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PRIMARY RESEARCH PAPER

Growth phase-dependent allelopathic effects of cyanobacterial exudates on *Potamogeton crispus* L. seedlings

Runbing Xu · Sabine Hilt · Ying Pei · Lijuan Yin · Xiaolong Wang · Xuexiu Chang

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Abstract Cyanobacterial exudates are known to allelopathically inhibit submerged macrophytes, but the influence of the cyanobacteria growth phase on this effect is yet unknown. We compared the effect of exudates of the exponential growth phase of *Microcystis aeruginosa* Kütz. Elenk with exudates during the decline phase on seedlings of the macrophyte species *Potamogeton crispus* L. Biomass, chlorophyll content, the ratio of variable–maximum fluorescence (F_v/F_m), and light response capacity of *P. crispus* seedings were significantly inhibited when affected by *M. aeruginosa* exudates of the exponential growth phase but promoted by exudates of the decline phase. Tiller numbers of *P. crispus* increased by 350% under the influence of exponential phase exudates, but decreased by 60% when decline phase exudates were applied. Both exudates increased the malondialdehyde contents and decreased the activity of superoxide dismutase and peroxidase in *P. crispus* seedlings. We conclude that the exponential growth phase of cyanobacteria rather than the decline phase is important for disrupting photosynthesis and for inducing oxidative stress in submerged macrophytes. Planting *P. crispus* should thus not be applied in summer but during the cyanobacteria decline phase.

Keywords Allelopathic effects · Cyanobacterial exudates · Exponential phase · Decline phase · *Potamogeton crispus* L.

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Introduction

The occurrence of harmful cyanobacterial blooms in freshwater lakes is often accompanied by the production of various allelochemicals that affect the growth of other co-inhabiting microorganisms and plants (Carmichael, 2008; Paerl & Huisman, 2008; Corbel et al., 2014). Specifically, cyanobacterial allelochemicals have been shown to affect the germination (Zheng et al., 2013), growth, and several physiological and biochemical processes of aquatic plants (Baszynski et al., 1988; Mitrovic et al., 2004; Ha & Pflugmacher, 2013). Allelochemicals of cyanobacteria also induced oxidative stress in chloroplasts of *Lemna gibba* L. (Saqrane et al., 2007) and *Chlorella vulgaris* Beij. (Qian et al., 2009). Overall, cyanobacterial allelochemicals have been proposed to negatively affect macrophyte vegetation (Zheng et al., 2013) and potentially result in a slower recovery of damaged vegetation in eutrophic lakes (Heisler et al., 2008).

Bloom-forming cyanobacteria have an annual life cycle with dormancy in winter, recruitment in spring, growth, and floating to the water surface in summer and sinking to the sediment in the end of autumn (Kong & Fao, 2005; Shao et al., 2013). Their allelochemicals are synthesized in cells and released into the aquatic environment either by exudation or during cell lysis (Zheng et al., 2013). Previous studies assumed that decomposing cyanobacterial cells are a key source for inhibiting compounds (Sivonen & Jones, 1999; Song et al., 2007). Microcystins (MCs), one of the main cyanobacterial secondary metabolites, were shown to have negative effects on the growth and photosynthetic oxygen production of some submerged macrophyte species (Pflugmacher, 2002). However, Sukenik et al. (2002) suggested that retardation of photosynthesis and growth of the dinoflagellate *Peridinium gatunense* Nyg. by *Microcystis* sp. were due to a thermally stable, relatively hydrophobic component with a molecular weight <5 kDa, not the presence of MCs. More researches demonstrated that the influence of MCs was restricted because of their unstability both in situ and ex situ (Casanova et al., 1999; Rojo et al., 2013). Zheng et al. (2013) also showed that exudates of *Microcystis aeruginosa* Kütz., one of the most common and harmful bloom-forming cyanobacterial species (e.g., Fogg, 1969; de Figueiredo et al., 2004), have more negative effects on submerged plants than their extracts. This suggests that cyanobacterial blooms could have stronger allelopathic effects during active growth than during breakdown. First, indications for this assumption come from Lyck (2004) who showed a coupling between MC production and cell division for *M. aeruginosa* cultures. Suikkanen et al. (2004) found that cell-free filtrates of exponentially growing *Nodularia spumigena* Mertens inhibited algae monocultures more than filtrates obtained during the stationary growth phase. The question during which developmental stage cyanobacteria exert the highest allelopathic effect on submerged macrophytes, however, has remained open.

We hypothesized that allelopathic effects on submerged macrophytes are higher during the exponential growth phase of cyanobacteria than during their decline. To test this hypothesis, we compared the effects of these two stages' exudates from *M. aeruginosa* (toxic strain FACHB-905) on the early seedling growth of a submerged macrophyte, *Potamogeton crispus* L. *P. crispus* is a dicotyledonous submerged macrophyte commonly found in shallow lakes, ponds, ditches, and slow-flowing streams. In particular, this plant is one of the few species that survives in very nutrient-rich Chinese lakes (Lu et al., 2012).

We tested the effect of *M. aeruginosa* exudates on axenic *P. crispus* biomass, tiller number, chlorophyll (Chl) content, the ratio of variable–maximum fluorescence (F_v/F_m) in leaves and light response capacity, malondialdehyde (MDA) content, and antioxidative enzymes. To minimize functional and structural damages, plants have developed different mechanisms to cope with cyanobacterial allelochemicals such as accumulation (Romero-Oliva et al., 2014), biotransformation (Pflugmacher et al., 1998), antioxidative enzymes [e.g., superoxide dismutase (SOD), and peroxidase (POD)]. MDA is a cytotoxic product of lipid peroxidation and a good indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979).

Materials and methods

Microcystis aeruginosa cultivation

Microcystis aeruginosa (FACHB-905) was obtained from the Freshwater Algae Culture Collection of the Institution of Hydrobiology (FACHB-Collection) at the Chinese Academy of Sciences. This strain originated from Lake Dianchi, a typical plateau shallow lake in China (Lu et al., 2012), and produces MC-LR (Sun et al., 2012). It was axenically kept in a modified M III nutrient solution (Körner & Nicklisch, 2002). The prepared M III nutrient solution was filtered through a glass-fiber filter (0.45 μm) and put into a sterilized 500-ml flask (0.1 MPa, 30 min). Cultures were grown semi-continuously (daily additions of fresh M III nutrient solution) in a climate-controlled room (Dongnan, GXZ-380B, made in Ningbo, China) at 25 ± 1°C in a 12:12 h light–dark cycle with light of 80 μmol quanta m^{-2} s^{-1} [measured as photosynthetic photon flux density (PPFD) by a quantum meter (Spectrum Technology, Inc., USA)], manually shaken twice daily. Cells were manually counted by hemocytometer measurement with a light microscope (Olympus, Japan) every day to confirm exponential growth of *M. aeruginosa*. The daily supplement of fresh nutrient solution was carried on until the *M. aeruginosa* cultures were grown up to exponential phase with a concentration of 1.6×10^6 cells ml^{-1}, which is in the range reported for cyanobacteria

blooming periods in Lake Dianchi (1–13 × 10^6 cells ml^{-1}, Luo, 2002; Li et al., 2005; Wan et al., 2008). The cultures in the exponential growth phase with a concentration of 1.6 × 10^6 cells ml^{-1} were divided into two parts: one for obtaining the exudates of the exponential phase and the other remained without supplement to enter the stationary phase. To obtain exudates of the exponential phase, *M. aeruginosa* cultures were centrifuged at 6000 rpm for 10 min, and the supernatant was filtered through a glass-fiber filter (0.45 μm) under axenic conditions. To obtain exudates of the decline phase, *M. aeruginosa* cultures were harvested 2 weeks after the beginning of the decline phase (more than 6 months after cultivation) with a concentration of 1.6 × 10^6 cells ml^{-1} and treated as described above.

Subsequently, *M. aeruginosa* exudates were diluted into five different concentrations [100, 75, 50, 25, and 0% (control)] using fresh M III nutrient solution. The concentrations of nitrate and phosphate were adjusted to the same level as in the M III growth medium. The pH value of all media was modified to 8.0 using NaOH and HCl. At the end of the experiments, nitrate and phosphate concentrations were measured and showed no significant differences between treatments and control.

Axenic culture of *Potamogeton crispus*

Healthy mature plants of *P. crispus* were collected from a pond at Yunnan University in July 2011 and kept in aquaria containing tap water for 2 weeks before the experiments started. Stem tips (with nodes) of about 10 cm long were cut and soaked in tap water for 1 h. After that, the leaves were thoroughly cut off. The remaining stems were cleaned thrice with deionized water and then sterilized by two identical steps in a clean bench. In each step, stems were sterilized with 0.01% mercuric chloride for 4 min and then washed thrice in sterile deionized water. Between the two steps, stems were immersed in sterile deionized water for 10 min. These surface-sterilized stems were sectioned into fragments of about 1 cm long with one node each. These fragments were placed onto M III solid medium containing 6 g l^{-1} agar, 20 g l^{-1} sucrose, and plant hormones (1 mg l^{-1} 6-benzylaminopurine and 0.1 mg l^{-1} 1-naphthaleneacetic acid), in sterilized glass bottles (pH 6.0) and incubated at 25 ± 1°C, in a 12:12 h light–dark cycle with 80 μmol quanta m^{-2} s^{-1}, for 4–6 weeks. During cultivation, stem samples were checked daily, and only healthy survivals without microorganisms were kept until roots emerged. Young seedlings with roots were carefully transferred to sterilized M III nutrient solution without sugar and were cultured under the same growth conditions as above. Ten ml of nutrient solutions was replaced every 3 days to account for evaporation and nutrient absorption.

Seedling growth test

Growth tests started when seedling lengths reached more than 5 cm. Seedlings were tested in two separate experiments. For each experiment, young seedlings (without any tiller and root) with a length of 4 ± 0.1 cm were cut from plants. 15 seedlings were grown in a glass bottle without agar containing 150 ml of nutrient solution (controls, 0%), or exudates. Growth conditions were the same as mentioned in "Axenic culture of *Potamogeton crispus*" section. Each treatment had three replicates. In the first experiment, *P. crispus* plants were harvested after 15 days to measure fresh weight, shoot length, leaf number, tiller number, and root length. The second experiment was carried out to test the response of *P. crispus* Chl content, the F_v/F_m ratios [maximum quantum yield of photosystem II (PSII)], and rapid light curves (RLCs) of leaves. The F_v/F_m ratios of leaves were determined every other day, while the RLCs were measured on the seventh and the last day (day 15); Chl content was measured only on the last day. Ten ml of fresh nutrient solution without or with exudates for controls and treatments, respectively, was added into each bottle every day, to supply the evaporated and absorbed solution.

Chlorophyll fluorescence and chlorophyll content measurements

The F_v/F_m ratios of leaves were determined by Junior-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany), employing the principle described in Schreiber et al. (1994) and the methods for aquatic macrophytes from Hanelt & Roleda (2009). Samples were mounted at a distance of 2 mm from the end of the fiber optics probe of the fluorometer using the magnetic sample holder. After application of a 5 s far red pulse (~30 μmol m^{-2} s^{-1}), used to oxidize the electron transport chain, the sample was darkened for 5 min. Then, Chl fluorescence yield in the quasi-dark state (F_0)

was measured with a pulsed, red measuring light ($\sim$0.3 µmol m^{-2} s^{-1}, 650 nm), and the F_m was determined with a 800 ms completely saturating white light pulse ($\sim$5000 µmol m^{-2} s^{-1}), and the F_v was calculated as $F_m - F_0$.

RLCs were measured automatically by the Junior-PAM under the control of an internal program providing a sequence of actinic illumination periods, with light intensities ranging from eight steps: 17, 49, 104, 176, 248, 342, and 506 µmol quanta m^{-2} s^{-1}. Each illumination period lasted 10 s, and subsequently eight data of electron transport rate (ETR) were obtained for each leaf. The light response of *P. crispus* leaves was characterized by fitting the model of Platt et al. (1980) to ETR versus *E* [spectrally averaged photon irradiance of PAR (400–700 nm) (µmol quanta m^{-2} s^{-1})] curves and by estimating the parameters: ETR_{mPot} (maximum potential light-saturated ETR), alpha (α, initial slope which is related to quantum efficiency of photosynthesis), and beta (β, parameter for photoinhibition). An empirical function which has been introduced by Platt et al. (1980) was used to estimate these cardinal data: $ETR = ETR_{mPot} \times (1 - \exp(-\alpha \times PPFD/ETR_{mPot})) \times \exp(-\beta \times PPFD/ETR_{mPot})$ (*PPFD* photosynthetic photon flux density). The model was fitted iteratively using IBM SPSS statistics 19. Curve fit was very good ($r > 0.90$) in all cases.

The Chl (*a* and *b*) content was determined after ethanol extraction (Yang, 2002).

Malondialdehyde content and enzyme activity determination

For extraction of enzymes, 1 g of leaf tissues was homogenized in 6 ml ice-cold 50 mM phosphate buffer (pH 7.8) containing 0.2 M EDTA and 2% polyvinylpyrrolidone (w/v). The homogenates were centrifuged at 4°C for 20 min at 15,000 rpm, and the resulting supernatants were used for the determination of enzymatic activity. SOD activity was assayed by measuring the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction rate of NBT as monitored at 560 nm (Giannopolitis & Ries, 1977). POD activity was determined at 470 nm following the method published by Cakmak & Marschner (1992). The reaction mixture contained 25 mM PBS (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and enzyme extract.

MDA was assayed as an end product of lipid peroxidation by the 2-thiobarbituric acid (TBA) reaction. Leaf samples (0.5 g) were homogenized in 5 ml of 10% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3000 rpm for 10 min, and 4 ml of 20% TCA containing 0.65% (w/v) TBA was added to 1 ml of supernatant. The mixture was heated in a hot water bath at 95°C for 25 min and immediately placed in an ice bucket to stop the reaction. Then samples were centrifuged at 3000 g for 10 min, and the absorbance of the supernatant was recorded at 440, 532, and 600 nm. MDA equivalents were calculated according to Hodges et al. (1999).

Statistical analysis

The effects of *M. aeruginosa* exudates on *P. crispus* were presented by "change to control" and "inhibition rate" (IR). The "change to control" was calculated with the formula: "change (%) = $(N/C) \times 100$," IR for all parameters was calculated with the formula: "IR (%) = $(1 - N/C) \times 100$," with *N* being the treatment data and *C* being the control data.

Data were expressed as mean ± standard deviation. Comparisons between different concentrations [0% (control) and 25–100%] or between different treatments (exudates of the exponential and decline phase) were performed by one-way analysis of variance (ANOVA) and subsequent Fisher LSD comparison tests. A two-way ANOVA was performed to assess the overall differences for each parameter (except for data of F_v/F_m and RLCs) of *P. crispus* between different concentrations of two types of *M. aeruginosa* exudates. When a significant interaction in the between-subjects' variables (exudates type and concentration) was determined, a subsequent one-way ANOVA was performed. The tests were performed using SPSS 17.0 after homoscedasticity of the variance was checked with $P < 0.05$ considered statistically significant.

Results

Effect of *Microcystis aeruginosa* exudates on biomass of *Potamogeton crispus*

The effects of *M. aeruginosa* exudates of the exponential growth phase on fresh weight of *P. crispus* seedlings were not significant at low concentrations

(25%) but were significantly negative at levels higher than 50% (Fig. 1; Table 1). Maximum IR was 93% ($P < 0.01$) when the *M. aeruginosa* cell density increased to 100%. Exudates of the decline phase promoted fresh weight of seedlings when applied at a concentration of 25% (42% higher than controls, $P < 0.05$) but showed no significant effects at higher levels (≥50%). Shoot lengths of *P. crispus* seedlings were only significantly reduced by the highest concentration of extracts of the decline phase (IR = 17%, $P < 0.05$; Fig. 1; Table 1). Tiller numbers of *P. crispus* were rarely affected by exudates of the decline phase but were significantly increased in all treatments with exudates of the exponential phase (Fig. 1; Table 1). Root numbers were significantly increased in all treatment with exudate concentrations higher than 50%. Exudates of the exponential phase showed a much stronger promotion effect than those of the decline phase (Fig. 1; Table 1). The inhibitory effects of all exudate concentrations of the exponential phase and those of higher concentrations (≥50%) of the decline phase on leaf numbers of *P. crispus* were highly significant (Fig. 1; Table 1).

Effect of *Microcystis aeruginosa* exudates on photosynthesis of *Potamogeton crispus*

Higher concentrations (≥50%) of exudates of the exponential phase resulted in significant decreases of the total Chl, Chl *a*, and Chl *b* content of *P. crispus* seedlings after 15 days, whereas exudates of the decline phase had an opposite effect (Fig. 2A, B; Table 1). With the increasing exposure time, lower exudate concentrations also induced a significant decrease of F_v/F_m ratios. Exudates of the decline phase had lower effects on F_v/F_m ratios of *P. crispus* (Fig. 3B). F_v/F_m ratios were only significantly inhibited by high concentrations (75, 100%) after an exposure time of 11 days but were significantly increased by the lowest concentration (25%) at the 9th and 13th day and by higher concentrations (75, 100%) at the 3rd day.

RLCs of *P. crispus* leaves strongly differed between treatments with exudates of the exponential as compared to the decline phase (Fig. 4). ETR_{max} was highest in controls and progressively decreased with the increasing concentrations of exudates of the exponential phase. This effect was more pronounced after 15 days of treatment as compared to 7 days (Fig. 4). ETR_{mPot}, α and β showed a response similar to that of the ETR_{max} values (Table 2).

In the treatments with exudates of the decline phases, ETR_{max} values of leaves treated with exudates were higher than those of controls after 7 days (Fig. 4). On the 15th day, the curves were different from those of the 7th day. Only the ETR_{max} values of the 25% treatment were higher than the control, whereas those of treatments with 75 and 100%

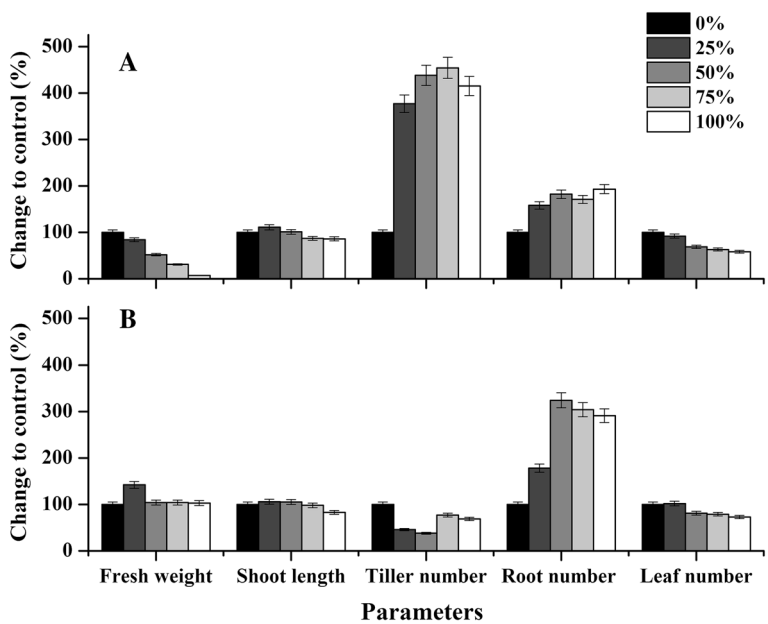

Fig. 1 Effects of *M. aeruginosa* exudates [0% (controls), 25, 50, 75, and 100%] obtained from cultures in the exponential (**A**) and the decline phase (**B**) on fresh weight, shoot length, tiller number, root number, and leaf number of seedlings of *P. crispus* after 15 days of treatment (*error bars* indicate standard deviation)

Table 1 Results of one-way ANOVA and Fisher LSD post hoc test comparing relative change based on different parameters of seedlings of *P. crispus*, treated with *M. aeruginosa* exudates of different concentrations (ETR_{max}-7, ETR_{max}-15: the maximum value of ETR of the 7th day and the 15th day) obtained from cultures in the exponential and the decline phase

Parameters	Treatments	df	*F*	*P*	0%	25%	50%	75%	100%
Fresh weight	Exponential phase	4	11.5	<0.01	a	ab	bc	cd	d
	Decline phase	4	2.8	<0.05	b	a	b	b	b
Shoot length	Exponential phase	4	2.5	<0.05	a	a	a	b	b
	Decline phase	4	3.7	<0.05	a	a	a	a	b
Tiller number	Exponential phase	4	5.2	<0.05	b	a	a	a	a
	Decline phase	4	0.5	>0.05	a	a	a	a	a
Root number	Exponential phase	4	2.1	<0.05	ab	ab	ab	ab	a
	Decline phase	4	7.1	<0.01	c	bc	a	a	ab
Leaf number	Exponential phase	4	178.7	<0.01	a	b	c	d	d
	Decline phase	4	84.1	<0.01	a	a	d	b	c
Chl	Exponential phase	4	12.0	<0.01	a	ab	b	c	c
	Decline phase	4	1.6	<0.05	b	ab	a	ab	ab
Chl *a*	Exponential phase	4	15.5	<0.01	a	ab	b	b	b
	Decline phase	4	1.8	<0.05	b	ab	ab	a	ab
Chl *b*	Exponential phase	4	4.9	<0.05	a	ab	b	c	c
	Decline phase	4	2.1	>0.05	a	a	a	a	a
ETR_{max}-7	Exponential phase	4	323.4	<0.01	a	b	c	d	e
	Decline phase	4	41.7	<0.01	e	d	c	b	a
ETR_{max}-15	Exponential phase	4	946.8	<0.01	a	b	c	d	e
	Decline phase	4	189.7	<0.01	b	a	b	c	d
MDA	Exponential phase	4	2.0	<0.05	b	ab	ab	ab	a
	Decline phase	4	3.8	<0.05	b	b	ab	ab	a
SOD	Exponential phase	4	163.3	<0.01	a	b	c	c	d
	Decline phase	4	937.5	<0.01	b	a	c	d	e
POD	Exponential phase	4	57.1	<0.01	a	a	a	b	c
	Decline phase	4	28.9	<0.01	b	a	b	c	d

Different letters indicate significant differences at $P < 0.05$

exudates were lower. All fitted parameters (ETR_{mPot}, α, β) showed the same effects as the ETR_{max} values (Table 2).

Effect of *Microcystis aeruginosa* exudates on membrane lipid peroxidation and antioxidant defense system of *Potamogeton crispus*

After 15 days treatment, the MDA content of *P. crispus* seedlings was increased when treated with the highest concentrations of both exudate types ($P < 0.05$), whereas low concentrations had no significant effect (Fig. 5; Table 1). The activities of both SOD and POD were significantly lowered by both exudate types ($P < 0.01$), with a stronger effect by exudates of the exponential phase (Fig. 5A, B; Table 1). SOD was more sensitive to cyanobacterial exudates than POD, with the greatest IR of 77% at the highest concentration of exudates of the exponential phase.

Discussion

Potential reasons of differences in the allelopathic effects of cyanobacteria exudates of different growth phases on macrophytes

Negative allelopathic effects of *M. aeruginosa* exudates of the exponential growth phase on the seedling

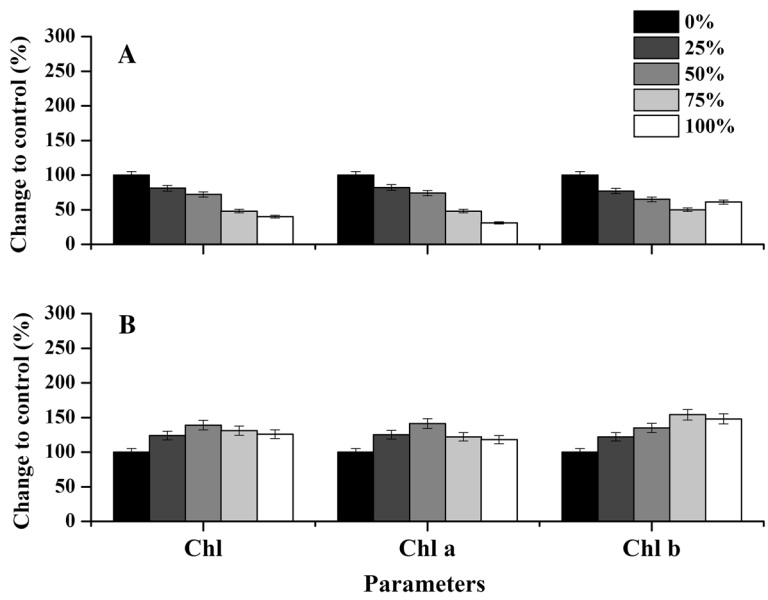

Fig. 2 Effects of *M. aeruginosa* exudates [0% (controls), 25, 50, 75, and 100%] obtained from cultures in the exponential (**A**) and the decline phase (**B**) on chlorophyll contents (Chl, Chl *a*, and Chl *b*) of seedlings of *P. crispus* after 15 days of treatment (*error bars* indicate standard deviation)

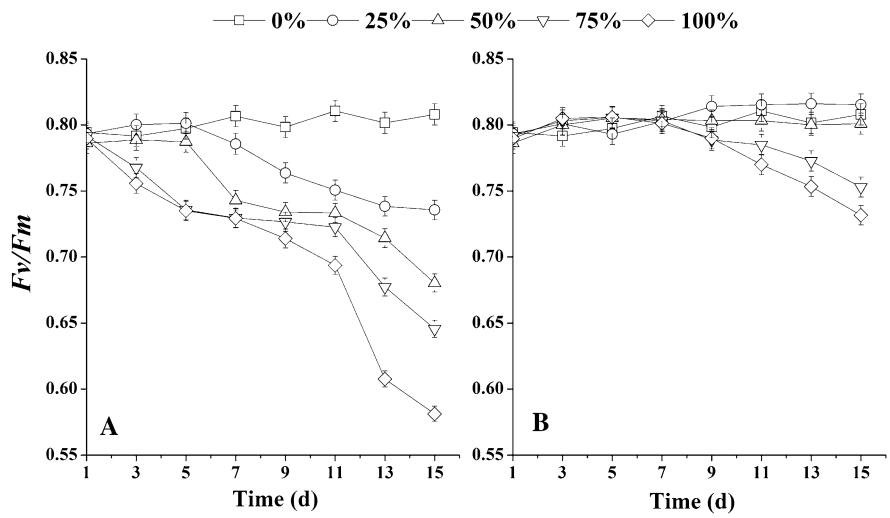

Fig. 3 Effects of *M. aeruginosa* exudates obtained from cultures in the exponential (**A**) and the decline phase (**B**) of different densities [0% (controls), 25, 50, 75, and 100%] on F_v/F_m ratios of *P. crispus* (*error bars* indicate standard deviation)

growth of *Potamogeton malaianus* Miq. and *Ottelia acuminata* (Gagnep.) Dandy. have already been observed in our previous studies (Zheng et al., 2013; Xu et al., 2015). To our knowledge, there are only a limited number of studies that investigated and compared effects of extracellular metabolites from different growth stages. In the present study, allelopathic effects of *M. aeruginosa* exudates of the exponential growth phase on *P. crispus* were significantly stronger than those of the decline phase.

Suikkanen et al. (2004) also found that cell-free filtrates of exponentially growing *N. spumigena* Mertens ex Bornet & Flahault inhibited algae monocultures more than those in the stationary growth phase. Thus, the release of extracellular compounds during the exponential growth phase might play a role in interspecific competition and contribute to cyanobacterial bloom maintenance. Our preliminary analysis by GC–MS showed that saturated alkanes were the major component of volatile organic compounds produced by cyanobacteria in the exponential growth phase (unpublished data from Xuexiu Chang). These findings were consistent with earlier studies reporting that massive accumulations of cyanobacteria

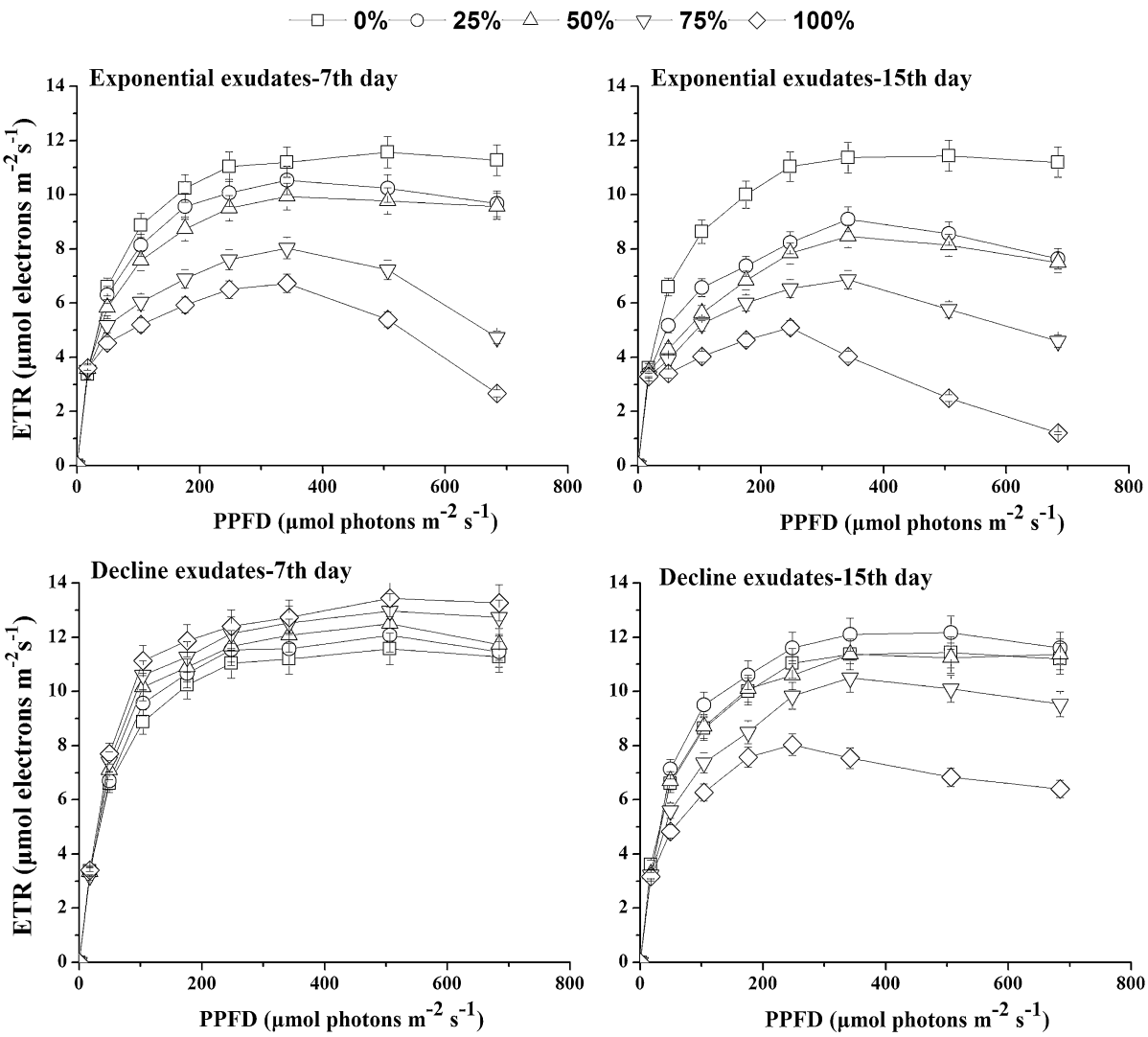

Fig. 4 Rapid light curves of *P. crispus* on the 7th (*left*) and 15th day (*right*) when treated with *M. aeruginosa* exudates obtained from cultures in the exponential (*above*) and the decline phase (*below*) (*error bars* indicate standard deviation). *ETR* electron transport rate, *PPFD* photosynthetic photon flux density

in most lakes cause an increase in the precursor of trihalomethane (Pu et al., 1998; Codd, 2000; Paerl, 2006). A recent analysis of GC–MS also indicated that some extracellular allelopathic compounds produced by *M. aeruginosa*, such as D-limonene and 1-chlorine heptacosane, might play important roles in the competition among species (Zhai et al., 2013). However, analyses of the chemical nature of the substances involved in the observed effects in our study are still needed.

Few studies have been carried out on the biodegradation of dead cyanobacteria (Lim et al., 1996; Zhou et al., 2001). Some studies point to a dominance of nutrient release and assume higher dissolved phosphorus concentrations in cyanobacteria-dominated lakes due to decomposing cyanobacteria (Sun et al., 2007; He et al., 2009; Chuai et al., 2011). In our experiment, however, the amounts of nitrate and phosphate were measured at the end and showed no significant differences as compared to controls. Some others infer that toxins, such as MCs, should be the dominant chemicals released by dead cells (Sivonen & Jones, 1999; Song et al., 2007). However, Rojo et al. (2013) found that MC only limited the initial number

Table 2 Fitted parameters of rapid light curves (ETR_{mPot}, α and β) of *P. crispus*, treated with *M. aeruginosa* exudates of different densities (same concentrations as in Fig. 1) obtained from cultures of the exponential and the decline phase and inhibition rate (IR)

Days	Exudate concentrations (%)	Exponential growth phase						Decline phase					
		ETR_{mPot}	IR (%)	α	IR (%)	β	IR (%)	ETR_{mPot}	IR (%)	α	IR (%)	β	IR (%)
7	0	10.574	–	0.204	–	−0.001	–	10.574	–	0.204	–	−0.001	–
	25	10.040	5.05	0.200	1.96	0	100	11.339	−7.23	0.207	−1.47	−0.001	0
	50	8.949	15.37	0.197	3.43	−0.001	0	11.63	−9.99	0.222	−8.82	−0.001	0
	75	8.536	19.27	0.167	18.14	0.005	600	11.827	−11.85	0.23	−12.75	−0.002	−100
	100	8.145	22.97	0.152	25.49	0.009	1000	12.122	−14.64	0.247	−21.08	−0.002	−100
15	0	10.422	–	0.206	–	−0.002	–	10.422	–	0.206	–	−0.002	–
	25	8.046	22.80	0.174	15.53	0	100	11.426	−9.63	0.21	−1.94	−0.001	50
	50	7.458	28.44	0.139	32.52	−0.001	50	10.205	2.08	0.209	−1.46	−0.002	0
	75	7.243	30.50	0.135	34.47	0.004	300	9.657	7.34	0.16	22.33	−0.001	50
	100	7.194	30.97	0.128	37.86	0.015	850	8.412	19.29	0.158	23.30	0.003	250

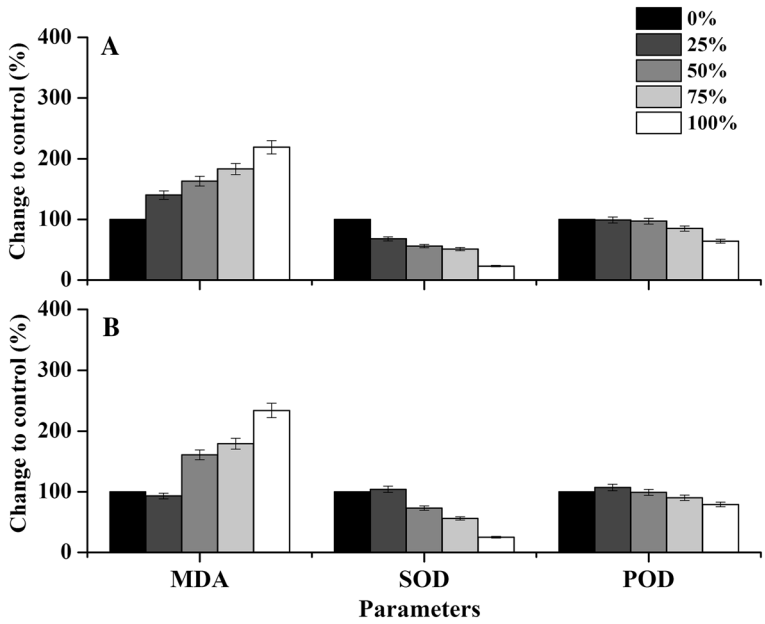

Fig. 5 Effects of *M. aeruginosa* exudates obtained from cultures in the exponential (**A**) and the decline phase (**B**) on malondialdehyde (MDA) content, activity of superoxide dismutase (SOD) and peroxidase (POD) of *P. crispus* (*error bars* indicate standard deviation)

of aquatic macrophyte germlings (over the first days) but did not affect final densities and lengths of germlings. In nature, degradation of MC is very common in water (Lahti et al., 1997; Casanova et al., 1999). Also, no free MC could be detected in the water of Lake Dianchi during most of 2011, except for May (0.081 g l^{-1}) and December (0.195 g l^{-1}), whereas intracellular MC concentrations were high between April and December, with maximum concentrations of 7.19 g l^{-1} in June (Bao, 2012). Exudates used in the present study were applied at in situ concentrations.

Previous studies showed that cyanobacteria could also benefit plants by producing growth-promoting regulators/phytohormones similar to IAA, cytokinin, gibberellin, ethylene, jasmonic acid, or abscisic acids (Rodgers et al., 1979; Yadav et al., 2011). The

concentrated culture filtrates of three cyanobacterial strains (*Calothrix ghosei* Bharadw., *Hapalosiphon intricatus* West, W. and G. S. and *Nostoc* sp.) enhanced germination percentage, radicle, and coleoptile length in studies with wheat seeds (Karthikeyan et al., 2009). Thus, we suppose that phytohormones might have relevance for the positive effects of decaying cyanobacteria on macrophytes in the present study. The wide utilization of cyanobacteria as biofertilizers in management of agroecosystem depends not only on the improved nitrogen, phosphorus, potassium, iron, and other mineral content in the soils but also on phytohormones facilitating plants to make better use of such minerals in plant growth promotion for enhanced crop production (Prasanna et al., 2010; Kumar et al., 2015). However, more work is needed to investigate the composition of exudates released by decaying cyanobacteria and the identification of key factors inducing their positive effects on submerged plants.

Survival strategy of *Potamogeton crispus* in response to stress by cyanobacterial exudates

A significant decrease in F_v/F_m ratios of *P. crispus*, as well as a significant dose–response and time–effect relationship was observed in the allelopathic effects of *M. aeruginosa* exudates of the exponential growth phase in the present study. Compared with other tested parameters (seedling biomass, Chl content, RLCs, antioxidant enzymes), F_v/F_m ratios of seedling leaves were the most sensitive parameter to indicate deleterious effects of *M. aeruginosa* exudates of the exponential growth phase on *P. crispus*. Similar results were obtained by Zheng et al. (2013), showing that the reduction in PSII activity was a major mechanism responsible for the allelopathic effects of *M. aeruginosa* on *P. malaianus*. Cyanobacterial allelochemicals have already been shown to inhibit the electron transport either by blocking PSII or between PSII and PSI (Huang et al., 1997; Kummerova et al., 2006; Váňová et al., 2009). Our results of the decreasing ETR_{max} with the increasing exudate concentrations indicate an inhibition of the photochemical processes related to the electron transport chain in the thylakoid (Váňová et al., 2009). Our results also showed that only exudates of the exponential growth phase of *M. aeruginosa* decreased the Chl in *P. crispus*.

Microcystis aeruginosa can completely dominate the summer phytoplankton in lakes (e.g., Liu et al., 2011). In many lakes, eutrophication was associated with a major decline in submerged vegetation (Phillips et al., 1978; Gong et al., 2009; Hilt et al., 2013). *P. crispus*, one of the few species that can persist in small stands in very nutrient-rich Chinese lakes (Lu et al., 2012), may have developed an adaptive mechanism to germinate from turions in later autumn and subsequently grow fast during winter. Consequently, new turions emerge from April to July and go dormant from July to September (Chen, 1985).

Cyanobacterial exudates in our study suppressed the majority of parameters of biomass, photosynthesis, and antioxidant system of *P. crispus*. However, tiller and root number were increased when treated by exudates of the exponential and the decline phase, respectively. *P. crispus* might have developed an adaptive strategy to survive cyanobacterial blooms like many other plants who often survive under severe stress by rapid cloning, or increasing tillers and roots (Cheplick & Grandstaff, 1997; Deng et al., 2008; Liu et al., 2008). Wu et al. (2012) found that *P. crispus* could still produce turions even under strong cutting. Vegetative reproduction resulting in propagules of high survival rate, growth vigor, and competitive capacity is the main way of aquatic plants to spread in eutrophied water bodies (Cheng et al., 2004). *P. crispus* completes its vegetation reproduction by bulblets, which produce many turions at the base of tillers (Chen, 1985). Therefore, plant biomass allocation patterns of *P. crispus* were changed by *M. aeruginosa* exudates (Fig. 1; Table 1). The trade-offs of biomass in allocation or optimization between leaf, tiller, stem, and root to maximize growth rate are common in plants (Dewar, 1993; Génard et al., 1998; McConnaughay & Coleman, 1999; McCarthy & Enquist, 2007). First, plant-cyanobacteria feedback effects on biomass allocation can optimize the ability of plants to compete for limiting resources (Te Beest et al., 2009). Indeed, we found increased biomass allocation to roots, which may enhance the competitive ability of plants for nutrients (Poorter & Nagel, 2000). At the same time, more biomass allocation to tillers increases the competitive abilities of plants, which may relieve the reproduction limit under stress. Alternatively, less biomass allocation to leaves may occur when allelopathic stress is higher.

Conclusion

The study demonstrated that *M. aeruginosa* exudates derived from the exponential growth phase showed stronger allelopathic effects on *P. crispus* seedlings than those of the decline phase. Allelopathic effects of *M. aeruginosa* were responsible for a decrease of biomass, damages in photosynthesis, and oxidative stress of *P. crispus* seedlings. Lake management measures such as planting *P. crispus* should thus not be applied in summer when cyanobacteria grow exponentially and exude inhibitive chemicals, but during the cyanobacteria decline phase when potentially stimulating chemicals are released. Further experiments are needed to unravel allelochemical uptake and phytotoxic effects observed in *P. crispus* in order to better understand the fate and effects of cyanobacterial exudates in aquatic environments.

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