Introduction

Field evidence and laboratory studies indicate that allelopathy occurs in all aquatic habitats (marine, brackish, and freshwater) and that all primary producing organisms (cyanobacteria, micro- and macroalgae as well as angiosperms) are capable of producing and releasing allelopathically active compounds (Tang, 2011). Molisch (1937) was the first who defined the term allelopathy in a broad sense to describe either positive or negative biochemical interactions among plants. After a few decades, Inderjit and Dakshini (1995) gave an overview of allelopathic activities in aquatic habitats with particular emphasis on algae. Currently, it is believed that allelopathy is a prevalent natural phenomenon in aquatic ecosystem (Omezzine et al., 2009).

Macroalgae from the genus *Ulva* are cosmopolitan organisms, and in nutrient-rich coastal waters, they are often dominant and even bloom-forming species (Rybak, 2018a,b; Rybak, Gąbka, 2018). Several species of *Ulva* have distributions that extend into the Baltic Sea (Leskinen, 2004). *Ulva intestinalis* L. (synonym: *Enteromorpha intestinalis* (L.) Nees) is the principal marine and benthic macroalga growing in isolated rockpools, on rocks, and even on artificial substrates (breakwaters, jettys, etc.) (Björk, 2004). Some researchers have demonstrated that *Ulva* can reduce eutrophication and promote the productivity, survival rate, and feeding coefficient of the culture species, e.g., prawn and shrimp, by means of polyculture (Wang et al., 2001). Many papers have reported that *Ulva* can take up nutrients from mariculture waters and improve water quality (Jin, Dong, 2003).

Harmful cyanobacterial blooms (CyanoHABs) are a significant threat to fisheries, economies around the world, and public health (Paerl, 2018). On the other hand, allelopathy in aquatic environments may provide a competitive advantage of selected macroalgae relative to other primary producers, including cyanobacteria (Gross, 2010).
Thus, allelopathic macroalgae may be a promising mitigation strategy for CyanoHABs (Tang, 2011). However, we have still little knowledge about the allelopathic interactions between the macroalgae and cyanobacteria. In the present study, we performed a series of laboratory experiments under controlled conditions to examine the allelopathic interactions between the *Ulva intestinalis* and *Nostoc* sp. in order to verify the hypothesis of allelopathy between macroalgae and cyanobacteria.

**Material and methods**

The experiments were conducted with the strain BA-81 of cyanobacterium *Nostoc* sp. (Fig. 1). This strain was isolated from the coastal zone of the Gulf of Gdańsk (southern Baltic Sea) and was maintained as monocultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, the University of Gdańsk, Poland (Latała et al., 2006). Brackish water adapted macroalgae *U. intestinalis* was selected, based on allelopathic potential of species from the order Ulvales (e.g., Nan et al., 2004). Analysed green alga was collected manually from the coastal zone of the Gulf of Gdańsk (54°30’16”N 18°33’26”E) and immediately and carefully washed with distilled water to remove attached organisms.

The cyanobacteria culture used in the experiments was maintained in 25-mL glass Erlenmeyer flasks at 17°C and a 16:8 h of light : dark cycle at a irradiance of 20 μmol photons (PAR) m⁻²s⁻¹. The culture was acclimated to these conditions for 7 days, and these growth conditions were used for the experiments. Fluorescent lamps (Cool White 40W, Sylvania, USA) were used as a source of irradiance. 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

![Fig. 1. *Nostoc* sp. strain BA-81 used in this study: left panel depicts cyanobacterial filaments from the light microscope (A), whilst right panel illustrates target species under an epifluorescence microscope (B). Scale bars = 10 µm (Photo. S. Śliwińska-Wilczewska)](image-url)
glass fibre filters (Whatman GF/C) and autoclaved. The salinity was 8 PSU as measured with a salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany).

Allelopathic effects were tested according to a method proposed by Ghobrial et al. (2015) with modifications. The cyanobacteria cultures were exposed to the macrophyte extracts obtained from *U. intestinalis*. Dried plant materials were homogenised in a mortar grinding machine. For the bioassay experiment, 2 g dry weight of dried plants was extracted with 40 mL of f/2 medium for 10 minutes. Extracts were filtered through glass fibre filters (Whatman GF/C) using a vacuum pump (400 mbar) to remove plant particles for bioassay experiments. The concentrations of major nutrients in the controls and all treatments were adjusted to the same level as in the f/2 growth medium. Therefore, the effects of major nutrients, microelements, and vitamin limitations in the control and allelochemical treatments can be excluded.

Sterilised Erlenmeyer flasks 25-mL contained 10 mL f/2 medium with a cyanobacterial initial inocula (the final chlorophyll a (Chl a) concentration in the experimental cultures was 0.4 µg Chl a mL⁻¹) and different volumes of green alga extract treatments. Experimental treatments were prepared by adding 100, 500, and 1000 µL of these extracts to 25-mL Erlenmeyer flasks containing 10 mL of cell suspensions of the targeted cyanobacteria. The final concentrations of extract were 10, 50, and 100 µL mL⁻¹. The selection of these concentrations was based on previous introductory experiments to determine the effective-range broadly. Controls consisted in the addition of 100, 500, and 1000 µL of filtrated f/2 medium to 25-mL Erlenmeyer flasks containing 10 mL of cell suspensions of the same cyanobacteria species. The aliquots of the target species inoculated in the experimental flasks came from exponentially growing cultures. The flasks with cyanobacteria were swirled daily. Tests were conducted in triplicate. The experiments lasted 7 days.

The number of cells (N) in *Nostoc* sp. cultures was estimated with previously determined linear correlations between cell abundance (N mL⁻¹) and optical density (OD). N was counted using a Bürker chamber (48 squares per count) and a light microscope (LM) following a procedure according to Guillard and Sieracki (2005), 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 *Nostoc* sp. was $y = 39.8 \cdot 10^6 x - 1.1 \cdot 10^4$ ($r^2 = 0.95$), where $y = N$ (mL⁻¹) and $x = OD$. OD measurements were performed on the 0th, 1st, 3rd, and 7th days of experiment and control.

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 1st, 3rd, and 7th days of experiment. Samples were filtered through 13-mm glass fibre filters (Whatman GF/C). Before measurement, the filtered
sample was kept in the dark for approximately 5 min. The maximum PSII quantum efficiency (Fv/Fm) was calculated (Campbell et al., 1998).

Repeated measures ANOVA was used to test the effect of macroalgae extract on the growth and fluorescence of the targeted cyanobacteria during the following days of experiment. A post-hoc Tukey’s test was used to determine significant differences between the control and the other treatment levels. Data are reported as the means ± standard deviations (SD). The statistical analyses were performed using Statistica® 13.1 software.

**Results**

Our experiment demonstrated that the dry powder of *U. intestinalis* contains water-soluble allelochemical(s) and is capable of restricting the growth and fluorescence of filamentous cyanobacterium *Nostoc* sp. The addition of 10, 50, and 100 µL mL⁻¹ of extracts obtained from *Ulva intestinalis* significantly decreased the number of cells of *Nostoc* sp. (ANOVA, F₃,₁₆ = 11.2, p < 0.001, ANOVA, F₃,₁₆ = 25.4, p < 0.001 and ANOVA, F₃,₁₆ = 26.3, p < 0.001, respectively), whereas the control sample showed ac-
tive growth (Fig. 2). After the addition of extracts obtained from *U. intestinalis*, the highest decline in growth for *Nostoc* sp. was observed on the third and seventh day of the experiment. The growth of target cyanobacterium after the third day and the addition of 10 and 50 µL mL⁻¹ of extracts obtained from *U. intestinalis* was reduced to 59% (Tukey, *p* < 0.001) and 71% (Tukey, *p* < 0.01), respectively, compared to the control. Moreover, the addition of 50 and 100 µL mL⁻¹ extracts inhibited the growth of cyanobacterium; and, after seven days of exposition, the reduction was 35% (Tukey, *p* < 0.001) and 81% (Tukey, *p* < 0.01), respectively, of the initial amount of *Nostoc* sp. The effect of macrophytes extracts on chlorophyll fluorescence parameter *Fv/Fm* after 1, 3, and 7 days of incubation is shown in figure 3. The cyanobacterium showed statistically significant different responses to 10, 50, and 100 µL mL⁻¹ of extract additions obtained from *U. intestinalis* (ANOVA, *F*₂,₁₂ = 6.9, *p* < 0.001, ANOVA, *F*₂,₁₂ = 0.9, *p* > 0.05 and ANOVA, *F*₂,₁₂ = 44.5, *p* < 0.0001, respectively). It was found that, on the third day of the experiment, the values of *Fv/Fm* of target cyanobacterium after the addition of 10 µL mL⁻¹ extracts was reduced to 75% (Tukey, *p* < 0.05), compared to control treatment. The highest decrease in *Fv/Fm* for *Nostoc* sp. was observed after the first, third, and seventh day of experiment, after the addition of 100 µL mL⁻¹ extracts obtained from *U. intestinalis* with a magnitude of 69% (Tukey, *p* < 0.01), 59% (Tukey, *p* < 0.001) and 49% (Tukey, *p* < 0.001), respectively, compared to the control.

Discussion

Aquatic macroalgae and macrophytes have long been suspected of suppressing phytoplankton growth through the excretion of chemical substances that inhibit phytoplankton growth (Hutchinson, 1975). In addition, the production and excretion of allelochemicals by aquatic macroalgae and macrophytes could be an effective defence strategy against other photosynthetic organisms competing for nutrients and light (Wium-Andersen et al., 1982; Gopal, Goel, 1993; Elakovich, Wooten, 1995). Some of the results confirmed that *Ulva* sp. is able to suppress the growth of different species by allelopathy (Friedlander et al., 1996). Nan et al. (2008) demonstrated that green macroalgae *Ulva* sp. has allelopathic effects on the three species of red tide microalgae. Although toxic properties are rarely associated with the bloom-forming green macroalgae, there is evidence that *Ulva* sp. produce chemical defences against herbivores (Van Alstyne et al., 2001), and their extracts have allelopathic properties on other organisms, such as larval oyster, *Fucus gardneri* P.C. Silva zygote and epiphytic diatom (Nelson et al., 2002). On the other hand, Nan et al. (2004) demonstrated that in the nutrient replete semicontinuous co-cultures, *Ulva* sp. may have continuously released growth-inhibiting allelochemicals throughout the cultivation period; whereas, in the initial addition culture solution, the allelochemicals may inhibit growth only
at the start of cultivation, because they are quickly degradable. Furthermore, Nakai et al. (1999) showed that initial addition culture solution might underestimate the allelopathic inhibitory effects as compared with coexistence assays. Moreover, allelochemicals could exhibit positive effects on the target organism at lower concentrations and exhibit negative effects at higher concentrations (Van Aller, 1985).

Other studies have shown that macrophytes may also exhibit allelopathic effects on different phytoplankton species, including cyanobacteria (Van Donk, Gulati, 1995; Mjelde, Faafeng, 1997). Mjelde and Faafeng (1997) found that Ceratophyllum demersum L. hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. Moreover, Van Donk and Gulati (1995) suggested that C. demersum can inhibit the growth of both epiphytes and phytoplankton species. Additionally, the impact of different submerged macrophytes or their extracts on natural phytoplankton assemblages was studied under experimental conditions by Jasser (1995). This author also concluded that the release of organic compounds by C. demersum apparently contributed to a decline of cyanobacteria Oscillatoria limnetica Lemm. by changing the phytoplankton dominance

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**Fig. 3.** The fluorescence parameter $F_i/F_m$ of Nostoc sp. cells for controls (C) and experiments (Ex) with addition of extracts: 10, 50 and 100 ($\mu$L mL$^{-1}$) obtained from Ulva intestinalis L. after 1, 3, and 7 days of exposure; the values refer to means ($n = 3$, mean ± SD); asterisks indicates statistically significant difference compared with control obtained with the ANOVA and Tukey’s post hoc test; levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
structure. Natural phytoplankton assemblages, in which cyanobacteria were abundant, were incubated in the field in bags together with intact plants or plant extracts. Live *C. demersum, Myriophyllum spicatum* L., *Potamogeton lucens* L., *Statioites aloides* L., and *Chara fragilis* Desv. and its extract, had quite similar effects on the phytoplankton; whereas, the biomass and percentage contribution of cyanobacteria to total algal biomass declined, and those of green algae increased. Wium-Andersen et al. (1983) isolated a sulphur compound with allelopathic properties from *C. demersum*. Moreover, Gross and Sütfeld (1994) found that *M. spicatum* L. was able to release allelopathic polyphenols into the surrounding water and thereby strongly suppressed the growth of cyanobacterium *Anacystis* sp. Some authors noted that the identified allelochemicals belong to different chemical classes, such as sulphur compounds, polyacetylenes, polyphenols, and oxygenated fatty acids; however, most of the allelochemicals are still not identified (Gross, 1999).

In this study, it has been demonstrated that *U. intestinalis* can release some kind of allelopathic substances and effectively inhibit the growth and fluorescence parameter of cyanobacterium *Nostoc* sp. Our results not only provide insight into the interactions between macroalgae and cyanobacteria but also lead us to isolate and characterize these allelopathic substances in the future. Moreover, our results may provide new insight into the ecological role of macroalgae, such as *U. intestinalis*, in the occurrence of CyanoHABs.

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**References**


Allelopathy is a prevalent natural phenomenon in aquatic ecosystems. We reported the effects of the green macroalga Ulva intestinalis L. collected from estuaries of the Baltic Sea (Poland) on the growth and chlorophyll fluorescence of common filamentous cyanobacterium Nostoc sp. It was found that the addition of 50 μL mL⁻¹ extracts obtained from U. intestinalis inhibited the growth of cyanobacterium, and, after one week of exposition, the reduction was 35% of initial amount of Nostoc sp. In addition, we demonstrated that, on the seventh day of the exposition, the values of Fv/Fm of target cyanobacterium after the addition of 100 μL mL⁻¹ extracts obtained from U. intestinalis was reduced to 49%, compared to control treatment. These results showed for the first time the allelopathic activity of U. intestinalis on Baltic filamentous cyanobacteria Nostoc sp.

**Key words:** allelopathy, Chlorophyta, cyanobacteria, extract, fluorescence, green algae, growth, macroalgae, Ulvophyceae

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wyników badań stwierdzono, że po dodaniu ekstraktów uzyskanych z *U. intestinalis*, najmniejszy wzrost *Nostoc* sp. obserwowano w siódmym dniu eksperymentu. Dodanie 50 µL mL⁻¹ ekstraktu z *U. intestinalis* zahamowało rozwój sinicy *Nostoc* sp. i po 7 dniu ekspozycji jej wzrost osiągnął poziom 35% w stosunku do warunków kontrolnych. Ponadto największy spadek wartości parametru fluorescencji Fv/Fm dla *Nostoc* sp. obserwowano po tygodniu trwania eksperymentu. Stwierdzono, że po 7 dniach ekspozycji wartość Fv/Fm dla badanej sinicy po dodaniu 100 µL mL⁻¹ ekstraktu z *U. intestinalis* spadła o 49%. Przeprowadzone badania po raz pierwszy wykazały, że *U. intestinalis* wpływa allelopatycznie na bałtyckie sinice nitkowate z rodzaju *Nostoc*.

Słowa kluczowe: allelopatia, Chlorophyta, ekstrakt, fluorescencja, makroglony, sinice, Ulvophyceae, wzrost, zielenice

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