99.37107	99.00249	101.0025	99.79202	1.064384
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.673	0.66	0.658	0.663667	0.008145
% of control	84.65409	82.29426	82.45614	83.13483	1.318202
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.5	0.556	0.444	0.5	0.056
% of control	62.89308	69.32668	55.6391	62.61962	6.847889
Skeletonema marinoi					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
F_v/F_m	0.652	0.631	0.645	0.642667	0.010693
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
F_v/F_m	0.664	0.646	0.654	0.654667	0.009018
% of control	101.8405	102.3772	101.3953	101.871	0.491626
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
F_v/F_m	0.689	0.676	0.671	0.678667	0.009292
% of control	105.6748	107.1315	104.031	105.6125	1.551206
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
F_v/F_m	0.333	0.333	0.333	0.333	0
% of control	48.33091	49.26036	49.62742	49.0729	0.668273

Table S3. Value of photosynthetic pigments (chlorophyll *a* and carotenoid pigments) of studied cyanobacteria and microalgae obtained after 7th day of the experiment for control and culture with the addition of filtrate obtained from cultures of cyanobacteria *Synechococcus* sp. (BA-120, BA-124 and BA-132).

***Planktolyngbya* sp.**					
	Control$_1$	**Control$_2$**	**Control$_3$**	**Mean**	**SD**
Chl *a* (ug/ml)	0.19474	0.243425	0.2186	0.218922	0.024344
Car (ug/ml)	0.192	0.226	0.178	0.198667	0.024685
Chl *a* (pg/cell)	0.018354	0.02262316	0.019501	0.02016	0.002209
Car (pg/cell)	0.018096	0.021003735	0.015879	0.018326	0.00257
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD

Chl *a* (ug/ml)	0.24746	0.32097	0.35483	0.307753	0.054892
Car (ug/ml)	0.12	0.13	0.122	0.124	0.005292
Chl *a* (pg/cell)	0.024719	0.032549	0.035181	0.030817	0.005442
Car (pg/cell)	0.011987	0.013183	0.012096	0.012422	0.000661
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.39755	0.37869	0.3379	0.37138	0.030489
Car (ug/ml)	0.14	0.136	0.126	0.134	0.007211
Chl *a* (pg/cell)	0.042241	0.039606	0.034795	0.038881	0.003776
Car (pg/cell)	0.014875	0.014224	0.012975	0.014025	0.000966
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.40062	0.31211	0.389655	0.367462	0.048248
Car (ug/ml)	0.136	0.11	0.128	0.124667	0.013317
Chl *a* (pg/cell)	0.044702	0.034537	0.041734	0.040324	0.005227
Car (pg/cell)	0.015175	0.012172	0.01371	0.013686	0.001502
Aphanizomenon sp.					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.195705	0.255355	0.303075	0.251378	0.053795
Car (ug/ml)	0.11	0.13	0.11	0.116667	0.011547
Chl *a* (pg/cell)	0.559251	0.689979384	0.804324	0.684518	0.122627
Car (pg/cell)	0.314339	0.351265179	0.291926	0.319177	0.029964
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.27825	0.267285	0.348865	0.298133	0.044276
Car (ug/ml)	0.148	0.158	0.148	0.151333	0.005774
Chl *a* (pg/cell)	0.795134	0.684924	0.878849	0.786302	0.097263
Car (pg/cell)	0.422928	0.404879	0.372836	0.400214	0.02537
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.43141	0.4522	0.52378	0.46913	0.048456
Car (ug/ml)	0.124	0.138	0.142	0.134667	0.009452
Chl *a* (pg/cell)	0.971704	0.921	1.112441	1.001715	0.099186
Car (pg/cell)	0.279297	0.281066	0.30159	0.287317	0.012392
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.434305	0.50492	0.52185	0.487025	0.046435
Car (ug/ml)	0.124	0.152	0.15	0.142	0.01562
Chl *a* (pg/cell)	1.024732	1.028375	1.108341	1.053816	0.047255
Car (pg/cell)	0.292575	0.30958	0.31858	0.306912	0.013206
***Nostoc* sp.**					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.8001	1.337915	0.83203	0.990015	0.301713
Car (ug/ml)	0.242	0.268	0.2	0.236667	0.034312
Chl *a* (pg/cell)	-0.43701	-0.69469	-0.41279	-0.51483	0.156233
Car (pg/cell)	0.118188	0.20363195	0.125872	0.149231	0.047269
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.139125	0.15009	0.144125	0.144447	0.00549
Car (ug/ml)	0.048	0.054	0.046	0.049333	0.004163
Chl *a* (pg/cell)	-0.08361	-0.07893	-0.07618	-0.07957	0.003757
Car (pg/cell)	0.067455	0.072772	0.071258	0.070495	0.002739
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.08737	0.1093	0.1093	0.10199	0.012661
Car (ug/ml)	0.03	0.04	0.04	0.036667	0.005774
Chl *a* (pg/cell)	-0.07922	-0.06986	-0.06986	-0.07298	0.005404
Car (pg/cell)	0.040038	0.046675	0.046675	0.044463	0.003832
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD

Chl *a* (ug/ml)	0.09044	0.05658	0.02272	0.05658	0.03386
Car (ug/ml)	0.032	0.028	0.014	0.024667	0.009452
Chl *a* (pg/cell)	-0.05143	-0.05529	-0.05915	-0.05529	0.00386
Car (pg/cell)	0.03929	0.026912	0.010225	0.025476	0.014586

***Synechocystis* sp.**

	$Control_1$	$Control_2$	$Control_3$	Mean	SD
Chl *a* (ug/ml)	0.186845	0.2286	0.210705	0.208717	0.020948
Car (ug/ml)	0.092	0.122	0.082	0.098667	0.020817
Chl *a* (pg/cell)	0.014521	0.01732481	0.015035	0.015627	0.001493
Car (pg/cell)	0.00715	0.009245961	0.005851	0.007416	0.001713
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
Chl *a* (ug/ml)	0.031755	0.04465	0.074475	0.050293	0.021912
Car (ug/ml)	0.026	0.038	0.032	0.032	0.006
Chl *a* (pg/cell)	0.004668	0.007076	0.011219	0.007654	0.003313
Car (pg/cell)	0.003822	0.006022	0.004821	0.004888	0.001101
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
Chl *a* (ug/ml)	0.038685	0.050615	0.07158	0.053627	0.016653
Car (ug/ml)	0.028	0.028	0.04	0.032	0.006928
Chl *a* (pg/cell)	0.006294	0.008699	0.011965	0.008986	0.002846
Car (pg/cell)	0.004555	0.004812	0.006686	0.005351	0.001163
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
Chl *a* (ug/ml)	0.026755	0.03272	0.050615	0.036697	0.012417
Car (ug/ml)	0.028	0.028	0.03	0.028667	0.001155
Chl *a* (pg/cell)	0.005728	0.007533	0.011229	0.008163	0.002805
Car (pg/cell)	0.005994	0.006447	0.006656	0.006365	0.000338

***Phormidium* sp.**

	$Control_1$	$Control_2$	$Control_3$	Mean	SD
Chl *a* (ug/ml)	1.05177	1.12721	1.059665	1.079548	0.041465
Car (ug/ml)	0.242	0.264	0.244	0.25	0.012166
Chl *a* (pg/cell)	0.025945	0.026573032	0.028843	0.02712	0.001524
Car (pg/cell)	0.230088	0.234206581	0.230261	0.231519	0.002329
	$BA\text{-}120_1$	$BA\text{-}120_2$	$BA\text{-}120_3$	Mean	SD
Chl *a* (ug/ml)	0.181845	0.173863	0.16588	0.173863	0.007983
Car (ug/ml)	0.064	0.066	0.068	0.066	0.002
Chl *a* (pg/cell)	0.011803	0.01079	0.013873	0.012155	0.001571
Car (pg/cell)	0.351948	0.37961	0.409935	0.380498	0.029004
	$BA\text{-}124_1$	$BA\text{-}124_2$	$BA\text{-}124_3$	Mean	SD
Chl *a* (ug/ml)	0.146055	0.12316	0.134608	0.134608	0.011448
Car (ug/ml)	0.064	0.046	0.055	0.055	0.009
Chl *a* (pg/cell)	0.013292	0.009911	0.01185	0.011684	0.001697
Car (pg/cell)	0.438191	0.373498	0.408595	0.406761	0.032386
	$BA\text{-}132_1$	$BA\text{-}132_2$	$BA\text{-}132_3$	Mean	SD
Chl *a* (ug/ml))	0.15202	0.19281	0.17588	0.17357	0.020493
Car (ug/ml)	0.06	0.08	0.074	0.071333	0.010263
Chl *a* (pg/cell)	0.011822	0.01863	0.019169	0.01654	0.004095
Car (pg/cell)	0.394685	0.414916	0.420741	0.410114	0.013676

***Pseudanabaena* sp.**

	$Control_1$	$Control_2$	$Control_3$	Mean	SD
Chl *a* (ug/ml)	0.56764	0.62229	0.594965	0.594965	0.027325
Car (ug/ml)	0.252	0.254	0.253	0.253	0.001
Chl *a* (pg/cell)	0.027752	0.029336156	0.028048	0.028379	0.000842

Car (pg/cell)	0.01232	0.011974134	0.011927	0.012074	0.000215
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.49185	0.44334	0.343865	0.426352	0.075441
Car (ug/ml)	0.146	0.198	0.148	0.164	0.029462
Chl *a* (pg/cell)	0.027057	0.025267	0.024115	0.025479	0.001483
Car (pg/cell)	0.008032	0.011284	0.010379	0.009898	0.001679
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.52685	0.44141	0.341935	0.436732	0.092546
Car (ug/ml)	0.208	0.214	0.192	0.204667	0.011372
Chl *a* (pg/cell)	0.023842	0.020325	0.01593	0.020032	0.003964
Car (pg/cell)	0.009413	0.009854	0.008945	0.009404	0.000454
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.34097	0.3579	0.353865	0.350912	0.008843
Car (ug/ml)	0.12	0.124	0.13	0.124667	0.005033
Chl *a* (pg/cell)	0.020621	0.022505	0.020766	0.021297	0.001049
Car (pg/cell)	0.007257	0.007797	0.007629	0.007561	0.000276
Monoraphidium convolutum* var. *pseudosabulosum					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	2.30897	1.646325	2.45055	2.135282	0.429325
Car (ug/ml)	0.704	0.6	0.752	0.685333	0.0777
Chl *a* (pg/cell)	0.285330823	0.182113	0.265939	0.244461	0.054858
Car (pg/cell)	0.086996756	0.066371	0.081609	0.078325	0.010698
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.96115	0.78185	0.904395	0.882465	0.09164
Car (ug/ml)	0.368	0.31	0.362	0.346667	0.031896
Chl *a* (pg/cell)	0.132224	0.102293	0.115315	0.11661	0.015008
Car (pg/cell)	0.050625	0.040559	0.046157	0.04578	0.005044
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.735095	0.6972	0.261845	0.564713	0.262975
Car (ug/ml)	0.298	0.28	0.134	0.237333	0.089941
Chl *a* (pg/cell)	0.094631	0.090333	0.031674	0.072213	0.035173
Car (pg/cell)	0.038363	0.036278	0.016209	0.030283	0.012233
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.117545	0.10851	0.102545	0.109533	0.007552
Car (ug/ml)	0.08	0.078	0.07	0.076	0.005292
Chl *a* (pg/cell)	0.020188	0.018478	0.016889	0.018519	0.00165
Car (pg/cell)	0.01374	0.013283	0.011529	0.012851	0.001167
Chl*orella fusca*					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.097545	0.202985	0.124475	0.141668	0.054782
Car (ug/ml)	0.078	0.104	0.098	0.093333	0.013614
Chl *a* (pg/cell)	0.029856	0.055963942	0.036491	0.04077	0.01357
Car (pg/cell)	0.023874	0.028673301	0.028729	0.027092	0.002787
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.114475	0.097545	0.068685	0.093568	0.023153
Car (ug/ml)	0.102	0.088	0.07	0.086667	0.016042
Chl *a* (pg/cell)	0.034135	0.030667	0.020748	0.028517	0.006948
Car (pg/cell)	0.030416	0.027666	0.021146	0.026409	0.004761
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	-0.005	0.05772	0.04772	0.03348	0.033698
Car (ug/ml)	0.054	0.064	0.066	0.061333	0.006429

Chl *a* (pg/cell)	0.016609	0.01874	0.014478	0.016609	0.002131
Car (pg/cell)	0.01737	0.020779	0.020024	0.019391	0.001791
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.04772	0.046755	0.058685	0.051053	0.006627
Car (ug/ml)	0.062	0.068	0.08	0.07	0.009165
Chl *a* (pg/cell)	0.01423	0.013535	0.018704	0.01549	0.002805
Car (pg/cell)	0.018488	0.019686	0.025497	0.021223	0.003749

Kirchneriella obesa

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	1.40405	1.64914	1.53361	1.528933	0.122612
Car (ug/ml)	0.446	0.552	0.564	0.520667	0.064941
Chl *a* (pg/cell)	0.295612994	0.322839	0.287687	0.302047	0.018438
Car (pg/cell)	0.093902208	0.108061	0.1058	0.102588	0.007606
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.542725	0.622375	0.554655	0.573252	0.042958
Car (ug/ml)	0.268	0.294	0.244	0.268667	0.025007
Chl *a* (pg/cell)	0.136687	0.164429	0.144647	0.148588	0.014284
Car (pg/cell)	0.067497	0.077673	0.063632	0.069601	0.007253
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.3286	0.29974	0.278775	0.302372	0.025017
Car (ug/ml)	0.206	0.212	0.198	0.205333	0.007024
Chl *a* (pg/cell)	0.106681	0.097311	0.085689	0.09656	0.010516
Car (pg/cell)	0.066878	0.068826	0.060861	0.065522	0.004153
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.16544	0.227195	0.119475	0.170703	0.054053
Car (ug/ml)	0.086	0.126	0.088	0.1	0.022539
Chl *a* (pg/cell)	0.068931	0.089149	0.038326	0.065469	0.025588
Car (pg/cell)	0.035832	0.049441	0.028229	0.037834	0.010747

***Monoraphidium* sp.**

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	1.568875	1.850895	1.673525	1.697765	0.142564
Car (ug/ml)	0.496	0.634	0.55	0.56	0.069541
Chl *a* (pg/cell)	0.361472	0.376386055	0.36464	0.367499	0.007858
Car (pg/cell)	0.114279	0.128926146	0.119838	0.121014	0.007394
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.459215	0.47018	0.315705	0.415033	0.086195
Car (ug/ml)	0.296	0.312	0.254	0.287333	0.029956
Chl *a* (pg/cell)	0.118724	0.119532	0.076194	0.104817	0.024791
Car (pg/cell)	0.076527	0.079318	0.061302	0.072382	0.009697
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.570795	0.48711	0.55983	0.539245	0.045482
Car (ug/ml)	0.336	0.312	0.348	0.332	0.01833
Chl *a* (pg/cell)	0.144628	0.135595	0.143279	0.141168	0.004873
Car (pg/cell)	0.085136	0.086851	0.089065	0.087017	0.00197
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.05772	0.073685	0.073685	0.068363	0.009217
Car (ug/ml)	0.084	0.08	0.102	0.088667	0.011719
Chl *a* (pg/cell)	0.017541	0.02137	0.022215	0.020375	0.002491
Car (pg/cell)	0.025527	0.023202	0.030752	0.026494	0.003867

Chl*orella* sp.

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.06693	0.044738	0.010965	0.040878	0.028181

Car (ug/ml)	0.056	0.032	0.036	0.041333	0.012858
Chl *a* (pg/cell)	0.039255079	0.039255	0.039255	0.039255	0
Car (pg/cell)	0.03284453	0.016554	0.01936	0.02292	0.008709
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.020965	0.026843	0.025	0.024269	0.003006
Car (ug/ml)	0.018	0.026	0.026	0.023333	0.004619
Chl *a* (pg/cell)	0.015679	0.016913	0.018147	0.016913	0.001234
Car (pg/cell)	0.013461	0.018674	0.018873	0.017003	0.003068
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.020965	0.02386	0.020965	0.02193	0.001671
Car (ug/ml)	0.016	0.008	0.024	0.016	0.008
Chl *a* (pg/cell)	0.017455	0.017121	0.016787	0.017121	0.000334
Car (pg/cell)	0.013321	0.006368	0.019217	0.012969	0.006432
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.015965	0.008948	0	0.008304	0.008002
Car (ug/ml)	0.016	0.014	0.012	0.014	0.002
Chl *a* (pg/cell)	0.013499	0.013499	0.013499	0.013499	0
Car (pg/cell)	0.013529	0.011934	0.010339	0.011934	0.001595
Oocystis* cf. *submarina					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.20623	0.185265	0.187195	0.192897	0.011587
Car (ug/ml)	0.21	0.18	0.178	0.189333	0.017926
Chl *a* (pg/cell)	0.206347	0.18537	0.177731	0.189816	0.014817
Car (pg/cell)	0.210119	0.180102	0.169001	0.186408	0.021272
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.04079	0.06965	0.039825	0.050088	0.016948
Car (ug/ml)	0.058	0.09	0.078	0.075333	0.016166
Chl *a* (pg/cell)	0.05534	0.090767	0.054529	0.066879	0.020692
Car (pg/cell)	0.078689	0.117287	0.106798	0.100925	0.019958
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.046755	0.039825	0.034825	0.040468	0.005991
Car (ug/ml)	0.066	0.068	0.05	0.061333	0.009866
Chl *a* (pg/cell)	0.071235	0.057439	0.049985	0.059553	0.010782
Car (pg/cell)	0.100556	0.098075	0.071766	0.090132	0.015954
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.068685	0.056755	0.073685	0.066375	0.008698
Car (ug/ml)	0.07	0.074	0.072	0.072	0.002
Chl *a* (pg/cell)	0.105186	0.091141	0.115826	0.104051	0.012382
Car (pg/cell)	0.1072	0.118835	0.113178	0.113071	0.005818
Cyclotella meneghiniana					
	Control$_1$	Contro$_{l2}$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.875625	0.954135	1.062645	0.964135	0.09391
Car (ug/ml)	0.216	0.23	0.272	0.239333	0.029143
Chl *a* (pg/cell)	1.613067	1.534442398	1.733412	1.626974	0.100211
Car (pg/cell)	0.397913	0.3698866	0.443693	0.403831	0.037257
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.7015	0.7065	0.74922	0.719073	0.026227
Car (ug/ml)	0.186	0.21	0.216	0.204	0.015875
Chl *a* (pg/cell)	1.541497	1.44131	1.402884	1.461897	0.071563
Car (pg/cell)	0.408722	0.428415	0.404451	0.413863	0.012782
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.47369	0.556235	0.59106	0.540328	0.06028

Car (ug/ml)	0.138	0.16	0.174	0.157333	0.018148
Chl *a* (pg/cell)	1.040901	1.246321	1.298813	1.195345	0.136303
Car (pg/cell)	0.303245	0.358502	0.382353	0.348033	0.040579
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.448865	0.4179	0.39097	0.419245	0.028971
Car (ug/ml)	0.134	0.122	0.11	0.122	0.012
Chl *a* (pg/cell)	0.932409	0.900933	0.827226	0.886856	0.053986
Car (pg/cell)	0.278353	0.263015	0.232741	0.258036	0.02321
Amphora coffeaeformis					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.54641	0.430795	0.52676	0.501322	0.061863
Car (ug/ml)	0.176	0.13	0.166	0.157333	0.024194
Chl *a* (pg/cell)	4.524376	4.173169	4.361671	4.353072	0.175761
Car (pg/cell)	1.457313	1.259327	1.374511	1.363717	0.099433
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.003945	-0.01246	0.014735	0.002073	0.013694
Car (ug/ml)	0.014	0.018	0.02	0.017333	0.003055
Chl *a* (pg/cell)	0.085347	0.239389	0.39343	0.239389	0.154041
Car (pg/cell)	0.30288	0.430233	0.534007	0.422373	0.115764
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.046755	0.029735	0.07351	0.05	0.022067
Car (ug/ml)	0.02	0.02	0.026	0.022	0.003464
Chl *a* (pg/cell)	0.812285	0.386576	1.237993	0.812285	0.425709
Car (pg/cell)	0.336823	0.260014	0.43787	0.344902	0.089203
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.04772	0.0107	0.041755	0.033392	0.019877
Car (ug/ml)	0.006	0.014	0.008	0.009333	0.004163
Chl *a* (pg/cell)	1.663738	0.32358	1.114874	1.034064	0.673723
Car (pg/cell)	0.209187	0.423376	0.213603	0.282055	0.122407
Navicula perminuta					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.357285	0.253775	0.30553	0.30553	0.051755
Car (ug/ml)	0.136	0.098	0.116	0.116667	0.019009
Chl *a* (pg/cell)	1.277276	0.848855	1.067777	1.064636	0.214228
Car (pg/cell)	0.486193	0.327801	0.405401	0.406465	0.079201
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.438865	0.39211	0.460795	0.43059	0.035082
Car (ug/ml)	0.16	0.148	0.156	0.154667	0.00611
Chl *a* (pg/cell)	1.230439	1.079937	1.247082	1.185819	0.092074
Car (pg/cell)	0.448589	0.407617	0.422194	0.426133	0.020768
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.196055	0.18509	0.201055	0.194067	0.008166
Car (ug/ml)	0.074	0.07	0.074	0.072667	0.002309
Chl *a* (pg/cell)	0.752648	0.661687	0.702654	0.705663	0.045555
Car (pg/cell)	0.284083	0.250246	0.258618	0.264316	0.017623
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.1393	0.12237	0.106405	0.122692	0.01645
Car (ug/ml)	0.06	0.048	0.042	0.05	0.009165
Chl *a* (pg/cell)	0.56246	0.494101	0.418795	0.491785	0.071861
Car (pg/cell)	0.242266	0.193813	0.165306	0.200461	0.038908
Nitzschia fonticola					

	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.253775	0.236845	0.277635	0.256085	0.020493
Car (ug/ml)	0.078	0.07	0.084	0.077333	0.007024
Chl *a* (pg/cell)	0.80384	0.731558	0.81692	0.784106	0.045975
Car (pg/cell)	0.247067	0.216213	0.247164	0.236815	0.017841
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.05772	0.08658	0.075615	0.073305	0.014568
Car (ug/ml)	0.026	0.03	0.026	0.027333	0.002309
Chl *a* (pg/cell)	0.308844	0.410248	0.332894	0.350662	0.052986
Car (pg/cell)	0.139119	0.142151	0.114465	0.131911	0.015185
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.097545	0.06965	0.133335	0.100177	0.031924
Car (ug/ml)	0.046	0.032	0.058	0.045333	0.013013
Chl *a* (pg/cell)	0.232049	0.1539	0.28947	0.22514	0.068049
Car (pg/cell)	0.109429	0.070708	0.125918	0.102018	0.028341
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.022895	0.022895	0.01693	0.020907	0.003444
Car (ug/ml)	0.014	0.014	0.014	0.014	1.73E-18
Chl *a* (pg/cell)	0.112788	0.112788	0.086847	0.104141	0.014977
Car (pg/cell)	0.068968	0.068968	0.071816	0.069918	0.001644
Fistulifera saprophila					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	0.75922	0.800975	0.79001	0.783402	0.021648
Car (ug/ml)	0.238	0.256	0.256	0.25	0.010392
Chl *a* (pg/cell)	1.914883	1.91408	1.921521	1.916828	0.004084
Car (pg/cell)	0.600277	0.61176	0.622662	0.611566	0.011194
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.267635	0.24974	0.23281	0.250062	0.017415
Car (ug/ml)	0.104	0.104	0.096	0.101333	0.004619
Chl *a* (pg/cell)	0.847249	0.849727	0.879862	0.858946	0.018156
Car (pg/cell)	0.329232	0.353855	0.362814	0.348633	0.017389
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.313425	0.301495	0.260705	0.291875	0.027645
Car (ug/ml)	0.11	0.11	0.098	0.106	0.006928
Chl *a* (pg/cell)	1.093677	1.079652	0.887035	1.020121	0.115469
Car (pg/cell)	0.383838	0.393909	0.33344	0.370396	0.032398
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.03579	0.034825	0.034825	0.035147	0.000557
Car (ug/ml)	0.02	0.018	0.02	0.019333	0.001155
Chl *a* (pg/cell)	0.220898	0.205642	0.214942	0.213827	0.007689
Car (pg/cell)	0.123441	0.10629	0.123441	0.117724	0.009902
Skeletonema marinoi					
	Control$_1$	Control$_2$	Control$_3$	Mean	SD
Chl *a* (ug/ml)	1.368525	1.16361	1.28598	1.272705	0.1031
Car (ug/ml)	0.454	0.426	0.468	0.449333	0.021385
Chl *a* (pg/cell)	0.352225	0.272738	0.334258	0.31974	0.041685
Car (pg/cell)	0.116849	0.09985	0.121645	0.112781	0.011453
	BA-120$_1$	BA-120$_2$	BA-120$_3$	Mean	SD
Chl *a* (ug/ml)	0.718605	1.05203	1.120715	0.963783	0.21509
Car (ug/ml)	0.256	0.368	0.402	0.342	0.076394
Chl *a* (pg/cell)	0.241883	0.300208	0.408683	0.316925	0.084647
Car (pg/cell)	0.08617	0.105013	0.146594	0.112592	0.030917

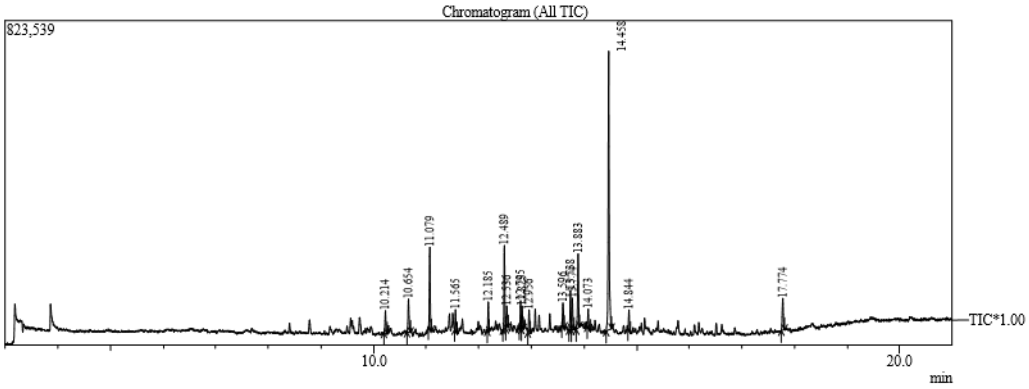
	BA-124$_1$	BA-124$_2$	BA-124$_3$	Mean	SD
Chl *a* (ug/ml)	0.36325	0.32246	0.35132	0.345677	0.020972
Car (ug/ml)	0.11	0.112	0.13	0.117333	0.011015
Chl *a* (pg/cell)	0.272622	0.217168	0.249404	0.246398	0.027849
Car (pg/cell)	0.082556	0.075429	0.092288	0.083424	0.008463
	BA-132$_1$	BA-132$_2$	BA-132$_3$	Mean	SD
Chl *a* (ug/ml)	0.05772	0.05272	0.02886	0.046433	0.015423
Car (ug/ml)	0.024	0.02	0.012	0.018667	0.00611
Chl *a* (pg/cell)	0.068954	0.060239	0.042152	0.057115	0.013671
Car (pg/cell)	0.028671	0.022852	0.017527	0.023017	0.005574

Table S4. Phytochemicals identified in different phenotypes of picocyanobacteria from the genus *Synechococcus* Type 1 (green strain), Type 2 (red strain), and Type 3a (brown strain) by GC-MS. The compounds found in both the f/2 medium and picocyanobacterial cultures are highlighted in italics.

Name of compound	**RT**	**Molecular**	**MW**	**Type 1**		**Type 2**		**Type 3a**		**f/2 medium**	
				Peak Area %	**SI**	**Peak Area %**	**SI**	**Peak Area %**	**SI**	**Peak Area %**	**SI**
Silanediol, dimethyl-	4.301	$C_2H_8O_2Si$	92	ND	ND	ND	ND	13.03	97	ND	ND
Oxime-, methoxy-phenyl-	8.444	$C_8H_9NO_2$	151	0.79	83	12.91	83	4.51	83	ND	ND
Cyclotetrasiloxane, octamethyl-	9.554	$C_8H_{24}O_4Si_4$	296	ND	ND	3.52	91	ND	ND	ND	ND
trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]-	9.563	$C_9H_{28}O_3Si_4$	296	ND	ND	ND	ND	3.53	93	ND	ND
Decane, 3,7-dimethyl-	10.214	$C_{12}H_{26}$	170	ND	ND	ND	ND	ND	ND	2.53	93
Cyclotrisiloxane, hexamethyl-	10.325	$C_6H_{18}O_3Si_3$	222	ND	ND	5.52	92	ND	ND	ND	ND
Nonanal	10.654	$C_9H_{18}O$	142	ND	ND	ND	ND	ND	ND	3.96	89
Cyclopentasiloxane, decamethyl-	11.079	$C_{10}H_{30}O_5Si_5$	370	1.03	84	7.39	83	6.75	83	8.16	83
Acetoxyacetic acid, 5-tetradecyl ester	11.323	$C_{18}H_{34}O_4$	314	ND	ND	2.74	79	ND	ND	ND	ND
Decanal	11.565	$C_{10}H_{20}O$	156	ND	ND	ND	ND	ND	ND	1.88	93
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	11.692	$C_{10}H_{14}O$	150	ND	ND	4.65	94	3.99	96	ND	ND
Artedouglasia oxide A	11.800	$C_{15}H_{22}O_3$	250	ND	ND	3.66	78	ND	ND	ND	ND
Nonane, 5-(2-methylpropyl)-	12.185	$C_{13}H_{28}$	184	ND	ND	ND	ND	ND	ND	2.72	93
3-tert-Butyl-2-pyrazolin-5-one	12.271	$C_7H_{12}N_2O$	140	0.77	86	ND	ND	ND	ND	ND	ND
Cyclohexasiloxane, dodecamethyl-	12.489	$C_{12}H_{36}O_6Si_6$	444	1.89	90	14.67	90	12.39	90	8.06	90
Nonane, 5-(2-methylpropyl)-	12.536	$C_{13}H_{28}$	184	ND	ND	ND	ND	ND	ND	2.05	91
2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.1(1,7)]decan-5-one	12.748	$C_{13}H_{20}O_2$	208	0.80	79	ND	ND	ND	ND	ND	ND
Naphthalene, decahydro-1,4a-dimethyl-7-(1-methylethyl)-,[1S-(1.alpha.,4a.alpha.,7.alpha., 8a.beta.)]-	12.750	$C_{15}H_{28}$	208	ND	ND	ND	ND	4.03	77	ND	ND

Octanoic acid-tert butyl ester	12.795	$C_{12}H_{24}O_2$	200	ND	ND	ND	ND	ND	ND	2.76	74
8,10-Dioxaheptadecane	12.797	$C_{15}H_{32}O_2$	244	0.86	81	8.76	81	6.26	80	ND	ND
3,4-Dimethylpent-2-en-1-ol	12.820	$C_7H_{14}O$	114	ND	ND	1.90	63	ND	ND	ND	ND
Propanoicacid, 2-methyl-, octyl ester	12.825	$C_{12}H_{24}O_2$	200	ND	ND	ND	ND	ND	ND	2.24	74
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	12.956	$C_{12}H_{24}O_3$	216	ND	ND	ND	ND	ND	ND	2.52	90
2,6,10-Trimethyltridecane	13.519	$C_{16}H_{34}$	226	0.48	94	ND	ND	ND	ND	ND	ND
1-Tetradecanol	13.596	$C_{14}H_{30}O$	214	ND	ND	ND	ND	ND	ND	2.76	91
Cycloheptasiloxane, tetradecamethyl-	13.738	$C_{14}H_{42}O_7Si_7$	518	1.59	70	7.59	83	6.76	85	4.24	83
Silane, trichlorooctadecyl-	13.770	$C_{18}H_{37}Cl_3Si$	386	ND	ND	4.09	78	ND	ND	ND	ND
Pentadecane	13.772	$C_{15}H_{32}$	212	5.51	96	ND	ND	ND	ND	ND	ND
Nonane, 5-(2-methylpropyl)-	13.774	$C_{13}H_{28}$	184	0.56	83	ND	ND	3.84	87	3.53	90
2,4-Di-tert-butylphenol	13.883	$C_{14}H_{22}O$	206	ND	ND	ND	ND	ND	ND	8.61	96
9-Octadecene, (E)-	13.886	$C_{18}H_{36}$	252	0.60	94	ND	ND	ND	ND	ND	ND
Tetradecane, 4-methyl-	14.073	$C_{15}H_{32}$	212	ND	ND	ND	ND	ND	ND	2.11	90
Pentadecane, 6-methyl-	14.097	$C_{16}H_{34}$	226	0.70	94	ND	ND	ND	ND	ND	ND
Cycloundecane, 1,1,2-trimethyl-	14.164	$C_{14}H_{28}$	196	0.93	92	ND	ND	ND	ND	ND	ND
9-Octadecene, (E)-	14.225	$C_{18}H_{36}$	252	0.87	91	ND	ND	ND	ND	ND	ND
Pentadecafluorooctanoic acid, dodecyl ester	14.315	$C_{20}H_{25}F_{15}O_2$	582	0.59	90	ND	ND	ND	ND	ND	ND
Pentadecane	14.433	$C_{15}H_{32}$	212	4.97	95	ND	ND	ND	ND	ND	ND
Diethyl Phthalate	14.458	$C_{12}H_{14}O_4$	222	ND	ND	12.90	96	25.52	97	35.58	97
(-)-Globulol	14.611	$C_{15}H_{26}O$	222	0.46	93	ND	ND	ND	ND	ND	ND
Cyclooctasiloxane, hexadecamethyl-	14.844	$C_{16}H_{48}O_8Si_8$	592	ND	ND	2.96	85	2.88	83	1.94	85
3-Octadecene, (E)-	14.948	$C_{18}H_{36}$	252	2.33	96	ND	ND	ND	ND	ND	ND
Eicosane, 10-methyl-	15.060	$C_{21}H_{44}$	296	64.98	96	6.73	94	ND	ND	ND	ND
1-Decanol, 2-hexyl-	15.167	$C_{16}H_{34}O$	242	0.56	92	ND	ND	ND	ND	ND	ND
Tridecane, 6-methyl-	15.342	$C_{14}H_{30}$	198	2.74	93	ND	ND	ND	ND	ND	ND
2-Pentadecanone, 6,10,14-trimethyl-	15.929	$C_{18}H_{36}O$	268	1.98	95	ND	ND	ND	ND	ND	ND
Octadecane, 1-(ethenyloxy)-	16.183	$C_{20}H_{40}O$	296	0.39	84	ND	ND	ND	ND	ND	ND
Bromoacetic acid, octadecyl ester	16.370	$C_{20}H_{39}BrO_2$	390	0.41	83	ND	ND	ND	ND	ND	ND
1,19-Eicosadiene	17.126	$C_{20}H_{38}$	278	1.89	95	ND	ND	ND	ND	ND	ND
1-Tricosene	17.236	$C_{23}H_{46}$	322	0.64	95	ND	ND	ND	ND	ND	ND
Phenol, 4,4′-(1-methylethylidene)bis-	17.774	$C_{15}H_{16}O_2$	228	0.68	89	ND	ND	6.53	88	4.34	91

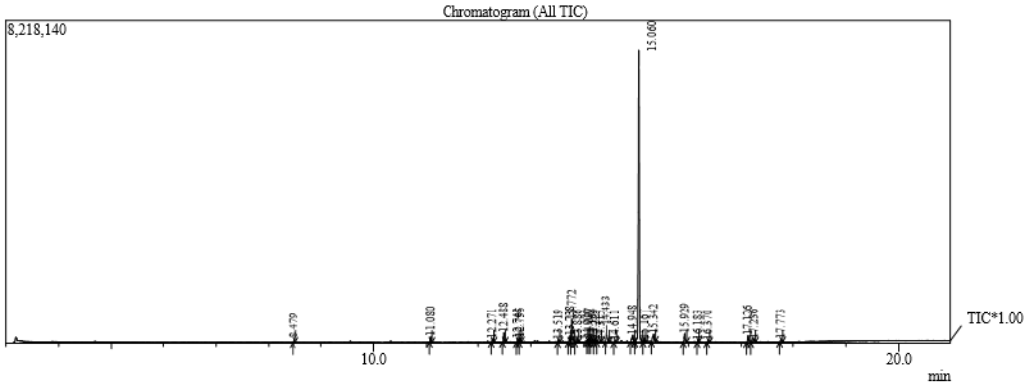
RT=Retention Time, MW=Molecular Weight, ND=Not Detected.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	10.214	10.190	10.250	69902	2.53	55407	2.55	1.26		Decane, 3,7-dimethyl-
2	10.654	10.625	10.690	109612	3.96	81159	3.73	1.35		Nonanal
3	11.079	11.050	11.110	225846	8.16	201143	9.26	1.12		Cyclopentasiloxane, decamethyl-
4	11.565	11.550	11.600	51987	1.88	51874	2.39	1.00		Decanal
5	12.185	12.165	12.205	75401	2.72	72100	3.32	1.05		Nonane, 5-(2-methylpropyl)-
6	12.489	12.460	12.515	223184	8.06	204250	9.40	1.09		Cyclohexasiloxane, dodecamethyl-
7	12.536	12.515	12.560	56786	2.05	53057	2.44	1.07	V	Nonane, 5-(2-methylpropyl)-
8	12.795	12.770	12.810	76330	2.76	67804	3.12	1.13		Octanoic acid-tert butyl ester
9	12.825	12.810	12.850	62072	2.24	55830	2.57	1.11	V	Propanoic acid, 2-methyl-, octyl ester
10	12.956	12.930	12.980	69707	2.52	56253	2.59	1.24		Propanoic acid, 2-methyl-, 3-hydroxy-2,2,
11	13.596	13.575	13.625	76305	2.76	57645	2.65	1.32		1-Tetradecanol
12	13.738	13.710	13.755	117346	4.24	96032	4.42	1.22		Cycloheptasiloxane, tetradecamethyl-
13	13.774	13.755	13.805	97769	3.53	77493	3.57	1.26	V	Nonane, 5-(2-methylpropyl)-
14	13.883	13.845	13.910	238339	8.61	181225	8.34	1.32		2,4-Di-tert-butylphenol
15	14.073	14.055	14.115	58319	2.11	46594	2.14	1.25		Tetradecane, 4-methyl-
16	14.458	14.415	14.515	984641	35.58	686330	31.58	1.43		Diethyl Phthalate
17	14.844	14.825	14.875	53806	1.94	50713	2.33	1.06		Cyclooctasiloxane, hexadecamethyl-
18	17.774	17.745	17.810	120168	4.34	78231	3.60	1.54		Phenol, 4,4'-(1-methylethylidene)bis-
				2767520	100.00	2173140	100.00			

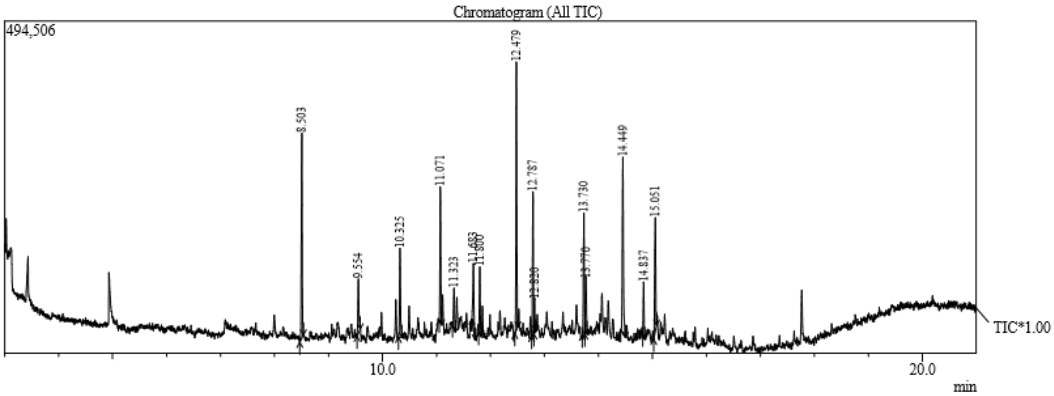
Figure S1. Chromatogram that shows the results of the analysis of sample of f/2 medium prepared using HS-SPME.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.479	8.460	8.510	109565	0.79	100914	0.93	1.09		Oxime-, methoxy-phenyl-_
2	11.080	11.055	11.105	142683	1.03	139764	1.29	1.02		Cyclopentasiloxane, decamethyl-
3	12.271	12.245	12.300	106787	0.77	86870	0.80	1.23		3-tert-Butyl-2-pyrazolin-5-one
4	12.488	12.460	12.515	261646	1.89	237503	2.20	1.10		Cyclohexasiloxane, dodecamethyl-
5	12.748	12.720	12.775	110567	0.80	92521	0.86	1.20		2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.
6	12.795	12.775	12.820	119074	0.86	109172	1.01	1.09		8,10-Dioxaheptadecane
7	13.519	13.500	13.545	66497	0.48	62444	0.58	1.06		2,6,10-Trimethyltridecane
8	13.738	13.700	13.750	219489	1.59	154910	1.43	1.42		Cycloheptasiloxane, tetradecamethyl-
9	13.772	13.750	13.825	762651	5.51	574786	5.31	1.33	V	Pentadecane
10	13.886	13.825	13.905	82975	0.60	59412	0.55	1.40	V	9-Octadecene, (E)-
11	14.070	14.055	14.085	76843	0.56	51582	0.48	1.49		Nonane, 5-methyl-5-propyl-
12	14.097	14.085	14.120	96506	0.70	89318	0.83	1.08	V	Pentadecane, 6-methyl-
13	14.164	14.120	14.180	129221	0.93	66978	0.62	1.93		Cycloundecane, 1,1,2-trimethyl-
14	14.225	14.180	14.245	120983	0.87	50342	0.47	2.40	V	9-Octadecene, (E)-
15	14.315	14.245	14.330	82310	0.59	31012	0.29	2.65	V	Pentadecafluorooctanoic acid, dodecyl est
16	14.433	14.410	14.505	687530	4.97	413712	3.83	1.66		Pentadecane
17	14.611	14.585	14.640	63053	0.46	45878	0.42	1.37		(-)-Globulol
18	14.948	14.900	14.985	322886	2.33	175156	1.62	1.84		3-Octadecene, (E)-
19	15.060	14.985	15.140	8995736	64.98	7406639	68.49	1.21		Eicosane, 10-methyl-
20	15.167	15.140	15.195	77462	0.56	59817	0.55	1.29		1-Decanol, 2-hexyl-
21	15.342	15.300	15.375	379007	2.74	183550	1.70	2.06		Tridecane, 6-methyl-
22	15.929	15.900	15.970	273889	1.98	222897	2.06	1.23		2-Pentadecanone, 6,10,14-trimethyl-
23	16.183	16.160	16.200	54042	0.39	45069	0.42	1.20		Octadecane, 1-(ethenyloxy)-
24	16.370	16.345	16.400	56711	0.41	46035	0.43	1.23		Bromoacetic acid, octadecyl ester
25	17.126	17.095	17.175	261973	1.89	173481	1.60	1.51		1,19-Eicosadiene
26	17.236	17.175	17.265	88715	0.64	68786	0.64	1.29	V	1-Tricosene
27	17.773	17.745	17.805	94748	0.68	66256	0.61	1.43		Phenol, 4,4'-(1-methylethylidene)bis-

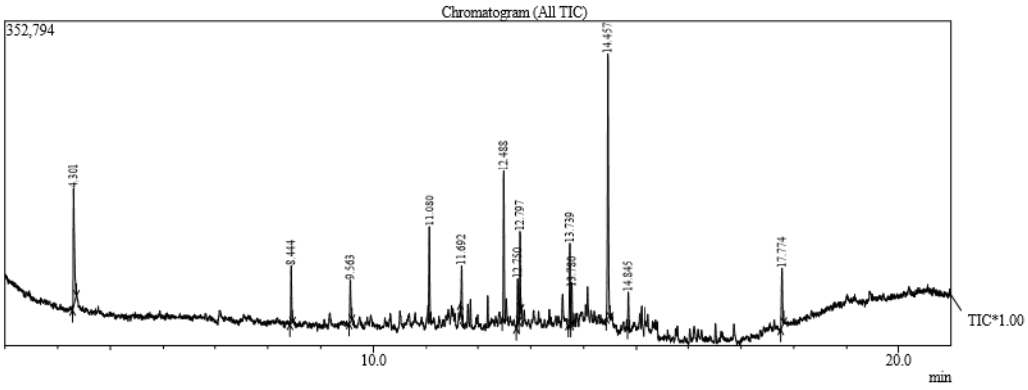
Figure S2. Chromatogram that shows the results of the analysis of sample of phenotype Type 1 of *Synechococcus* sp. prepared using HS-SPME.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.503	8.470	8.535	274400	12.91	226454	12.66	1.21		Oxime-, methoxy-phenyl-_
2	9.554	9.530	9.585	74777	3.52	59586	3.33	1.25		Cyclotetrasiloxane, octamethyl-
3	10.325	10.295	10.355	117447	5.52	98154	5.49	1.20		Cyclotrisiloxane, hexamethyl-
4	11.071	11.050	11.095	157021	7.39	149491	8.36	1.05		Cyclopentasiloxane, decamethyl-
5	11.323	11.295	11.350	58178	2.74	44555	2.49	1.31		Acetoxyacetic acid, 5-tetradecyl ester
6	11.683	11.655	11.715	98945	4.65	71697	4.01	1.38		Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trim
7	11.800	11.780	11.825	77750	3.66	74115	4.14	1.05		Artedouglasia oxide A
8	12.479	12.450	12.510	311915	14.67	295893	16.54	1.05		Cyclohexasiloxane, dodecamethyl-
9	12.787	12.760	12.805	186198	8.76	157707	8.82	1.18		8,10-Dioxaheptadecane
10	12.820	12.805	12.840	40488	1.90	36890	2.06	1.10	V	3,4-Dimethylpent-2-en-1-ol
11	13.730	13.700	13.750	161416	7.59	134128	7.50	1.20		Cycloheptasiloxane, tetradecamethyl-
12	13.770	13.750	13.795	86950	4.09	60397	3.38	1.44	V	Silane, trichlorooctadecyl-
13	14.449	14.415	14.500	274337	12.90	193430	10.82	1.42		Diethyl Phthalate
14	14.837	14.820	14.860	62992	2.96	59963	3.35	1.05		Cyclooctasiloxane, hexadecamethyl-
15	15.051	15.025	15.075	143095	6.73	125993	7.04	1.14		Eicosane, 10-methyl-
				2125909	100.00	1788453	100.00			

Figure S3. Chromatogram that shows the results of the analysis of sample of phenotype Type 2 of *Synechococcus* sp. prepared using HS-SPME.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.301	4.280	4.355	180520	13.03	115843	10.57	1.56		Silanediol, dimethyl-
2	8.444	8.420	8.470	62420	4.51	56571	5.16	1.10		Oxime-, methoxy-phenyl-_
3	9.563	9.540	9.590	48911	3.53	41209	3.76	1.19		trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(tri
4	11.080	11.060	11.115	93593	6.75	87709	8.01	1.07		Cyclopentasiloxane, decamethyl-
5	11.692	11.665	11.720	55252	3.99	48764	4.45	1.13		Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trim
6	12.488	12.460	12.515	171635	12.39	147370	13.45	1.16		Cyclohexasiloxane, dodecamethyl-
7	12.750	12.725	12.775	55771	4.03	45121	4.12	1.24		Naphthalene, decahydro-1,4a-dimethyl-7-
8	12.797	12.775	12.815	86707	6.26	81979	7.48	1.06		8,10-Dioxaheptadecane
9	13.739	13.710	13.755	93707	6.76	80077	7.31	1.17		Cycloheptasiloxane, tetradecamethyl-
10	13.780	13.755	13.795	53196	3.84	36340	3.32	1.46	V	Nonane, 5-(2-methylpropyl)-
11	14.457	14.425	14.500	353563	25.52	260864	23.81	1.36		Diethyl Phthalate
12	14.845	14.830	14.870	39854	2.88	36108	3.30	1.10		Cyclooctasiloxane, hexadecamethyl-
13	17.774	17.750	17.810	90417	6.53	57570	5.26	1.57		Phenol, 4,4'-(1-methylethylidene)bis-
				1385546	100.00	1095525	100.00			

Figure S4. Chromatogram that shows the results of the analysis of sample of phenotype Type 3a of *Synechococcus* sp. prepared using HS-SPME.

AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

Konarzewska, Z., Śliwińska-Wilczewska, S., Barreiro Felpeto A., Vasconcelos, V., Latała, A., 2020. Assessment of the allelochemical activity and biochemical profile of different phenotypes of picocyanobacteria from the genus *Synechococcus*. *Marine Drugs*, *18*(4), 179. DOI: 10.3390/md18040179.

were as follows:

Konarzewska Zofia 60%:

Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing

Śliwińska-Wilczewska Sylwia 15%:

Formal analysis, Investigation, Methodology, Supervision

Barreiro Felpeto Aldo 15%:

formal analysis, Supervision,

Vasconcelos Vitor 5%:

Supervision

Latała Adam 5%:

Supervision

Konarzewska Zofia

Śliwińska-Wilczewska Sylwia

Barreiro Felpeto Aldo

Vasconcelos Vitor

Latała Adam

9.4 PUBLIKACJA 4

Konarzewska, Z., Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Vasconcelos, V., Latała, A., 2025. Take this Waltz: allelopathy induces oscillatory coexistence in marine phytoplankton. Aim at: *The American Naturalist.* **IF = 2.4; 100 MNiSW**, (in elaboration).

Take this Waltz: allelopathy induces oscillatory coexistence in marine phytoplankton

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Abstract

Chemical interactions have been subject of exploration as a mechanism that allows us to explain the immense and paradoxical diversity observed in microbial communities, where from dozens to thousands of species can coexist in environments with non or limited spatial heterogeneity, and few potentially limiting resources. Theoretical works dominate the existing literature, and there is clear deficit of experimental evidence to support them. Models based on chemical interactions already established that either with rock-paper-scissors as the basic motif for species interactions, or with other similar intransitive interactive relationships with additional processes interfering, such as antibiotic degradation, it is possible to obtain robust coexistence and stabilization of highly diverse microbial communities, even in homogenous environments. Here we present long-term experimental and modeling results with two sets of four co-occurring phytoplankton species, which exhibit an a priori parameterized net of chemical interaction that combines inhibitory, and stimulatory effects of different strength. These species also differ strongly in their competitive abilities for the limiting resource that was determined in the long-term competition experiments. Our results show that, contrary to predictions by classical resource competition theory, both four species sets exhibited a consistent outcome of oscillatory coexistence of all the species, This outcome is predicted by a mechanistic model in which the competitive parameters for the limiting resource are fixed, and the allelopathic parameters need very light optimization from the a prior estimated. These results highlight

the enormous potential of chemical interactions *per se* as drivers of the fascinating diversity of microbial communities, particularly in the difficult context of homogenous environments.

Keywords: microbial diversity, allelopathy, resource competition, coexistence

1. Introduction

Explaining the immense diversity of microbial communities has been a fascinating challenge for microbial and plankton ecologists during decades (Hutchinson, 1961; Armstrong and McGehee, 1980; Czarán et al., 2001). This challenge is even greater for microbial communities in homogenous environments (Hutchinson, 1961; Kelsic et al., 2013).

In planktonic ecosystem, various mechanisms have been successfully invoked for the explanation of diversity: permanent lack of equilibrium due to seasonality (Grenney et al., 1973), spatiotemporal niche segregation by nutrients (Tilman, 1977), light (Stomp et al., 2004) and turbidity (Huisman et al., 2004), differential predation (Sommer and Sommer, 2006), distribution of populations in spatial patching with demographic rates higher than mixing rates (Richerson et al., 1970), life cycle strategies to avoid competitive exclusion (McQuoid and Hobson, 1996), interactions with viruses (Flynn et al., 2022). Others, with much less evidence were also proposed, such as deterministic chaos (Benincà et al., 2008). Very recently, it has been demonstrated experimentally in a two-species phytoplankton system that allelopathy, a chemical interaction, is a mechanism inducing diversity (Barreiro et al., 2018). This hypothesis had the support of a long and old list of predecessor theoretical works (Chao and Levin, 1981; Durret and Levin, 1997; Roy, 2009). In the case of soil bacterial communities, there is also experimental evidence of enhanced coexistence by chemical interactions similar to allelopathy (Chao and Levin, 1981). In soil microbial communities, cyclic dominance of killer-resistant-sensitive species (also known as the rock-paper-scissors game, RPS) has been theoretically shown as a robust mechanism to enhance diversity, in microbial communities in which the existence of this kind of relationship between their components can be assumed (Czarán et al., 2001). However, this mechanism involves certain degree of spatial heterogeneity. For microbial communities in homogeneous environments, the opposing action of antibiotic production and degradation has been theoretically proven to be a robust stabilization mechanism of microbial diversity (Kelsic et al., 2013).

In phytoplankton, it is conceivable the existence of asymmetric chemical interactions constituting networks with analogous properties as those shown by Czarán et al. (2001) and Kelsic et al. (2013). Allelopathy, although not yet fully demonstrated to have a significant

role in the field (Jonsson et al., 2009), has the potential to be widespread among phytoplankton species (Śliwińska-Wilczewska et al., 2021), potentially creating motifs of multi-species interactions involving inhibitory, stimulatory and neutral effects.

In the present work, we employed five co-occurring phytoplankton species from the Baltic sea. Among them, two strains belonging to different phenotypes of the picocyanobacterial genus *Synechococcus*, well known to be potentially allelopathic (Konarzewska et al., 2020). With each of the five species, we performed monoculture growth experiments in order to determine their competitive abilities for nitrate. We also performed allelopathy bioassays with all the pairwise combinations of species (except the two *Synechococcus* sp. strains between them). Our final aim was to determine if the species were able to coexist in the long term in continuous culture experiments in which we employed nitrate as the only limiting resource. For these experiments, we divided the five species in two sets of four, on each of them we used a different *Synechococcus* strain and the other three species were the same. We expected that the asymmetric pairwise allelopathic interactions shown between them could enable this coexistence, as shown by some theoretical models (Chakraboty et al., 2015) when otherwise exploitative inter-specific competition for nitrate predicts that only the best competitor for nitrate will prevail (Tilman, 1977). These experiments were performed with different initial abundances of one of the species, known to be allelopathic, since these initial conditions were shown, theoretically and experimentally, to determine the outcome of inter-specific competition in the presence of an allelopathic interaction (Chao and Levin, 1980; Roy, 2009; Barreiro et al., 2018).

2. Materials and Methods

2.1 Phytoplankton strains and cultures conditions

Laboratory experiments were conducted using selected phytoplankton species: *Synechocystis* sp. (BA-121), *Monoraphidium* sp. (BA-165), and *Chlorella* sp. (BA-167), along with two phenotypes of picoplanktonic cyanobacteria isolated from the southern Baltic Sea: *Synechococcus* strain BA-120 (phenotype Type 2) and strain BA-124 (phenotype Type 1). Both *Synechococcus* strains are known for their allelopathic effects on coexisting phytoplankton (Śliwińska-Wilczewska et al., 2016, 2018; Konarzewska et al., 2020, 2022). These strains originate from the Culture Collection of Baltic Algae (CCBA) at the University of Gdańsk (Latała et al., 2006). Long-term experiments were cultivated at 18°C under photosynthetically active radiation (PAR) of 10 µmol photons m^{-2} s^{-1} with a 16:8 h light:dark photoperiod in modified f/2 medium (Guillard, 1975), maintaining a constant nitrate (NO_3^-) concentration of 120 µM to induce nitrate limitation.

Experiments designed to develop the interspecific competition model for nitrate with allelopathic interactions—including nitrate competition assays and pairwise short-term allelopathic assays—were conducted under PAR of 25 µmol photons m^{-2} s^{-1}, with a 16:8 h light:dark photoperiod, at 23°C and a salinity of 8 ‰. In these experiments, the medium composition was modified following Barreiro et al. (2018), with a nitrate concentration of 320 µM.

2.2 Growth experiments

In order to study the competitive abilities for nitrate of each of our species, we performed experiments with nitrate as limiting nutrient. The population growth rate was assumed to follow the equation:

$$\mu = \frac{\mu_{max_i} N}{K + N}$$

Where: µ - the growth rate, µmax - the maximum growth rate, K - the half saturation constants for growth with the limiting nutrient, N - the concentration of nitrate.

The culture medium had an initial nitrate concentration of 80 µM and a phosphate concentration of 200 µM. Light was provided continuously for 24 hours per day throughout the duration of the experiments. Cell abundances and nitrate concentrations were monitored every 24 hours using the same procedures described earlier, with two replicates taken for nitrate analysis. The experiments lasted approximately 11 to 16 days. Results of daily growth rate and nitrate concentration were fir to the above equation with the function *nls* from *stats* package (R Development Core Team, 2023).

2.3 Short-term allelopathic assays

The allelopathic effect between selected species was conducted using all possible pairwise combinations of them. Cell-free filtrate from exponentially grown cultures of the donor species was obtained through filtration using 0.45 µM filter (Macherey-Nagel MN GF-5) and was administrated in 4 levels, with 5 replicates each: 25%, 50% and 75% of the total volume of the vials, plus a negative control without filtrate. The final nutrient concentration was adjusted to 640:40 µmols N:P. The experiments were performed in 5 mL vials, and placed in culture chambers in the same conditions as above. Allelopathy effects were estimated as the slopes of linear regressions between growth rate of the target species and the % of filtrate.

2.4 Long-term experiments in chemostats

The long-term continuous culture systems (chemostats) consisted of 1 L Erlenmeyer flasks containing 400 mL of culture, with a dilution rate of approximately 0.3 day^{-1}. The experimental design included six runs (three per *Synechococcus* strain), each inoculated with different cell densities of the corresponding *Synechococcus* strain, along with a uniform initial concentration of *Synechocystis* sp. (120,000 cells mL^{-1}), *Chlorella* sp. (2,000 cells mL^{-1}), and *Monoraphidium* sp. (3,000 cells mL^{-1}). Each strain was tested at three different inoculum levels: 1,200,000 cells mL^{-1} (L), 2,800,000 cells mL^{-1} (M), and 4,000,000 cells mL^{-1} (H). The experiment was conducted over a total duration of 90 days.

Throughout the experiment, daily samples were collected from each run for microscopic counts (using Bürker chambers), as well as for nitrate concentration measurements (10 mL samples, in duplicate per chemostat). pH values were regularly monitored to ensure that CO_2 availability was not a limiting factor for growth. Additionally, light intensity was measured daily to confirm the absence of light limitation. Moreover, in the same conditions, the continues culture monoculture experiments were conducted to exclude other factors influencing the growth of studied species.

2.5 Model formulation

A mechanistic four-species competition model was used to describe (i) the classical competition for a limiting resource (nitrate) in the continuous culture and (ii) the all the pairwise allelopathic interactions between the species. The model formulation is as follows:

$$\frac{dN}{dt} = \partial(N_0 - N) - F_1(N)\frac{1}{\eta_1}P_1 - F_2(N)\frac{1}{\eta_2}P_2 - F_3(N)\frac{1}{\eta_3}P_3 - F_4(N)\frac{1}{\eta_4}P_4$$

$$\frac{dP_1}{dt} = F_1(N)P_1 - \varphi_1(P_2)\varphi_2(P_2)\gamma_{21}P_1 - \varphi_1(P_3)\varphi_2(P_3)\gamma_{31}P_1 - \varphi_1(P_4)\varphi_2(P_4)\gamma_{41}P_1 - \partial P_1$$

$$\frac{dP_2}{dt} = F_2(N)P_2 - \varphi_1(P_1)\varphi_2(P_1)\gamma_{12}P_2 - \varphi_1(P_3)\varphi_2(P_3)\gamma_{32}P_2 - \varphi_1(P_4)\varphi_2(P_4)\gamma_{42}P_2 - \partial P_2$$

$$\frac{dP_3}{dt} = F_3(N)P_3 - \varphi_1(P_1)\varphi_2(P_1)\gamma_{13}P_3 - \varphi_1(P_2)\varphi_2(P_2)\gamma_{23}P_3 - \varphi_1(P_4)\varphi_2(P_4)\gamma_{43}P_3 - \partial P_3$$

$$\frac{dP_4}{dt} = F_4(N)P_4 - \varphi_1(P_1)\varphi_2(P_1)\gamma_{14}P_4 - \varphi_1(P_2)\varphi_2(P_2)\gamma_{24}P_4 - \varphi_1(P_3)\varphi_2(P_3)\gamma_{34}P_4 - \partial P_4$$

Where:

$$F_i(N) = \frac{\mu_{max_i}N}{K_i + N}$$

$$\varphi_1(P_i) = e^{-aP_i} + b$$

$$\varphi_2(P_i) = max\left[\frac{P_i - n_0}{P_i}, 0\right]$$

The change in nitrate concentration is given by the first equation, where N is the nitrate concentration in the culture, N_0 is the inflowing nitrate concentration, and ∂ is the dilution rate of the system. $F_i(N)$ are Monod growth functions for the species *Synechococcus* sp. (i = 1), *Chlorella* sp. (i = 2), *Monoraphidium* sp. (i = 3) and *Synechocystis* sp. (i = 4), where μ_i is the maximum growth rate of species i and K_i is the half-saturation constants for nitrate of species i. The parameters η_i are the yield coefficients of species i. P_i are the population abundances of *Synechococcus* sp. (i = 1), *Chlorella* sp. (i = 2), *Monoraphidium* sp. (i = 3) and *Synechocystis* sp. (i = 4). The natural mortality of phytoplankton is insignificant relative to the dilution rate and is neglected in similar models (Passarge et al., 2006; Fussmann et al., 2000), so this process was not included in our model. The allelopathic effect of species j on species i is determined by the parameter γ_{ji} and its dynamics depends on the functions $\varphi_1(P_j)$ and $\varphi_2(P_j)$. By these two functions, the functional form of the allelopathic effect becomes sigmoidal (concentration-dependent). In $\varphi_1(P_j)$, the parameter a is the velocity of the process and b is a lower threshold. In $\varphi_2(P_j)$, the parameter n_0 is the minimum concentration of the allelochemical required to produce an effect.

This model formulation is closely related to the most successful one among a series of candidate models tested in the same experimental system (Barreiro et al., 2018).

2.6 Model parameterization and optimization

Parameters ∂ and N_0 were fixed at 0.3 d^{-1} and 120 µM, respectively. The values of the parameters η_i, μ_i and K_i were determined by the specific experiments reported above and were also fixed. The initial values for a, b and n_0 were determined by a heuristic fit to the results of the experiments. The initial values for the state variables were the observed cell abundances for each species and 120 µM for nitrate. For the initial values of the allelopathy parameters (γ_i), we first calculated heuristically the suitable dimension of the parameters and then established their values based on the relationships shown in Table 2.

We optimized only the allelopathy parameter (γ_i) and also the initial conditions. This optimization was performed in a first step with a global optimization by simulated annealing using the *GenSA* function from the R package *GenSA* (Xiang et al., 2013). In a second step, local optimization was performed using a Nelder-Mead simplex (Nelder and Mead, 1962) with the *optim* function from the R package *stats* (R Core Team, 2022). In both global and local optimization, the objective functions were set according to a version of the Levenberg-Mardquart minimization criterion (Levenberg, 1944):

$$E = \sum_{t=1}^{t=n} \left[\left(\frac{\left| P_{1\,pred} - P_{1\,obs} \right|}{P_{1\,obs}} \times \frac{1}{CV_{P_1}} \right) + \left(\frac{\left| P_{2\,pred} - P_{2\,obs} \right|}{P_{2\,obs}} \times \frac{1}{CV_{P_2}} \right) + \left(\frac{\left| P_{3\,pred} - P_{3\,obs} \right|}{P_{3\,obs}} \times \frac{1}{CV_{P_3}} \right) + \left(\frac{\left| P_{4\,pred} - P_{4\,obs} \right|}{P_{4\,obs}} \times \frac{1}{CV_{P_4}} \right) \right]$$

The parameter set providing the minimum value of E is considered the best estimate. In this formula, P_{ipred} (being i = 1 for the corresponding *Synechococcus* sp. strain, 2 for *Chlorella* sp., 3 for *Monoraphidium* sp. and 4 for *Synechocystis* sp.) correspond to the abundances of each species predicted by the model for times $t = 1$ to $t = n$, and P_{iobs}, the same abundances from the real data. CV_{Pi} is the coefficient of variation of the observed population abundances.

Independent optimization was performed for each long-term test run. To obtain the model solutions, we used the *lsoda* function from the R package *deSolve* (Soetaert et al., 2010).

2.7 Model fit to the data series

Model fits to the real data were assessed by several quantitative probes, calculated over an equilibrium period of 50 days in the model (days 40 to 90 in the real data). These probes were average total number of cells mL^{-1} (considering all species), average total number of cells mL^{-1} for each species, and a series of features of the oscillations: cycle amplitude, cycle period and phase relationship. Cycle amplitude and period were calculated only for *Synechococcus* sp. in order to simplify and because it was the best species fit (see Results). The phase relationship was calculated as the average phase relationship of the peaks of each of the three species in each single run, relative to *Synechococcus* adjacent peak, in absolute value. For a better estimation of the probes related to the oscillatory pattern in the real data series, the data series were previously smoothed with the Savitzky-Golay filter, implemented with *sgolayfilt* function, from R package *signal* (signal developers, 2023).

2.8 Model linear equilibrium analysis

To examine the behavior of the model at equilibrium, the model was simulated with a grid of initial species abundances that included, for *Synechococcus* sp., 100 equally distanced values within a range from approximately the minimum to the maximum initial abundance of *Synechococcus* employed. For the rest of the species, it was obtained the mean

initial species abundance and its standard deviation from all runs. The range of values was obtained from 100 random samples of a normal distribution with the calculated means and standard deviations. To avoid transitional phases, the results of these simulations at day 100 were used as initial values for calculating equilibrium points using the *multiroot* function of the R package *rootSolve* (Soetaert, 2009, Soetaert and Herman, 2009). The Jacobian matrices and their stability modules were obtained using the *eigen* and *jacobian.full* functions from the *base* (R Core Team, 2022) and *rootSolve* R packages, respectively. An equilibrium solution was classified as "unstable" if at least one of the eigenvalues had a positive real part, and "stable" otherwise. For all equilibria showing coexistence, the equilibrium was classified as "oscillatory" if the eigenvalues have an imaginary part, and "stable" otherwise (in which case it means non-oscillatory).

3. Results

3.1 Competitive ability for nitrate

Table 1 shows the results of the competitive ability for nitrate of each species. None of them are strong competitors for nitrate. Further model simulations (see below) showed that the picocyanobacterium *Synechoccocus* sp. strain BA-120 was the best competitor on its species set of the long-term experiments, and the Chlorophyte *Monoraphidium* sp. on the species set with *Synechococcus* strain BA-124.

Table 1 Estimated parameters for nitrate growth. Parameter names such as in the model.

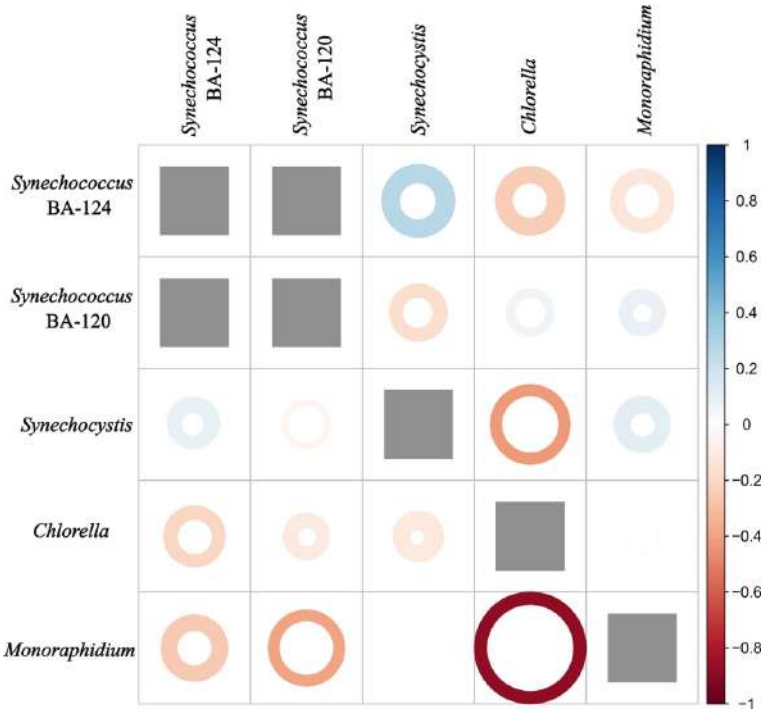
	μ_{max} (day^{-1})	K (*f*mol cell^{-1} h^{-1})	η
Synechococcus BA-120	0.59	83.66	182382.5
Synechococcus BA-124	0.83	168.91	999401.6
Monoraphidium	0.86	142.44	224534.6
Chlorella	0.59	66.37	88780.03
Synechocystis	0.78	222.37	120796.4

3.2 Pairwise allelopathic interactions

Figure 1 shows a matrix of the pairwise allelopathic interactions determined *a priori* in the two species sets conformed for the long-term experiments. As we ca see, there is a whole variety of strong and weak interactions, some of them eventually neutral, some inhibitory and others stimulatory. Overall, the chlorophyte *Monoraphidium* sp. showed the strongest inhibitory effects, and together with the other Chlorophyte, *Chlorella* sp., were the only

strains showing only inhibitory effects. The three picocyanobacteria showed in general weaker effects, and some of them stimulatory rather than inhibitory. *Synechocystis* sp. and *Monoraphidium* sp. were the species showing more resistance to inhibitory allelopathic effects, and *Chlorella* sp. the most strongly affected.

Figure 1. Pairwise allelopathic interactions within our species set. donor organisms on the vertical axis and target organisms on the horizontal axis. Growth stimulatory effects are represented by positive values and inhibitory effects by the negative ones. The width of the ring is proportional to the 95% Confidence Interval of the estimate of the linear allelopathy coefficient.



3.3 Long-term competition experiments

Monocultures of each of the five species in continuous culture conditions (Fig. S1) showed a stable steady state, therefore discarding the presence of endogenous viruses or other factors that could generate endogenous oscillations. The competition experiments with the two species sets showed identical outcomes, irrespective of the initial abundances of each of the *Synechococcus* sp. strains (Fig. 2).

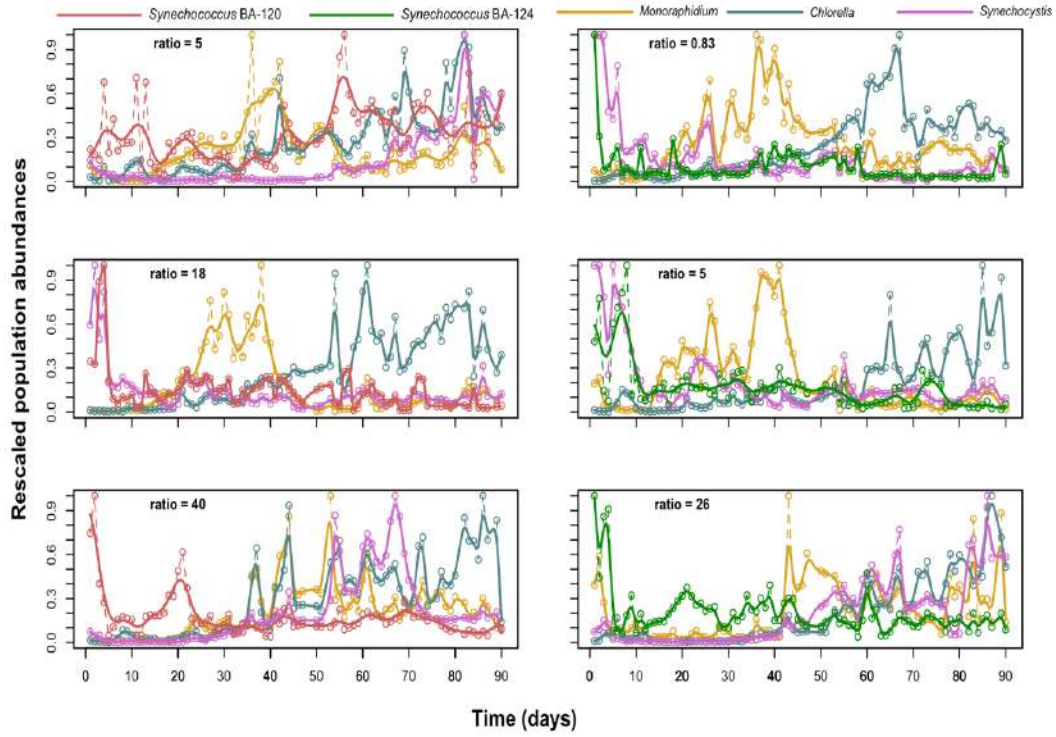


Fig. 2. Population dynamics data of the long-term competition experiments. Ratios indicate are the initial proportion of cells of each *Synechococcus* sp. strain relative to the sum of all other species. The point and the dashed-dotted line indicate the rescaled population abundances, while the solid line is the lowest smooth of the data. The values are mean ± SD (n = 3).

The steady state of biomass in equilibrium was reached between 20 to 30 days, according to nitrate data (Fig. 3). Regarding the dynamics of the species, the transient phase is, as usual, a bit longer (30-40 days) and an oscillatory equilibrium with coexistence of all the four species was reached in all the experimental runs, irrespective of the initial *Synechococcus* abundance (Fig.2). Despite the observed coexistence, biomass was overall dominated by the two chorophytes, with an initial dominance by *Monoraphidium* sp., later replaced by *Chlorella* sp. The nitrate data in steady state showed some oscillations, reflecting the biomass oscillations of the species.

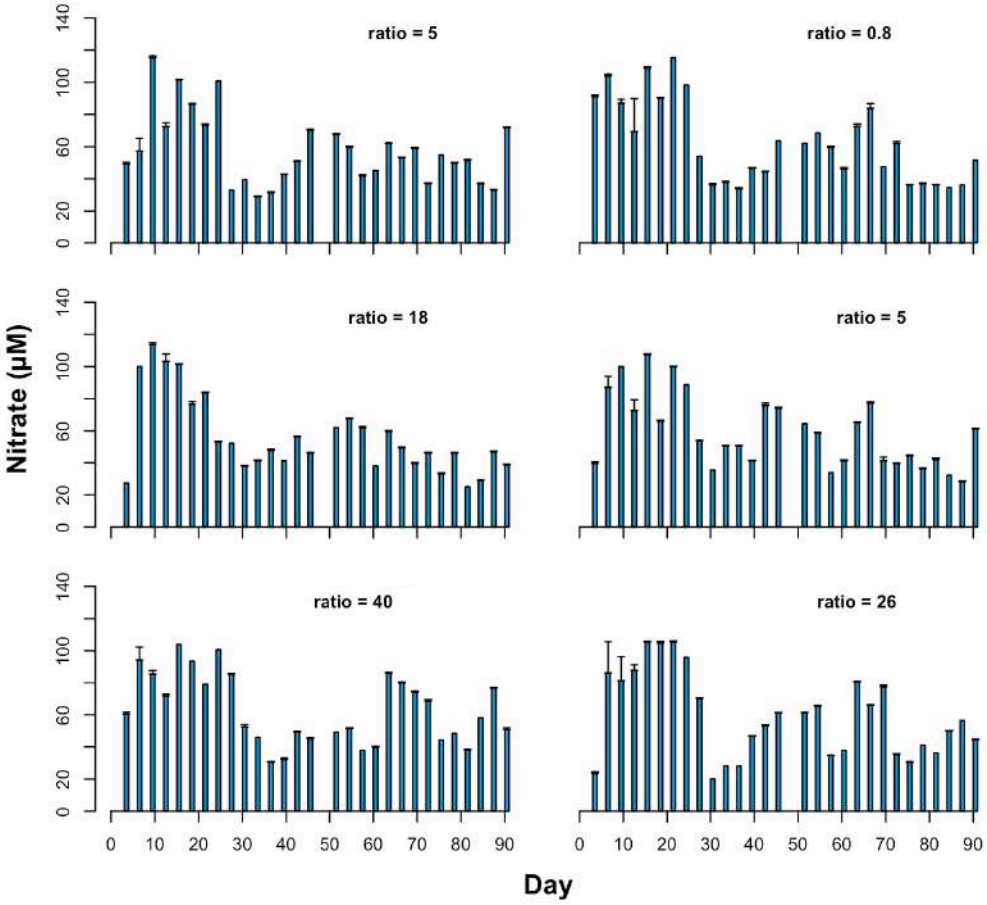


Fig. 3. Nitrate concentration (µM) in the long-term competition experiments.

3.4 Model results and comparison to the real data series

Our model optimization to the data showed that it was possible to obtain robust oscillatory coexistence with our two sets of species (Fig. 4). With fixed values, calculated independently, for the nitrate competition parameters, and optimizing only the allelopathy parameters γ_{ji}, Figure 4 shows the difference in the outcomes between our model with the optimized γ_{ji} values and therefore the effect of allelopathic interactions (oscillatory coexistence, Fig. 4) and our model if the allelopathic interactions were removed by making all $\gamma_{ji} = 0$ (Fig. 4) and therefore only competition for nitrate is at play.

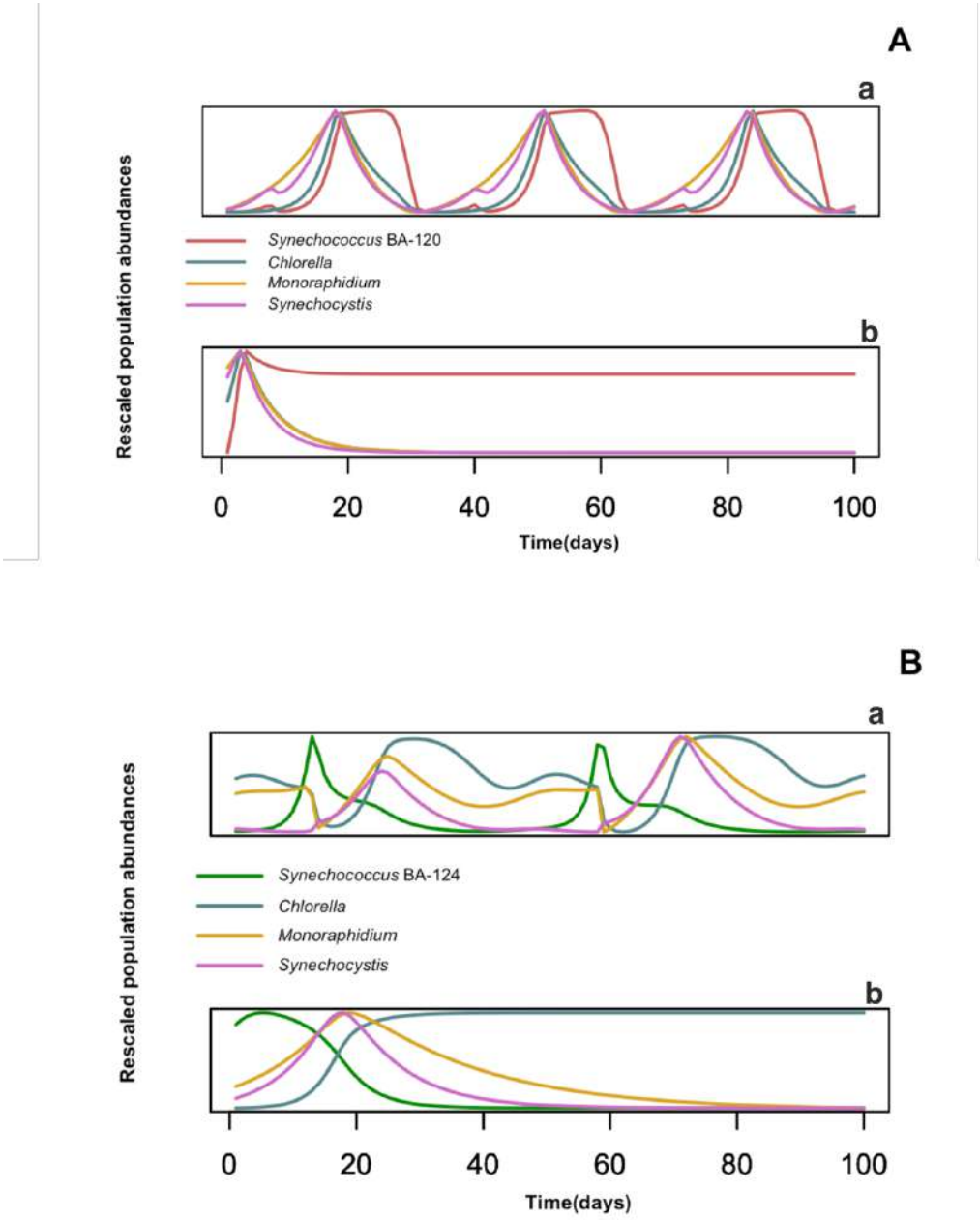


Fig. 4. Model simulations with optimized parameter values with *Synechococcus* BA-120. (A) and *Synechococcus* BA-124 (B). (a) Simulation results for equilibrium of the model including the allelopathic interactions. (b) Simulation results for the model including only nitrate competition (all $\gamma_{ji} = 0$) Values are rescaled population abundances over time.

The averages of the optimized values of the allelopathy parameters (γ_{ji}) are shown in Figure 5, with the aim to be compared to the *a priori* values shown in Figure 1. Despite minor changes, the overall relationships were not strongly altered relative to the a priori estimated interactions (Figure 5).

Fig. 5. Pairwise allelopathic interactions estimated by the average optimized values of γ_{ji} parameters.

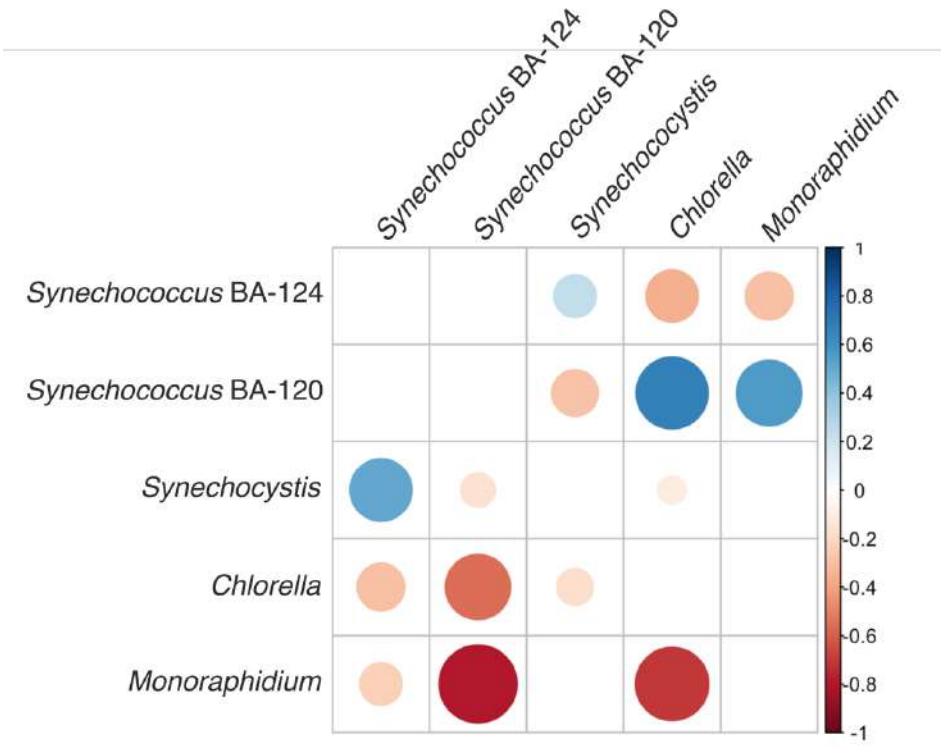


Figure 6 shows more in detail the oscillatory patterns of the experimental data at equilibrium. The oscillations are of relatively varying amplitude, but the period and phase seem relatively constant.

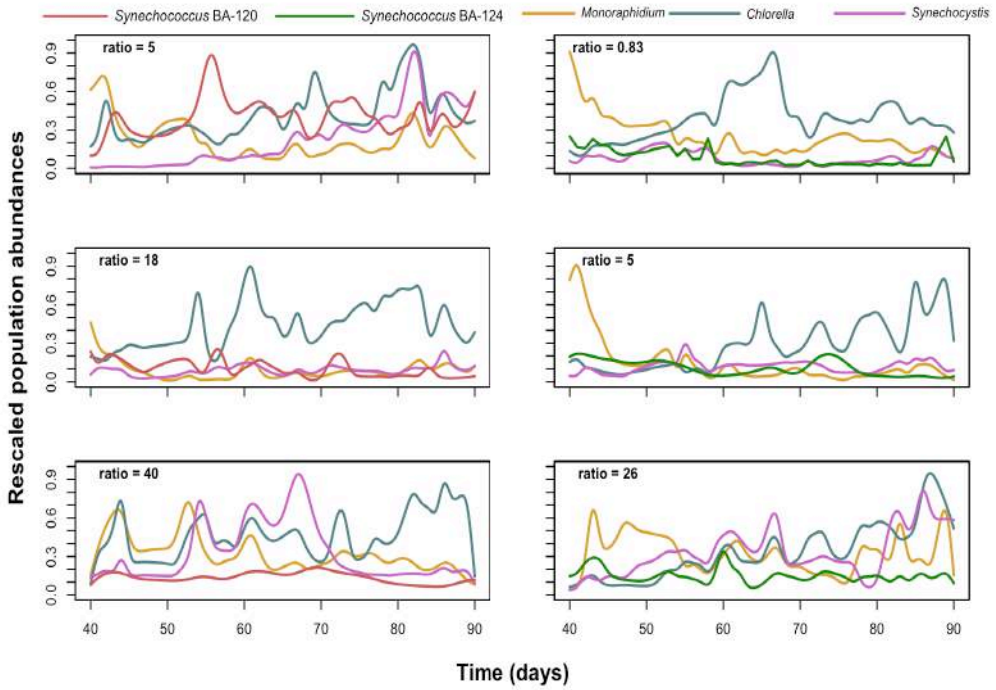


Fig. 6. Detail on the long-term competition experiments, showing only the smoothed population abundances for the equilibrium oscillations (days 40-90).

Table 4 shows a comparison of several quantitative probes obtained from our model and real data series for the experiments performed with the species set including *Synechococcus* sp. strain BA-120. The species set with strain BA-124 was not analyzed with this purpose since the linear equilibrium analysis failed to detect oscillatory equilibrium (see below). Although the overall cell numbers are not very accurately predicted, the values of the model are still in a realistic range. The model fails more strongly at predicting the period of the oscillations, whereas the average phase relationship was perfectly matched, indicating an almost in-phase oscillation of all the species.

Table 4. Model performance compared to the real data assessed by several quantitative probes. Values of cells and cycle amplitude are in cells mL^{-1}. The cycle period was measured in days. % difference accounts for the difference between real values and model fits.

Probe	BA-120	Real data	% difference
Total cells	8.4×10^6	15.8×10^6	46.9
Cells *Synechococcus*	8.3×10^6	9.6×10^6	13.6
Cells *Chlorella*	6.7×10^4	8×10^5	91.6
Cells *Monoraphidium*	5.6×10^4	1.7×10^5	67.3
Cells *Synechocystis*	3×10^4	5.3×10^6	99.4
Cycle amplitude	15×10^6	7.7×10^6	96.9
Cycle period	24	10	144.5
Phase relationship	0.14	0.14	0

3.5 Model equilibrium analysis

The linear equilibrium analysis results are shown only for the model fit to the species set that included the *Synechococcus* sp. strain BA-120 (Figs. 7,8), since the analysis failed to detect oscillatory equilibrium for the other species set, characterizing it as always stable and non-oscillatory (data not shown).

Although in the ranges employed in our experiments, we only detected stable oscillatory coexistence of all four species, the stability analysis results (Fig. 7) showed a clear dependence on the initial *Synechococcus* sp. abundance, although only manifested at very large initial values. At low initial values, the outcome in equilibrium is stable, with coexistence of all the species, and as initial values increase, this equilibrium becomes unstable. Finally, at the highest initial *Synechococcus* sp. abundances simulated, extinction of only one of the species (*Chlorella* sp.) was predicted.

The transition from oscillatory to stable coexistence in equilibrium was also a function of initial *Synechococcus* sp. abundances, being the lower range of them the necessary condition for the oscillations (Fig. 8).

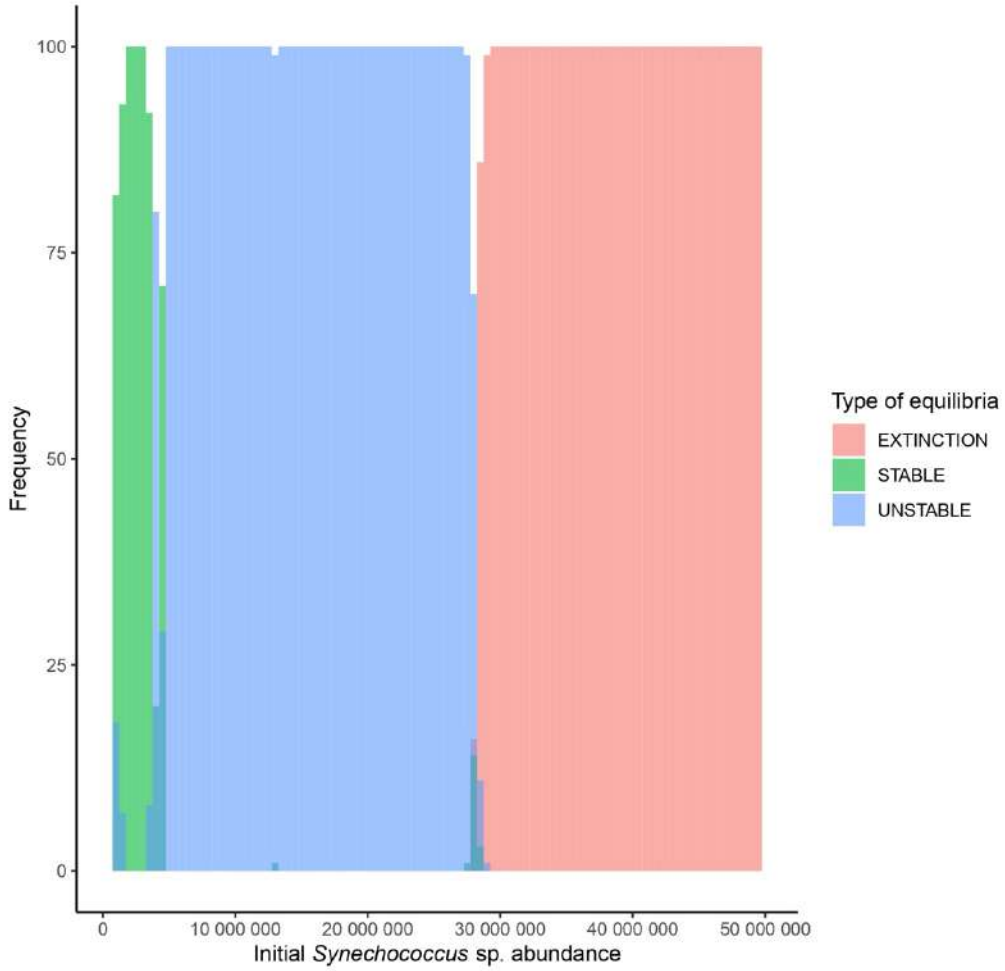


Fig. 7. Stability analysis of the model equilibrium as a function of initial *Synechococcus* sp. abundance, in the species set including *Synechococcus* BA-120.

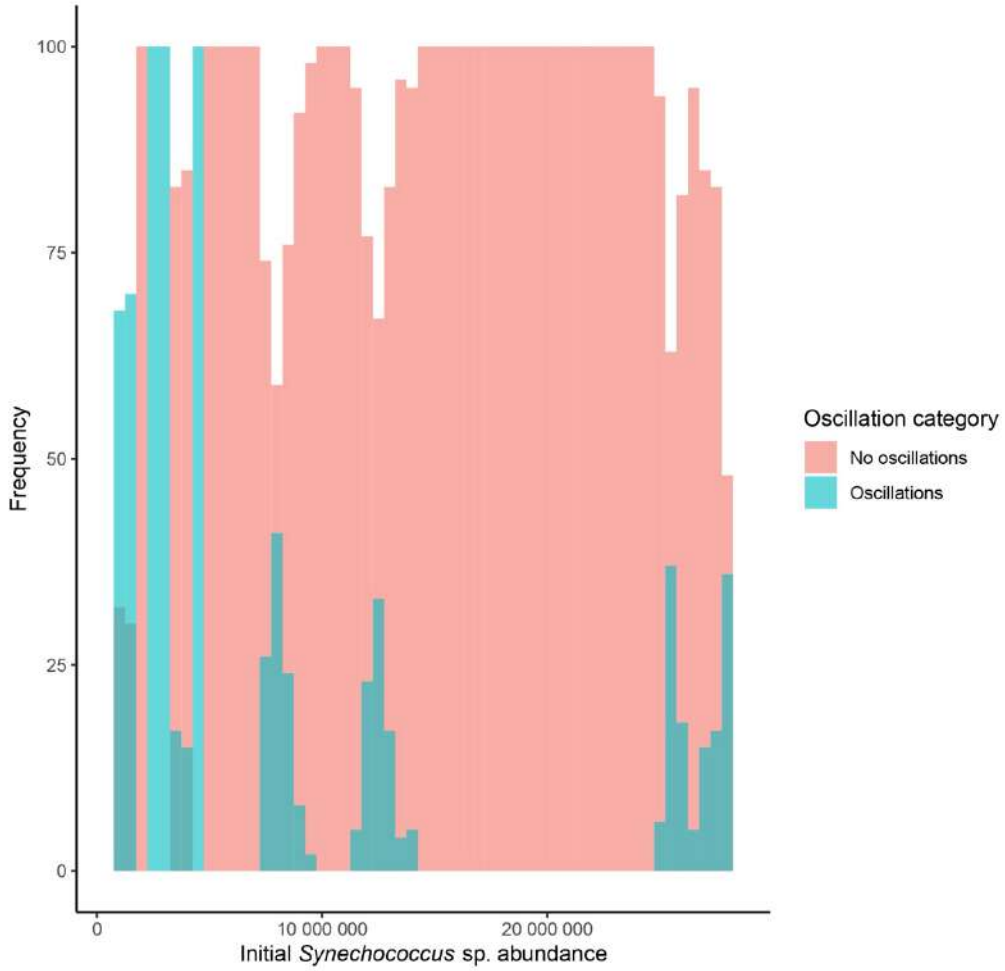


Fig. 8. Analysis of the presence/absence of oscillatory equilibrium in the stable and unstable equilibria from Fig. 5.

4. Discussion

Our experiments showed very consistent results, that can be solely attributed to allelopathy. By controlling all other potential limiting factors for this kind of experiments (light, phosphate, and CO_2, monitored by pH) it is guaranteed that only nitrate is limiting our experiments. However, in nitrate limited conditions, classical resource competition (Tilman, 1977) predicts dominance by a single species, the best competitor for nitrate (*Synechococcus* sp. strain BA-120 in its species set and *Monoraphidium* sp. in the species set with BA-124). This was clearly not the outcome of our experiments, leaving no room for other explanation, under these conditions, as allelopathy, in a similar way as Barreiro et al. (2018) for a two species system.

The model fit to the real data left several probes with poor fits (Table 4). However, overall, the biomass values were realistic, and some parameters, such as *Synechococcus* biomass and phase relationship showed very good match with the real data series. Anyways, we should clarify that improving the fit to real data is possible, with a more systematic parameter search through sensitive analysis, but, moreover, by allowing to optimize as well the growth parameters. However, this was not the aim of the present work, and in general, must be understood that in works comparing real data with model predictions, priority should be given to the qualitative aspects of model behavior. Many relevant works in ecological dynamics do not show any realistic match of model predictions to real data, and focus on fit to model qualitative behavior and model tractability with the aim of performing extrapolations and equilibrium analysis (Fussmann et al., 2000, Yoshida et al., 2003, McCauley et al., 2008). Compared to these examples, our model still stands a comparison with the dimensions of real data.

In addition, we should point out that, even after optimizing the allelopathic interactions, (Table 3) the difference with regard to the a priori estimates (Table 2) is very little, suggesting the robustness of our model, which does not need to optimize growth parameters and a light optimization of allelochemical interactions is enough to obtain fits to the real data that are qualitatively almost identical and quantitatively reasonable.

The oscillatory coexistence was clearly a robust outcome of our experiments. However, the model equilibrium analysis showed that, for the range of initial abundances of *Synechococcus* sp. employed ($8 – 15 \times 10^6$ mL^{-1}) it is most likely to find unstable coexistence (Fig 4) and not always oscillatory (Fig. 5). The observed overlay of oscillatory and non-oscillatory equilibria in the range were unstable equilibria are mostly found (Fig 5) may indicate the existence of bi-stability. In any case, the tendency to instability of the equilibria shown by the model may not be completely real, considering that our experimental data showed consistently the same outcome despite the differences in initial conditions, and the existence of eventual random perturbations. It is very likely that in the real experiments, the allelopathic interactions played a more complex role than what the model formulation presents. The model assumes a non-linear effect of the allelochemicals but does not consider the dynamics of production of these compounds, which can be subjected to feed-backs with population growth and complex hormetic effects (Calabrese, 2008) as a function of allelochemical concentration. This more dynamic and multi-faceted role of allelochemicals could stabilize the system towards the observed pattern of short-period and almost-in-phase oscillations.

The prediction of four species coexistence (whether stable or unstable, oscillatory or not) was consistent in our model, and was only disrupted with the extinction of *Chlorella* sp. for simulated high initial abundances of the *Synechococcus* sp. strain BA-120, which were not tested in the real experiments. This strong trend to stabilize community diversity, against the prediction determined by resource competition (Fig. 2) suggest that our model has certain relationship with those of Czarán et al. (2001) and Kelsic et al. (2013). These three models share the assumption of asymmetric inhibitory interactions mediated by chemicals but differ in other two aspects: Czaran et al. (2001) present these interactions exclusively in an intransitive way, determining cyclic dominance killer-resistant- sensitive. Similar intransitive relationships can be found among some of our species: *Monoraphidium* sp. (killer) – *Synechocystis* sp. (resistant) *Chlorella* sp. (sensitive), but the intransitive motifs are blurred by the presence of stimulatory effects and interference with other non-intransitive motifs among our species. There might be, however, traces of some cyclical dominance in our data (between *Monoraphidium* and *Chlorella*) although it is difficult to explain how they actually emerged from our complex net of chemical interactions. In addition, Czarán et al. (2001) model implies the existence of some spatial structure. Kelsic et al. (2013) model differ from ours in the presence of a net of degradation of antibiotics, which the mechanism that, in the absence of spatial structure, enables to model to exhibit strong stabilization of community diversity.

The present work, although it constitutes an extension of the theoretical and experimental work by Barreiro et al. (2018) to more than two species, it shows the property of more robust coexistence, in particular oscillatory coexistence, emerging with the larger species set employed. In Barreiro et al. (2018, in preparation) coexistence in a two-species system with allelopathic interaction was shown to be stable or oscillatory, but also alternative exclusion of one species was possible, suggesting more sensitivity to initial conditions than the present system.

Our results present an exciting finding, because they showed that robust coexistence and stabilization of diversity can be obtained as well, simply with a relatively random set of asymmetric inhibitory and stimulatory allelopathic effects, without the need of additional constrains such as spatial structure, exclusively intransitive relationships or the existence of additional processes interfering (degradation of the allelochemicals). Compared to previous works in two species systems, this robust coexistence seems to be an emergent property of increasing the number of species in the system, provided that all of them show some sort of allelopathic interaction with the majority of the other species.

In essence, our results demonstrate the enormous potential of chemical interactions by themselves as drivers of the fascinating diversity of microbial communities. In particular, in the difficult context of homogenous environments, with few limiting resources, such as the aquatic environment.

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Supplementary material

Take this Waltz: allelopathy induces oscillatory coexistence in marine phytoplankton

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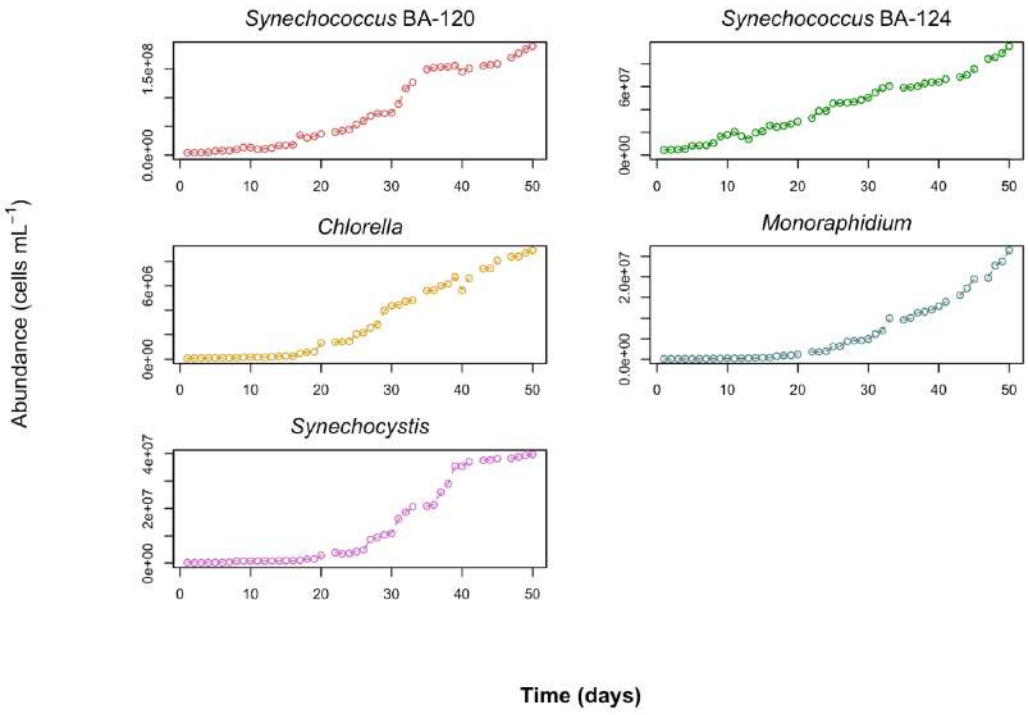

Fig. S1. Abundance over time (50 days) in continuous monoculture of *Synechococcus* (strains BA-120 and BA-124), *Synechocystis*, *Chlorella*, and *Monoraphidium*.

AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

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were as follows:

Konarzewska Zofia 50%:

Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - Original draft preparation

Barreiro Felpeto Aldo 35%:

Conceptualization, Investigation, Methodology, Supervision, Formal analysis, Writing - Review & Editing

Śliwińska-Wilczewska Sylwia 5%:

Supervision, Writing - Review & Editing

Morais Joao 5%:

Data curation

Vasconcelos Vitor 5%:

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Konarzewska Zofia

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9.5 PUBLIKACJA 5

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Allelopathy of *Synechococcus* increases diversity of coexisting phytoplankton communities

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Abstract

Picocyanobacteria of the genus *Synechococcus* are a widespread component of marine planktonic communities and are seasonally dominant in the Baltic Sea. It has been shown that light, salinity, temperature, nutrients and grazing influence niche segregation of *Synechococcus* in the seasonal succession. Many *Synechococcus* strains have allelopathic properties, but the role of allelopathy remains unknown. Recent studies in two-species systems provided experimental evidence for increased diversity at intermediate levels of

allelopathy, while extreme levels (low or high) led to reduced diversity. Here we studied the role of *Synechococcus* allelopathy in structuring coexisting phytoplankton communities. We brought natural phytoplankton communities from the Baltic Sea to equilibrium in semi-continuous cultures under nitrate limitation. These communities were inoculated with two Baltic *Synechococcus* strains (BA-124; BA-132) known to have allelopathic properties, with different inoculum sizes: low, medium and high, as a proxy of the strength of allelopathy. With this design, according to classical resource competition theory, the dominant species at equilibrium should be the same irrespective of the initial inoculum of any species. Our equilibrium communities were dominated by 2-4 diatom genera at all *Synechococcus* levels. Community diversity was significantly higher only at the medium size of *Synechococcus* inoculum, with strain BA-124. This was the expected effect of allelopathy, according to previous works in two-species systems. There is no other clear factor that could produce such an effect considering our experimental design. Therefore, we state that allelopathy is the best hypothesis to explain these results. In strain BA-132, the allelopathic effect appeared to be weaker and had no effect on community diversity, with a constant abundance of this strain in the equilibrium communities, a pattern consistent with exploitative resource competition as the main driver of community structure. These results suggest that allelopathy could eventually be a driving factor of phytoplankton succession in the Baltic Sea.

Running head: *Synechococcus influences coexisting phytoplankton communities*

Keywords: *Synechococcus*, allelopathy, Baltic Sea, diversity, community

Introduction

In recent decades, coastal ecosystems worldwide have experienced an increase in picocyanobacterial blooms (O'Neil et al., 2012; Hunter-Cevera et al., 2020). These blooms occur in various subtropical and tropical marine coastal waters (Phlips et al., 1999; Doré et

al., 2022), as well as in brackish waters of temperate regions (Sorokin and Zakuskina, 2010; Caroppo, 2015), and have also been reported in the Baltic Sea (Kuosa et al., 1991; Mazur-Marzec et al., 2013; Zufia et al., 2022). Human-induced environmental changes such as coastal eutrophication, ocean acidification and rising water temperatures favor the spread of bloom-forming toxic phytoplankton and cyanobacteria in particular (Heisler et al., 2008; Rost et al., 2008; Suikkanen et al., 2013; Paerl, 2018). These blooms pose a threat to the environment, partly due to the toxic compounds produced by the species associated with them and to oxygen depletion in the water column (Allen et al., 2006).

Picoplanktonic cyanobacteria of the genus *Synechococcus* play a crucial role in marine ecosystems due to their contribution to primary production and wide distribution (Sorokin et al., 2004; Mühling et al., 2008; Rii et al., 2016; Zufia et al., 2022). These globally distributed picocyanobacteria are integral components of marine planktonic communities and can adapt to different light, temperature, and nutrient conditions (Flombaum et al., 2013; Bemal and Anil, 2018; Gin et al., 2021). They can form large blooms, which are favored by the current context of increasing eutrophication of coastal ecosystems and climate change (Sorokin et al., 2004; Flombaum et al., 2013; Dutkiewicz et al., 2015; Li et al., 2019).

In the Baltic Sea, the dominance of picocyanobacteria is seasonally determined by factors such as the availability of nutrients and the water temperature (Zufia et al., 2022). The cyanobacterial bloom in the Baltic Sea comprises various species, including mainly *Synechococcus* spp. with peak values of up to 10^6 cells mL^{-1} (Haverkamp, 2009; Aguilera et al., 2023) as well as filamentous, diazotrophic species (Kuosa, 1991; Mazur-Marzec et al., 2013; Eigemann et al., 2018; Zufia et al., 2021).

There is a classification of *Synechococcus* phenotypes based on the composition of photosynthetic pigments (phycobiliproteins) in their phycobilisomes. Six et al. (2007) identified three main phenotypes of marine picoplanktonic cyanobacteria within this genus: type 1, type 2 and type 3. Recent studies have confirmed the occurrence of all three in the

Baltic Sea (Aguilera et al., 2023). Type 1 and type 3 *Synechococcus* strains show remarkable differences in pigment composition and ecological distribution, which affects their ecological niches. Type 1, which is rich in phycocyanin (PC), is predominantly found in coastal waters, estuaries and freshwater systems. In contrast, type 3, which contains both PC and two forms of phycoerythrin (PE-I and PE-II), is prevalent in oligotrophic marine environments. These pigment compositions lead to ecological niche segregation and expansion (Haverkamp, 2009; Six et al., 2007).

Recent work suggests that certain *Synechococcus* strains produce toxins with potentially allelopathic effects against competitors (Śliwińska-Wilczewska et al., 2017; Konarzewska, 2020). Allelopathic substances can affect the growth and development of target species and selectively influence phytoplankton community structures (Fistarol et al., 2003; Suikkanen et al., 2005). Despite the crucial role that the genus *Synechococcus* plays in aquatic ecosystems, the influence of its allelopathic effect on natural plankton communities has not been studied.

The diversity of plankton communities is maintained by temporal (seasonal) heterogeneity, which places the communities in a permanent non-equilibrium state. Nutrients, turbulence, temperature and light are among the most important seasonal drivers of community change. Certain species dominate in each season, but equilibrium conditions are not maintained long enough to exclude poor competitors (Malchow, 1993; Scheffer et al., 2003). The result is a constant succession of seasonally and spatially differentiated communities. For decades, there has been extensive research aimed at deciphering the relevant factors for structuring plankton communities and maintaining their diversity. It is evident that several groups with life cycle strategies such as the formation of resting stages can avoid exclusion if optimal spatio-temporal conditions do not prevail. There is strong experimental and theoretical evidence for the potential of nutrient competition and light to structure phytoplankton communities (Tilman, 1977; Huisman et al., 1999). Other potentially structuring factors such

as self-organized dynamics (deterministic chaos), differential predation or chemical interactions have been considered but less thoroughly studied in real communities (Benincà et al., 2008; Weiss and Vasseur, 2013; Brown et al., 2019). Allelopathy is considered a potential driver of plankton community structure (Keating 1977; Legrand et al., 2003; Barreiro et al., 2018). According to theoretical models, the effect of allelochemicals depends mainly on factors such as species abundance and turbulence (Hulot and Huisman 2004; Roy 2009; Jonsson et al. 2009). Studies by Barreiro et al. (2018) provided joint theoretical and experimental evidence that allelopathy can promote the coexistence of two phytoplankton species in a single limited resource. This contradicts the principle of competitive exclusion as predicted by the classical theory of resource competition (Tilman, 1977). This behavior in the system of Barreiro et al. (2018) is due to the existence of the following trade-off: the worse competitor for the single limiting nutrient produces allelopathic substances that inhibit the growth of the best competitor. In this system, coexistence (and thus higher diversity) was shown at intermediate levels of allelopathic effect, while extreme (low or high) effects of allelopathy showed the alternative exclusion of each species. This predicted "hump shape" effect of allelopathy on diversity is consistent with a broad interpretation of the intermediate disturbance hypothesis (Wilkinson, 1999), which considers allelopathy as a disturbance. However, an extension of the Barreiro et al. (2018) system has never been applied to complex plankton communities.

Based on resource competition theory, when nitrate is the only limiting factor and initial *Synechococcus* abundance is the only manipulated variable, this should not influence community composition at equilibrium; thus, diversity should remain consistent across all runs. However, we hypothesize that allelopathy of two strains of the genus *Synechococcus* affects the diversity of coexisting natural phytoplankton communities at equilibrium, extending the system described by Barreiro et al. (2018) to more complex phytoplankton communities. Long-term experiments were performed in semi-continuous cultures with

natural phytoplankton communities originating from the same environment as the *Synechococcus* strains (southern Baltic Sea). These natural communities were inoculated with different initial *Synechococcus* amounts to determine the strength of allelopathy (as in Barreiro et al., 2018). Nitrate was set as the only limiting resource, therefore excluding the effect of other factors (light, CO_2, other macro-or micronutrients) as potential drivers of the community structure observed. This method makes it possible to analyze the effect of allelopathy in relatively complex communities, in a situation more similar to actual environmental conditions. This experimental method of equilibrating natural plankton communities in laboratory systems has also been successfully used to investigate other relevant ecological hypotheses (Burton et al., 2018; Papanikolopoulou et al., 2018).

Materials and Methods

Cultures and Phytoplankton Sampling

Laboratory experiments were carried out with two phenotypes of picoplanktonic cyanobacteria isolated from the southern Baltic Sea: *Synechococcus* strain BA-124 (PC-rich strain, type 1) and strain BA-132 (PC-, PE-I- and PE-II-containing, type 3). Both strains are known for their allelopathic properties against coexisting phytoplankton (Śliwińska-Wilczewska et al., 2016, 2018; Konarzewska et al., 2020, 2022). These cyanobacteria originate from the Culture Collection of Baltic Algae (CCBA) at the University of Gdańsk (Latała et al., 2006). The picocyanobacteria were cultivated at 18°C under photosynthetically active radiation (PAR) of 10 μmol photons $m^{-2}s^{-1}$ with a photoperiod of 16:8 light in f/2 medium (Guillard, 1975).

The field community was sampled from the surface waters of the Gulf of Gdańsk (54°50′52.1″ N, 18°55′72.0″ E) in September 2022. For this purpose, 5 L of water was collected and passed through a 55 μm fine sieve to remove zooplankton and large aggregates.

The sample was then acclimatized to the culture room conditions (as described above) for 24 hours.

Experimental Setup

The microcosms consisted of semi-continuous culture systems with 1 L Erlenmeyer flasks filled with 400 mL of culture. In each culture, 260 mL of the medium was replaced to ensure a dilution rate of 0.3 day^{-1}. The culture medium was based on f/2 medium (Guillard, 1975) with a constant nitrate (NO_3^-) concentration of 120 µM to induce nitrate limitation. All runs were inoculated with 250 mL of the field community obtained as described above. We used 250 mL to ensure consistency between runs and to prevent the initial population from influencing the final community. The experimental design included two control runs without the addition of *Synechococcus* and six treatment runs (three per strain), each inoculated with different cell densities of the corresponding *Synechococcus* strain. The three cultures of each strain were inoculated with the corresponding *Synechococcus* sp. strain at densities of 250,000 (L), 1,000,000 (M) and 2,500,000 (H) cells mL^{-1}. The experiment lasted a total of 79 days.

Samples (10 mL) were taken from each culture to quantify the phytoplankton using microscope counts in Ütermöhl and Bürker chambers. The samples were preserved with a drop of Lugol's solution before counting. In addition, nitrate concentrations were monitored by taking 10 mL samples (in duplicate per chemostat). Both microscopic counts and nitrate monitoring were performed on days 3 and 7 and then every two weeks until day 79. The pH values were monitored regularly to ensure that CO_2 was not limiting growth.

Microscopic phytoplankton counts

Cell counts were monitored daily by microscopy using either Ütermöhl or Bürker counting chambers. Prior to counting, samples were sonicated to remove large aggregates.

For the Ütermöhl chamber, 10 mL or 5 mL samples were sedimented for 24 hours, depending on phytoplankton abundance. Counts were performed by examining 10 fields of view at 20x magnification. At this sample intensity, the saturation curves were saturated in all samples. Taxa were identified to genus level.

Nitrate Analysis

For the analysis of nitrate (NO_3^-), 10 mL samples (2 replicates) were filtered with 0.22 µm PES syringe filters. NitraVer® 5 nitrate reagent pads were used to generate the color reaction. The absorbance was measured with a DR6000 spectrophotometer (Hach-Lange, Loveland, USA).

Statistical analysis

Identifying homogenous equilibrium communities

Within each experimental community (culture), we tested the dissimilarity of communities between sampling time points, assuming that the time point after which communities were found to be homogeneous would indicate that equilibrium had been reached. To test this homogeneity of communities by dissimilarity, we performed a hierarchical cluster analysis with Bray-Curtis distance implemented using the hclust function from the R package stats (R Core Team, 2023). Cluster significance was determined with 1000 bootstrap repetitions using the pvclust function from the R package pvclust (Suzuki et al., 2019), modified from Hanson (2014). We selected as time points with homogeneous equilibrium communities the largest significant cluster that contained the latest sampling data.

Correspondence analysis and dominance analysis

With the selected homogeneous communities, we performed correspondence analysis (CA) as an exploratory tool to reveal relationships among and within taxa and

treatments. We used the function fviz_ca_biplot from the R package factoextra (Kassambara and Mundt, 2020).

Based on the homogeneous communities selected with the cluster analysis, we performed an analysis of dominant taxa. We considered as dominant those whose average abundance was higher than the overall average.

Contrast of hypothesis over indexes

We then performed an analysis of variance (ANOVA) and Tukey post-hoc to test the effects of our experimental treatments on species richness, evenness and Shannon-Wiener diversity. Within each treatment level (culture), we considered each of the days selected as homogeneous according to the cluster analysis as a technical replicate. Due to the relatively limited total number of replicates, we used an approach based on the Monte Carlo framework for this statistical analysis, adapting Grason and Miner (2012) to our context. We obtained the critical value of the ANOVA and Tukey's contrast statistics (Fisher's *F* and Tukey's *t*) with a $\propto = 0.95$ from their distribution computed over 10000 null communities. To create each of these null communities, the real taxa abundances from the entire pool of replicates were randomly distributed among each replicate to obtain an expected equal distribution of taxa among treatment levels. We then calculated Fischer's *F* and Tukey's *t* values for each of these 10000 null communities based on species richness, Pielou evenness and the Shannon-Wiener diversity index. The Shannon-Wiener index was calculated using the diversity function implemented in the R package vegan (Oksanen et al, 2022). Species richness was calculated using the specnumber function implemented in the vegan R package. The Pielou-Evenness index was calculated using the following formula:

$$\frac{\text{Shannon} - \text{Wiener index}}{\text{LN}(\text{species richness})}$$

We then ran 1000 one-way ANOVA and Tukey tests on the original data. For each of these 1000 tests, we randomized the respective index values between replicates within each

treatment level. We considered significant differences if in more than 50% of these tests the p-value was significant according to our null distributions.

Results

pH levels

The results of the pH measurements are shown in Table 1S. During the 79 days of the experiments, the pH did not exceed 7.6, indicating that there was no CO_2 limitation. The lowest values on day 79 were recorded in the two controls, Control 1 (7.32) and Control 2 (7.42).

Nitrate analysis

As it is inevitable in continuous or semi-continuous cultures, the limiting resource drops until reaching an equilibrium (whether stable or oscillatory) according to the renewal rate (dilution rate) of the system. This equilibrium in limiting resource level is reflected in the total biomass, which reaches the same equilibrium. This drop till an equilibrium is clearly shown for all of our experimental runs, although not all reached it at the same time (Figure 1). The mean nitrate values for the equilibria were not the same in all the runs, being lowest in Control 2 (12.50 µM), BA-132 L (14.92 µM) and Control 1 (15.12 µM). In the experiments with strain BA-124, the nitrate concentrations were higher than in the experiments with strain BA-132 and correlated with the size of the *Synechococcus* sp. inoculum, with the highest average nitrate values for BA-124 H being 45.16 µM. In the experiments with strain BA-132, the highest average nitrate concentration at equilibrium was observed for BA-132 H, with a value of 17.54 µM.

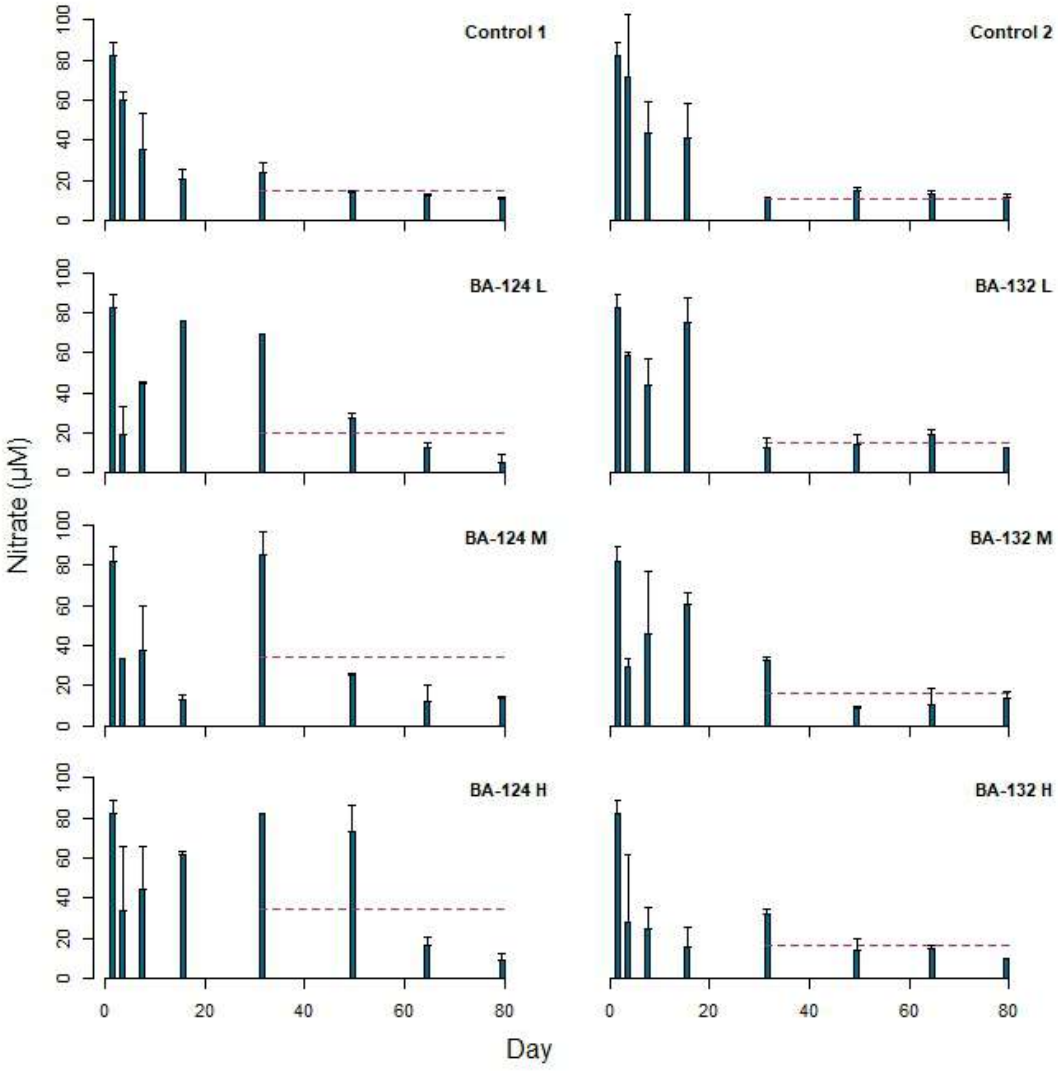

Fig. 1. Nitrate concentration (µM) in controls and cultures with different inoculum sizes of the tested *Synechococcus* phenotypes: BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 cells mL^{-1}). The dashed-dotted line indicates the average nitrate concentration during equilibrium. The values are mean ± SD (n = 2).

Community composition

The results of the hierarchical clustering are shown in Figure S1. Days 31 to 79 were isolated in a single significant cluster in all experimental cultures and were therefore considered as homogenous equilibrium communnities. Figure 2 shows the relative

abundances of the different taxa at equilibrium. The relative abundance as biomass (panel A) shows that *Nitzschia* and *Navicula* were the dominant taxa in all experiments. BA-124 L showed a similar community structure to the control cultures. BA-124 M had the largest number of species at equilibrium. Among the different taxa present in BA-124 M at equilibrium, *Pleurosigma*, *Cyclotella*, and *Fistulifera* were the most abundant. There was also a notable presence of *Cyclotella* in BA-124 H. In the BA-132 runs, BA-132 L showed a similar structure to the controls, with a high occurrence of *Navicula* and *Nitzschia*. In BA-132 M, an increase of *Cyclotella* is noticeable, while in BA-132 H *Cyclotella* and *Fistulifera* occur in low but constant abundance. Regarding the cell concentrations (panel B), *Navicula* and *Nitzschia* were the predominant taxa in Control 1 and Control 2. In all other runs, the predominant taxon was *Synechococcus*. In BA-124 runs, there was a correlation between *Synechococcus* abundance at equilibrium and initial inoculum size, with *Chlorella*, *Cyclotella*, *Fistulifera*, and *Pleurosigma* also consistently relevant at low levels. In BA-124 H, *Synechococcus* dominated overwhelmingly. In the BA-132 runs, *Synechococcus* accounted for over 75% of the cells in all inoculum sizes with no correlation to initial inoculum size. *Navicula* and *Nitzschia* were other consistently relevant taxa, as was *Fistulifera* in the BA-132 H run.

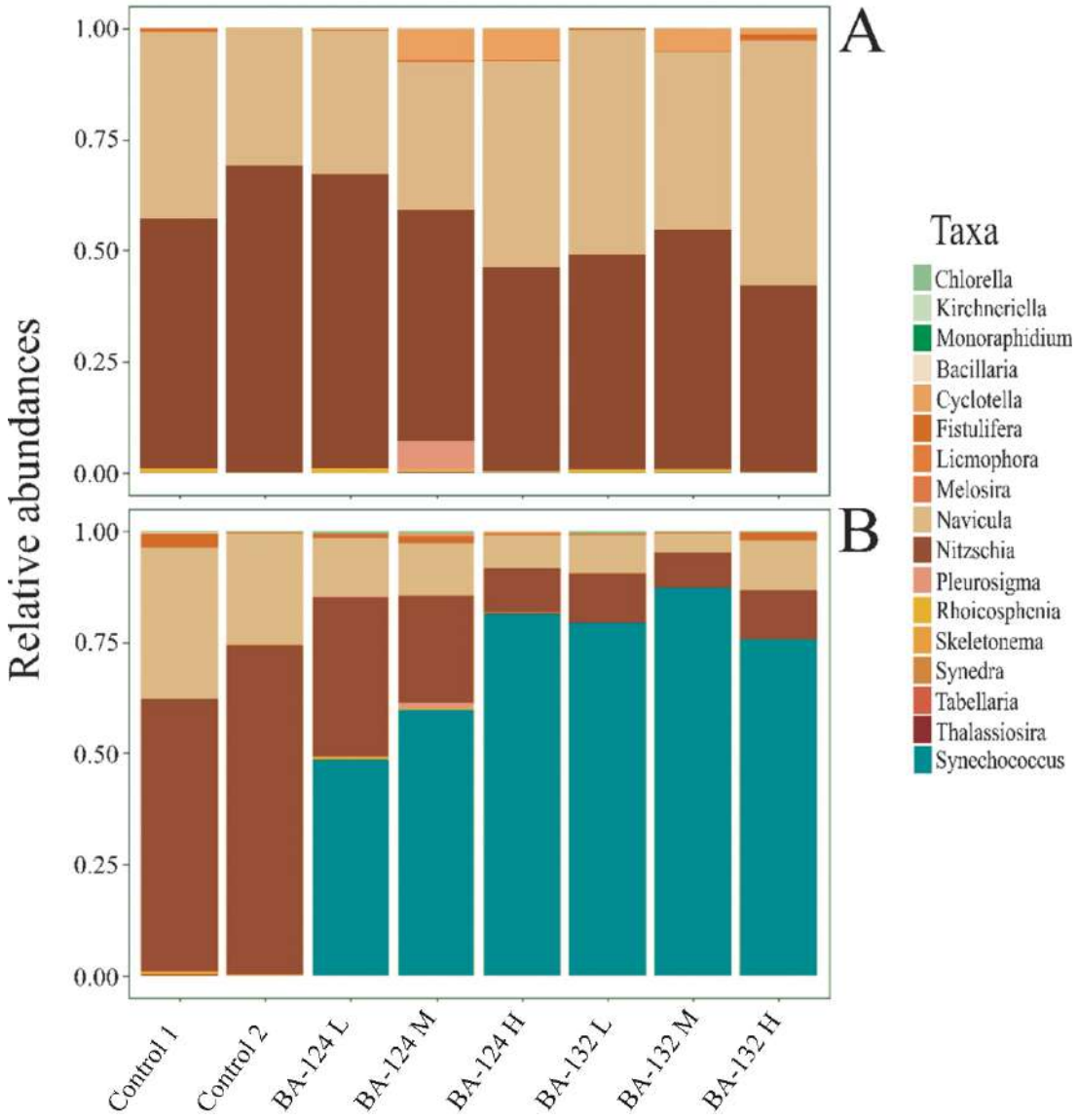

Fig. 2. Average relative abundances in equilibrium (day 31-79) in terms of biomass (A) and cells mL^{-1} (B) of taxa identified in controls and cultures with different inoculum sizes of *Synechococcus* phenotypes BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 initial *Synechococcus* cells mL^{-1}).

Correspondence analysis

The results of the CA ordination are shown in Fig. 3. The first two main components cumulatively explained 85.3% of the total variance.

The first dimension clearly shows a separation between the equilibrium community of BA-124 M and the rest. The taxa associated with this difference are the diatoms *Thalassiosira*, *Bacillaria*, *Cyclotella*, *Synedra*, *Licmophora*, *Tabelaria*, and *Pleurosigma*, although some of them were present at very low levels (Fig. 2). Roughly speaking, the second dimension orders a pattern of equilibrium communities leading from controls to increasing *Synechococcus* inoculum sizes. The taxa most strongly correlated with this pattern are *Skeletonema*, *Nitzschia*, and *Roicosphenia*, which are associated with control or low *Synechococcus* inoculum, while *Kirchneriella* and, of course, *Synechococcus* are associated with higher *Synechococcus* inoculum size. Overall, it is clear that the equilibrium community in the experimental series with smaller inoculum size of *Synechococcus* is most similar to the controls. On the other hand, the equilibrium communities of the BA-124 phenotype with high and medium inoculum size were found to be most different from those of the controls. In particular, the equilibrium community of BA-124 M was the most distant compared to all others.

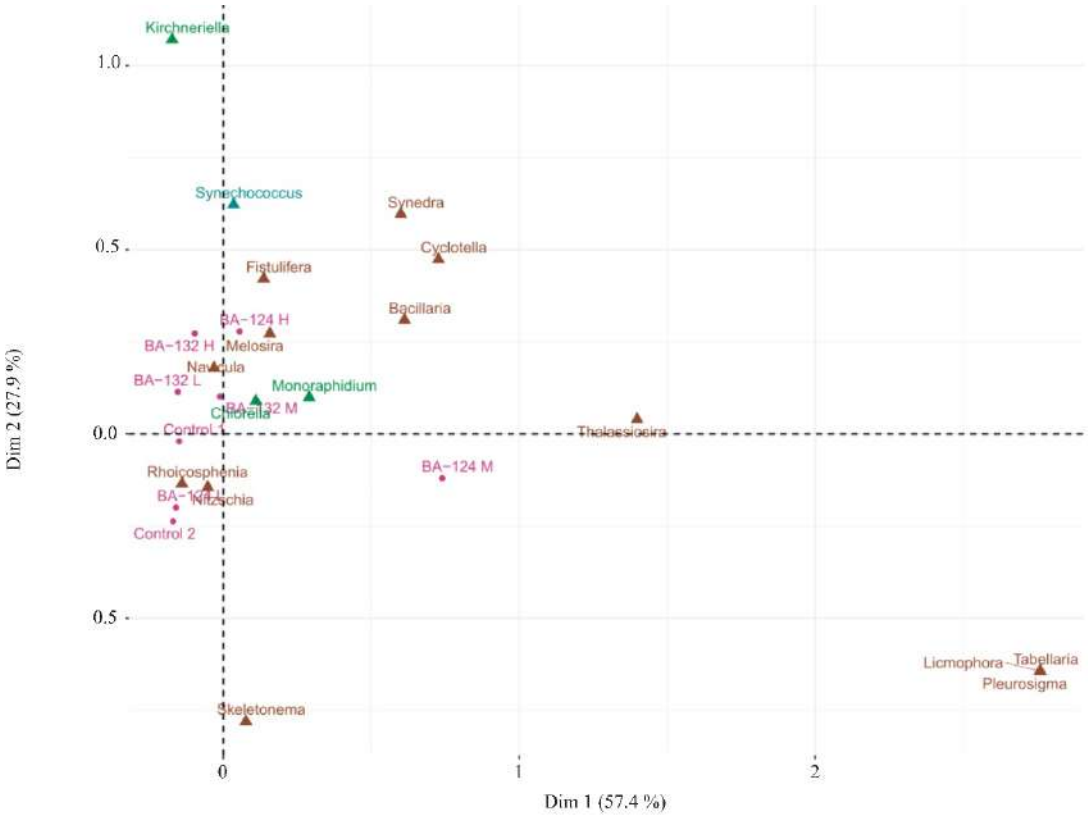

Fig. 3. Correspondence analysis of phytoplanktonic taxa in equilibrium communities in the controls and cultures with different inoculum sizes of *Synechococcus* phenotypes BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 initial *Synechococcus* cells mL^{-1}).

Taxa dominance

Both control groups (Control 1 and Control 2), the low inoculum sizes of *Synechococcus* (BA-124 L and BA-132 L) and the medium inoculum size of strain BA-132 showed *Nitzschia* and *Navicula* as dominant taxa (Fig. 4). The BA-124 M run showed the greatest diversity of dominant taxa, including *Nitzschia*, *Navicula*, *Cyclotella*, and *Pleurosigma*. In chemostats with high inoculum sizes of *Synechococcus* (both BA-124 H and BA-132 H), the dominance shifts from *Nitzschia* to *Navicula*. In the BA-124 H run, *Cyclotella* also appeared as the dominant species.

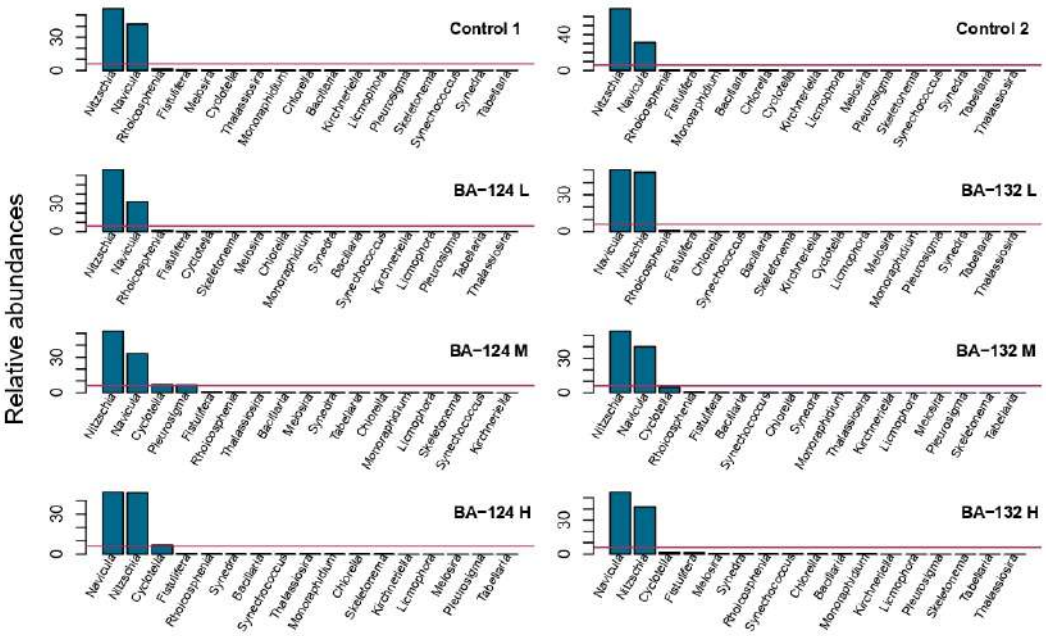

Fig. 4. Dominance analysis on each of the experimental runs: Controls and with different inoculum sizes of *Synechococcus* phenotypes BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 initial *Synechococcus* cells mL^{-1}).

Analysis of community diversity

The indices calculated for the equilibrium communities are shown in Fig. 5. The percentage of significant one-way ANOVAs was 60.2% for evenness, 99% for species richness and 87.7 for Shannon-Wiener diversity. Table 1 shows the results of the corresponding post-hoc Tukey tests, with significance presented as letters in the Fig.5. In these pairwise comparisons, evenness showed no significant differences. The control group had a slightly higher evenness, while the other experimental series were relatively similar (Fig. 5).

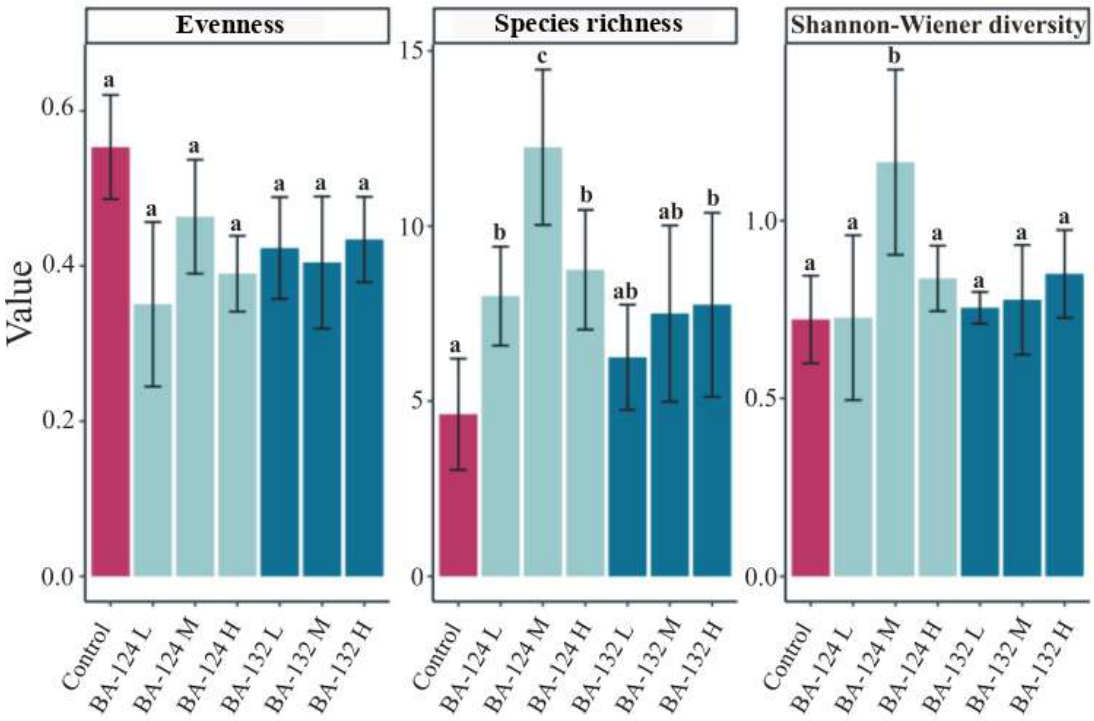

Fig 5. Evenness, Species richness, and Shannon-Wiener diversity index in the Control and with different inoculum sizes of *Synechococcus* phenotypes BA-124 and BA-120 (L-250000, M-1000000, H-2500000 initial *Synechococcus* cells mL^{-1}). Letters on top of the bars represent homogenous subsets according to Tukey tests (Table 1). Values are means ± SD.

Species richness showed the clearest pairwise differences between treatments. The control group had the lowest richness and was significantly different from all the BA-124 and BA-132 H treatments. BA-124 M had the highest richness and was significantly different from all other treatments. The other treatments showed relatively intermediate values and similarities between them.

Shannon-Wiener diversity showed a higher and significantly different value for trial BA-124 M. All other runs showed very similar average values and no significant differences, with a slight tendency to increase with the size of the *Synechococcus* inoculum for BA-132.

Table 1. Percentage of significant pairwise comparisons resulting from the 1000 post-hoc Tukey tests performed for Shannon-Wiener diversity index, Species richness, and Evenness for the Control and with different inoculum sizes of *Synechococcus* phenotypes BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 initial *Synechococcus* cells mL^{-1}).

Shannon-Wiener diversity	**Control**	**BA-124 L**	**BA-124 M**	**BA-124 H**	**BA-132 L**	**BA-132 M**
BA-124 L	6.1					
BA-124 M	59.1	61.7				
BA-124 H	7.5	10.9	62			
BA-132 L	0.1	2.9	69.7	0.2		
BA-132 M	2	6	65.8	1	0.1	
BA-132 H	11.6	14.8	58.5	0.4	1.3	3.3
Species richness						
BA-124 L	59.7					
BA-124 M	60.6	68.4				
BA-124 H	73.4	1.6	50.6			
BA-132 L	19.6	6.6	88.4	20.7		
BA-132 M	46.2	3.4	73.5	5.6	7.9	
BA-132 H	51.8	1.6	71.7	3.5	13	5.4
Pielou eveness						
BA-124 L	48.2					
BA-124 M	7	12.4				
BA-124 H	27.2	2	2.2			
BA-132 L	15.8	7.1	1.2	0.5		
BA-132 M	24	4.8	2.5	0.1	0.5	
BA-132 H	12.6	8.1	0.3	0.4	0	0.6

Discussion

According to resource competition theory, diversity and community composition at equilibrium should remain constant in each run and should not depend on *Synechococcus* sp. abundance. However, our data showed that the inoculum size of *Synechococcus* sp. influenced community diversity and species dominance for strain BA-124. At low and high inoculum sizes, communities did not differ much from the controls. At medium inoculum size, diversity and dominant species showed a clear change. Then, for strain BA-124, we observed the expected 'hump-shaped' effect of the Intermediate Disturbance Hypothesis

(IDH) on diversity (Shannon-Wiener index, Fig. 5, Table 1). We hypothesize that the best explanation, given the experimental design and previous works, is the effect of allelopathy from BA-124 strain. The nitrate data also supported the conclusion that this pattern is related to the presence or absence of an allelopathic effect in these two strains. A stronger allelopathic effect would mean that more biomass (cells) need to be removed from the cultures and therefore less nitrate is consumed. The medium and high inoculum sizes of BA-124 showed significantly higher average nitrate concentrations during equilibrium (Fig. 1, see Results). In BA-124 H, where the allelopathic effect is expected to be strongest, it takes longer than in all other experiments for nitrate to stabilize at the equilibrium level (around day 60 compared to day 30 in the experiments without BA-124), whereas the community composition in all cultures remained stable since day 31. Another observation consistent with this conclusion is that the abundance of BA-124 during equilibrium is proportional to the size of the inoculum (i.e., its ability to survive depends on the strength of the allelopathic effect), whereas BA-132 achieves near-stable abundance regardless of the size of the inoculum. This pattern in BA-132 would correspond to a community that is in equilibrium in terms of competition for resource exploitation, in agreement with classical resource competition theory (Tilman 1977). Accordingly, BA-132 would be expected to be a better competitor for nitrate than BA-124 (Konarzewska et al. in prep.), so it could be excluded non-competitively in a community composed of strong competitors. In other situations, *Synechococcus* strains such as BA-132 could survive in equilibrium with better competitors due to their specialization in light niches (Burton et al., 2018). However, this was not possible here, as there was not a single culture near the light limitation. It cannot be completely ruled out that a small degree of allelopathy by BA-132 is also at play in our experiments. This strain is known to be allelopathic under certain conditions determined by nutrient concentration, light and salinity (Śliwińska-Wilczewska et al., 2016, 2018; Konarzewska et al., 2022), which may not have been optimal to produce allelopathy in our current

experiments. In addition, there is a small and non-significant but consistent pattern of increase in diversity as a function of inoculum size for BA-132 (Fig. 5), which could be interpreted as an incipient effect of allelopathy that does not reach the "hump shape" due to its weakness.

Less likely, to our opinion, but still foreseeable alternative hypotheses for the explanation of the BA-124 results could be the existence of a mutualistic-like collaborative network between the dominant species and this *Synechococcus* sp. strain. These would be of the kind of a microbial consortium, allowing species to grow in syntropy with respect to different molecular forms of the limiting resource (nitrogen). However, it would be difficult to explain by this mechanism the inoculum density-dependence pattern observed in our results. In addition, this kind of interaction is more frequent in the context of the breakdown of complex substrates (Morris et al., 2013) which does not match very well with our system.

Barreiro et al. (2018) and Barreiro et al. (in preparation) also reported that intermediate levels of allelopathy are associated with increased diversity, while both low and high levels lead to a decrease in diversity. The "hump shape" associated with the effect of allelopathy could be understood as an extension or generalization of the IDH. In the allelopathy case, diversity would not necessarily be promoted by an increase in resource heterogeneity that allows the coexistence of a maximum number of K-strategists and r-strategists. Instead, it would be the coexistence of a maximum number of good competitors that are sensitive to allelopathy and of poor competitors that are not sensitive to allelopathy, as well as the allelopathic species. Our data suggest that the genera that clearly benefit from allelopathy are the diatoms *Cyclotella* and *Navicula*. Some chlorophytes such as *Kirchneriella*, *Monoraphidium*, and *Chlorella* were also favored by the effect of *Synechococcus*, especially by BA-124 (Fig. 2-4). Among the taxa that are more clearly negatively affected by *Synechococcus* and its allelopathy is *Nitzschia*, which clearly loses its dominance only in BA-124 and BA-132 H (Fig. 4). All this does not necessarily mean that

the species that benefit are completely resistant to allelopathy and the species that do not benefit are sensitive to allelopathy. In a complex community, resistance to allelopathy needs to be understood relative to individual taxa, so that, for example, a sensitive species might appear to be “favored" by allelopathy while its stronger competitors are still more sensitive. We also need to consider the possible interaction with abiotic factors that influence allelopathy. Strains of some of these chlorophyte genera, which appear here as resistant, were found to be sensitive to *Synechococcus* allelopathy under certain conditions in bioassays with individual target species, and the opposite is true for some of the diatom genera (Śliwińska-Wilczewska et al., 2016, 2017; Konarzewska et al., 2020). Moreover, in complex communities such as those used here, other species could also be allelopathic, making these relationships even more complex.

Our experimental approach of natural communities driven to equilibrium is relatively new in experimental ecology, existing to our knowledge, only two works published in ecology journals (Burton et al. 2018, Papanikolopoulou et al. 2018). This approach needs clarification of several aspects unfamiliar to most experimental ecologists working on similar systems. The first one is the absence of culture replication, which is often a logistic limitation, but also it is not strictly possible to replicate long-term data series, and at the same time, not strongly necessary due to the fact of the existence of time-dependent replicates. This feature is typical however, of similar works in continuous or semi-continuous culture systems that addressed similar questions to ours (Tilman 1977, Huisman et al 1999, Bénincà et al. 2008, Barreiro et al. 2018). Because of working with natural communities, for researchers unfamiliar with autotrophic growth in optimal culture conditions with inorganic nutrients, it could be argued the effect of organisms using different sources of energy and matter than the autotroph way, like heterotrophic bacteria and mixotroph phytoplankton. The heterotrophic bacteria, however, they are almost inexistent or absolutely irrelevant once the large mass of autotrophs is developed. At the same time, despite being heterotrophs, they

would be still subjected to the effect of nitrate limitation, since their only source of nitrogen would come from organic compounds released from the autotrophic phytoplankton, which is nitrate limited. This same applies to the potential presence of mixotrophic phytoplankton, which otherwise does not seem to be important given the dominant species present.

The classical models of resource competition (Tilman, 1977) predict that with a single limited resource, only the best competitor remains in equilibrium. In the experiments conducted by Tilman (1977), there were only two species. In our experiments, we believe that we can neglect the presence of species below the average abundance at equilibrium. Despite their low abundances, they are consistently present and show no trend towards exclusion. However, this presence is probably the consequence of two factors: 1) the impossibility of experimentally establishing a perfect equilibrium, even more difficult in complex communities and 2) the use of large sample sizes in sedimentation chambers (Ütermöhl), which allowed us to detect species at very low abundances, that would not be possible if we sampled in smaller volume chambers (like Sedgewick-Rafter) as it was the case of Burton et al. (2018). To summarize, in our case we can say that at least 2 species coexist in equilibrium in most of our experiments, with the exception of 3 species in BA-124 H and BA-132 M and 4 species in BA-124 M (Fig. 4). Barreiro et al. (2018) have already experimentally demonstrated that the existence of allelopathy may contradict Tilman's prediction for a single limiting resource. In the present case, given the conditions of our experimental design, and despite the limitation of not having measured allelochemical compounds (which are still unknown for this species) we consider that the most likely explanation for the coexistence of 3-4 species in the experimental run BA-124 M, which are responsible for a significantly higher diversity, would be the effect of *Synechococcus* sp. allelopathy. In order to explain the coexistence of 2-3 species at equilibrium in all the other runs, there are two main possibilities. First, it cannot be completely ruled out the existence of an allelopathic interaction between the dominant species. Second, it is very likely that the

two dominant species are almost competitively equivalent with regard to the limiting nutrient. This could be the case since the two species that dominate in those experimental runs (*Navicula* sp., *Nitzschia* sp.) are pennate diatoms of similar size, and might differ only slightly in their competitive ability for nitrate, and/or may show slightly different patterns of resource utilization, such as a different preference for the different forms of nitrogen. Although the nitrogen was provided in the form of nitrate, the presence of other forms derived from the biological transformation of the nitrate provided cannot be excluded. Other studies with natural communities driven to equilibrium also showed that this prediction of the classical theory of resource competition does not hold (Burton et al., 2018). In this case, the authors attributed the coexistence of more species than expected to light niche segregation. But this was not possible in the cases without light limitation in Burton et al. (2018) and, as mentioned above, is not possible at all in our experiments due to the absence of light limitation. Other work driving natural communities to equilibrium in the laboratory (Papanikolopoulou et al., 2018) showed few dominating species (around 3) but applying nutrient pulses, therefore there was not a long-term limiting resource.

The initial cell concentrations we used in our experiments are consistent with the cell abundances observed in the natural environment. Previous studies have shown that the highest *Synechococcus* cell concentrations in the Baltic Sea, ranging between 10^5 and 10^6 cells mL^{-1}, occur during the summer season, which is characterized by higher temperatures (Kuosa, 1991; Tamm et al., 2018; Zufia et al., 2021). The abundance of *Synechococcus* is usually higher in coastal waters, with maximum concentrations reaching 1.7×10^6 cells mL^{-1} (Mazur-Marzec et al., 2013). In winter, the maximum abundance of *Synechococcus* decreases to about 10^2 cells mL^{-1} (Zufia et al., 2022). The abundance of *Synechococcus* also varies depending on the pigment phenotype. While earlier studies by Legrand et al. (2003) indicated an even distribution of strains with dominant phycocyanin (PC) and phycoerythrin (PE), more recent studies indicated a higher abundance of PE-rich strains in the Baltic Sea

(Zufia et al. 2021). These authors reported that PE-rich strains dominated with abundances ranging from 8.2×10^2 cells mL^{-1} in winter to 3.8×10^5 cells mL^{-1} in summer, while PC-rich strains showed a maximum abundance of 2.1×10^5 cells mL^{-1} during the summer season, especially near the coast. Strain BA-124 is classified as PC-rich. Strain BA-132 is divided into several subgroups that have both PC- and PE-containing organisms, and their abundance in the environment is more difficult to determine. Repeated studies by Konarzewska et al. (under revision) show that the abundance of *Synechococcus* type BA-132 is similar to the abundance of PE-rich strains. Due to the relatively low abundance of phycocyanin (PC)-rich types in the natural environment, their population levels do not reach the mean abundances used in this study with strain BA-124. In contrast, the type represented by strain BA-132 in our study is more dominant in the real environment. However, due to the diversity of *Synechococcus* phenotypes, accurately determining the exact abundances of each strain in natural environments presents significant challenges.

The potential of allelopathy to influence species dynamics under real environmental conditions must be studied in interaction with other important factors. One of these factors is the grazing of zooplankton. Zufia et al. (2024) have shown that flagellates and ciliates can effectively control the abundance of *Synechococcus*. However, depending on the sensitivity of grazers to allelopathic substances secreted by cyanobacteria, their effect could result in the reduction or enhancement of cyanobacterial blooms by selective grazing (Scotti et al., 2015; Leitão et al., 2018). Species dynamics are also strongly influenced by abiotic factors such as light (Haverkamp et al., 2009), temperature (Suikkanen et al., 2013; Paerl, 2018), and nutrient loading (Granéli and Johansson, 2003a, b), and all these factors may also interact with allelopathy. Following the present theoretical and relatively simple experimental evidence of the potential effect of allelopathy on phytoplankton dynamics, further research efforts in complex plankton communities need to be undertaken to gain a more comprehensive understanding of how this mechanism works through its interactions

with other factors and using different allelopathic species. We will then significantly improve our understanding of phytoplankton allelopathy and define a greatly improved framework for it, which is essential to accurately address the study of its role under real field conditions.

Additional Supporting Information may be found in the online version of this article.

Authors Contribution Statement: Z.K.: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - Original draft preparation, A.B.F.: Conceptualization, Methodology, Supervision, Writing - Review & Editing, S.Ś-W.: Supervision, Writing - Review & Editing, J.M.: Data curation,V.V.: Supervision, A.L.: Supervision, Writing - Review & Editing. All authors contributed to the discussion of the results, supported manuscript preparation, and approved the final submitted manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest

None declared.

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Supplementary material

Allelopathy of *Synechococcus* increases diversity of coexisting phytoplankton communities

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Table 1S. pH values for the Control 1, Control 2 and with different inoculum sizes of *Synechococcus* phenotypes BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 initial *Synechococcus* cells mL^{-1}).

Day	Control 1	Control 2	BA-132 L	BA-132 M	BA-132 H	BA-124 L	BA-124 M	BA-124 H
3	7.10	7.35	7.36	7.45	7.60	7.37	7.33	7.54
7	7.02	7.07	7.15	7.21	7.24	7.16	7.19	7.26
11	6.91	6.92	7.10	7.15	7.27	7.12	7.13	7.22
15	7.02	7.13	7.15	7.20	7.48	7.15	7.20	7.24
19	7.10	7.17	7.34	7.35	7.46	7.25	7.35	7.34
23	6.69	6.94	7.26	7.26	7.32	7.24	7.26	7.36
27	6.79	6.95	7.25	7.32	7.40	7.24	7.42	7.46
31	7.15	7.05	7.18	7.22	7.32	7.20	7.30	7.35
36	7.17	7.24	7.31	7.35	7.38	7.23	7.33	7.37
40	7.14	7.22	7.34	7.40	7.45	7.28	7.40	7.44
44	7.07	7.12	7.35	7.38	7.39	7.26	7.33	7.35
49	7.20	7.14	7.24	7.36	7.50	7.23	7.35	7.27
52	7.23	7.23	7.30	7.29	7.40	7.19	7.40	7.33
56	7.18	7.20	7.29	7.33	7.39	7.20	7.44	7.31
60	7.20	7.28	7.35	7.29	7.42	7.30	7.31	7.41
64	7.15	7.24	7.39	7.30	7.40	7.26	7.32	7.45
71	7.28	7.29	7.40	7.40	7.41	7.33	7.33	7.50
75	7.19	7.22	7.33	7.45	7.50	7.44	7.46	7.49
79	7.32	7.42	7.55	7.51	7.55	7.43	7.46	7.59

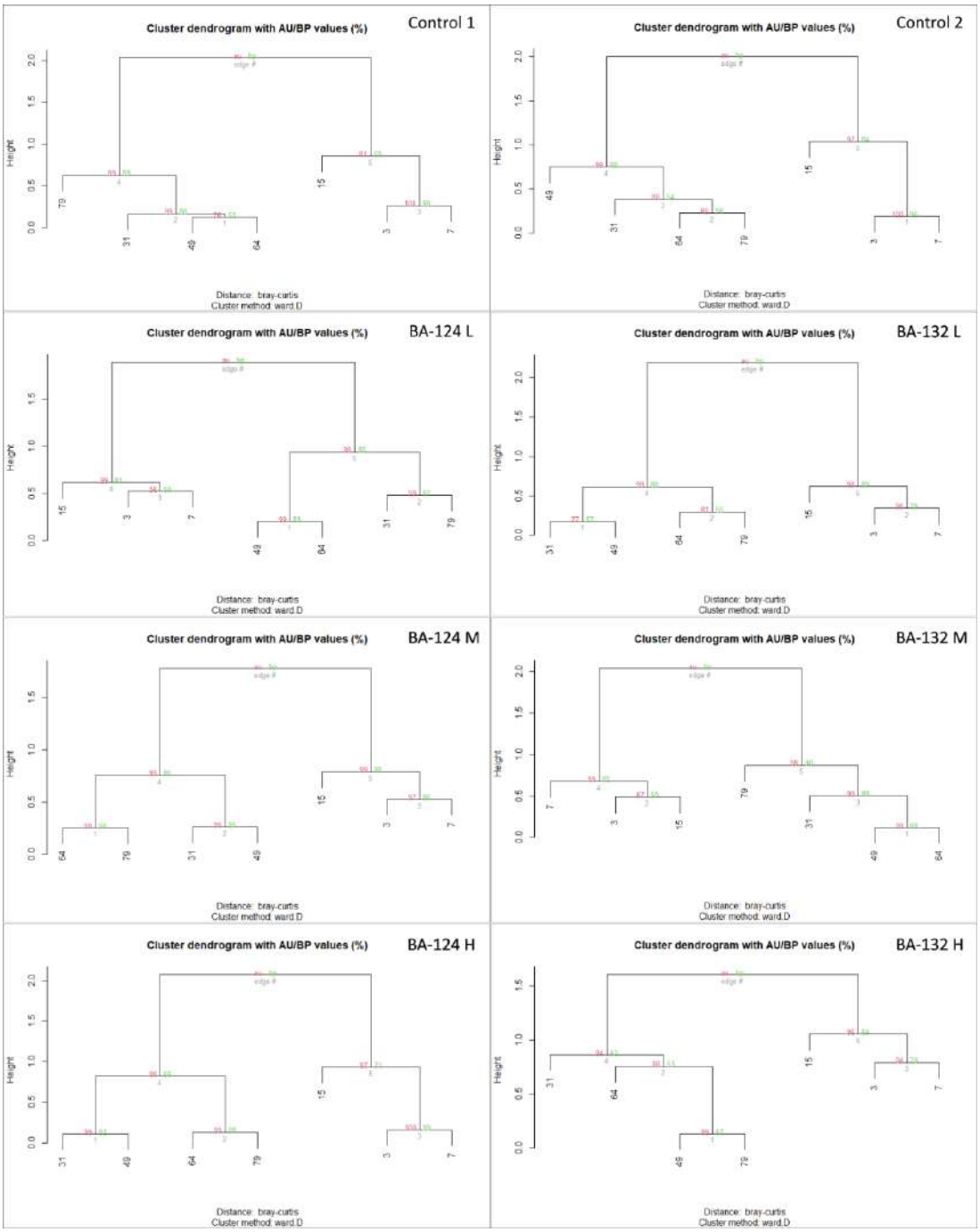

Fig. S1. The results of hierarchical clustering in controls and cultures with different inoculum sizes of tested *Synechococcus* phenotypes: BA-124 and BA-120 (L- 250000, M-1000000, H-2500000 cells mL^{-1}).

AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

Konarzewska, Z., Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Morais, J., Vasconcelos, V., Latała, A., 2025. Allelopathy of *Synechococcus* sp. phenotypes influences the structure of coexisting phytoplankton communities driven to equilibrium. *Marine Ecology Progress Series.*

Konarzewska Zofia 50%:

Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing - Original draft preparation

Barreiro Felpeto Aldo 30%:

Conceptualization, Methodology, Supervision, Writing - Review & Editing

Śliwińska-Wilczewska Sylwia 5%:

Supervision, Writing - Review & Editing

Morais Joao 5%:

Data curation

Vasconcelos Vitor 5%:

Supervision

Latała Adam 5%:

Supervision, Writing - Review & Editing

Konarzewska Zofia

Barreiro Felpeto Aldo

Śliwińska-Wilczewska Sylwia

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Vasconcelos Vitor

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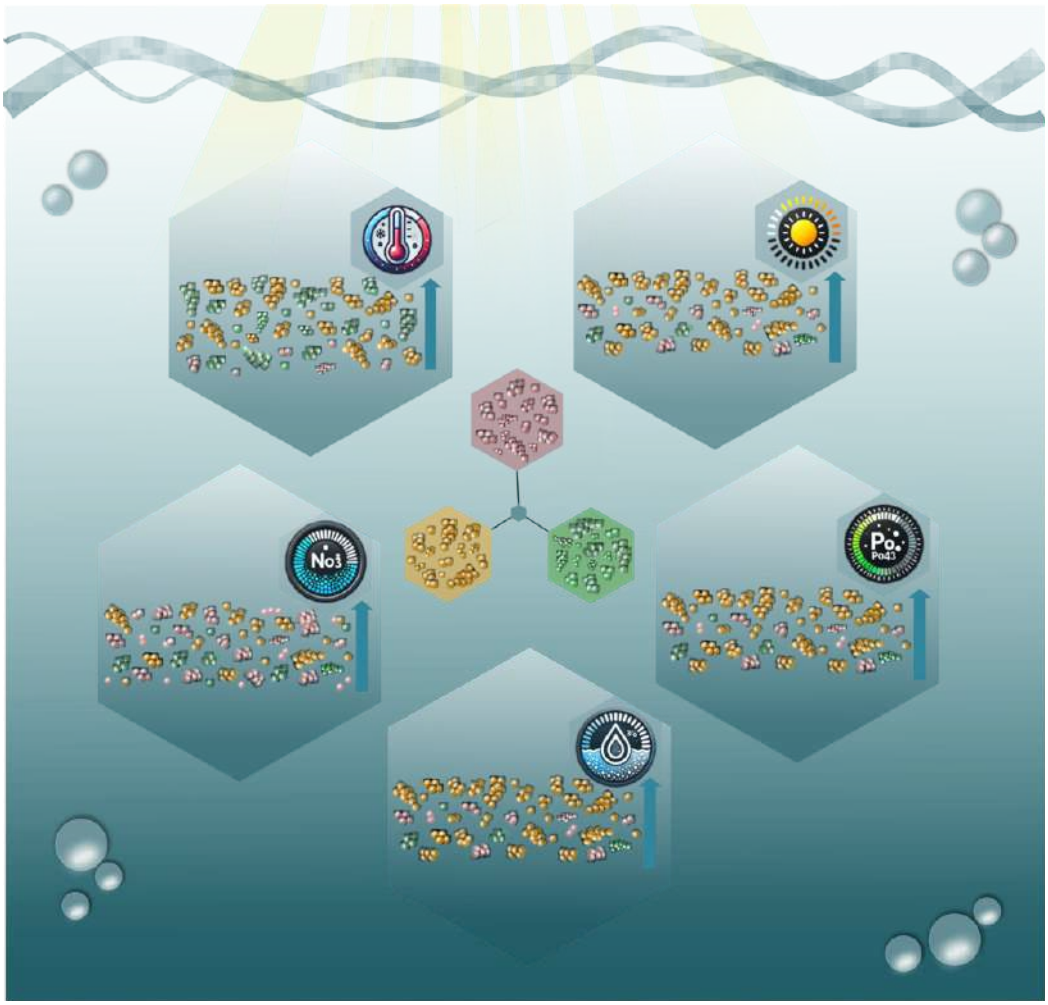
10 VERIFICATIONS OF TESTED HYPOTHESES

10 WERYFIKACJE HIPOTEZ

10.1 FIRST HYPOTHESIS (H1)

10.1 PIERWSZA HIPOTEZA (H1)



H1 (Publications 1 and 2): Abiotic factors that promote the growth of Synechococcus phenotypes enhance their allelopathic activity.

The occurrence of picocyanobacteria *Synechococcus* sp. in the environment strongly depends on the temperature (Berner et al., 2018; Śliwińska-Wilczewska et al., 2018c; 2020), light intensity (Mella-Flores et al., 2012; Eigman et al., 2018; Śliwińska-Wilczewska et al., 2025), salinity (Aguilera et al., 2023), and nutrient concentrations (Stal et al., 1999; Lagus et al., 2004). These factors collectively determine the *Synechococcus* growth and abundance, thriving particularly in warm, nutrient-rich, and moderate-to-low light environments (Zufia et al., 2021; Aguilera et al., 2023). However, it is important to note that the effects of selected

environmental factors on *Synechococcus* ecophysiology is phenotype-depended (Śliwińska-Wilczewska et al., 2018c; 2020; 2025).

What is more, the cyanobacterial allelopathy could be also modulated by environmental factors (Śliwińska-Wilczewska et al. 2016; Barreiro Felpeto et al., 2019). In some organisms, allelopathic activity is strongest under favorable growth conditions (Antunes et al., 2012), whereas in others, the production of allelochemicals is triggered by physiological stress, particularly under nutrient-limiting conditions (Krueger et al., 2012; Sim et al., 2023). Here, we hypothesize that the increase of allelopathic activity of different *Synechococcus* phenotypes is positively correlated with the optimal conditions for their growth.

Synechococcus phenotypes in marine environments exhibit distinct pigment profiles that allow them to occupy different ecological niches (Scanlan et al., 2009; Scanlan, 2012; Callieri et al., 2022). In the Baltic Sea, *Synechococcus* phenotypes have been classified into three main groups: Type 1, Type 2, and Type 3a (Six et al., 2007; Haverkamp et al., 2009). However, the Baltic Sea is highly dynamic ecosystem, with strong mixing and fluctuating environmental conditions (Holtermann et al., 2014), which may disrupt clear niche separation. This resulting in coexistence of different picocyanobacteria phenotypes in the same area and overlapping their spatial and temporal distributions rather than strictly occupying different light niches. Thus, we assumed that in the Baltic Sea different factors than color of light, could influence the spatial and horizontal distribution of *Synechococcus* phenotypes. While adaptations to different environmental conditions provide important mechanisms for *Synechococcus* niche differentiation, they cannot fully explain the coexistence of multiple phenotypes in such a fluctuating system. We hypothesize that allelopathy plays a crucial role in structuring *Synechococcus* distributions by allowing certain phenotypes to outcompete others under specific environmental conditions. Since temperature, light intensity, nutrient concentrations, and salinity modulate allelopathic interactions, their effects may further reinforce *Synechococcus* phenotypes in the aquatic ecosystem.

Publication 1 and **2** examine how abiotic factors - temperature, light intensity, nutrient concentration, or salinity affect the strength of allelopathic interactions among three distinct *Synechococcus* phenotypes. The experimental approach involves assessing their growth after addition of cell-free filtrate and in mixed culture experiments under different environmental conditions. Assuming that the strength of allelopathic activity is highly phenotype-dependent, this study aims to characterize intra-genus allelopathic interactions, which may play a crucial role in the diversity of *Synechococcus* phenotypes in brackish ecosystems. This is particularly important in predicting whether projected climate-driven changes in aquatic environments may favor the prevalence of specific picocyanobacterial phenotypes in coastal areas.

Higher temperature intensifies the allelopathic effects of *Synechococcus* phenotypes

Higher temperature generally increases the number of *Synechococcus* cells which is correlated with the peak of their abundances in the Baltic Sea during the summer period, when the water temperature is the highest (Zufia et al., 2021). Furthermore, laboratory studies conducted by Śliwińska-Wilczewska et al. (2018c; 2020) determined that temperature strongly influences the growth of *Synechococcus* phenotypes, with

differences shown between the phenotypes. That works indicated that Type 1 and Type 2 thrive in warmer environments, with their highest cell concentrations observed at the 30°C. In contrast, Type 3a was more flexible, exhibiting greater resistance to temperature fluctuations, and highest cell abundance at 22.5°C (Śliwińska-Wilczewska et al., 2020).

Results from **Publication 1** indicate that temperature had a significant impact on allelopathic activity, with the strongest effects observed at the highest tested temperature (23°C), which also corresponded to the highest growth obtained for all studied phenotypes in our work. At this optimal temperature, all phenotypes exhibited the highest allelopathic activity.

Our results align with previous research indicating that allelopathic activity in cyanobacteria is strongest under optimal growth conditions (Antunes et al., 2012; Ma et al., 2015; Śliwińska-Wilczewska et al., 2016). The findings support H1, demonstrating that high temperature, as a factor promoting growth, enhances the allelopathic activity of *Synechococcus* phenotypes.

The observed increase in allelopathic activity of *Synechococcus* phenotypes at higher temperatures suggests that projected climate-driven changes could intensify allelopathic interactions in the Baltic Sea region. With the increase of temperature, the enhanced allelopathic strength of *Synechococcus* phenotypes, particularly Type 1 and Type 3a, may lead to increased suppression of competing phytoplankton species. Meanwhile, *Synechococcus* Type 2, which was the most sensitive to allelopathy at high temperatures, could experience greater competitive disadvantages, leading to shifts in population structures. Type 1, in addition to its allelopathic ability, showed high resilience at 23°C, as it was the least affected by the allelopathic interactions of other phenotypes. This suggests that *Synechococcus* Type 1 may persist under warming conditions by combining its allelopathic influence with greater tolerance to competitive interactions. In **Publication 1** we demonstrated that during the summer period, when surface temperatures rise, Type 1 and Type 3a, which exhibit higher growth and stronger allelopathic activity under elevated temperatures, may be favored, in the upper layers in the Baltic Sea. In contrast, Type 2, being more sensitive to temperature stress and exhibiting weaker allelopathic interactions at higher temperatures, may be disadvantaged in the Baltic Sea during the summer season.

Higher light intensity increases the allelopathic effects of *Synechococcus* phenotypes

Light intensity is another key factor influencing the growth of *Synechococcus*. Previous works have shown that three main *Synechococcus* phenotypes respond differently to varied light intensity (Śliwińska-Wilczewska et al., 2018c). **Publication 1** showed that Type 1 thrived under low to moderate light intensities (10–55 µmol photons m^{-2} s^{-1}). *Synechococcus* Type 2 showed the highest cell-abundance at 55 µmol photons m^{-2} s^{-1}. *Synechococcus* Type 3a exhibited the highest adaptation to low-light conditions, with optimal growth at 10 µmol photons m^{-2} s^{-1} (Śliwińska-Wilczewska et al., 2020). However, in the most recent studies, Śliwińska-Wilczewska et al. (2025) investigated the growth responses of *Synechococcus* phenotypes belonging to Type 1 and Type 2 under varied light intensities and photoperiods. The study indicated that both Type 1 and Type 2 strains achieved their highest growth rates under high light conditions around 180–300 µmol photons m^{-2} s^{-1}, confirming that these strains can thrive in high-light conditions. Notably, one of the Type 2 strains reached one of the highest recorded growth rates noted for

cyanobacteria at 180 µmol photons m^{-2} s^{-1}. These findings indicate that *Synechococcus* can successfully grow under high light conditions, supporting their potential to dominate in surface waters during summer where the light intensity is the highest and the duration of its occurrence is the longest.

Results obtained in **Publication 1** showed that higher light intensity (about 50 - 100 µmol photons m^{-2} s^{-1}) enhanced allelopathic activity of tested *Synechococcus* phenotypes in both cell-free filtrates and mixed cultures experiments. Under higher light conditions, *Synechococcus* Type 3a exhibited the strongest allelopathic activity in both filtrate and mixed culture experiments. In filtrate experiments, Type 3a caused more than 50% growth inhibition of two other tested phenotypes. *Synechococcus* Type 2 also showed strong allelopathic activity under the highest light intensity, but its effect varied depending on the target phenotype. Type 1 exhibited strong allelopathic activity at higher irradiance, inhibiting Type 2 at both 50 and 100 µmol photons m^{-2} s^{-1} but affecting Type 3a only at 100 µmol photons m^{-2} s^{-1}. In mixed culture experiments, Type 2 and Type 3a had the strongest allelopathic effects under the highest studied light conditions (190 µmol photons m^{-2} s^{-1}), while for *Synechococcus* Type 1, the allelopathic activity was the strongest at low light of 10 µmol photons m^{-2} s^{-1}.

Previous studies have also shown increase of negative allelopathic activity in higher light conditions (Antunes et al., 2012; Śliwińska-Wilczewska et al., 2016). Moreover, it was noted that light intensity influences both allelopathic activity of studied cyanobacteria and the response of the target organism to allelopathy (Barreiro Felpeto et al., 2018b).

Our findings in **Publication 1** provided strong support for H1, demonstrating that higher light intensity between 50 and 100 µmol photons m^{-2} s^{-1} enhances the allelopathic activity of *Synechococcus* phenotypes. The results from the cell-free filtrate experiments revealed that allelopathic effects increased with higher light intensity across all phenotypes, suggesting that light intensity could not only influences vertical distribution of these organisms but also modulates their competitive interactions. The study further revealed that under high light conditions, Type 2 and Type 3a, showed stronger allelopathic activity, with Type 3a exhibiting the strongest allelopathic effects on coexisting *Synechococcus* phenotypes. In contrast, the Type 1 displayed a more variable response, exhibiting weaker allelopathy under high light in mixed cultures. As a result, during summer period, when light availability is the highest, the Type 3a and Type 2, highly allelopathic strains (may gain a competitive advantage, potentially dominating in phytoplankton communities. Given their higher growth and stronger allelopathic activity under elevated light conditions, these phenotypes are likely to be favored in the upper layers of the Baltic Sea, where they can inhibit competing phytoplankton and influence ecosystem structure to the greatest extent.

Elevated nutrient availability intensifies the allelopathic effects of *Synechococcus* phenotypes

Nutrient availability, particularly nitrate (NO_3^-) and phosphate (PO_4^{3-}), plays a critical role in cyanobacterial growth, with elevated nutrient concentrations promoting biomass accumulation (Mills et al., 2004; Schindler, 2006; Lagus et al., 2007). **Publication 2** showed that all three *Synechococcus* phenotypes exhibited the highest growth rates at moderate nitrate and phosphate conditions.

Nitrate concentration significantly influenced allelopathy, particularly for *Synechococcus* Type 2 and Type 3a. In cell-free filtrate experiments under high nitrate conditions, both Type 2 and Type 3a exhibited stronger allelopathic activity Type 1. *Synechococcus* Type 1 also exhibited allelopathic activity against Type 2 in high-nitrate conditions, though its strongest effect was observed in moderate nitrate. In mixed cultures, allelopathic activity of all phenotypes increased with high nitrate availability, However, for Type 1 and Type 2 higher allelopathic activity was noted in in low phosphate conditions, compared to Type 3 which increased allelopathy with the increase of phosphate.

Phosphate concentration also had a significant impact on *Synechococcus* allelopathy, particularly for Type 1 and Type 3a in low phosphate conditions. In mixed cultures, in higher phosphate concentrations Type 1's allelopathy is weaker, while Type 2 and Type 3a increased their allelopathic activity with rising phosphate concentrations.

Previous works have shown that nitrate and phosphate concentrations strongly influence the allelopathic activity of *Synechococcus* (Śliwińska-Wilczewska and Latała, 2018). They reported an increase in allelopathic activity with the increase of both nutrients. On the other hand, works with different cyanobacteria reported stronger allelopathic activity under phosphorus limitation (Antunes et al. 2012; Krueger et al., 2012; Sim et al., 2023).

Our findings in **Publication 2** support H1 only for nitrate, showing that increased nitrate enhanced allelopathic activity in *Synechococcus*, with phenotype-specific responses. However, phosphate limitation intensified allelopathy in Type 1 and Type 3a in cell-free filtrate experiments and for Type 1 and Type 2 in mixed cultures. These results suggest that nutrient availability modulate *Synechococcus* allelopathy. It is important to highlight that under moderate nutrient conditions, corresponding to the f/2 growth medium, nutrients are already present in excess to support proper picocyanobacterial growth. Additionally, clear conclusions from this study regarding the effect of nutrients on *Synechococcus* growth may be limited by the short duration of the experiments. The delayed response of growth to the nutrient composition of the medium could also affect the observed outcomes. The authors hypothesize that the differences in allelopathic activity between moderate and high nitrate conditions may not be substantial, indicating that factors beyond nitrate concentration could be influencing the observed patterns.

In the Baltic Sea, where coastal eutrophication results in high nutrient availability, the allelopathic impact of *Synechococcus* is expected to be strongest under nitrogen-rich conditions. However, strong allelopathic activity was also observed under phosphate-limited conditions, suggesting that nutrient availability alone may not fully predict allelopathic effects. Moreover, phosphate limitation intensified allelopathy especially in *Synechococcus* Type 1 and Type 3a, indicating that during periods of phosphorus scarcity, common in stratified summer conditions, these phenotypes may exert stronger suppressive effects on competing species. With ongoing eutrophication trends increasing nitrogen inputs while phosphate remains a limiting factor, Type 1 is expected to be the favored phenotype, as it maintains both high growth and strong allelopathic potential under phosphate limitation. Meanwhile, Type 3a may gain a competitive advantage in more phosphate-enriched zones, such as areas of upwelling or external phosphorus release from sediments. These findings suggest that nutrient-driven shifts in allelopathic interactions could further favor certain *Synechococcus* phenotypes in the Baltic Sea, potentially altering phytoplankton community composition and ecosystem functioning in response to ongoing eutrophication. With the most notable changes in *Synechococcus* allelopathic interactions in coastal areas,

where nutrient fluctuations are most significant, particularly due to the river runoff. These regions experience significant nitrogen enrichment due to anthropogenic activity, leading to conditions that strongly favor the growth and allelopathic activity of Type 2 and Type 3.

Salinity enhances allelopathy within the optimal range for each phenotype

The Baltic Sea exhibits a strong salinity gradient, ranging from approximately 20 PSU in the Kattegat to 2 PSU in the Bay of Bothnia, due to the continuous exchange of saline water from the North Sea and freshwater inflows from rivers (Lehmann et al., 2022). Salinity plays a important role in shaping the spatial distribution and diversity of *Synechococcus* (Aguilera et al., 2023), with each phenotype exhibiting distinct salinity preferences. *Synechococcus* Type 1 showed optimal growth at 8 and 13 PSU, whereas Type 2 and Type 3a reaching their highest concentrations at 18 PSU (Śliwińska-Wilczewska et al., 2018c). Our data presented in **Publication 1** showed that salinity also significantly affected *Synechococcus* allelopathy. Type 1 had the strongest allelopathic effect at low salinity of 3 PSU, while Type 2 showed the highest inhibitory effect at 8 PSU. The allelopathic effect of Type 3a was strongest against Type 1 at 3 PSU and Type 2 at 13 PSU. On the other hand, in mixed cultures, the strongest allelopathic effect of all phenotypes was observed at 8 PSU.

Our results partially support hypothesis H1, indicating that while allelopathic activity is influenced by salinity, it does not always peak within the optimal growth range of each phenotype. Instead, moderate salinity levels (8 PSU) appear to enhance interspecific allelopathic interactions, regardless of individual growth optima. Type 1 exhibited optimal growth at 8–13 PSU but showed its strongest allelopathic effect at 3 PSU, suggesting that sometimes stress-induced allelopathy may play a role in lower-salinity environments. Type 2 reached peak abundance at 18 PSU, but its strongest allelopathic effect occurred at 8 PSU, indicating that allelopathy may be maximized in intermediate rather than optimal growth conditions. Similarly, Type 3a thrived at 18 PSU but had the highest allelopathic activity at 3 PSU against Type 1 and 13 PSU against Type 2, suggesting salinity-dependent target specificity rather than a direct alignment with growth optima. Additionally, in mixed cultures, the strongest allelopathic effects for all phenotypes were observed at 8 PSU, reinforcing the idea that moderate salinity conditions favor allelopathic interactions, possibly due to heightened competition and resource limitation in brackish waters. Nearshore environments with lower salinity (3 PSU) are likely to favor Type 1, whereas Type 3a, with its strongest allelopathic effects against Type 2 at higher salinity levels (13 PSU), is expected to have a competitive advantage in offshore waters. The highest allelopathic activity at 8 PSU suggests that Type 2 is well-adapted to estuarine or brackish environments, where salinity fluctuates but generally stays within its optimal range. These conditions likely provide a competitive advantage for Type 2, allowing it to thrive and exert strong allelopathic effects against other phytoplankton species. Similarly to our findings, previous studies have shown that salinity influences allelopathic activity, with stronger effects observed in 8 PSU (Śliwińska-Wilczewska et al., 2016). Thus, while salinity does modulate allelopathic activity in *Synechococcus*, our results indicate that the strongest allelopathy occurs either within the optimal salinity range for growth or under moderate salinity conditions where interspecific competition is highest.

However, the study has shown that *Synechococcus* phenotypes are highly adapted to small differences in salinity, making it difficult to establish a clear correlation between

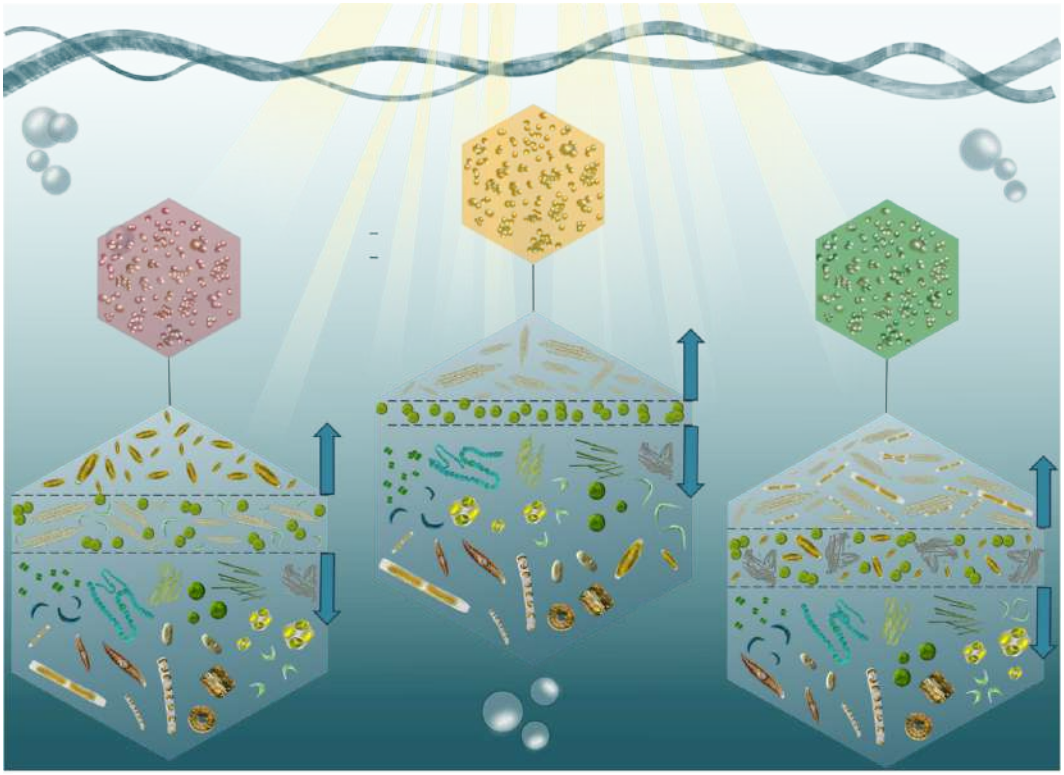
growth and allelopathy. The authors hypothesize that the range of salinities used may not have been optimal for testing the intended hypothesis, as the differences between salinity steps were too small to produce a noticeable and clear effect. These findings indicate that the influence of salinity changes on allelopathic activity requires further investigation.

H1 Summary and implications:

Publications 1 and **2** confirmed that abiotic factors—specifically temperature and light intensity—that enhance the growth of *Synechococcus* also intensify their allelopathic activity. Our findings suggest that nutrient availability influences *Synechococcus* allelopathy in a phenotype-specific manner, though the extent of this impact remains uncertain due to the short experimental duration and potential delayed responses. However, optimal salinity does not consistently enhance allelopathic properties. Instead, moderate salinity conditions (8 PSU) appeared to intensify interspecific allelopathic interactions. Thus, the hypothesis (H1) cannot be fully confirmed. Nonetheless, the findings indicate that the relationship between growth-promoting conditions and allelopathic activity may depend on the environmental conditions in which *Synechococcus* naturally occurs and could be particularly pronounced during the summer, with allelopathy likely serving as a competitive strategy. Moreover, each *Synechococcus* phenotype responds differently to these factors, highlighting the role of allelopathy in shaping their ecological success, distribution, and niche differentiation.

The strongest allelopathic effects were observed under high temperature and light conditions, suggesting that allelopathy plays its most significant ecological role in the surface waters. This effect is expected to be intensified during summer, when increased irradiance and temperature enhance *Synechococcus* growth and allelopathic activity. Based on these results, in surface waters during the summer period, where both light and temperature are high, Type 3a is expected to gain dominance due to its strong allelopathy and high growth potential. In nutrient-enriched, phosphate-limited coastal waters, Type 1 and Type 2 will likely be favored. Offshore, where salinity is higher, Type 3a may be more competitive, particularly in areas with elevated phosphate availability. Additionally, the seasonal increase in nitrogen fixation by filamentous cyanobacteria could further favor allelopathic *Synechococcus* phenotypes.

These findings suggest that climate-driven changes, such as rising temperatures and increased water stratification, along with human-induced factors like nutrient enrichment, may further enhance the impact of allelopathy in shaping phytoplankton communities. Previous studies suggest that global environmental changes tend to favor PE-rich *Synechococcus*, (Type 2 and Type 3a) a trend already observed in the Baltic Sea (Haverkamp et al., 2009; Mazur-Marzec et al., 2013; Zufia et al., 2021; Śliwińska-Wilczewska et al., 2025). Our findings align with this pattern, particularly for Type 3a. These expected shifts in *Synechococcus* dominance and allelopathic activity could have long-term implications for phytoplankton community composition and ecosystem functioning in the Baltic Sea.



H2 (Publication 3): Allelopathy exhibited by different Synechococcus sp. phenotypes affects co-occurring phytoplankton species.

Synechococcus sp. is widely distributed in marine ecosystems, where it interacts with co-occurring phytoplankton species through allelopathy (Śliwińska-Wilczewska et al., 2017a). Previous studies have demonstrated the allelopathic activity of *Synechococcus* Type 1 on co-occurring phytoplankton species (Śliwińska-Wilczewska et al., 2018b), as well as in short-term experiments on natural phytoplankton communities (Śliwińska-Wilczewska et al., 2017a). However, allelopathic interactions among cyanobacteria and microalgae have been shown to be highly species- and strain-specific (Suikkanen et al., 2004; Barreiro Felpeto et al., 2018). Given the species- and strain-specific nature of allelopathic interactions among cyanobacteria and microalgae, this study aims to investigate the allelopathic potential of different *Synechococcus* phenotypes present in the Baltic Sea. This study examines the effects of cell-free filtrates from three distinct *Synechococcus* phenotypes—Type 1, Type 2 and Type 3a — on co-occurring phytoplankton species. Specifically, it evaluates their impact on the growth, chlorophyll fluorescence, and pigment composition of 18 phytoplankton strains from three major taxonomic groups: Heterokontophyta (in this work - diatoms), Chlorophyta (green algae), and Cyanophyta (cyanobacteria). By analyzing these responses,

this study aims to determine how allelopathic activity differs among *Synechococcus* phenotypes.

The allelopathic effect is different depending on the *Synechococcus* phenotype

Publication 3 demonstrated that allelopathic activity varied among *Synechococcus* phenotypes, with *Synechococcus* Type 3a exhibiting the strongest allelopathic activity on tested strains (Table 3).

We demonstrated that *Synechococcus* Type 3a significantly reduced the number of cells in diatoms and green algae. In **Publication 3**, *Skeletonema marinoi* and *Amphora coffeaeformis* showed the highest growth inhibition (20% and 29% of growth compared to control, respectively). Type 3a filtrate addition had a negative effect on the growth of target green algae species, with the highest inhibition noted for *Kirchneriella obesa* (53% of growth compared to control). In cyanobacteria a negative effect on growth was observed, with the exception of *Aphanizomenon* sp. showing 29% stimulation of growth, compared to control. **Publication 3** also showed that *Synechococcus* Type 1 has the strongest allelopathic activity against green algae, with *K. obesa* showing the strongest growth inhibition (62% growth compared to control). After addition of the cell-free filtrate, diatoms and cyanobacteria exhibited both inhibition and stimulation of growth. What is more, *Synechococcus* Type 2 had a moderate allelopathic effect across diatoms, green algae, and cyanobacteria. *A. coffeaeformis* was the most inhibited diatom (36% growth compared to control), while *Navicula perminuta* exhibited 29% increase of growth. Type 2 had a negative impact on growth of cyanobacteria, with only *Aphanizomenon* sp. showing no significant response.

Table 3. Allelopathic effect of *Synechococcus* phenotypes on total number of tested diatoms, green algae, cyanobacteria. +; indicate stimulation of growth, 0; indicate no effect on growth, -; indicate inhibition of growth, ND; indicate no recorded data.

***Synechococcus* phenotype**	**Diatoms**			**Green algae**			**Cyanobacteria**		
	0	**+**	**-**	**0**	**+**	**-**	**0**	**+**	**-**
Type 1	1	1	4	1	ND	5	1	1	4
Type 2	ND	1	5	2	ND	4	1	0	5
Type 3a	ND	ND	6	1	ND	5	0	1	5

Previous studies align with our conclusions, reporting variations in the strength and effects of allelopathy depending on *Synechococcus* strains (Paz-Yepes et al., 2013; Śliwińska-Wilczewska et al., 2017b; Barreiro Felpeto et al., 2018b). Overall, these findings suggest that *Synechococcus* Type 3a, with its allelopathic effects, may have the strongest competitive advantage over coexisting species, inhibiting particularly diatoms and green algae.

The allelopathic effect is different depending on the target phytoplankton species

The response to *Synechococcus* allelopathy varied significantly among target phytoplankton groups. We have shown in **Publication 3** that diatoms and green algae are the most sensitive groups, while cyanobacteria exhibited both inhibitory and stimulatory effects.

Among diatoms, the strongest inhibition rates were recorded in *S. marinoi* (20% and 35% of control after addition of cell free filtrate obtained from Type 3a and Type 2 phenotypes, respectively) and *A. coffeaeformis* (29% and 36% after addition of cell free filtrate obtained from Type 3a and Type 2, respectively). However, *N. perminuta* exhibited stimulation as a result of Type 2 allelopathy (126% growth compared to control), highlighting species-specific variability. Green algae were also highly sensitive to *Synechococcus* allelochemicals, *K. obesa* was the most inhibited species (with 53%, 63% and 48% growth compared to control, as a result of allelopathic activity of Type 3a, Type 1 and Type 2, respectively). *Chlorella fusca* was not sensitive to *Synechococcus* phenotypes allelopathy.

Cyanobacteria showed the most variable responses, with some species experiencing inhibition while others were stimulated. *Aphanizomenon* exhibited a strong positive response after the addition of Type 3a cell-free filtrate (with 26% growth stimulation) but showed no effect after the addition of Type 2 filtrate. *Nitzschia fonticola* displayed the highest stimulation (120% and 90% as a result of allelopathic activity of Type 1 and Type 2, respectively), while *Nostoc* sp. and *Pseudanabaena* sp. were inhibited under all treatments.

Multiple studies have demonstrated the effects of *Synechococcus* Type 1 filtrates on various phytoplankton species, including the diatoms *N. perminuta* (Śliwińska-Wilczewska et al., 2016), *Bacillaria paxillifer* and *S. marinoi* (Śliwińska-Wilczewska and Latała, 2018), as well as the cyanobacteria *Nostoc, Phormidium* sp., and *Aphanizomenon flos-aquae* (Śliwińska-Wilczewska et al., 2017b), *Nodularia spumigena* (Barreiro et al., 2018b), and the green algae *Chlorella vulgaris* and *Oocystis submarina* (Śliwińska-Wilczewska and Latała, 2018). These studies have reported varying responses, including inhibition, stimulation, or resilience to allelopathic activity, as seen in **Publication 3**.

These observations highlight the species-specific nature of allelopathic interactions. The differential sensitivity of diatoms, green algae, and cyanobacteria to *Synechococcus* allelopathy suggests that if these interactions persist in natural environments, allelochemicals can act as a selective force, influencing which species are favored or suppressed in phytoplanktonic community.

The addition of *Synechococcus* cell-free filtrates resulted in significant alterations in pigment content (chlorophyll *a*; Chl *a*, carotenoid; Car) and the maximum quantum yield of PSII (F_v/F_m), highlighting disruptions in the photosynthetic processes of co-occurring phytoplankton species. We showed that Type 3a caused the strongest decline in Chl *a*, Car, and F_v/F_m in diatoms and green algae, indicating a suppression of their photosynthetic efficiency. In cyanobacteria significant inhibition of Chl *a* and Car content was observed, with the exception of *Planktolyngbya* and *Aphanizomenon.*

These findings align with previous studies demonstrating that allelopathic compounds released by cyanobacteria can negatively impact fluorescence and photosynthetic activity (Figueredo et al., 2007; Gantar et al., 2008; Śliwińska-Wilczewska et al., 2016, 2017a, 2018, 2019; Barreiro Felpeto et al., 2019). The decline in F_v/F_m observed in **Publication 3** suggests significant impairment of PSII function, a key indicator of

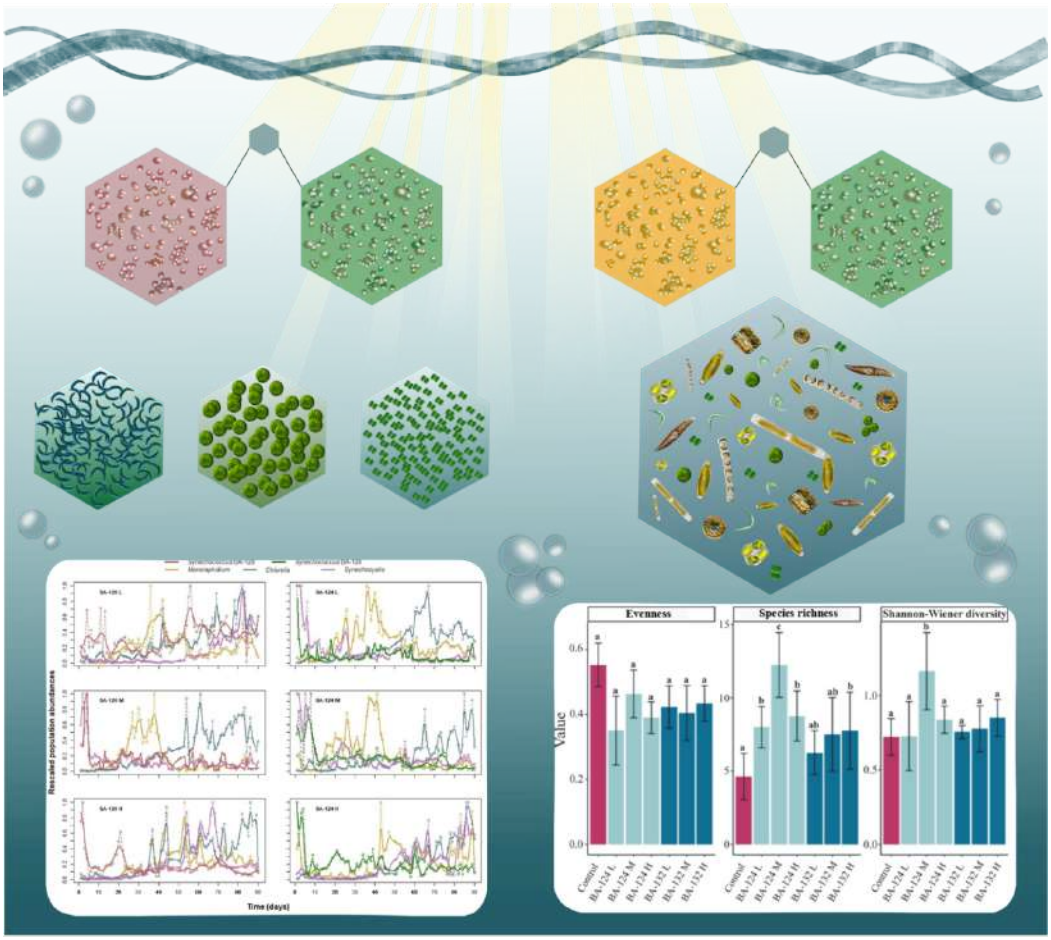
photosynthetic stress (Qian et al., 2010; Machado et al., 2015). Allelopathic compounds from cyanobacteria have been shown to suppress photosynthesis and reduce the number of affected species cells (Śliwińska-Wilczewska et al., 2019).

The effect of *Synechococcus* allelopathy on Chl *a*, Car, and F_v/F_m was weaker than its effect on the growth of the studied phytoplankton species. Some species exhibited growth inhibition without impact fluorescence and photosynthetic activity. This discrepancy suggests that growth integrates multiple metabolic and physiological processes, as well as compensation mechanisms. Some phytoplankton species may employ compensation strategies, such as energy reallocation, where cells prioritize photosynthetic activity over biomass accumulation. These findings highlight the complexity of allelopathic interactions and suggest that *Synechococcus* allelopathy can influence phytoplankton community structure not only through direct growth suppression but also by disrupting key physiological functions. The long-term consequences of these effects may be particularly relevant in seasonal succession patterns, where prolonged exposure to allelochemicals could weaken certain phytoplankton groups over time, favoring *Synechococcus* in the ecosystem.

H2 summary and implications:

The findings from **Publication 3** confirm that allelopathy exhibited by different *Synechococcus* phenotypes affects differentially co-occurring phytoplankton species. The strength of allelopathic interactions was highly phenotype-dependent, with *Synechococcus* Type 3a exhibiting the strongest inhibitory effect on co-occurring phytoplankton species, particularly diatoms and green algae. In contrast, *Synechococcus* Type 1 and Type 2 displayed weaker allelopathic effect.

The seasonal dynamics of phytoplankton communities may be influenced by allelopathic interactions among *Synechococcus* phenotypes. Diatoms, which typically dominate in nutrient-rich spring conditions, were the most suppressed by allelopathy, suggesting that *Synechococcus* could influence the spring diatom blooms. This effect may be particularly pronounced during early warming events, where *Synechococcus* populations expand earlier in the season and exert stronger allelopathic pressure. During the summer season, increasing temperature and light intensity, along with decreasing nutrient concentrations, create favorable conditions for *Synechococcus* and filamentous cyanobacteria. The higher abundance of *Synechococcus*, and thus the stronger allelopathic effect can inhibit the filamentous cyanobacteria. However, *Aphanizomenon* may benefit from *Synechococcus* allelopathy, reinforcing its presence in the environment or even caused their booms. Allelopathic activity of *Synechococcus* may also limit green algae abundance through the year.



H3 (Publications 4 and 5): The strength of allelopathy from Synechococcus sp. phenotypes influences plankton community diversity:

Low allelopathic strength is associated with lower diversity due to dominance by strongest competitors. Intermediate allelopathic intensity increases community diversity due to coexistence between allelopathic weak competitors and sensitive stronger competitors. High allelopathic intensity reduces diversity due to dominance by Synechococcus sp. phenotypes.

Since decades ago, theoretical works suggested that allelopathy could influence phytoplankton dynamics (Chao and Levin, 1981; Durret and Levin, 1997; Roy and Chattopadhyay, 2007; Roy, 2009). Allelopathy would work as an additional dimension for niche differentiation, and it was pointed out that, under certain conditions, it could increase community diversity. However, there was no experimental support for this suggestion, until Barreiro et al. (2018) provided both theoretical and experimental evidence that allelopathy

can facilitate the coexistence of two phytoplankton species competing for a single limiting nutrient. This work contradicts with the mechanistic competitive exclusion principle demonstrated experimentally by Tilman (1977). The phytoplankton coexistence was facilitated by the susceptibility of the superior competitor to allelopathic effects exerted by the weaker competitor. Notably, diversity followed a "*hump-shaped*" response, where intermediate allelopathic effect promoted coexistence, while extreme (low or high) allelopathy led to competitive exclusion. This aligns with an extended interpretation of the Intermediate Disturbance Hypothesis (IDH), where allelopathy would be considered a form of disturbance (Wilkinson, 1999). This effect of increasing diversity at intermediates levels of allelopathy is also a theoretical prediction from other works (Chao and Levin, 1981; Durret and Levin, 1997; Hulot and Huisman, 2004; Roy, 2009).

Based on these previous works, we hypothesized that allelopathy of *Synechococcus* phenotypes in interaction with nitrate competition will show an analogous result, i.e., increase the community diversity at intermediate levels, both in experimental systems with selected co-occurring phytoplankton species and in natural phytoplankton communities. Confirming this hypothesis would suggest that allelopathy could have a role in community structure and dynamics of Baltic phytoplankton communities where *Synechococcus* is a key element of this ecosystem.

The Effect of *Synechococcus* allelopathy in small laboratory communities

This study provides experimental evidence with theoretical support for the coexistence of selected species on a single limiting resource (nitrate) by the interplay between nutrient competition and allelopathy. However, the overall results did not conform to the predicted influence of different strength of allelopathy under the working hypotheses. This was because the same outcome of competition (oscillatory coexistence of all the species) was found under all the intended levels of allelopathy (low, intermediate, and high, as a function of initial *Synechococcus* abundance).

Based on our analysis of competitive abilities for nitrate (**Publication 4**), *Synechococcus* Type 2 (BA-120) was identified as the strongest competitor for nitrate. The pairwise allelopathic assays indicated significant allelopathic interactions (both inhibitory and stimulatory) between most of the pairs, with the strongest effect observed in *Monoraphidium* sp. against *Chlorella* sp. Among the two *Synechococcus* phenotypes, Type 1 (BA-124) exhibited stronger allelopathic effects, particularly against *Chlorella*. This situation, although posing a very interesting example of a network of chemical interactions, blurs our initial aim of using *Synechococcus* abundance as a proxy for the strength of allelopathy.

There could be three reasons, some of them mutually exclusive, to explain the lack of conformity of these results with our *a priori* hypothesis: i) due to the above-mentioned limitation, the long-term competitive experiments did not represent well the intended low, intermediate, and high levels of allelopathy, ii) the dynamic nature of allelopathy would have induced changes in the strength of it during the long-term experiments, which would not be a big problem if there was a single allelopathic species, but this was not the case, and iii) it is possible that the chosen range of abundances of *Synechococcus* did not represent well the intended three levels of allelopathy (although we had prior information from **Publication 5**).

The theoretical model constructed supports that the allelopathic interactions are responsible for the observed oscillatory coexistence. In a nitrate-limited system, the prediction of the classical mechanistic resource competition theory (Tilman, 1977) is that the best competitor will always dominate, excluding all others, irrespective of the initial conditions. This is the case if we construct a model for our system with growth based on nitrate as limiting resource as the only mechanism (**Publication 4**). However, including in the model the allelopathic interactions observed, we demonstrated that oscillatory coexistence is possible, and therefore this is due to the allelopathic interactions.

To the best of our knowledge, these findings constitute the first experimental demonstration of a real multispecies (more than two species) microbial community whose long-term coexistence is based on chemical interactions, confirming what was previously suggested based on thorough theoretical analysis by Kelsic et al. (2015).

The strength of *Synechococcus* allelopathy influences diversity of natural plankton communities

As a test for this hypothesis scaling up the degree of community complexity, we performed long-term experiments with natural phytoplankton communities. The hypothesized outcome was supported, but only for *Synechococcus* Type 1. At both low and high inoculum sizes, plankton communities remained similar to the controls. However, at a medium inoculum size, a distinct increase in diversity and dominant species composition was observed for *Synechococcus* Type 1. Diversity exhibited the expected '*hump-shaped*' pattern according to the IDH (Fig. 5 and Table 1 in **Publication 5**).

At low *Synechococcus* inoculum levels (BA-124_L or BA-132_L), community structure closely resembled control groups, with *Nitzschia* sp. and *Navicula* sp. as dominant taxa. The Shannon-Wiener diversity index did not differ significantly from the controls, indicating that low levels of *Synechococcus* did not substantially alter the phytoplankton community. This supports the expectation that weak allelopathic interactions do not sufficiently disrupt competitive dynamics, leading to the dominance of a few strong competitors, as seen in control conditions.

A significant increase in diversity was observed at intermediate *Synechococcus* inoculum levels (BA-124_M), with the Shannon-Wiener diversity index significantly higher than in all other conditions. Correspondence analysis (CA) indicated that the community in BA-124_M was the most distinct from the control, and taxa dominance analysis revealed shifts with *Pleurosigma* sp. and *Cyclotella* sp. becoming dominant alongside *Nitzschia* and *Navicula*. However, no significant changes were detected for the brown strain of *Synechococcus* Type 3a (BA-132).

At high inoculum levels (BA-124_H and BA-132_H), diversity did not significantly differ from the controls. Dominant species analysis showed a shift in dominance from *Nitzschia* to *Navicula*, with *Cyclotella* also noted as a dominant species in BA-124_H. Although the Shannon-Wiener diversity index was higher than in the controls for both strains, the difference was not statistically significant ($p > 0.05$).

These results support the hypothesis that moderate allelopathic intensity disrupts the pattern predicted by competitive interactions, allowing a broader range of species to coexist but only for strain Type 1. We hypothesize that not observing these results for strain Type 3a means that the effect of allelopathy was very weak for this strain. Therefore, they were never

reached intermediated or high levels of it. This is suggested by the steady but still low increase in diversity correlated with increasing *Synechococcus* Type 3a inoculum size, suggesting a steady small increase in allelopathy.

H3 summary and implications:

Allelopathy of *Synechococcus*, and networks of allelopathic interactions between species, have been demonstrated to be an essential driver of the dynamics and structure of experimental communities. Both with selected multispecies communities and natural communities.

These findings suggest that, in Baltic phytoplankton communities in which different phenotypes of *Synechococcus* are present, allelopathic interactions could be a driver of community structure and diversity. The strength of allelopathy in those natural communities will depend on a variety of factors, including *Synechococcus* abundances. Hence, the predicted increase in *Synechococcus* abundance could enhance the role of allelopathic interactions as drivers of phytoplankton community structure and succession.

11 CONCLUSIONS

11 WNIOSKI

- Abiotic factors that promote growth or align with the optimal niche conditions of *Synechococcus* sp. phenotypes enhance their allelopathic activity.

- The allelopathic effect varies depending on the *Synechococcus* phenotype and the specific phytoplankton species targeted.

- Allelopathy, as an isolated factor, has the potential to facilitate and stabilize phytoplankton coexistence.

- The functional relationship between allelopathy and phytoplankton community diversity exhibits a peak at intermediate levels of allelopathic activity.

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Project ID: 539-O140-B416-20. Project title: Evaluation of the effects of environmental factors (light, temperature and salinity) on the growth of different strains of picoplankton cyanobacteria of the genus *Synechococcus* sp. in mixed cultures. Principal Investigator: **Konarzewska Z.**

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