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PRIMARY RESEARCH PAPER

Temperature-dependent impacts of allelopathy on growth, pigment, and lipid content between a subpolar strain of *Synechocystis* sp. CCBA MA-01 and coexisting microalgae

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Abstract Picocyanobacteria represent the main fraction of ocean primary production and, due to the effects of ocean acidification and an increase in seawater temperature, this group is expected to be favored in future scenarios. For this reason, we studied the ecophysiological response of picocyanobacteria to increases in water temperature by testing the allelopathic activity of a subpolar strain of *Synechocystis* sp., grown at a temperature range of 10–20°C, against coexisting microalgae. We showed that cell-free filtrates of *Synechocystis* sp. inhibited growth and the maximum quantum yields of PSII (F_v/F_m) of *Porphyridium purpureum*, *Fistulifera* sp., and *Chlorella vulgaris*, and the negative effect of the picocyanobacterium was stronger at the highest temperatures at which *Synechocystis* sp. was grown. Similarly, in *P. purpureum*, the effect of filtrates reduced chlorophyll *a* (Chl *a*) and carotenoids (Car) content, and the effect depended on the temperature at which the picocyanobacterium was grown. The lipid content was observed to increase in all species, and the allelopathic effect was the strongest at the highest temperature in which *Synechocystis* sp. was grown. The results of the present study predict a stronger allelopathic effect of picocyanobacteria against competitors with rising temperatures. They also suggest that the potential effect of climate change would benefit this group in future phytoplankton communities.

Keywords Allelopathy · Climate change · Harmful algal bloom · Microalgae · Picocyanobacteria

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Introduction

Picocyanobacteria are cosmopolitan organisms which dominate primary production in the open ocean (Callieri, 2010; Worden & Wilken, 2016). Picocyanobacteria also are able to produce a variety of bioactive and allelopathic compounds, which could have a negative impact in many organisms of the ecosystem, from microbes to plants and animals (Śliwińska-Wilczewska et al., 2018a). They also form

harmful blooms in marine ecosystems, which may span over large areas (Beardall, 2008; Sorokin & Dallocchio, 2008; Sorokin & Zakuskina, 2010). These blooms could negatively impact seagrass beds and corals communities (Hall et al., 1999). Sorokin & Zakuskina (2010) also reported that blooms of picocyanobacteria can cause mortality of bottom vegetation and benthic fauna. Furthermore, picocyanobacterial blooms are also known to inhibit zooplankton grazing (Goleski et al., 2010). Specific studies are needed to describe the drivers of these blooms. Currently, these are considered complex events, caused by multiple factors occurring simultaneously (Heisler et al., 2008).

According to the literature, allelopathy is a potential driver of the development of blooms and plankton community dynamics (Kubanek et al., 2005; Prince et al., 2008; Hattenrath-Lehmann & Gobler, 2011; Franzè et al., 2018). The number of reports on the allelopathic activity of cyanobacteria has been steadily increasing (e.g., Brutemark et al., 2015; Costa et al., 2015; Dias et al., 2017). There are some reports of allelopathic effects such as growth inhibition or stimulation caused by picocyanobacterium *Synechococcus* sp. C.Nägeli (e.g., Barreiro Felpeto et al., 2018; Śliwińska-Wilczewska & Latała, 2018), but no information about the allelopathic activity of *Synechocystis* sp. on coexisting microalgae has been found.

Synechocystis sp. Wislouch, alongside *Synechococcus* sp. and *Prochlorococcus* sp. Chisholm, Frankel, Goericke, Olson, Palenik, Waterbury, West-Johnsrud & Zettler, is an important primary producer, especially in oceanic waters during summer (Lochte & Turley, 1988). *Synechocystis* sp. occurs numerously, e.g., in the Baltic Sea and the Atlantic Ocean (Möller & Jansson, 1997; Martins et al., 2005, 2007, 2008). Moreover, Vareli et al. (2012) noted that the cyanobacterial community was found to be dominated almost exclusively by the cosmopolitan species *Synechococcus* and *Synechocystis* in the Mediterranean Sea. According to the best knowledge of the authors, there are no literature reports about the blooms of *Synechocystis* sp. It was argued that allelopathy could only be effective at cell abundances close to blooming conditions (Jonsson et al., 2009) mainly due to the large cell-to-cell distances that occur in the real ecosystems. However, there are still many unknown facts regarding the mechanism of action of allelochemicals, which might not need to reach high concentrations in order to be effective (Lewis, 1986). Recent papers showed significant effects of diatom allelochemicals, at low concentrations, in field planktonic food webs (Franzè et al., 2018).

Among secondary metabolites, there are many different active compounds that have an allelopathic effect. Inhibition of growth is the most frequently described mode of action. Some studies indicate that allelopathic compounds can also affect lipid content of target organisms. Poulson-Ellestad et al. (2014) demonstrated that the fatty acid syntheses of two competing phytoplanktons, *Asterionellopsis glacialis* (Castracane) Round and *Thalassiosira pseudonana* Hasle & Heimdal, were disrupted due to *Karenia brevis* (C.C.Davis) Gert Hansen & Moestrup allelopathy. Also, Poulin et al. (2018) showed that allelochemicals induced changes in lipid composition, in part due to the use of various lipids as signal molecules during stress. An indirect effect of picocyanobacterial allelochemicals in photosynthesis is by altering the pigment content. Śliwińska-Wilczewska et al. (2017) showed that filtrates from picocyanobacterium *Synechococcus* sp. caused significant reductions in the chlorophyll content of *Phormidium* sp. Kützing ex Gomont and *Rivularia* sp. C.Agardh ex Bornet & Flahault cells and increased the carotenoid contents of *Anabaena flos-aquae* Ralfs ex Bornet & Flahault and *Nostoc* sp. Vaucher ex Bornet & Flahault. Similarly, Barreiro Felpeto et al. (2018) demonstrated that *Synechococcus* sp. allelochemicals caused reductions of chlorophyll content of *Nodularia spumigena* Mertens ex Bornet & Flahault. Considering all this knowledge, we decided to consider the following ones as response variables: growth in terms of cell numbers, ratio F_v/F_m, and pigment and lipid compositions.

It has also been shown that an increase in surface water temperature, an indicator of global change, could have a positive effect in the development of cyanobacterial blooms (Paul, 2008; Paerl & Huisman, 2009). In the current century, average global temperatures are expected to increase in the range of 1.5–5°C (Houghton et al., 2001; IPCC, 2007). As surface water temperatures approach and exceed 20°C, the growth rates of eukaryotic phytoplankton generally stabilize or decrease, while growth rates of many cyanobacteria keep increasing (Paerl & Huisman, 2009). Focusing on picocyanobacteria, these usually achieve maximal growth rates at higher temperatures than other cyanobacteria (Moore et al., 1995) and thus will

potentially be favored by global warming. Moreover, it was reported that the ability of picocyanobacterium *Synechococcus* sp. to produce allelopathic compounds is being affected by temperature (Śliwińska-Wilczewska et al., 2016). Thus, considering all the knowledge available, it is expected that massive blooms of picocyanobacteria will be more frequent under future scenarios predicted by global change, and allelopathy of these species should also be a factor to be taken into account. However, the allelopathic activity of the picocyanobacterium *Synechocystis* sp. targeted at coexisting microalgae has never been investigated, although some previous works have suggested that these species produce biologically active compounds, i.e., microcystins (Oudra et al., 2002), *β*-Methylamino-L-alanine (Cianca et al., 2012), and lipopolysaccharides (Schmidt et al., 1980).

The main aim of this work was to study the allelopathic activity, and its temperature dependence, of the picocyanobacterium *Synechocystis* (MA-01) against the coexisting eukaryote microalgae *Porphyridium purpureum* (Bory) K.M.Drew & R.Ross, *Fistulifera* sp. S.Mayama, M.Matsumoto, K.Nemoto & T.Tanaka, and *Chlorella vulgaris* Beyerinck [Beijerinck]. We tested the influence of cell-free filtrate obtained from *Synechocystis* cultures grown under different temperatures on the following parameters: growth, the maximum quantum yield of PSII, as well as pigment and lipid contents. This study will be useful to better understand the extent to which rising temperatures, as a global change indicator, could influence picocyanobacterial dominance in high northern latitudes.

Materials and methods

Material and culture conditions

The strains employed in the experiments were, as a donor of allelochemicals, the picocyanobacterium *Synechocystis* sp. (MA-01), and as targets: the red algae *Porphyridium purpureum* (MA-04), the diatom *Fistulifera* sp. (MA-14) and the green algae *Chlorella vulgaris* (MA-24). All these strains were isolated from the coastal zone of Norway, in the Norwegian Sea or the Greenland Sea. They were maintained as unispecies cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk, Poland.

Cultures were grown in f/2 medium (Guillard, 1975) in 25-ml glass Erlenmeyer flasks that were swirled daily during the experimental period. Culture media was prepared with artificial seawater filtered through glass fiber filters (Whatman GF/C) and autoclaved. The salinity was 32 PSU, measured with salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). The conditions in the culture room were 16:8 h light:dark cycle with Photosynthetically Active Radiation (PAR) irradiance of 20 µmol photons m^{-2} s^{-1} and 10°C. Target microalgae were grown during 7 days under constant conditions of 10°C and 32 PSU, under a 16:8 h light:dark cycle at 20 µmol photons m^{-2} s^{-1}. These growth conditions were used for control. The donor species, the picocyanobacteria *Synechocystis* sp. was grown at three different temperatures: 10, 15, and 20°C. All picocyanobacterial cultures were maintained for 7 days in order to allow them to acclimate and reach the exponential growth phase when they will be employed in the experiments.

Test of the allelopathic effect of cell-free filtrates

After the 7 days of acclimation, the concentrations of macronutrients (i.e., nitrate, N-NO_3, and P-PO_4 orthophosphates) were measured in the donor picocyanobacterial cultures, and then they were readjusted to the standard levels of the f/2 medium. Nutrients were determined using spectrophotometric methods as described by Grasshoff (1976). Then, cultures from each temperature condition were filtered through a 0.45-µm filter (Macherey–Nagel MN GF-5) using a vacuum pump (400 mbar). The filtrates were then analyzed on an epifluorescence microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) to confirm the absence of picocyanobacteria. These cell-free filtrates were then employed to test allelopathic activity, following a similar protocol to that of Suikkanen et al. (2004). We tested the allelopathic activity of *Synechocystis* sp. on coexisting microalgae by a single addition of cell-free filtrate. 10 ml of the cell-free filtrate obtained from each of the temperature regimes were separately added to 25-ml Erlenmeyer flasks containing 10 ml of cell suspensions of the target microalgae species, obtained from the exponentially growing cultures referred in the section above.

Controls consisted of adding 10 ml of filtered f/2 medium to 25-ml Erlenmeyer flasks containing 10 ml of cell suspensions of the same microalgae species and kept in the same culturing conditions as described above for the target species. Three replicates were employed in all treatments and control. The ratio of donor picocyanobacteria to target species was adjusted to 1:1 based on the chlorophyll *a* (Chl *a*) content (the initial Chl *a* concentration in the experimental cultures was 0.4 µg Chl *a* ml^{-1}). The experiments lasted for 7 days. On the first and the last days of the experiment, the pH values of the treatments and controls were measured using pH-meter (Elmetron CP-401, Zabrze, Poland). pH values were similar between treatments and the initial and final time points, which ranged from 7.9 to 8.3.

Determination of cell abundances

Cell abundances were estimated with previously determined linear regression models between the number of cells (N ml^{-1}) and optical density (OD). Cyanobacterial and microalgal cells were counted using flow cytometer BD Accuri™ C6 Plus (BD Biosciences, San Jose, CA, USA) calibrated with SPHERO™ Rainbow Calibration Particles (BD, San Jose, USA). OD was measured spectrophotometrically at 750 nm with a Multiskan GO UV–Vis spectrophotometer (Thermo Scientific, Massachusetts, USA). These data were used to fit a linear regression model between the variables N and OD. The linear correlation between N and OD for *Synechocystis* sp. was $y = 78.3 \times 10^6 x + 21.2 \times 10^4$; ($r = 0.97$), where y = N (ml^{-1}) and x = OD. For *P. purpureum*, *Fistulifera* sp., and *C. vulgaris*, the correlation coefficients for their respective linear regression models were $r = 0.99$, $r = 0.99$, and $r = 0.98$, respectively, and the model equations: $y = 52.9 \times 10^{5-} x + 8.4 \times 10^3$, $y = 23.0 \times 10^6 x - 7.7 \times 10^4$ and $y = 18.3 \times 10^6 x + 6.5 \times 10^4$, respectively, where y = N ($m\ l^{-1}$) and x = OD. Cell abundances were estimated in all the experiments performed at following days: zeroth day (1 h) and 1st, 3rd, and 7th days of the experiment and control.

Measurements of the maximum quantum yield of PSII

The maximum PSII quantum efficiency was calculated as F_v/F_m (where F_v—the difference between the maximum and minimum fluorescence; and F_m—the maximum fluorescence) (Campbell et al., 1998). Chlorophyll *a* fluorescence was measured using a Pulse Amplitude Modulation (PAM) fluorometer (FMS1, Hansatech), equipped with a 594-nm amber modulating beam with a 4-step frequency control serving as a measuring light. Samples were taken at the following days: 0 (1 h), the 1st, the 3rd, and the 7th days of the experiment. Samples were filtered through 13-mm glass fiber filters (Whatman GF/C). Before measurements, the filtered sample was kept in the dark for approximately 10 min (Śliwińska-Wilczewska et al., 2016).

Measurements of pigment content

The concentration of photosynthetic pigments of target microalgae was measured by spectrophotometry on the 7th day of the experiment. Chl *a* and Car concentrations were extracted with cold 90% acetone in the dark for 2 h at − 60°C. To remove cell debris and filter out particles, these extracts were centrifuged at 13,000 rpm (14,359×*g*) for 2 min (Sigma 2-16P, Osterode am Harz, Germany). The extinction values were determined as 480, 664, and 750 nm using a DU530 UV–VIS Life Science spectrophotometer (Beckman, California, USA). The concentrations of Chl *a* and Car were calculated according to Strickland & Parsons (1972).

Measurements of lipid content

The total lipid content of target microalgae was measured on the 7th day of the experiment. Quantitative measurements of lipids were performed using the optimized colorimetric sulphophospho-vanilin method proposed by Klin et al. (2018). Bligh and Dyer (1959) demonstrated that the results of separation of lipids and nonlipids in this method were reproducible to within ± 2%; therefore, loss of lipids through incomplete partitioning in our experiment can be excluded. The concentration of lipids was estimated based on the calibration curve that was developed using high-quality soybean oil as a source of fatty

acids, which has a similar composition of lipids compared to algae (Grama et al., 2014). For the extraction, 1 ml aliquots of microalgal cultures were centrifuged at 10,000 rpm (8496×*g*) for 5 min (Sigma 2-16P, Osterode am Harz, Germany) and resuspended in 0.5 ml of methanol. Subsequently, 1 ml of chloroform was added. The resuspended samples were centrifuged (8496×*g*, 5 min), and the supernatant was collected and supplemented with 0.2 ml of 0.8% NaCl solution. At that point, samples were allowed to remain at room temperature, until two layers were formed, and then the upper layer was collected and discarded. The lower layer was dried under N_2 at 50°C. Next, 0.3 ml of concentrated sulfuric acid was added to the samples and then heated at 90°C for 10 min. 1 ml of SPV reagent (1.2 g vanillin per 1 l of 68% phosphoric acid) was added in order to fully stain the fatty acids. Then, the samples were incubated at 36°C for 5 min. The absorbance of the final solution was measured at 525 nm.

Statistical analyses

A general linear model was used to test the effect of picocyanobacterial filtrates obtained from cultures grown under different temperatures on growth and the maximum quantum yield of PSII of the targeted microalgae. The treatment (temperature) was used as a categorical factor and time (day) as a covariate. A post hoc least significant distance (LSD) test was used to test significant differences between combinations of treatment levels (comparisons were reported only for the pairs control *vs* treatment within the same day). Independent sample *t* tests assuming equal variances were applied to determine the effect of cell-free filtrates on pigment and lipid contents of the target species. The statistical analyses were performed using *R* (R Core Team 2017) and Statistica® software.

Results

Allelopathic effect of cell-free filtrates on growth

The addition of cell-free filtrate obtained from *Synechocystis* sp. significantly affected the number of cells of *P. purpureum*, *Fistulifera* sp. and *C. vulgaris* (Table 1). The target species had positive growth during the seven days of the experiments, in the majority of treatments (Fig. 1). The factors 'Treatment' and 'Days' showed a significant interaction in all three species (Table 1). This could be expected for two main reasons: (1) the effect of time must be significant in the model (the algae are growing), and then, the effects of the treatment would be apparent mostly in the last days of the experiments; and (2) if there is a temperature-dependent effect, there will always be an interaction between the two factors. Because of this interaction, the significance values of the other main effects ('Treatment,' 'days') are not directly interpretable. For these reasons, in order to compare the effect of different temperatures, we should look at the results of the pairwise comparisons between the control and the different levels of temperature, within each time step, performed with LSD (see asterisks in Fig. 1). In *P. purpureum*, cell-free filtrates from all three temperatures produced a significant effect. It was found that the addition of cell-free filtrates obtained from *Synechocystis* sp. significantly affected the number of cells of *P. purpureum,* and the negative effect was amplified with the increasing temperature at which picocyanobacterium was grown. In *Fistulifera* sp., this effect was only significant for the filtrate from 20°C, but in *C. vulgaris*, it was significant for the filtrate from all three temperatures, without distinction between them. At the end of the experiment, the growths of *P. purpureum* relative to the control were 87, 84, and 73%, observed for the cell-free filtrates from 10, 15, and 20°C, respectively. All these constituted significant differences, according to the LSD test (Fig. 1). For *Fistulifera* sp., the corresponding amounts were 95, 88, and 44% for 10, 15, and 20°C, respectively, constituting significant differences only for the two highest temperatures (Fig. 1). And, for *C. vulgaris*, they were 74, 74, and 69%, for 10, 15, and 20°C, respectively, constituting significant differences for all of them.

Allelopathic effect of cell-free filtrates on the maximum quantum yield of PSII

The cell-free filtrates obtained from *Synechocystis* sp. had a negative effect in the maximum PSII quantum efficiency of all the three species (Table 2, Fig. 2). For *C. vulgaris*, in the pairwise comparisons by LSD, there were no significant differences between any of the control–treatment pairs. In these data, because there

Table 1 Results of the analysis of variance applied to the linear model fitted to the growth data in the three species

Independent variable	*F*	*df* (factor)	*df* (residual)	*P*
Porphyridium purpureum				
Temperature	14.8	3	40	< 0.001
Days	493	1		< 0.001
Temperature × days	9.7	3		< 0.001
Fistulifera sp.				
Temperature	44.7	3	40	< 0.001
Days	447	1		< 0.001
Temperature × days	45.1	3		< 0.001
Chlorella vulgaris				
Temperature	12.9	3	40	< 0.001
Days	228	1		< 0.001
Temperature × days	9.6	3		< 0.001

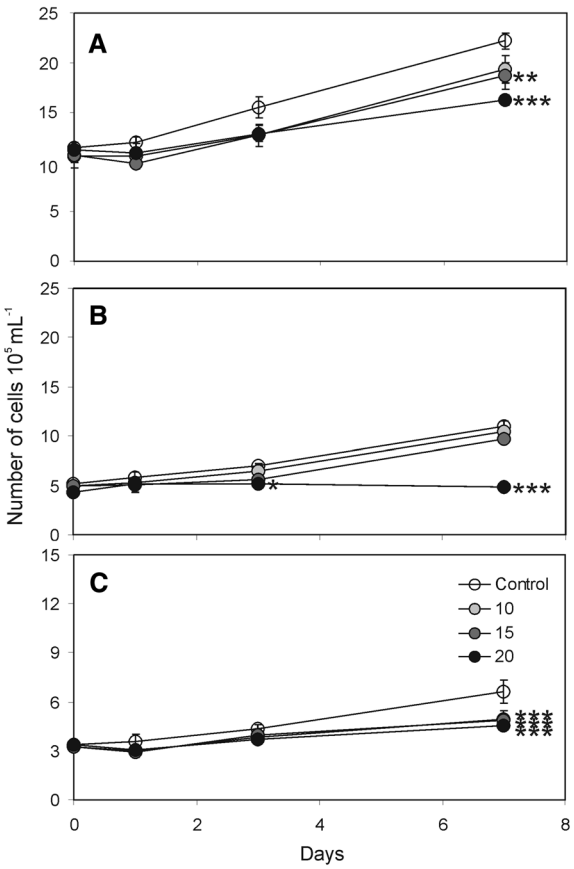

Fig. 1 The number of cells (N) of *P. purpureum* (**A**), *Fistulifera* sp. (**B**), and *C. vulgaris* (**C**) employed as a target for the *Synechocystis* sp. cell-free filtrate obtained from cultures grown under different temperatures. Values are mean ± SD ($n = 3$). Asterisks indicate level of significance from post hoc LSD tests of single temperature level compared to control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

was no interaction between 'Temperature' and 'Days,' it was possible to perform a post hoc Dunnett test to detect significant differences between the control and the different levels of temperature. In this test, significant differences were found between the control and both 15°C ($t = -3.8$, $P < 0.01$) and 20°C ($t = -4.7$, $P < 0.001$). For *P. purpureum* and *Fistularia* sp., the existence of the interaction between the factors 'Temperature' and 'Days' prevents a straightforward interpretation of the significance of each main effect individually. By comparing the control against the different temperature levels by LSD, significant effects of the filtrates from 20°C treatment on days 1, 3, and 7 for *P purpureum*, were found with the fluorescence parameter F_v/F_m values on these days being 87, 84, and 69% of the control, respectively. In the same species, the filtrate from 15°C also showed a significant difference on the seventh day. In *Fistularia* sp., filtrates from 15°C and 20°C only showed significant differences on the seventh day, the F_v/F_m parameter values in those cases being 93% and 84% of the control, respectively. All these results indicate a stronger allelopathic effect of the filtrates of cultures from higher temperatures.

Allelopathic effects of cell-free filtrates on pigment content

In *P. purpureum*, the addition of cell-free filtrate obtained from *Synechocystis* sp. reduced both the Chl *a* and Car contents per cell (Fig. 3A). This reduction was temperature dependent—the stronger the reduction, the higher the temperature. In comparison with

Table 2 Results of the analysis of variance applied to the linear model fitted to the maximum quantum yield of PSII data in the three species

Independent variable	*F*	*df* (factor)	*df* (residual)	*P*
Porphyridium purpureum				
Temperature	38.6	3	40	< 0.001
Days	16.5	1		< 0.001
Temperature × days	20.4	3		< 0.001
Fistulifera sp.				
Temperature	11.6	3	40	< 0.001
Days	102	1		< 0.001
Temperature × days	14.9	3		< 0.001
Chlorella vulgaris				
Temperature	8.9	3	40	< 0.001
Days	9.1	1		< 0.01
Temperature × days	0.58	3		0.63

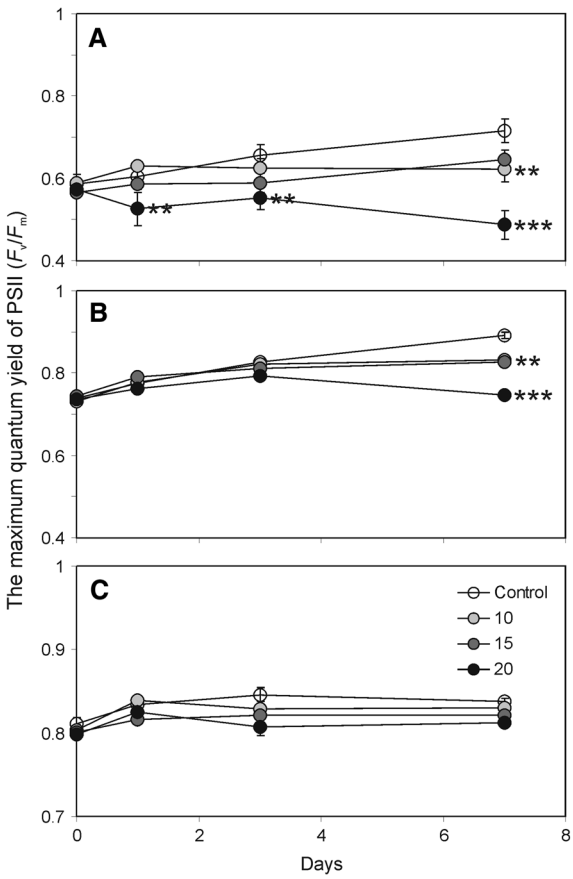

Fig. 2 The maximum quantum yields of PSII (F_v/F_m) of *P. purpureum* (**A**), *Fistulifera* sp. (**B**), and *C. vulgaris* (**C**) employed as a target for the *Synechocystis* sp. cell-free filtrate obtained from cultures grown under different temperatures. Values are mean ± SD ($n = 3$). Asterisks indicate level of significance from post hoc LSD tests of single temperature level compared to control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

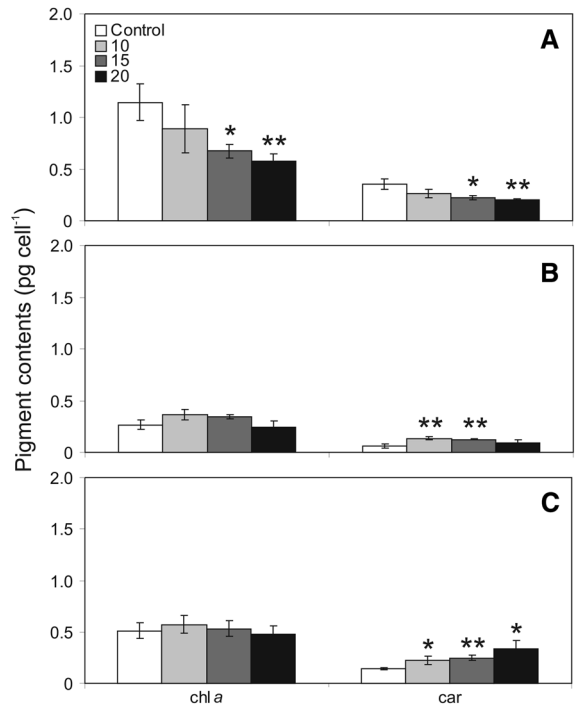

Fig. 3 Chl *a* and Car contents (pg cell^{-1}) of *P. purpureum* (**A**), *Fistulifera* sp. (**B**), and *C. vulgaris* (**C**) for controls and experiments with the addition of cell-free filtrate obtained from *Synechocystis* sp. cultures grown under varied temperatures: 10, 15, and 20°C after 7 days of exposure. Values are mean ± SD ($n = 3$). Asterisks indicate significant difference compared with control obtained with the *t* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

the control, only the treatment levels of 15 and 20°C showed significant differences for both Chl *a* and Car. For Chl *a*, the reduction was, relative to control, 60% (*t* test, $P < 0.05$) and 52% (*t* test, $P < 0.01$),

respectively, for each temperature level. For the Car content, the reductions were of 64% (t test, $P < 0.05$) and 57% (t test, $P < 0.01$), respectively. On the other hand, *Fistulifera* sp. and *C. vulgaris* did not show any significant effect of filtrates on the Chl *a* content. Regarding Car content, both species showed relatively small, but statistically significant, increases. These increases were positively related with temperature, in particular for *C. vulgaris*.

Allelopathic effect of cell-free filtrates on lipid content

The effects of cell-free filtrate from *Synechocystis* sp. on lipid contents are shown in Fig. 4. The lipid content of the microalgae employed was significantly altered in all the species, but not for all the temperature levels. In *P. purpureum*, for the filtrates obtained from 10, 15, and 20°C, the lipid content constituted, relative to the control, 151% (t test, $P < 0.01$), 145% (t test, $P < 0.01$) and 464% (t test, $P < 0.001$), respectively (Fig. 4A). For *Fistulifera* sp. and *C. vulgaris*, significant differences relative to the control were found only for the filtrates from 20°C. At the seventh day of experiment, in that treatment level, the lipid content constituted, relative to the control, 300% (t test, $P < 0.05$) and 130% (t test, $P < 0.01$), respectively, for each species (Fig. 4B, C).

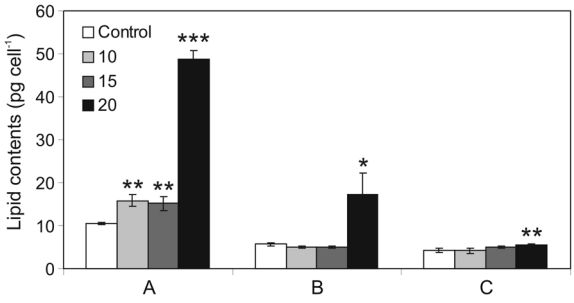

Fig. 4 Lipid contents (pg cell^{-1}) of *P. purpureum* (**A**), *Fistulifera* sp. (**B**), and *C. vulgaris* (**C**) for controls and experiments with addition of cell-free filtrates obtained from *Synechocystis* sp. cultures grown under varied temperatures: 10, 15, and 20°C after 7 days of exposure. Values are mean ± SD ($n = 3$). Asterisks indicate significant difference compared with control obtained with the t test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

Discussion

In the present study, we showed that *Synechocystis* sp. filtrate negatively affected several strains of microalgae. The effects were positively dependent on the temperature in which picocyanobacterium was grown, and not all the target species employed were sensitive. There are previous reports of allelopathic effects of cyanobacteria against different groups of algae: red algae (García-Espín et al., 2017), diatoms (Suikkanen et al., 2004) and green algae (Antunes et al., 2012; Żak et al., 2012; Żak & Kosakowska, 2015). Very little information is available about allelopathic effects of picocyanobacteria (i.e., *Synechococcus* sp.) (Śliwińska-Wilczewska et al., 2016, 2017; Śliwińska-Wilczewska & Latała, 2018), and none about *Synechocystis* sp. although one strain was already reported to show biological activity (Costa et al., 2014).

In our experiments, the most sensitive species were, depending on the parameter studied, the diatom *Fistulifera* sp. or the Rhodophyte *Porphyridium purpureum*. There is extensive information in the literature showing that species differ in their sensitivity to allelopathy (Suikkanen et al., 2004; Barreiro Felpeto & Hairston, 2013; Barreiro Felpeto & Vasconcelos, 2014). In general, smaller species are more sensitive, which is probably due to a higher surface to volume relationship, which may cause higher allelochemical uptake rates (Lyczkowski & Karp-Boss, 2014). However, this is not always the case (Barreiro Felpeto & Vasconcelos, 2014). Then, allelochemicals should have a differential impact in phytoplankton community structure.

Our experiments showed a strong negative effect of *Synechocystis* sp. filtrates on the maximum quantum yield of PSII of all target microalgae. There was a decrease in F_v/F_m for *P. purpureum* and *C. vulgaris* already after the first day of exposure to cell-free filtrates. The effect in growth, however, was not evident so rapid, and significant growth decreases were observed only after three and seven days of exposure. According to this, the efficiency of PSII may be more sensitive as an indicator of the allelopathic effect than growth measured over a few days. This was also pointed out by other authors (Prince et al., 2008). This reduction of PSII efficiency may be an effect of the allelopathic mechanism for cyanobacteria (Gross, 2003).

The changes in pigment content caused by allelochemicals seem to vary considerably across the different microalgal species. In this work, we showed significant reductions in the Chl *a* and Car contents of *P. purpureum*. This reduction of pigment content is probably one of the main reasons for the inhibition of photosynthesis detected, as suggested by Sukenik et al. (2002) and Śliwińska-Wilczewska et al. (2017). Car contents for *Fistulifera* sp. and *C. vulgaris* increased, while their growths were inhibited. Carotenoid pigments have a protective function (e.g., Jodłowska & Latała, 2013); therefore, increased Car contents may indicate a protective response against the stress caused by allelochemical compounds. Changes in lipid composition are known to have an important role in cell survival and reproduction under different stress effects (Lu et al., 2012). In this work, we observed that the cell-free filtrate from *Synechocystis* sp. affected lipid contents of *P. purpureum, Fistulifera* sp., and *C. vulgaris*, by increasing it with a positive temperature-dependent effect. Microalgae synthesize and accumulate neutral lipids in their cytoplasm which account for 20-50% of dry biomass (Rawat et al., 2011). Lipids are synthesized and used as a reserve for situations of stress caused by nutrient depletion (Mendes & Vermelho, 2013), salinity (Lu et al., 2012) or UV radiation (Guihéneuf et al., 2010). Detailed studies on how the lipid composition is affected by the stress caused by allelopathic compounds have been made by Poulson-Ellestad et al. (2014) and Poulin et al. (2018). Poulson-Ellestad et al. (2014) noted that allelopathic compounds produced by *K. brevis* altered cell membrane components, inhibited osmoregulation, and increased the oxidative stress of *T. pseudonana*. Poulin et al. (2018) showed that allelopathic compounds produced by dinoflagellate *K. brevis* altered the structure of the lipidomes of *A. glacialis* and *T. pseudonana*. These authors demonstrated that membrane-associated lipids of living *T. pseudonana* cells became permeable after exposing them to the compounds produced by *K. brevis*. The conclusion was that allelopathic compounds may affect lipid biosynthesis. Our findings suggest that the effect of *Synechocystis* allelopathy in lipid production is opposed since in general, lipid production increased. Probably, these differences have to do with the mechanism of action. While in *K. brevis*, allelochemicals disrupt membranes, in *Synechocystis* they seem to affect cellular metabolic processes, and thus, the increase of lipid production could be a reaction similar to what was observed under other stress conditions.

Also, it is surprising to note the increase in lipid production after clearly reduced efficiency in photosynthesis-related processes. Since lipids are costly to produce, we hypothesize that they are produced as a response to stressful conditions due to reallocating much of the energy budget, which otherwise could be used for growth (which was consequently inhibited in all the species).

The influence of abiotic factors on the production and release of allelopathic compounds is far from being understood. Our study demonstrated a positive effect of temperature on the allelopathic effect of *Synechocystis* sp. which was detected in growth, the maximum quantum yield of PSII, pigment, and lipid contents. Furthermore, the observed changes in our experiment may be more pronounced in co-culturing experiments, as noted by, e.g., Paul et al. (2009, 2013), Dunker et al. (2017) and Śliwińska-Wilczewska et al. (2018b). Temperature conditions influenced the production of cylindrospermopsin (CYN) and unrecognized allelopathic compounds in the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju (Antunes et al., 2012). In this case, the allelopathic activity of filtrates from *C. raciborskii*, grown at 30°C, exhibited the highest inhibitory activity and this effect coincided with the optimal growth of analyzed cyanobacterium. Similarly, Śliwińska-Wilczewska et al. (2016) showed that higher temperature enhanced the allelopathic activity of picocyanobacterium *Synechococcus* sp. What actually seems is that allelopathic compounds in these species are produced at higher rates under growth conditions close to their optimal. Future studies should examine in detail the mechanisms behind temperature effect.

Our work contributes to elucidate the effects of allelochemicals at a scale of physiological processes. However, more effort needs to be made yet to elucidate the mechanisms of actions of these compounds at a metabolic level, for which it is mandatory to identify the allelopathic compounds.

The current status of knowledge suggests that climate change will enhance the frequency and strength of harmful cyanobacterial blooms in aquatic ecosystems because higher carbon dioxide concentrations in the waters and warmer temperatures would benefit these species (Paerl & Huisman, 2008).

Allelopathic interactions were considered to be a potential driver of these blooms by some authors (Kubanek et al., 2005; Prince et al., 2008). Here, we have demonstrated the existence of a positive effect of temperature on the allelopathic effect of a species of picocyanobacteria. This effect, in the context of rise in sea temperature caused by climate change, could strengthen the allelopathic activity of these species in the subpolar oceanic regions. Depending on the extent to which allelopathy is important in community dynamics, as blooms of allelopathic cyanobacteria become more frequent and stronger due to climate change, stronger allelopathy should be an additional factor to be taken into account as an indirect effect of climate change.

In the present work, we conclude that picocyanobacterial allelopathy has potential to influence plankton dynamics in subpolar latitudes. The allelopathic effect was detected in different physiological parameters, and species sensitivity was not uniform. Interestingly, we detected a positive effect of temperature on allelopathic activity. Hence, this allelopathic potential could be enhanced by rising sea temperatures in the context of climate change.

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