Allelopathic activity of the picocyanobacterium *Synechococcus* sp. on coexisting microalgae

Sylvia Śliwińska-Wilczewska^A, Aldo Barreiro Felpeto^{B,D}, Jakub Maculewicz^A, Amanda Sobczyk^A, Vitor Vasconcelos^{B,C} and Adam Latała^A

^AUniversity of Gdansk, Institute of Oceanography, Av. Pilsudskiego 46, 81-378 Gdynia, Poland

^BInterdisciplinary Center of Marine and Environmental Research–CIMAR/CIIMAR, University of Porto, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

^CDepartment of Biology, Faculty of Sciences, Porto University. Rua do Campo Alegre, 4069-007 Porto, Portugal

^DCorresponding author. Email: aldo.barreiro@gmail.com

Abstract. The production and release of allelopathic compounds is an important adaptation by which some species of cyanobacteria can achieve a competitive advantage over other primary producers. In this study, we tested the allelopathic activity of the picocyanobacterium *Synechococcus* sp. against the following coexisting microalgae: *Porphyridium purpureum*, *Stichococcus bacillaris*, *Prymnesium parvum* and *Nitzschia dissipata*. We demonstrated that both addition of *Synechococcus* sp. cell-free filtrate and co-culture inhibited the growth of *P. purpureum* and *S. bacillaris*. Conversely, the growth of *P. parvum* was positively affected. By contrast, *N. dissipata* was unaffected by either the picocyanobacterial filtrate or co-culture. These results showed that *Synechococcus* sp. allelopathy should be considered when estimating the potential interactions between picocyanobacteria and coexisting microalgae in aquatic ecosystems.

26

27 **Additional keywords:** allelopathy, cyanobacterial bloom, growth, microalgae,
28 picocyanobacteria, *Synechococcus* sp.

29

30 **Introduction**

31 | Allelopathy can be found in aquatic environments and primary producers can release active
32 compounds which can affect the functioning of the marine, brackish and freshwater
33 ecosystems (e.g., Fistarol *et al.* 2004; Ji *et al.* 2011; Poulson-Ellestad *et al.* 2014; Brutemark
34 *et al.* 2015). Allelopathy is also considered among the potential drivers of massive blooms
35 throughout many aquatic environments. The number of reports on the allelopathic effects of
36 cyanobacteria and microalgae has been steadily increasing (e.g., Barreiro and Hairston 2013;
37 Źak and Kosakowska 2015; Dias *et al.* 2017; Wang *et al.* 2017).

38 Picocyanobacteria play an important role in aquatic ecosystems (Beardall 2008; Callieri
39 2010; Costa *et al.* 2015; Worden and Wilken 2016) but little is known about their allelopathic
40 activity against coexisting microalgae. In previous works, we demonstrated the allelopathic
41 effect of *Synechococcus* sp. on diatom *Navicula perminuta* (Śliwińska-Wilczewska *et al.*
42 | 2016) and we also showed a significant inhibitory effect of cell-free filtrate obtained from
43 *Synechococcus* sp. against the filamentous cyanobacteria *Nostoc* sp. and *Phormidium* sp.
44 (Śliwińska-Wilczewska *et al.* 2017a) and against different groups in a natural plankton
45 community (Śliwińska-Wilczewska *et al.* 2017b). There are also some reports of allelopathic
46 effects such as growth inhibition or stimulation caused by other species of cyanobacteria (e.g.,
47 Suikkanen *et al.* 2004, 2005; Antunes *et al.* 2012; Barreiro and Vasconcelos 2014; Rzymiski *et*
48 *al.* 2014) but there is no information about allelopathic potential of *Synechococcus* sp. on
49 coexisting microalgae like *Porphyridium purpureum*, *Stichococcus bacillaris*, *Prymnesium*

50 *parvum* and *Nitzschia dissipata*. The issue of picocyanobacterial allelopathy needs more
51 researcher's attention, since this group is the major contributor to marine primary production.

52 The effect of allelochemicals depends on the nature of the interaction between donor
53 and target organisms (direct cell to cell contact, no contact) and activity of the chemical
54 compounds responsible of this interaction. In aquatic ecosystems, donor organisms may affect
55 the target species in different ways, and, in most cases, allelopathic compounds can reduce the
56 growth rate and eventually cause the death of targeted organisms (Rzymiski *et al.* 2014).
57 However, it is difficult to find direct evidence of allelopathic interactions between
58 cyanobacteria and microalgae in natural communities (see Keating 1977, 1978). Before this
59 can be achieved, it is very important to characterize allelopathic interactions under controlled
60 laboratory conditions, in order to investigate in detail the nature of released substances and
61 their impact on target organisms.

62 The main aim of this study was to determine the influence of allelopathic compounds
63 produced by picocyanobacterium *Synechococcus* sp. on growth of *Porphyridium purpureum*,
64 *Stichococcus bacillaris*, *Prymnesium parvum* and *Nitzschia dissipata* in both co-cultures and
65 bioassays employing cell-free filtrates. These species are widely geographically distributed
66 and relevant in terms of abundance in phytoplankton communities, so allelopathic interactions
67 between them could play a significant role in aquatic ecosystems. Based on this, further
68 research could be done to identify the chemical structures of the allelopathic compounds
69 involved in the interaction, as well as their mechanisms and modes of action on target
70 organisms.

72 **Materials and methods**

73 *Material and culture conditions*

The experiments were conducted with the picocyanobacterium *Synechococcus* sp. (BA-124) and the following microalgae: the red algae *Porphyridium purpureum* (MA-03), the green algae *Stichococcus bacillaris* (BA-09), the golden algae *Prymnesium parvum* (AA-69) and the diatom *Nitzschia dissipata* (BA-40). These strains were isolated from the coastal zone of the Norwegian Sea and the Baltic Sea and 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 experiment. Culture media was prepared with Baltic Sea water filtered through glass microfiber filters (Whatman GF/C) and autoclaved. The salinity was 8 PSU, as measured with a salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). The picocyanobacteria and microalgae were incubated under a 16:8 h light:dark cycle at Photosynthetically Active Radiation (PAR) irradiance $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and temperature 20°C. The intensity of PAR was measured using a quantum-meter (LI-COR, Nebraska, USA) with a cosine collector.

Prior to the experiments, the concentrations of macronutrients (i.e., nitrate, N-NO₃ and P-PO₄ orthophosphates) in the picocyanobacterial 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). Then, the concentrations of N-NO₃ and P-PO₄ in the controls and all treatments were adjusted to the same level as in the f/2 growth medium. The microalgae in the controls showed active growth during the course of the experiment. Therefore, the effects of major nutrients, microelements and vitamin limitations in the control and allelochemical treatments can be excluded. On the first and the last days of the experiment, the pH of all experimental flasks was measured using a pH-meter (Elmetron CP-

401, Zabrze, Poland). pH values were similar across treatments and durations and ranged from 8.2 to 8.6.

Test of the allelopathic effect of cell-free filtrates

Allelopathic effects were tested following a modified version of the method proposed by Suikkanen *et al.* (2004). Cultures of the donor picocyanobacterium *Synechococcus* sp. and the target microalgae were maintained in active growth during 7 days in standard conditions (see above) and then employed in the experiments. The picocyanobacterium culture was gently filtered through a 0.45- μ m filter (Macherey-Nagel MN GF-5) using a vacuum pump (400 mbar). The filtrate obtained was analyzed under an epifluorescence microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) in order to confirm the absence of picocyanobacteria cells. Experimental treatments were prepared by adding 4 mL of this cell-free filtrate to 25-mL Erlenmeyer flasks containing 20 mL of cell suspensions of the target microalgae. These cell suspensions were obtained from culture aliquots. Controls were prepared by adding 4 mL of filtered f/2 medium to 25-mL Erlenmeyer flasks containing 20 mL of cell suspensions of the same microalgae species. These tests were conducted in triplicate. In all experiments, 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 0.8 μ g Chl *a* mL⁻¹). The experiments lasted 7 days.

Test of the allelopathic effect in co-cultures

Allelopathic interactions in co-cultures were determined following a modified version of the method proposed by Ji *et al.* (2011). Employed cultures maintained in the conditions described above, 4 mL aliquots from the donor picocyanobacteria were added to 25-mL Erlenmeyer flasks containing 20 mL of cell suspensions of the target microalgae. Controls

were prepared by adding 4 mL of filtered f/2 medium to 25-mL Erlenmeyer flasks containing 20 mL of cell suspensions of the target microalgae. These tests were conducted in triplicate. In all these experiments, the initial ratio of donor picocyanobacteria to target species in Erlenmeyer flasks was adjusted to 1:1 based on the Chl *a* content (the initial chlorophyll *a* concentration in the experimental cultures was 0.8 µg Chl *a* mL⁻¹ for donor and target species). The experiments lasted 7 days.

Determination of cell abundances

The number of cells (N) in cultures was counted with flow cytometer BD Accuri™ C6 Plus (BD Biosciences, San Jose, CA, USA). Events were recorded in list mode. Samples were run at a flow rate of approximately 14 µl min⁻¹. Flow was daily calibrated with Spherotech 6- and 8- Peak Validation Beads (BD, San Jose, USA). This ensures that the cytometer is working properly before running experimental samples. FITC, PE, and PE-Cy5 detectors were daily calibrated with SPHERO™ Rainbow Calibration Particles (BD, San Jose, USA), and the APC channel was calibrated with SPHERO 6-peaks Allophycocyanin Calibration Particles (APC). Detectors FL1, FL2, and FL3 read fluorescence emissions excited by the blue laser (480 nm), while detector FL4 reads emissions excited by the red laser (640 nm). In the co-cultures, the populations of *Synechococcus* sp. and the corresponding target microalgae were completely discriminated using a combination of red and orange fluorescences (Fig. 1). N was determined in all the experiments at times 0 (1h) and 1st, 3rd and 7th day of the experiments.

Fig. 1

Statistical analyses

Different ANOVAs were performed in order to test the effect of picocyanobacterial filtrates or co-culture on the growth of the targeted microalgae. A post-hoc Dunnett's test was used to

determine significant differences between the control and the other treatment levels. These statistical analyses were performed with Statistica® 13.1 software.

Results

Allelopathic effect of cell-free filtrates

The addition of cell-free filtrate obtained from *Synechococcus* sp. significantly affected the number of cells of *Porphyridium purpureum*, *Stichococcus bacillaris* and *Prymnesium parvum* (ANOVA, $F_{7,16} = 280.3$, $P < 0.001$, ANOVA, $F_{7,16} = 459.5$, $P < 0.001$ and ANOVA, $F_{7,16} = 279.5$, $P < 0.001$, respectively, Fig. 2A, B, C). Considering individual days, the allelopathic effect of the picocyanobacterium significantly reduced the number of cells of *P. purpureum* at day 7 (85% relative to the control; Dunnett, $P < 0.01$), and of *S. bacillaris* at day 3 (87% relative to the control; Dunnett, $P < 0.05$) (Fig. 2A, B). In contrast, the addition of cell-free filtrate obtained from *Synechococcus* sp. had a significantly positive effect on the growth of *P. parvum*, which, at day 7 was higher than the control by 19% (Fig. 2C; Dunnett, $P < 0.001$). The addition of the cell-free filtrate obtained from *Synechococcus* sp. had no effect on the target diatom *Nitzschia dissipata* (ANOVA, $F_{7,16} = 0.5$, $P = 0.7$, Fig 2D).

Fig. 2

Allelopathic effects in co-cultures

After the addition of *Synechococcus* sp. cells, it was observed not only a reduction, but also a decline with time in the number of cells of *Porphyridium purpureum* (ANOVA, $F_{7,16} = 1526.6$, $P < 0.001$, Fig. 3A). Significant differences were found on the first, third and seventh day of the experiment, when cell numbers of *P. purpureum* constituted 38% (Dunnett, $P < 0.001$), 17% (Dunnett, $P < 0.001$) and 1% (Dunnett, $P < 0.001$), of control. For *Stichococcus*

bacillaris, it was observed a reduction in the number of cells (ANOVA, $F_{7,16} = 599.8$, $P < 0.001$, Fig. 3B). By the first, third and seventh day of the experiment the growths of *S. bacillaris* were reduced by 80% (Dunnett, $P < 0.001$), 86% (Dunnett, $P < 0.01$) and 94% (Dunnett, $P < 0.05$), respectively, relative to the control treatment. On the other hand, *Synechococcus* sp. cells had significantly positive effect on the growth of *Prymnesium parvum* (ANOVA, $F_{7,16} = 491.1$, $P < 0.001$, Fig. 3C). The number of cells of *P. parvum* significantly increased after third (Dunnett, $P < 0.01$) and seventh (Dunnett, $P < 0.001$) day of the experiment and constituted 114% and 131%, respectively, of control (Fig. 3C). No effects were detected in *Nitzschia dissipata* cultures (ANOVA, $F_{7,16} = 1.2$, $P = 0.3$, Fig. 3D).

Fig. 3

Discussion

There are very few reports of allelopathic compounds from phytoplankton that affect red algae. Just very recently, García-Espín *et al.* (2017) showed that both cyanobacteria extracts obtained from *Rivularia haematites* and *Rivularia biasolettiana* as well as pure microcystin affected the photosynthetic activity of *Chroothoece richteriana*. In this sense, our report of the strong negative effect of *Synechococcus* sp. on *P. purpureum*, particularly evident in the co-culture, is also a novel finding. *P. purpureum* was more strongly inhibited in co-culture compared to cell-free filtrate addition. This could be explained simply by the renewal of allelochemical compounds in the presence of cells.

Some studies have shown that green algae are a phytoplankton group that are particularly sensitivity to allelopathic compounds from cyanobacteria (Schlegel *et al.* 1999; Schagerl *et al.* 2002; Žak *et al.* 2012). The data presented here constitutes, to our knowledge, the first report of an allelopathic effect of a picocyanobacteria against a specific species of

chlorophyte. These inhibitory effects on *S. bacillaris* in monoculture and co-culture were stronger during the first days of exposition both with cell-free filtrates and co-culture. This suggests that this species may become resistant to these compounds through adaptation.

Previous studies did not find allelopathic effects from different cyanobacteria (*Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermannii*) against *Prymnesium parvum* (Suikkanen *et al.* 2004). Our study shows that *Synechococcus* sp. affects *P. parvum* positively both through cell-free filtrates and co-culture. Suikkanen *et al.* (2005) also noted that cyanobacterial filtrates could promote the growth of some microalgae due to stimulatory allelochemicals. This could explain our results in the cell-free filtrate treatment. Also, osmotrophic feeding by *P. parvum* on exudates from *Synechococcus* sp. could be an explanation. In the co-culture treatment, the stronger positive effect could be explained by the higher concentration of exudates in the presence of *Synechococcus* sp. cells, but, it is also possible the occurrence of phagotrophic feeding by *P. parvum*, (Tillmann 1998).

Many authors showed different sensitivity of diatoms for the allelopathic compounds produced by cyanobacteria. In this work, the diatom *Nitzschia dissipata* was found to be tolerant to *Synechococcus* sp. cell-free filtrate and co-culture. Suikkanen *et al.* (2004) showed that *Thalassiosira weissflogii* was inhibited by cell-free filtrates of three cyanobacteria: *N. spumigena*, *A. flos-aquae* and *A. lemmermannii*. The tolerance of our species could be due to its relatively large cell size, as suggested by Lyczkowski and Karp-Boss (2014).

Our results suggest that allelopathic effects of *Synechococcus* sp. may have a differential effect in phytoplankton communities, due to the contrasting responses found among target species. The effects of picocyanobacteria on coexisting phytoplankton species is very important, since they account for the majority of primary producer biomass in the oceans, and their relative abundance is even expected to increase in scenarios predicted by global change (Dutkiewicz *et al.* 2015).

224

225 **Acknowledgments**

226 This study was supported by BMN grants, Poland, No. 538-G245-B568-17 [and FCT Project](#)
227 [UID/Multi/04423/2013](#).

228

229 **References**

- 230 Antunes, J. T., Leão, P. N., and Vasconcelos, V. M. (2012). Influence of Biotic and Abiotic
231 Factors on the Allelopathic Activity of the Cyanobacterium *Cylindrospermopsis*
232 *raciborskii* Strain LEGE 99043. *Microbial Ecology* **64**, 584–592. doi: 10.1007/s00248-
233 012-0061-7
- 234 Barreiro, A., and Vasconcelos, V. M. (2014). Interactions between allelopathic properties and
235 growth kinetics in four freshwater phytoplankton species studied by model simulations.
236 *Aquatic Ecology* **48**, 191–205. doi: 10.1007/s10452-014-9475-2
- 237 Barreiro, A., and Hairston Jr, N. G. (2013). The influence of resource limitation on the
238 allelopathic effect of *Chlamydomonas reinhardtii* on other unicellular freshwater
239 planktonic organisms. *Journal of Plankton Research* **35**, 1339–1344. doi:
240 10.1093/plankt/fbt080
- 241 Beardall, J. (2008). Blooms of *Synechococcus*: An analysis of the problem worldwide and
242 possible causative factors in relation to nuisance blooms in the Gippsland Lakes.
243 Monash university, 1–8.
- 244 Brutemark, A., Vandelannoote, A., Engström-Öst, J., and Suikkanen, S. (2015). A less saline
245 Baltic Sea promotes cyanobacterial growth, hampers intracellular microcystin
246 production, and leads to strain-specific differences in allelopathy. *PloS one* **10**,
247 e0128904. doi: 10.1371/journal.pone.0128904

248 Callieri, C. (2010). Single cells and microcolonies of freshwater picocyanobacteria: A
 249 common ecology. *Journal of Limnology* **69**, 257–277. doi: 10.3274/JL10-69-2-08

250 Costa, M. S., Costa, M., Ramos, V., Leão, P. N., Barreiro, A., Vasconcelos, V., and Martins,
 251 R. (2015). Picocyanobacteria from a clade of marine cyanobium revealed bioactive
 252 potential against microalgae, bacteria, and marine invertebrates. *Journal of Toxicology*
 253 *and Environmental Health, Part A* **78**, 432–442. doi: 10.1080/15287394.2014.991466

254 Dias, F., Antunes, J. T., Ribeiro, T., Azevedo, J., Vasconcelos, V., and Leão, P. N. (2017).
 255 Cyanobacterial Allelochemicals But Not Cyanobacterial Cells Markedly Reduce
 256 Microbial Community Diversity. *Frontiers in Microbiology* **8**, 1495. doi:
 257 10.3389/fmicb.2017.01495

258 Dutkiewicz, S., Morris, J. J., Follows, M. J., Scott, J., Levitan, O., Dyhrman, S. T., and
 259 Berman-Frank, I. (2015). Impact of ocean acidification on the structure of future
 260 phytoplankton communities. *Nature Climate Change* **5**, 1002–1006. doi:
 261 10.1038/NCLIMATE2722

262 Fistarol, G. O., Legrand, C., Selander, K., Hummer, C., Stolte, W., and Granéli, E. (2004).
 263 Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal
 264 monocultures. *Aquatic Microbial Ecology* **35**, 45–56.

265 García-Espín, L., Cantoral, E. A., Asencio, A. D., and Aboal, M. (2017). Microcystins and
 266 cyanophyte extracts inhibit or promote the photosynthesis of fluvial algae. Ecological
 267 and management implications. *Ecotoxicology* **26**, 658–666. doi: 10.1007/s10646-017-
 268 1798-z

269 Grasshoff, K. (1976). Methods of seawater analysis, Verlag Chemie Weinheim, New York.

270 Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: W.
 271 L., Smith, M. H., Chanley, (eds), Culture of Marine Invertebrate Animals, pp. 26–60.
 272 Plenum Press, New York, USA.

273 Ji, X. Q., Han, X. T., Zheng, L., Yu, Z. M., Yang, B. J., and Zou, J. Z. (2011). Allelopathic
 274 interactions between *Prorocentrum micans* and *Skeletonema costatum* or *Karenia*
 275 *mikimotoi* in laboratory cultures. *Chinese Journal of Oceanology and Limnology* **29**,
 276 840–848. doi: 10.1007/s00343-011-0512-x

277 Keating, K. I. (1977). Allelopathic Influence on Blue-Green Bloom Sequence in a Eutrophic
 278 Lake. *Science* **196**, 885–887. doi: 10.1126/science.196.4292.885

279 Keating, K. I. (1978). Blue-green algal inhibition of diatom growth: transition from
 280 mesotrophic to eutrophic community structure. *Science* **199**, 971–973. doi:
 281 10.1126/science.199.4332.971

282 Lyczkowski, E. R., and Karp-Boss, L. (2014). Allelopathic effects of *Alexandrium fundyense*
 283 (Dinophyceae) on *Thalassiosira* cf. *gravida* (Bacillariophyceae): a matter of size.
 284 *Journal of Phycology* **50**, 376–387. doi: 10.1111/jpy.12172

285 Poulson-Ellestad, K., Mcmillan, E., Montoya, J. P., and Kubanek, J. (2014). Are offshore
 286 phytoplankton susceptible to *Karenia brevis* allelopathy? *Journal of Plankton Research*
 287 **36**, 1344–1356. doi: 10.1093/plankt/fbu064

288 Rzymiski, P., Poniedziałek, B., Kokociński, M., Jurczak, T., Lipski, D., and Wiktorowicz, K.
 289 (2014). Interspecific allelopathy in cyanobacteria: *Cylindrospermopsin* and
 290 *Cylindrospermopsis raciborskii* effect on the growth and metabolism of *Microcystis*
 291 *aeruginosa*. *Harmful Algae* **35**, 1–8. doi: 10.1016/j.hal.2014.03.002

292 Schagerl, M., Unterrieder, I., and Angeler, D.G. (2002). Allelopathy among cyanoprokaryota
 293 and other algae originating from Lake Neusiedlersee (Austria). *International Review of*
 294 *Hydrobiology* **87**, 365–374. doi: 10.1002/1522-2632(200207)87:4<365::AID-
 295 IROH365>3.0.CO;2-B

296 Schlegel, I., Doan, N. T., de Chazal, N., and Smith, G. D. (1999). Antibiotic activity of new
 297 cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria.
 298 *Journal of Applied Phycology* **10**, 471–479.

299 Suikkanen, S., Fistarol, G. O., and Granéli, E. (2004). Allelopathic effects of the Baltic
 300 cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena*
 301 *lemmermannii* on algal monocultures. *Journal of Experimental Marine Biology and*
 302 *Ecology* **308**, 85–101. doi: 10.1016/j.jembe.2004.02.012

303 Suikkanen, S., Fistarol, G. O., and Granéli, E. (2005). Effects of cyanobacterial
 304 allelochemicals on a natural plankton community. *Marine Ecology Progress Series* **287**,
 305 1–9.

306 Śliwińska-Wilczewska, S., Pniewski, F., and Latała, A. (2016). Allelopathic activity of the
 307 picocyanobacterium *Synechococcus* sp. under varied light, temperature and salinity
 308 conditions. *International Review of Hydrobiology* **101**, 69–77. doi:
 309 10.1002/iroh.201501819

310 Śliwińska-Wilczewska, S., Maculewicz, J., Barreiro Felpeto, A., Vasconcelos, V., and Latała,
 311 A. (2017a). Allelopathic activity of the picocyanobacterium *Synechococcus* sp. on
 312 filamentous cyanobacteria. *Journal of Experimental Marine Biology and Ecology* **496**,
 313 16–21. doi: 10.1016/j.jembe.2017.07.008

314 Śliwińska-Wilczewska, S., Maculewicz, J., Tuszer, J., Dobosz, K., Kalusa, D., and Latała, A.
 315 (2017b). First record of allelopathic activity of the picocyanobacterium *Synechococcus*
 316 sp. on a natural plankton community. *Ecohydrology & Hydrobiology* **17**, 227–234. doi:
 317 10.1016/j.ecohyd.2017.05.001

318 Tillmann, U. 1998. Phagotrophy by a plastidic haptophyte, *Prymnesium patelliferum*. *Aquatic*
 319 *Microbial Ecology* **14**, 155–160.

320 Wang, L., Zi, J., Xu, R., Hilt, S., Hou, X., and Chang, X. (2017). Allelopathic effects of
321 *Microcystis aeruginosa* on green algae and a diatom: Evidence from exudates addition
322 and co-culturing. *Harmful Algae* **61**, 56–62. doi: 10.1016/j.hal.2016.11.010

323 Worden, A. Z., and Wilken, S. (2016). A plankton bloom shifts as the ocean warms. *Science*
324 **354**, 287–288. doi: 10.1126/science.aaj1751

325 Żak, A., and Kosakowska, A. (2015). The influence of extracellular compounds produced by
326 selected Baltic cyanobacteria, diatoms and dinoflagellates on growth of green algae
327 *Chlorella vulgaris*. *Estuarine, Coastal and Shelf Science* **167**, 113–118. doi:
328 10.1016/j.ecss.2015.07.038

329 Żak, A., Musiewicz, K., and Kosakowska, A., 2012. Allelopathic activity of the Baltic
330 cyanobacteria against microalgae. *Estuarine, Coastal and Shelf Science* **112**, 4–10. doi:
331 10.1016/j.ecss.2011.10.007

332

333

Fig. 1. Cytograms obtained with a monocultures (a) and co-cultures with *Synechococcus* sp. (b) of four microalgae: *P. purpureum* (A), *S. bacillaris* (B), *P. parvum* (C) and *N. dissipata* (D) analyzed using a BD Accuri™ C6 Plus flow cytometer.

Fig. 2. The number of cells (N) for *P. purpureum* (A), *S. bacillaris* (B), *P. parvum* (C) and *N. dissipata* (D) for controls and experiments with additions of cell-free filtrate obtained from *Synechococcus* sp. cultures after 0 (1 h), 1, 3 and 7 days of exposure. The values refer to means (n = 3, mean $\pm$ SD). Asterisk indicates significant difference compared with control. Levels of significance were: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 3. The number of cells (N) for *P. purpureum* (A), *S. bacillaris* (B), *P. parvum* (C) and *N. dissipata* (D) for controls and experiments with additions of *Synechococcus* sp. cultures after 0 (1 h), 1, 3 and 7 days of exposure. The values refer to means (n = 3, mean $\pm$ SD). Asterisk indicates significant difference compared with control. Levels of significance were: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.