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Allelopathic activity of picocyanobacterium *Synechococcus* sp. on filamentous cyanobacteria

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ABSTRACT

Allelopathy of picocyanobacterium *Synechococcus* sp. may be involved in the formation of massive blooms and their subsequent global expansion in many aquatic ecosystems. However, the effect of the allelopathic activity of this species on filamentous cyanobacteria remains unknown. In this study, we tested the allelopathic activity of *Synechococcus* sp. on growth, pigment content and chlorophyll fluorescence of filamentous cyanobacteria *Aphanizomenon flos-aquae*, *Nostoc* sp., *Phormidium* sp. and *Rivularia* sp. by single and repeated addition of cell-free filtrate. Negative effects against *Nostoc* sp. and *Phormidium* sp. were amplified by repeated filtrate additions compared with single filtrate addition. A maximum reduction in growth and a maximum quantum yield of photosystem II (PSII) photochemistry (F_v/F_m) relative to controls were observed for *Phormidium* sp. However, the addition of picocyanobacterial filtrate stimulated the growth and F_v/F_m of *A. flos-aquae*. *Synechococcus* sp. filtrate had no allelopathic effects on the growth and F_v/F_m of *Rivularia* sp. Moreover, filtrates caused significant decreases in the chlorophyll *a* (Chl *a*) contents of *Phormidium* sp. and *Rivularia* sp. cells. The addition of filtrate also resulted in increased cell carotenoid (Car) content in *A. flos-aquae* and *Nostoc* sp. These results showed for the first time that picocyanobacterium *Synechococcus* sp. negatively and positively affected coexisting filamentous cyanobacteria.

1. Introduction

Picocyanobacteria belonging to the genus *Synechococcus* are extremely important ocean organisms. *Synechococcus* sp. is widely distributed in marine and freshwater ecosystems (Callieri, 2010; Worden and Wilken, 2016). Despite its association with open ocean systems, blooming events caused by species of *Synechococcus* have frequently been reported in recent years in Florida Bay, San Francisco Bay, the Mediterranean Sea, the Black Sea and the Baltic Sea (Beardall, 2008). An unusually dense bloom of picocyanobacteria caused the mortality of bottom vegetation and benthic fauna and the collapse of valuable eel, mullet and clam fisheries (Sorokin and Zakuskina, 2010). These events demonstrate the potentially dangerous properties of blooms from this picocyanobacterium. Such a dense bloom has been a relatively new phenomenon in Europe and requires careful investigation (Beardall, 2008) because this species is potentially toxic (Jakubowska and Szeląg-Wasielewska, 2015) and allelopathic (Śliwińska-Wilczewska et al., 2016a, 2016b). Picocyanobacterial proliferation appears to have stronger impacts on the environment than other blooming events, such as those from toxic dinoflagellates (Sorokin et al., 2004).

The number of reports on the allelopathic effects of cyanobacteria has steadily increased (e.g., Bar-Yosef et al., 2010; Antunes et al., 2012; Rzymski et al., 2014; Żak and Kosakowska, 2015; Wang et al., 2017). Allelopathy may be one of the key factors contributing to the formation and maintenance of cyanobacterial blooms (e.g., Antunes et al., 2012; Rzymski et al., 2014). Moreover, some cyanobacteria produce harmful compounds which can have negative effects on plants, animals and even humans (Granéli and Hansen, 2006). Cyanobacteria are effective producers of many bioactive metabolites, including both acute toxins and allelopathic compounds (Burja et al., 2001; Berry et al., 2008; Leão et al., 2012). Mazur-Marzec et al. (2015) described Baltic cyanobacteria as a rich source of novel metabolites, e.g., curacin A, apratoxin D, dolastatin 10 and largazole, and different peptides, e.g., anabaenopeptins, cyanopeptolins, aeruginosins, spumigins and nostocyclopeptides. However, many of cyanobacteria remain unknown.

Summer blooms of cyanobacteria in the Baltic Sea are generally

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composed of two distinct groups: large filamentous cyanobacteria (e.g., *Aphanizomenon flos-aquae*, *Nostoc* sp., *Phormidium* sp. and *Rivularia* sp.) and small-sized picocyanobacteria, mainly belonging to genus *Synechococcus* (Stal et al., 2003; Mazur-Marzec et al., 2013). In a blooming event in the Baltic Sea, the recorded contribution of picocyanobacteria to total cyanobacterial biomass ranged from 20% in the beginning of July to 97% in late July and August (Mazur-Marzec et al., 2013). However, due to their small size (0.2 to 2.0 μm), picocyanobacteria, in contrast to the filamentous cyanobacteria, remain a poorly studied fraction of phytoplankton (Jakubowska and Szeląg-Wasielewska, 2015). While scarce information on picocyanobacteria allelopathic activity has been reported (e.g., Śliwińska-Wilczewska et al., 2016a, 2016b), the number of reports concerning their presence in ecosystems has increased (Callieri, 2010; Sorokin and Zakuskina, 2010; Worden and Wilken, 2016). Thus, the issue of picocyanobacteria allelopathy requires further attention.

Allelopathy of picocyanobacteria *Synechococcus* sp. is considered a potential driver of massive blooms and may also be responsible for their increased global occurrence throughout many aquatic ecosystems (Beardall, 2008; Sorokin and Zakuskina, 2010). However, the influence of picocyanobacterial allelopathy and the mode of action of allelopathic compounds on coexisting cyanobacteria remain largely unknown. The main aim of this work was to study the allelopathic activity of picocyanobacterium *Synechococcus* sp. against coexisting filamentous cyanobacteria *A. flos-aquae*, *Nostoc* sp., *Phormidium* sp. and *Rivularia* sp. We tested the influence of cell-free filtrates from *Synechococcus* sp. on growth, pigment content and chlorophyll fluorescence of filamentous cyanobacteria species. The findings of these experiments will be useful in determining the extent to which picocyanobacterial allelopathy influences the worldwide emerging phenomenon of massive *Synechococcus* sp. blooms.

2. Materials and methods

2.1. Material and culture conditions

The experiments were conducted with the picocyanobacterium *Synechococcus* sp. (BA-124) and the following filamentous cyanobacteria: *Aphanizomenon flos-aquae* (BA-69), *Nostoc* sp. (BA-81), *Phormidium* sp. (BA-112) and *Rivularia* sp. (BA-66). These strains were isolated from the coastal zone of the Gulf of Gdańsk (southern Baltic Sea) and were maintained as unispecific and non-axenic cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk, Poland (Latała et al., 2006).

Donor and target cultures used in the experiments were maintained in 25-mL glass Erlenmeyer flasks at 20 °C and a 16:8 h light:dark cycle at 20 μmol photons·m^{-2}·s^{-1}. The intensity of Photosynthetically Active Radiation (PAR) was measured using a quantum-meter (LI-COR, Nebraska, USA) with a cosine collector. The culture medium employed was f/2 (Guillard, 1975). Culture media were prepared with Baltic Sea water filtered through glass fiber filters (Whatman GF/C) and autoclaved. The salinity was 8 PSU as measured with a salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). Cultures were acclimated to these conditions for 7 days.

2.2. Experimental setup

Allelopathic effects were tested with according to a method proposed by Suikkanen et al. (2004) with modifications. Filamentous cyanobacteria species were exposed to cell free-filtrates of the donor picocyanobacterial strain. Before filtration, the concentrations of macronutrients (i.e., nitrate, N-NO_3 and P-PO_4 orthophosphates) in the cyanobacterial cultures were measured to set them to the standard levels of f/2 medium. Nutrients were determined using spectrophotometric methods as described by Grasshoff (1976). The treatments and controls received the same amounts of N-NO_3 and P-PO_4 (N-NO_3 = 9.7 ± 0.3 mg L^{-1} and P-PO_4 = 3.0 ± 0.2 mg L^{-1}). The analyzed filamentous cyanobacteria in the control showed active growth over the duration of the experiment. Therefore, the effects of major nutrients, microelements and vitamin limitations in the control and allelochemical treatments can be excluded.

Synechococcus sp. culture was filtered through a 0.45-μm filter (Macherey-Nagel MN GF-5) using a vacuum pump (400 mbar). These filtrates were analyzed on an epifluorescence microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) to confirm the absence of cyanobacteria. Experimental treatments were prepared by adding 2 mL of this cell-free filtrate to 25-mL Erlenmeyer flasks containing 20 mL of cell suspensions of the targeted cyanobacteria. Controls consisted of adding 2 mL of filtered f/2 medium to 25-mL Erlenmeyer flasks containing 20 mL of cell suspensions of the same cyanobacteria species. The flasks with cyanobacteria were swirled daily. Tests were conducted in triplicate. The experiments lasted 14 days. In all experiments, the ratio of picocyanobacterium to target species was adjusted to 1:1 based on the Chl *a* content (the final Chl *a* concentration in the experimental cultures was 0.8 μg Chl *a* mL^{-1}). Aliquots of the target species inoculated in the experimental flasks were sampled from the exponentially growing cultures. To simulate the effects of continuous release of allelochemicals, in an additional set of experimental flasks, the filtrate was renewed daily. This daily renewal consisted of removing 2-mL volumes from the experimental cultures and replacing with an equal volume of fresh filtrate (for the experimental treatments) or filtered f/2 medium (for the controls). On the first and the last days of the experiment, the pH of the treatments and controls were measured using a pH-meter (Elmetron CP-401, Zabrze, Poland). pH values were similar across treatments and durations and ranged from 7.9 to 8.1.

2.3. Determination of the culture density

The number of cells (N) in cultures was estimated with previously determined linear correlations between cell abundance and optical density (OD). N was counted using a Bürker chamber, and the OD was measured spectrophotometrically at 750 nm with a Multiskan GO UV-VIS spectrophotometer (Thermo Scientific, Massachusetts, USA). The linear correlation between N and OD for *Synechococcus* sp. was $y = 92.7 \cdot 10^6 x - 4.0 \cdot 10^5$; ($r^2 = 0.992$), where y = N ($mL^{-1}$) and x = OD (Jodłowska and Śliwińska, 2014). For *A. flos-aquae*, *Nostoc* sp., *Phormidium* sp. and *Rivularia* sp., the correlation coefficients were $r^2 = 0.985$, $r^2 = 0.946$, $r^2 = 0.960$ and $r^2 = 0.957$, respectively, and the linear correlations were $y = 20.0 \cdot 10^6 x + 2.7 \cdot 10^4$; $y = 30.0 \cdot 10^6 x - 9.5 \cdot 10^4$; $y = 10.0 \cdot 10^7 x - 5.0 \cdot 10^5$ and $y = 30.0 \cdot 10^6 x - 1.2 \cdot 10^4$, respectively, where y = N and x = OD. Initially, the number of filament units in the filamentous cyanobacteria cultures was counted using the Bürker chamber, where 1 filament unit = 100 μm. Later, the filament units were converted to cell numbers and used in the linear regressions. Cell counts and OD measurements were performed on the 1st, 3rd, 7th and 14th days of experiment and control.

2.4. Chlorophyll fluorescence analysis

Chlorophyll *a* fluorescence was measured with a Pulse Amplitude Modulation (PAM) fluorometer (FMS1, Hansatech), using a 594 nm amber modulating beam with a 4-step frequency control as a measuring light. Samples were taken for chlorophyll fluorescence analysis after the 0th (1 h of exposure), 1st, 3rd, 7th and 14th days of experiment. Samples were filtered through 13-mm glass fiber filters (Whatman GF/C). Before measurement, the filtered sample was kept in the dark for approximately 15 min. The maximum PSII quantum efficiency (F_v/F_m) was calculated (Campbell et al., 1998).

2.5. Measurements of pigment content

The concentration of photosynthetic pigments of target filamentous cyanobacteria was measured by spectrophotometry on the 14th day of

the experiment. These analyses were performed only with the samples treated with repeated additions of filtrate. Chl *a* and Car 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 10,000 rpm for 5 min. The extinction values were determined at 630, 647, 664 and 750 nm with a DU530 UV-VIS Life Science spectrophotometer (Beckman, California, USA). The concentration of Chl *a* was calculated according to Strickland and Parsons (1972): Chl *a* [μg mL^{-1}] = 11.236 (E_{665} - E_{750}) V_a/V_b. The concentration of Car was estimated: Car [μg mL^{-1}] = 4 (E_{480} - E_{750}) V_a/V_b, as derived from the factor by Strickland and Parsons (1972), where: V_a is the extract volume [mL], V_b is the sample volume [mL], and E_x is the extinction (absorption) measured at wavelength x in a 1-cm cuvette.

2.6. Statistical analyses

Repeated measure ANOVA was used to test the effect of picocyanobacterial filtrates on the growth and fluorescence of the targeted cyanobacteria. A post hoc test (Tukey's HSD) was used to test differences between treatment levels. Independent sample *t*-tests assuming equal variances were applied to determine whether the pigment content of the target species, when treated with picocyanobacterial filtrates, differed from the control on the last day of the experiment. Data are reported as the means ± standard deviations (SD). The statistical analyses were performed using Statistica® 13.1 software.

3. Results

3.1. Effects of picocyanobacterial filtrates on the growth of cyanobacteria

Both single and repeated additions of cell-free filtrate obtained from *Synechococcus* sp. significantly decreased the number of cells of *Nostoc* sp. (ANOVA, $F_{1,4} = 21.4$, $p < 0.001$ and ANOVA, $F_{1,4} = 18.1$, $p < 0.001$, respectively), and *Phormidium* sp. (ANOVA, $F_{1,4} = 403.4$, $p < 0.001$ and ANOVA, $F_{1,4} = 254.8$, $p < 0.001$, respectively); the control showed active growth (Fig. 1). After the addition of cell-free filtrate, the highest decline in growth was observed for *Phormidium* sp. On the seventh day of the experiment, after single and repeated filtrate additions, the growths of *Phormidium* sp. of were reduced by 48% (Tukey HSD, $p < 0.001$) and 57% (Tukey HSD, $p < 0.001$), respectively, whereas on the 14th day, the growths were reduced by 53% (Tukey HSD, $p < 0.001$) and 53% (Tukey HSD, $p < 0.001$), respectively (Fig. 1D). The inhibition of *Nostoc* sp. growth after the single addition of cell-free filtrate was 67% by the 14th day (Tukey HSD, $p < 0.001$). For the repeated addition of cell-free filtrate, on the 14th day, the reduction was 44% (Tukey HSD, $p < 0.001$, Fig. 1A). However, the single addition of cell-free filtrate from *Synechococcus* sp. stimulated the growth of *A. flos-aquae*, and on the 14th day of experiment, the growth increased by 26% relative to control (Tukey HSD, $p < 0.05$). The repeated addition of filtrate did not significantly affect *A. flos-aquae* abundance (ANOVA, $F_{1,4} = 2.2$, $p > 0.05$, Fig. 1B). Additionally, the single and repeated additions of the filtrate did not significantly affect the target *Rivularia* sp. (ANOVA, $F_{1,4} = 0.3$, $p > 0.05$ and ANOVA, $F_{1,4} = 1.5$, $p > 0.05$, respectively, Fig. 1C).

3.2. Effects of picocyanobacterial filtrates on chlorophyll fluorescence

The effects of picocyanobacterial cell-free filtrate on chlorophyll fluorescence after 1 h and 1, 3, 7 and 14 days of incubation are shown in Fig. 2. The cyanobacterial species showed different responses. The single and repeated additions of cell-free filtrate from *Synechococcus* sp. cultures had significant effects on the values of the F_v/F_m of *A. flos-aquae* (ANOVA, $F_{1,4} = 5.9$, $p < 0.01$ and ANOVA, $F_{1,4} = 2.9$, $p < 0.05$, respectively) and *Phormidium* sp. (ANOVA, $F_{1,4} = 3.9$, $p < 0.05$ and ANOVA, $F_{1,4} = 3.1$, $p < 0.05$, respectively). The strongest negative response for *Phormidium* sp. after single filtrate

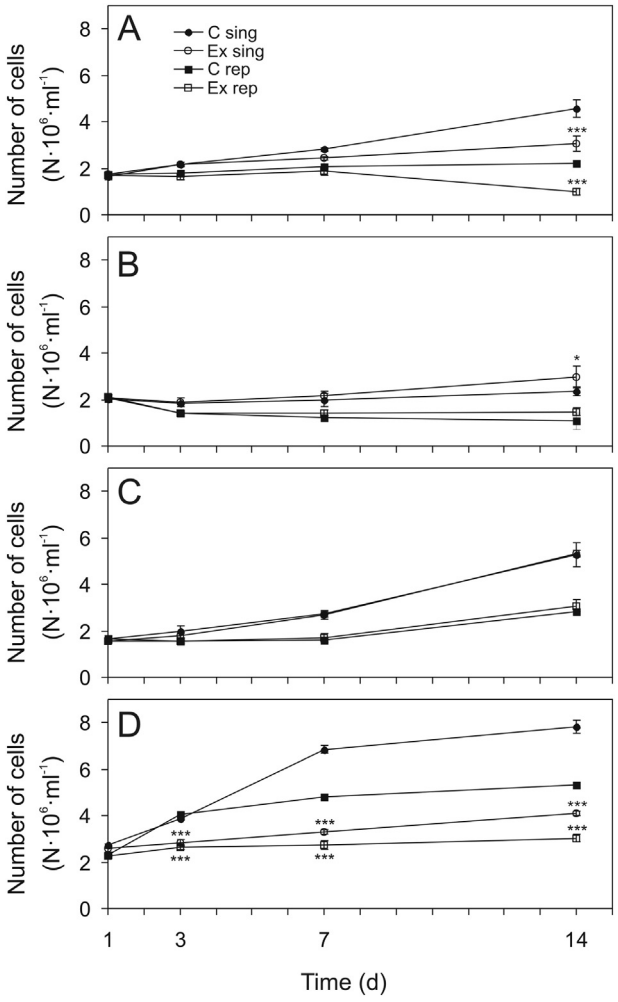

Fig. 1. The number of cells (N) for A) *Nostoc* sp., B) *A. flos-aquae*, C) *Rivularia* sp. and D) *Phormidium* sp. for controls and experiments with single and repeated additions of cell-free filtrate obtained from *Synechococcus* sp. cultures (referred to as C sing, Ex sing, C rep and Ex rep, respectively) after 1, 3, 7 and 14 days of exposure. The values refer to means ($n = 3$, mean ± SD). Asterisk indicates significant difference compared with control. Levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

addition was at 1 h and on the first and third days of exposure to the filtrates. Their magnitudes were 70% (Tukey HSD, $p < 0.001$), 80% (Tukey HSD, $p < 0.05$) and 74% (Tukey HSD, $p < 0.05$), respectively, compared with controls. Moreover, after the target cyanobacterium was exposed to a repeated addition of cell-free filtrate, the value of F_v/F_m was significantly inhibited on each day of the experiment. The highest decreases in F_v/F_m for *Phormidium* sp. were observed after 7 and 14 days of experiment, with magnitudes of 62% (Tukey HSD, $p < 0.001$) and 55% (Tukey HSD, $p < 0.001$), respectively, compared with controls (Fig. 2D). Furthermore, the single and repeated additions of the picocyanobacterial filtrate resulted in an increased F_v/F_m for *A. flos-aquae* after the third day of exposure, i.e., increases of 22% (Tukey HSD, $p < 0.05$) and 44% (Tukey HSD, $p < 0.05$), respectively, compared with controls (Fig. 2B). However, the single and repeated additions of cell-free filtrate from *Synechococcus* sp. cultures had no significant effects on F_v/F_m for *Nostoc* sp. (ANOVA, $F_{1,4} = 1.8$, $p > 0.05$ and ANOVA, $F_{1,4} = 0.1$, $p > 0.05$, respectively, Fig. 2A) and *Rivularia* sp. (ANOVA, $F_{1,4} = 1.5$, $p > 0.05$ and ANOVA, $F_{1,4} = 1.6$, $p > 0.05$, respectively, Fig. 2C).

3.3. Effects of picocyanobacterial filtrates on pigment content

The effects of repeated additions of cell-free filtrate from

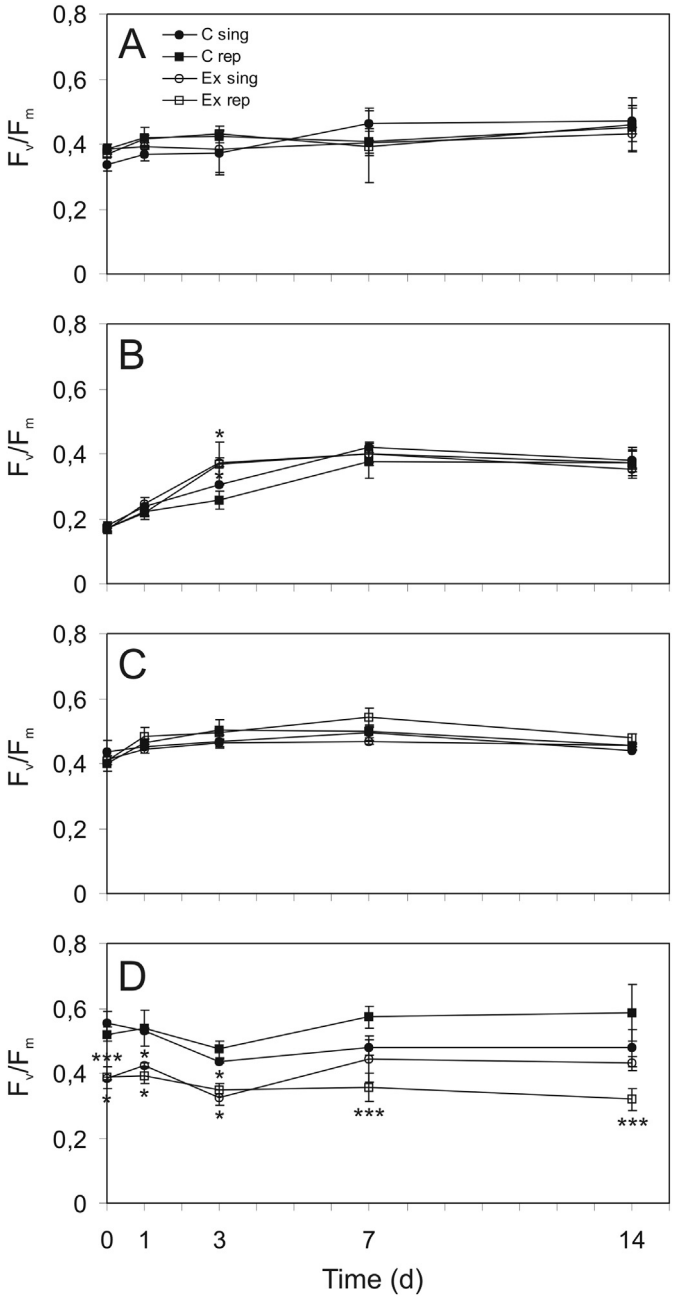

Fig. 2. The fluorescence parameter F_v/F_m for A) *Nostoc* sp., B) *A. flos-aquae*, C) *Rivularia* sp. and D) *Phormidium* sp. for controls and experiments with single and repeated additions of cell-free filtrate obtained from *Synechococcus* sp. cultures (referred to as C sing, Ex sing, C rep and Ex rep, respectively) after 0 (1 h), 1, 3, 7 and 14 days of exposure. The values refer to means (n = 3, mean ± SD). Asterisk indicates significant difference compared with control. Levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Synechococcus sp. cultures on the pigment contents of *A. flos-aquae*, *Nostoc* sp., *Phormidium* sp. and *Rivularia* sp. after two weeks of exposure are shown in Fig. 3. The pigment contents of all cyanobacteria were significantly altered. The Chl *a* contents of *Phormidium* sp. and *Rivularia* sp. were significantly lower than that of the control: 28% (*t*-test, $p < 0.05$) and 31% (*t*-test, $p < 0.01$), respectively. These two species also showed reduced Car contents per cell in the treatment with filtrate, although the differences here were not significant (Figs. 3C, D). However, *A. flos-aquae* and *Nostoc* sp. showed increased Car contents of 25% (*t*-test, $p < 0.05$) and 46% (*t*-test, $p < 0.05$), respectively, which were higher than the control treatment (Fig. 3A, B).

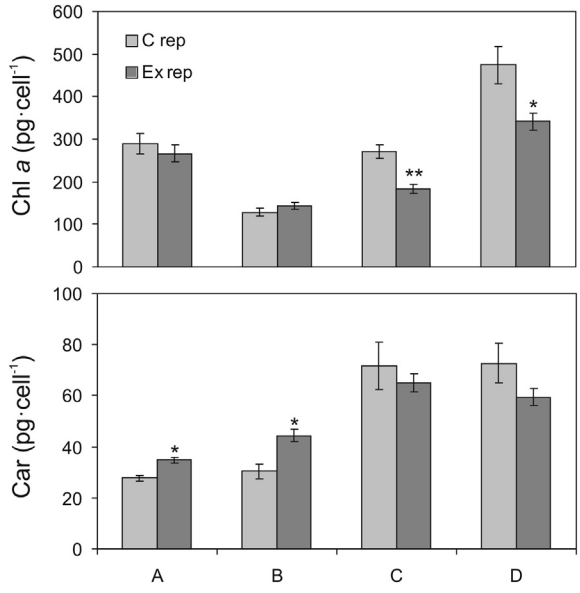

Fig. 3. Chl *a* and Car contents of A) *Nostoc* sp., B) *A. flos-aquae*, C) *Rivularia* sp. and D) *Phormidium* sp. for controls and experiments with repeated addition of cell-free filtrate obtained from *Synechococcus* sp. cultures (referred to as C rep and Ex rep, respectively) after 14 days of exposure. The values refer to mean ± SD (n = 3). Asterisk indicates significant difference compared with control. Levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

Picocyanobacteria, despite their ubiquity, are a group of poorly studied organisms. Although they are usually associated with open ocean systems, it has been increasingly evident in recent years that *Synechococcus* spp. were significant contributors to cyanobacterial blooms in many aquatic ecosystems (Beardall, 2008; Sorokin and Dallocchio, 2008; Sorokin and Zakuskina, 2010). However, knowledge about their allelopathic activity remains scarce. In this study, we demonstrated for the first time that the picocyanobacterium *Synechococcus* sp. showed allelopathic activity on several filamentous cyanobacteria: *A. flos-aquae*, *Nostoc* sp., *Phormidium* sp. and *Rivularia* sp.

The filtrate obtained from *Synechococcus* sp. caused a significant inhibition of growth of *Nostoc* sp. and *Phormidium* sp. A few authors have also reported negative allelopathic effects of cyanobacteria against other coexisting cyanobacteria species. Schagerl et al. (2002) reported that *Anabaena torulosa* and *Nostoc* sp. inhibited the growth of *Anabaena cylindrica* and *Microcystis flos-aquae*. Moreover, Valdor and Aboal (2007) showed that cell extracts of several filamentous cyanobacteria had inhibitory effects on the growths of other different cyanobacteria species. They showed that after four days, the growth of *Scytonema* sp. was inhibited by all employed cyanobacterial extracts, except those from *Rivularia biasolettiana*. *Pseudocapsa* sp. was inhibited by the extracts from *Rivularia haematites*, *Geitlerinema splendidum*, *Phormidium* sp. and *Oscillatoria* sp. After eight days, *Pseudocapsa* sp. continued to be inhibited by the extracts of *Oscillatoria* sp. and *Phormidium* sp. Authors also described *Pseudocapsa* sp. and *Nostoc* sp. as the most sensitive species, because their growths were completely inhibited by all cyanobacterial extracts employed.

In the present study, picocyanobacterial exudates had stimulatory effects on the growth, Car content and fluorescence of *A. flos-aquae*. Suikkanen et al. (2005) noted that cell-free filtrates from *Nodularia spumigena* stimulated both *N. spumigena* and other cyanobacteria (*Anabaena* sp.) from the natural plankton community. Monahan and Trainor (1970) showed that *Aphanizomenon* sp. filtrates caused an over 50-fold increase in the cell numbers of *Aphanizomenon* sp. in the natural community. This suggested that the cyanobacteria released auto-stimulatory or quorum-sensing compounds (Swift et al., 1994). The

compounds present in the picocyanobacterial filtrates may have been useful to other filamentous cyanobacteria because they share many primary metabolic pathways.

In the present work it was shown that single and repeated addition of the picocyanobacterial filtrate did not significantly affect the growth and chlorophyll *a* fluorescence of *Rivularia* sp. Specific features, e.g., membrane permeability, may contribute to differences in the sensitivity of phytoplankton species to cyanobacterial allelochemicals (Suikkanen et al., 2004). This may account for the resistance of *Rivularia* sp. This differential sensitivity to allelochemicals among species suggests that picocyanobacterial blooms may drive changes in the structure of phytoplankton communities (Suikkanen et al., 2004).

Despite the ecological importance of *Synechococcus* in marine, brackish and freshwater environments, very little is known about their toxic and allelopathic effects on other organisms. Martins et al. (2007) noted that a few Portuguese coastal *Synechococcus* strains produced substances with negative effects on invertebrates. Moreover, Hamilton et al. (2014) demonstrated a significant effect of a *Synechococcus* strain on dark preferences of black perch *Embiotoca jacksoni*. Recently, certain *Synechococcus* strains from Southern California have been reported to inhibit the growth of other picocyanobacteria, very likely due to secreted secondary metabolites (Paz-Yepes et al., 2013). In other works, Śliwińska-Wilczewska et al. (2016b) noted that picocyanobacterium *Synechococcus* sp. negatively affected coexisting filamentous, bloom-forming cyanobacteria *N. spumigena* and that the production of allelopathic substances was influenced by light intensity. However, the identities of these compounds remain unknown. The identification of allelopathic compounds is a difficult and time-consuming task. Similarly, the identification of allelopathic compounds released by *Synechococcus* sp. will require further investigation. Overall, our results confirmed the allelopathic activity of the picocyanobacterium *Synechococcus* sp. on filamentous cyanobacteria. These results showed that picocyanobacterial allelopathy should be considered when estimating the potential interactions between cyanobacteria in aquatic ecosystems.

We investigated the allelopathic activity of *Synechococcus* sp. on cyanobacteria using cultures under non-axenic conditions. However, the suitability of non-axenic cultures to allelopathic experiments has been previously debated. For one, the observed inhibition may be caused by accompanying heterotrophic bacteria. However, Suikkanen et al. (2004) demonstrated that a filtrate containing only compounds excreted by heterotrophic bacteria did not inhibit target microalgae *Rhodomonas* sp. Similarly, Tillmann and John (2002) demonstrated that extracellular compounds produced by heterotrophic bacteria did not cause any allelopathic effects. However, further studies should be carried out to determine whether accompanying heterotrophic bacteria have allelopathic effects on different species of filamentous cyanobacteria.

The precise mode of action of picocyanobacterial allelopathic compounds remains unknown. One possible mode of action is by altering the pigment content. In this work, we showed that filtrates from *Synechococcus* sp. caused significant reductions in the Chl *a* contents of *Phormidium* sp. and *Rivularia* sp. cells and increased the Car contents of *A. flos-aquae* and *Nostoc* sp. The Chl *a* content of *Rivularia* sp. significantly decreased, but this had no impact on growth. More time may have been necessary to see growth inhibition. The Chl *a* content of *Nostoc* sp. was unaffected, whereas the growth of this organism was strongly inhibited. This growth inhibition of *Nostoc* sp. caused by *Synechococcus* sp. filtrates and may have been associated with damage to the cell membranes rather than photosynthesis inhibition, as suggested by Suikkanen et al. (2006). Car contents increased in *A. flos-aquae*, which was stimulated by *Synechococcus* sp. allelochemicals, and *Nostoc* sp., which was inhibited. Carotenoid pigments have a protective function (e.g., Jodłowska and Latała, 2013). This increased Car contents of *A. flos-aquae* and *Nostoc* sp. may indicate a protective response. Suikkanen et al. (2006) also showed significant differences in the cellular Chl *a* contents of target *Rhodomonas* sp. between the cyanobacterial filtrate obtained from *A. flos-aquae* and *N. spumigena* and the control. Cyanobacteria produce allelopathic compounds affecting a wide range of biochemical processes. A few of these compounds are directed against photosynthesis (Sukenik et al., 2002). Therefore, light-dependent processes of filamentous cyanobacteria would be logical targets for competing and coexisting picocyanobacteria. These results supported the hypothesis that the growth inhibition caused by the *Synechococcus* sp. filtrates could be directly associated with the inhibition of *Phormidium* sp. photosynthetic processes.

Another mode of action described for allelopathic compounds is the effect on the chlorophyll *a* fluorescence of the target species. In this work, we observed decreased F_v/F_m in *Phormidium* sp. after single and repeated additions of filtrate after 1 h of exposure. However, significant growth decreases were only observed after three days of filtrate exposure. Prince et al. (2008) showed that *Karenia brevis* bloom extract decreased the F_v/F_m of *Skeletonema costatum*, *Akashiwo sanguinea*, *Amphora* sp., *Asterionellopsis glacialis*, and *Prorocentrum minimum* after 1 h of exposure. The authors indicated that the efficiency of *PSII* measured after 1 h of exposure to allelopathic compounds may have been a more sensitive indicator of stress rather than effects on growth measured over a few days. Our F_v/F_m results may provide more accurate information on a few cyanobacterial species regarding their sensitivity to picocyanobacterial allelopathy. The inhibition of *PSII* efficiency may be an effective strategy for cyanobacteria, as noted by Gross (2003). However, the picocyanobacterial filtrate has been shown to result in a decrease of *Nostoc* sp. growth. This did not affect the values of the fluorescence parameter F_v/F_m. Thus, the allelopathic effect is induced through a different mechanism not considered here. The F_v/F_m of *Nostoc* sp. was found to be unaffected. However, the growth of this cyanobacterium was strongly inhibited. This suggested that the mode of action of *Synechococcus* sp. allelochemicals for this species was not related to Chlorophyll *a* inhibition. Moreover, fluorescence parameters likely are not good representations of the physiological status for this cyanobacterium (Kruskopf and Flynn, 2006). Determining the allelopathic effects of picocyanobacteria on the fluorescence and photosynthesis of filamentous cyanobacteria would require further study.

The present study indicated that the picocyanobacterium *Synechococcus* sp. negatively and positively affected coexisting filamentous cyanobacteria. To better understand these allelopathic effects, the further study of the production and secretion of active allelopathic compounds is required. The identification of the allelopathic compounds is also necessary to better understand the molecular targets in the affected cyanobacteria species. Providing new information on the allelopathy of this species may also be important for a better understanding of the worldwide intensifying phenomenon of massive picocyanobacterial blooms.

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