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Succession and toxicity of *Microcystis* and *Anabaena* (*Dolichospermum*) blooms are controlled by nutrient-dependent allelopathic interactions

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ABSTRACT

Microcystis and *Anabaena (Dolichospermum)* are among the most toxic cyanobacterial genera and often succeed each other during harmful algal blooms. The role allelopathy plays in the succession of these genera is not fully understood. The allelopathic interactions of six strains of *Microcystis* and *Anabaena* under different nutrient conditions in co-culture and in culture-filtrate experiments were investigated. *Microcystis* strains significantly reduced the growth of *Anabaena* strains in mixed cultures with direct cell-to-cell contact and high nutrient levels. Cell-free filtrate from *Microcystis* cultures proved equally potent in suppressing the growth of nutrient replete *Anabaena* cultures while also significantly reducing anatoxin-a production. Allelopathic interactions between *Microcystis* and *Anabaena* were, however, partly dependent on ambient nutrient levels. *Anabaena* dominated under low N conditions and *Microcystis* dominated under nutrient replete and low P during which allelochemicals caused the complete suppression of nitrogen fixation by *Anabaena* and stimulated glutathione S-transferase activity. The microcystin content of *Microcystis* was lowered with decreasing N and the presence of *Anabaena* decreased it further under low P and high nutrient conditions. Collectively, these results indicate that strong allelopathic interactions between *Microcystis* and *Anabaena* are closely intertwined with the availability of nutrients and that allelopathy may contribute to the succession, nitrogen availability, and toxicity of cyanobacterial blooms.

1. Introduction

Over the last 140 years, toxic cyanobacterial blooms have been reported from fresh and brackish water bodies of temperate and tropical regions (Francis, 1878; Sivonen and Jones, 1999; Rantala et al., 2004). Cyanobacteria such as *Aphanizomenon, Radiocystis, Cylindrospermopsis, Microcystis, Anabaena,* and *Planktothrix* synthesize potent metabolites including hepatotoxins (e.g. microcystins and cylindrospermopsin) and neurotoxins (e.g. anatoxins and saxitoxins) that can kill wild and domestic animals (Krienitz et al., 2003; Zimba et al., 2006; Puschner et al., 2008), as well as humans (Jochimsen et al., 1998). Some of these cyanotoxins have been associated with elevated levels of human hepatocellular carcinoma cases reported in China (Kuiper-Goodman et al., 1999; Ren et al., 2017). There is evidence that the risks associated with cyanotoxins and cyanobacterial blooms will continue to increase globally because of climate change perturbations coupled with the eutrophication of aquatic ecosystems (Paerl, 2008; O'Neil et al., 2012).

The structure of phytoplankton communities can be influenced by chemical interactions between different species, a process referred to as allelopathy (Leão et al., 2009) whereby organisms synthesize and release bioactive compounds called allelochemicals into the surrounding environment to positively or negatively influence the growth of neighboring species (Rice, 1984). Allelopathy plays important roles in intra-species communication (Schatz et al., 2007), invasive fitness (Figueredo et al., 2007), resource and interference competition (De Nobel et al., 1997; Van Der Grinten et al., 2005), ecological succession, and bloom formation (Keating, 1978; Vardi et al., 2002; Legrand

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et al., 2003). Furthermore, cyanotoxins such as anatoxin-a (ATX-A), cylindrospermopsin, and microcystins (MCs) have been referred to as allelochemicals because of their effects on phytoplankton and macrophytes (Mitrovic et al., 2004; Leflaive and Ten-Hage, 2007; Campos et al., 2013; Rzymski et al., 2014; Chia et al., 2015, 2016).

While nutrient availability plays a key role in influencing the ecological dynamics of phytoplankton populations (Barreiro and Hairston, 2013a), the effect of abiotic and biotic factors on the production of allelochemicals and their allelopathic activity has not been extensively studied. Only a few studies have examined the effect of specific environmental factors such as nutrient, temperature, or pH variation on the production of allelochemicals or allelopathic activity (Legrand et al., 2003; Gomes et al., 2015). The excessive delivery of nitrogen (N) and phosphorus (P) has eutrophied marine and freshwater ecosystems worldwide (Cloern, 2001), skewing the stoichiometry of nutrients and driving phytoplankton communities into nutrient limitation (Graneli et al., 2009). Thus, producing allelochemicals may reduce competition in the immediate environment, allowing greater or longer access to a limiting resource (Legrand et al., 2003). Examples of N and P limitation abound in freshwaters (Gobler et al., 2016; Paerl et al., 2016). Some cyanobacteria outcompete other species in N-deficient waters by fixing nitrogen (i.e. diazotrophs) and producing antibiotic substances that are active against other cyanobacteria (Flores and Wolk, 1986). This suggests that nitrogen-fixing cyanobacteria could adopt either resource competitive or allelopathic strategies under different nutrient conditions against other phytoplankton species (Legrand et al., 2003). Studies on toxic and non-toxic strains of *Microcystis* spp. and *Anabaena* sp. revealed strain specific responses under low nitrogen, low phosphorus and excess nutrient conditions (Li and Li, 2012). Unfortunately, the impact of nutrient variations on the allelopathic interactions between *Microcystis* spp. and *Anabaena* spp, as well as the effects of allelopathy on the ability of diazotrophic cyanobacteria to fix nitrogen has not been previously researched. Furthermore, the physiological changes associated with individual species during allelopathic interactions have been poorly investigated in quantitative terms (Dunker et al., 2013; Song et al., 2017), as resolving strain/species-specific responses due to direct cell-to-cell contact in mixed culture experiments is complex.

The objectives of the present study were to investigate allelopathic interactions between strains of *Microcystis* and *Anabaena*. Experiments were performed using direct co-culture as well as cell-free culture filtrate and the effect of nutrient variation on the allelopathic interaction between *Microcystis* and *Anabaena* was also explored. Beyond observing changes in cell densities, experiments quantified photosynthetic efficiency, N_2-fixation rates, microcystin and anatoxin-a concentrations, and intracellular enzyme activity. The results obtained from these studies contribute to the current understanding of the role allelopathy plays in successional processes in aquatic ecosystems.

2. Materials and methods

2.1. Cyanobacterial strains

Six cyanobacterial strains of *Microcystis* and *Anabaena* were investigated in this study. While the genus *Anabaena* has been changed to or referred to as synonymous with other genera including *Dolichospermum* and *Trichormus* (Rajaniemi et al., 2005; Komárek and Zapomelova, 2007), recent whole genome comparisons of the order Nostocales indicates that the *Anabaena* and *Dolichospermum* genera should perhaps be collapsed back into a single genus complex since they share greater than 97.5% nucleotide identity at the 16S rRNA locus whereas 97% identity is the commonly used species cut-off and 92% identity is used to delineate genera (Li et al., 2016). Because of this and for the sake of continuity with prior studies with these same cultures, in the present study, the genus *Anabaena* is used to refer to *Anabaena* sp. 54, *Anabaena variabilis* UTEX B377, *A. flos-aquae* UTEX 1444, and *A. flos-aquae* CPCC 64. In addition, *Microcystis aeruginosa* LE 3 (*Microcystis* LE 3) and *Microcystis* sp. SR (*Microcystis* SR), were isolated from Lake Erie, USA (Brittain et al., 2000) and Sassafras River, MD, USA, respectively, and were the representative *Microcystis* strains used in the present study. Both *Microcystis* cultures produce microcystins and *Anabaena* sp. 54 produces ATX-A. The strains *A. variabilis* UTEX B377 (unknown origin), *A. flos-aquae* UTEX 1444 (isolated from Mississippi) and *A. flos-aquae* CPCC 64 (isolated from Lake Ontario) were obtained from the Greg L Boyer laboratory, ESF, State University of New York, and *Anabaena* sp. 54 (isolated from Finland) was obtained from HAMBI culture collection, Helsinki, Finland. The identity of the strains was confirmed using 16S rRNA gene (Nubel et al., 1997; Urbach et al., 2001) and the accession numbers were published under pending accession numbers in the NCBI GenBank. Stock cultures were maintained in BG11 medium under controlled light intensity (30 μmol m^{-2} s^{-1}), photoperiod (14:10 light:dark) and temperature (22 + 1 °C).

2.2. Mixed culture experiments of toxin-producing and non-toxin-producing cyanobacteria

The *Anabaena* sp. 54, *A. variabilis* UTEX B377, *A. flos-aquae* UTEX 1444 and *A. flos-aquae* CPCC 64 cultures were co-cultured with *Microcystis* LE-3 and *Microcystis* SR in 500 mL Erlenmeyer flasks containing 250 mL of BG-11 culture medium (Table S1). Since previous studies have shown that initial cell density influences species dominance, thereby giving an advantage to the species with higher cell density (Li and Li, 2012; Ma et al., 2015), *Anabaena* strains were co-cultured with *Microcystis* strains in a 1:1 biovolume ratio at 4.0×10^6 μM^3 mL^{-1} for each strain. The control treatments were established with only one strain in the culture medium. The cultures were incubated using the same conditions stated above for the stock cultures for 7 days and samples (10 mL) were obtained daily to quantify cell abundances, pH, and nutrient levels. All experimental treatments throughout this study were carried out in triplicate with cultures in exponential growth phase.

2.3. Effect of Microcystis LE 3 cell free medium on different Anabaena strains

Cell-free BG 11 culture medium was obtained from exponential growth phase cultures of *Microcystis* LE 3 after centrifugation (2000 G × 10 min), a process shown not to damage cells of this strain (Harke and Gobler, 2013). The N and P content of the *Microcystis* LE 3 cell free medium was supplemented to the nutrient levels found in freshly prepared BG11 medium, ensuring cyanobacterial growth was not nutrient limited. The initial cell density of *Anabaena* sp. 54, *A. variabilis* UTEX B377, *A. flos-aquae* UTEX 1444 and *A. flos-aquae* CPCC 64 was 5×10^5 cells mL^{-1} in 50 mL culture tubes containing 30 mL of *Microcystis* LE 3 cell free medium (see Table S2), and control cultures in 30 mL of BG11 medium were also established. The cultures were incubated for 3 days as described above. Samples for cell abundances (2 mL) and maximum quantum yield of photosystem II (Fv/Fm) (2 mL) were collected on days 1 and 3, and samples ATX-A content (15 mL) were collected on day 3 of the experiment.

2.4. Co-cultivation of A. variabilis UTEX B377 and Microcystis LE 3 using partitioned chambers under different nutrient conditions

Partitioned chambers were constructed to explore the concurrent growth and physiological condition of individual cyanobacterial strains during allelopathic interactions. The partitioned chambers were made up of two polystyrene Corning 200 mL U-shaped canted neck cell culture flasks separated by a 0.45 μm mesh size nylon membrane (Harke et al., 2017). Each compartment had a vented cap to enable sample retrieval without cross contamination. The *A. variabilis* UTEX B377 and *Microcystis* LE 3 strains were selected for co-cultivation experiments with partition chambers due to the significant reciprocal growth inhibition observed during the mixed culture experiments above. Furthermore, *A. variabilis* UTEX B377 was the only species capable of dominating the *Microcystis* spp. during mix cultivation, indicating it was the most competitive among the *Anabaena* spp. investigated. The strains were co-cultured using the partitioned chambers under high nutrient (+N + P), low nitrogen (-N + P) and low phosphorus (-P + N) conditions (Table S3). The high nutrient condition consisted of the N and P concentrations found in BG11 medium, while low N and low P treatments had 7.8 μM and 0.05 μM, respectively. The high nutrient treatments simulated the concentrations found in highly eutrophic aquatic ecosystems, while the low nitrogen and low phosphorus were similar to those of oligotrophic or nutrient limited water bodies (Reynolds, 2006; Harke et al., 2016). Prior to the commencement of this experimental phase, the cyanobacteria were acclimated to the respective nutrient conditions for a minimum of 2 weeks. Cultures of *Microcystis* LE 3 (4.0×10^6 μM^3 mL^{-1}, 150 mL) and *A. variabilis* UTEX B377 (4.0×10^6 μM^3 mL^{-1}, 150 mL) were added to the two compartments to give a total volume of 300 mL per treatment combination. The control treatments consisted of only one strain per pair of compartment chamber per nutrient condition. The co-cultures were incubated for 7 days under the same conditions as the stock cultures. Culture aliquots were (5 mL) collected daily during the experiments to quantify cell density, pH, and particulate (intracellular) MCs measurements. Samples (40 mL) for glutathione S-transferase activity were collected on day 7 of the experiment.

2.5. ***Data*** collection

pH measurements were made with pH strips (Merck, Germany) to avoid culture media contamination. Dissolved inorganic nutrients (nitrate, ammonia, and orthophosphate) concentrations were colorimetrically determined on a Lachat QuikChem 8500 flow injection analysis system (Lachat Instruments; Hach Company, Colorado, USA) (Table S4).

For the mixed cultures, Lugol's iodine preserved samples of *Microcystis* spp. were counted microscopically with a Hausser Scientific bright-line haemocytometer counting chamber, while those of the filamentous cyanobacterial strains were quantified with gridded Sedgwick rafter counting chamber. The gridded Sedgewick-Rafter chamber allowed for accurate assessment of filamentous cyanobacterial cell densities without the layering of cells and filaments. For all samples, a minimum of 200 cells was enumerated. Cell and filament dimensions were measured and biovolumes calculated according to Hillebrand et al. (1999).

Cell counts of *Microcystis* LE3 from the partition chamber co-culture experiments were quantified with a CytoFLEX flow cytometer (Beckman Coulter, USA) using fluorescence patterns and particle size derived from side angle light scatter (Olson et al., 1991). All data files were acquired and analyzed using the manufacturer supplied CytExpert Software for CytoFLEX (Beckman Coulter, USA).

Maximum quantum efficiency of photosystem II (Fv/Fm) and *in vivo* chlorophyll florescence were measured from *in vivo* (F_i) and DCMU (3,4-dichlorophenyl-1,1-dimethylurea)-enhanced *in vivo* fluorescence (Fm) of each replicate experimental sample on a Turner Designs TD-700 fluorometer (EX filter of 340–500 nm and EM filter of >665 nm), using blank corrections from BG11 media (Harke and Gobler, 2013; Parkhill et al., 2001).

For microcystin (MC) analyses, 10 mL culture samples were filtered through pre-combusted (2 h at 450 °C) glass fiber filters (GF/C) for extracellular (dissolved) and intracellular (particulate) MC analysis. The residue (cyanobacterial cells) and filtrate (dissolved MC) were kept at −20 °C until analysis. Intracellular MC was extracted with an Abraxis QuikLyse Cell Lysis kit for Microcystins/Nodularins ELISA Microtiter Plate according to the manufacturer's instructions. Subsequently, the lysed samples and filtrates were analyzed with an Abraxis Microcystins/Nodularins (ADDA) ELISA Kit according to the manufacturer's instructions.

For ATX-A analysis, 15 mL of *Anabaena* sp. 54 cultures were filtered onto pre-combusted (2 h at 450 °C) glass fiber filters (GF/C) and extracted using acetonitrile (ACN):water with formic acid solution as described by Dell'Aversano et al. (2005). The amount of MCs, homoanatoxin, and ATX-A (including derivatives) was assessed by liquid chromatography tandem mass spectrometry (LC–MS/MS) using an Agilent 1200 series HPLC and Agilent 6410 triple quadrupole mass spectrometer equipped with a Peak Nitrogen generator #NM30LA (Peak Scientific, Inc. Billerica, MA). The analytes were separated using a flow rate of 400 μL/min over a 57-min gradient. ATX-A was detected using appropriate qualifier and quantifier ions (Meriluoto and Codd, 2005; James et al., 2005) using Agilent MassHunter Qualitative Analysis software (version B.03.01). For each sample, the peak areas of the product ions were determined using the MS/MS integrator option. The amount of analyte was determined by normalization to the parent compound.

N_2-fixation by *A. variabilis* UTEX B377 from the partitioned chamber experiments was determined as a function of nitrogenase activity using the acetylene reduction assay (ARA) with gas chromatography and calculated as the amount of ethylene produced per hour (μg cell^{-1} h^{-1}) (Flett et al., 1976). Aliquots of *A. variabilis* UTEX B377 cultures (5 mL) were placed in 10 mL glass vials and sealed with rubber septa. Acetylene was added to the gas phase of the 10 mL vials and left to incubate for 4 h under the same growth conditions as the stock cultures. After the incubation period, a portion of the gas phase was withdrawn and analyzed for ethylene content using the Thermo Scientific Trace 1310 gas chromatography with a flame ionization detector (GC-FID) system (Thermo Scientific, Waltham, USA). An ethylene standard curve was used to calculate the concentration of ethylene produced by *A. variabilis* UTEX B377. The ratio of ethylene produced to the amount of nitrogen fixed by *A. variabilis* UTEX B377 was assumed to be 4:1 as was previously determined empirically for this species (Jensen and Cox, 1983). Peaks were viewed and analyzed with the Chromeleon version 7.2 Chromatography Data System (CDS) software (Thermo Scientific, Waltham, MA, USA).

For glutathione S-transferase (GST) activity analysis, total protein was extracted from 40 mL of partitioned chamber cultures of *A. variabilis* UTEX B377 in 0.1 M phosphate buffer (pH 6.5) containing 1% (w/v) polyvinylpyrrolidone (PVP). Glutathione S-transferase activity was analyzed using the Sigma GST activity assay kit following the manufacturer's instructions (Sigma-Aldrich, St. Louis, USA).

2.6. Statistical analysis

The data obtained were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Mauchly's sphericity tests, respectively. Significant differences in biomass production of the different strains during mixed and co-cultivation were determined using repeated measure one-way and two-way analysis of variance (ANOVA), respectively. The data obtained from the *Microcystis* LE 3 cell free medium experiment was analyzed using Student's *t*-test. When significant differences were detected in the ANOVA tests, Tukey's HSD post hoc test was used for multiple comparisons of means. All statistical analyses were done at 5% significance level using Statistica v.10 (StatSoft, Inc., Tulsa, OK, USA) for Windows.

3. Results

3.1. Mixed culture experiments of toxin-producing and non-toxin-producing cyanobacteria

In nearly all mixed culture experiments, *Microcystis* strongly inhibited the growth of *Anabaena* whereas the effects of *Anabaena* on *Microcystis* were trivial. The biovolume inhibition of *Microcystis* LE 3 and *Microcystis* SR biomass after exposure to *Anabaena* strains was 7–21% and 17–29%, respectively (Fig. 1a–b). There was greater and significant ($p < 0.05$) inhibition of *Anabaena* cultures by both *Microcystis* as *A. flos-aquae* UTEX 1444, *A. flos-aquae* CPCC 64 and *A. variabilis* UTEX B377 were inhibited by both *Microcystis* spp. by 27–75%, while *Anabaena* sp. 54 was inhibited by *Microcystis* LE 3 (43%;

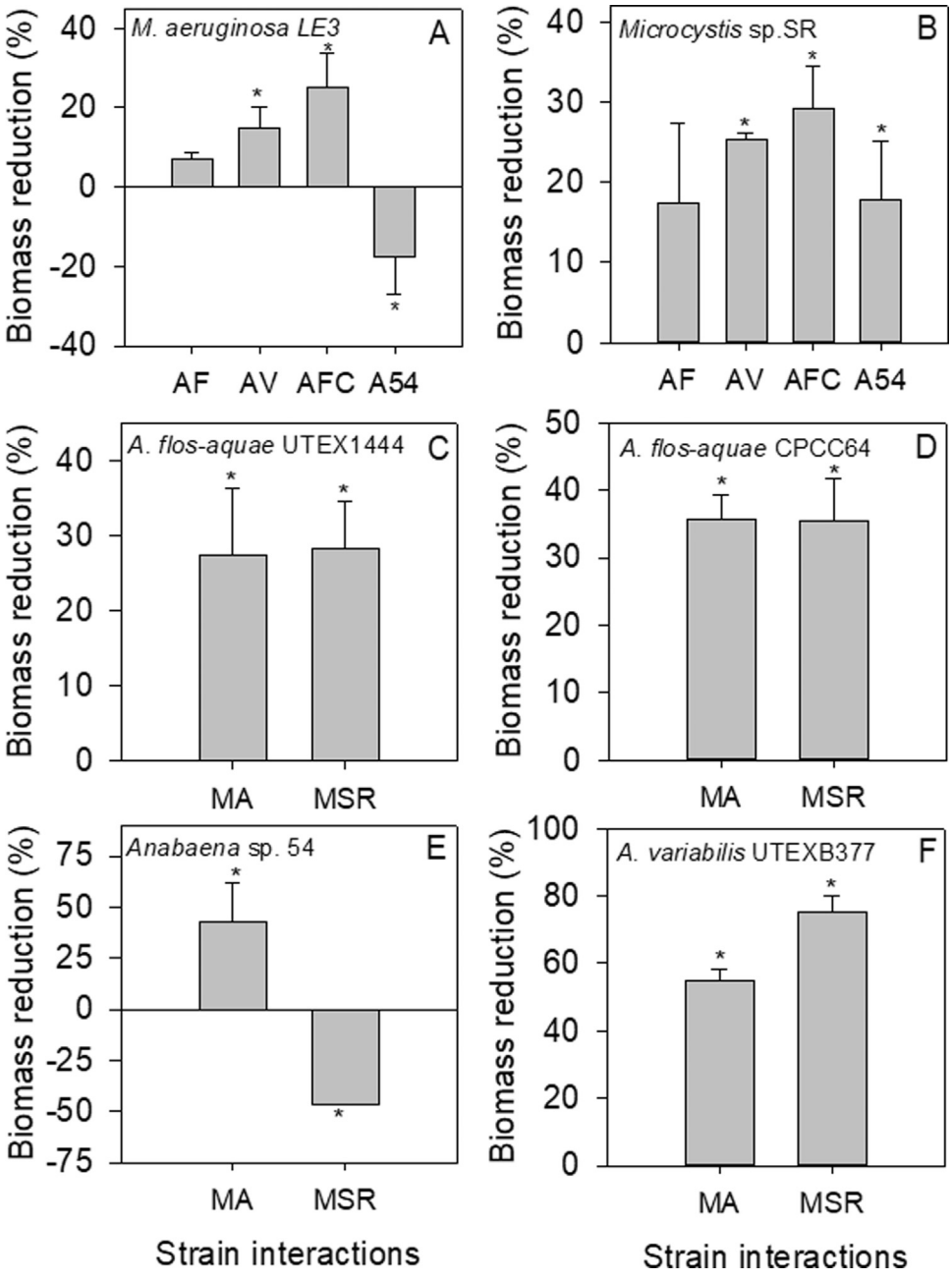

Fig. 1. Percentage reduction in the biomass of *Anabaena* sp. 54 (A54), *Anabaena flos-aquae* UTEX 1444 (AF), *A. flos-aquae* CPCC64 (AFC), *A. variabilis* UTEX B377 (AV), *Microcystis* LE 3 (MA), and *Microcystis* SR (MSR) during mixed culture experiments. Error bars represent standard deviation for $n = 3$. Values with asterisk are significantly different ($p < 0.05$) from the control.

$p < 0.05$; Fig. 1c–f). Relative to the monoculture controls, the most sensitive *Anabaena* strain to the presence of *Microcystis* spp. was *A. variabilis* UTEX B377, which experienced >50% inhibition of its biovolume at the end of the experiment (day 7).

3.2. Effect of Microcystis LE 3 cell free medium on different Anabaena strains

The cell free medium of *Microcystis* LE 3 had significant negative effect on the cell density of *A. flos-aquae* UTEX 1444, *A. flos-aquae* CPCC 64, *Anabaena* sp. 54 and *A. variabilis* UTEX B377, inhibiting biomass levels by 39–83% after 3 days (Fig. 2). Concurrently, the maximum quantum efficiency of photosystem II (Fv/Fm) of *A. flos-aquae* UTEX 1444, *A. flos-aquae* CPCC 64 and *A. variabilis* UTEX B377 was significantly ($p < 0.05$) inhibited while *Anabaena* sp. 54 was not (Fig. 3). The ATX-A content per cell of *Anabaena* sp. 54 was significantly inhibited by more than 50% when grown in *Microcystis* LE 3 cell free medium ($p < 0.05$; Fig. 4).

3.3. Co-cultivation of A. variabilis UTEX B377 and Microcystis LE 3 using partitioned chambers under different nutrient conditions

The highest biovolume of *Microcystis* LE 3 occurred under high nutrient and monoculture (control) conditions, while the lowest was observed under low P conditions (Fig. 5). On day 7, the biovolume of *Microcystis* LE 3 was significantly ($p < 0.05$) inhibited by 24% and 40% when co-cultured with *A. variabilis* UTEX B377 under high nutrient and low P conditions compared to the control and low P monocultures, respectively. For *Anabaena*, the biovolume of strain UTEX B377 in co-culture with *Microcystis* was significantly ($p < 0.05$) lower than the low N monocultures ($p < 0.05$) and high nutrient (control) condition, while the lowest value occurred under low P co-culture condition. Compared to the control, *Microcystis* caused 43% inhibition of the biovolume of *A. variabilis* UTEX B377 and >50% decline under P limitation. A significant ($p < 0.05$) interaction was recorded between the presence of *Microcystis* and changing nutrient conditions on the decline in the biomass of *A. variabilis* UTEX B377. The highest biovolume of *A. variabilis* UTEX B377 occurred under low N concentration with or without *Microcystis* LE 3 presence.

The cellular chlorophyll-*a* content of *Microcystis* LE 3 declined under low N and low P conditions while the presence of *A. variabilis* UTEX B377 only significantly ($p < 0.05$) inhibited the pigment content of *Microcystis* LE 3 under low P and high nutrient conditions (Table 1). For *A. variabilis* UTEX B377, the highest chlorophyll *a* concentration was detected under the low P treatment, while the lowest concentration was recorded under low N condition. Furthermore, the presence of *Microcystis* LE 3 resulted in significantly ($p < 0.05$) lower chlorophyll-*a* content under low N condition (Table 1).

The *Microcystis* LE 3 cultures grown under high nutrient conditions had the highest intracellular MC content, while the lowest concentration was observed during co-cultivation with *A. variabilis* UTEX B377 under low P (Fig. 6). The reductions in intracellular MC content of *Microcystis* LE 3 from monocultures were significant ($p < 0.05$) under low N and low P conditions. Furthermore, the presence of *A. variabilis* UTEX B377 significantly ($p < 0.05$) lowered the intracellular MC content of *Microcystis* LE 3 under high nutrient and low P condition only.

N_2-fixation by *A. variabilis* UTEX B377 was significantly ($p < 0.05$) reduced by the presence of *Microcystis* LE 3 at all nutrient levels (Fig. 7). These rates were reduced to undetectable

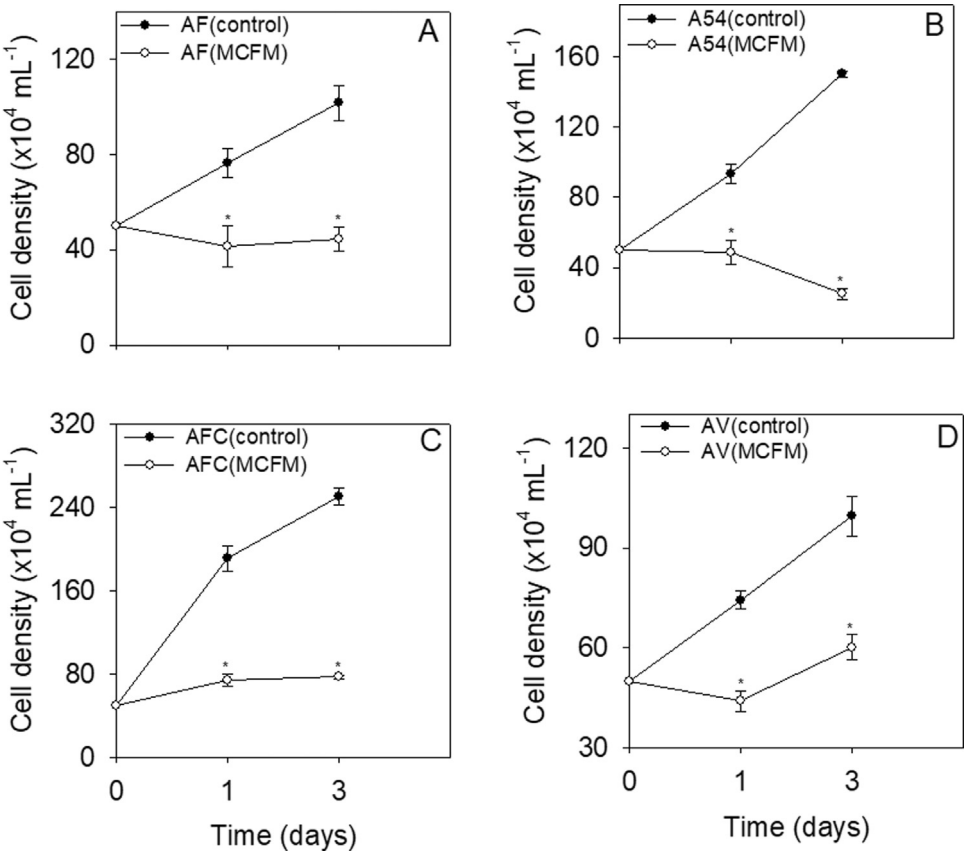

Fig. 2. Cell density variation of *Anabaena* sp. 54 (A54), *Anabaena flos-aquae* UTEX 1444 (AF), *A. flos-aquae* CPCC 64 (AFC), and *A. variabilis* UTEX B377 (AV) exposed to *Microcystis* LE 3 cell free medium (MCFM). Values with asterisk are significantly different ($p < 0.05$) from the control per time. Error bars represent standard deviation for $n = 3$.

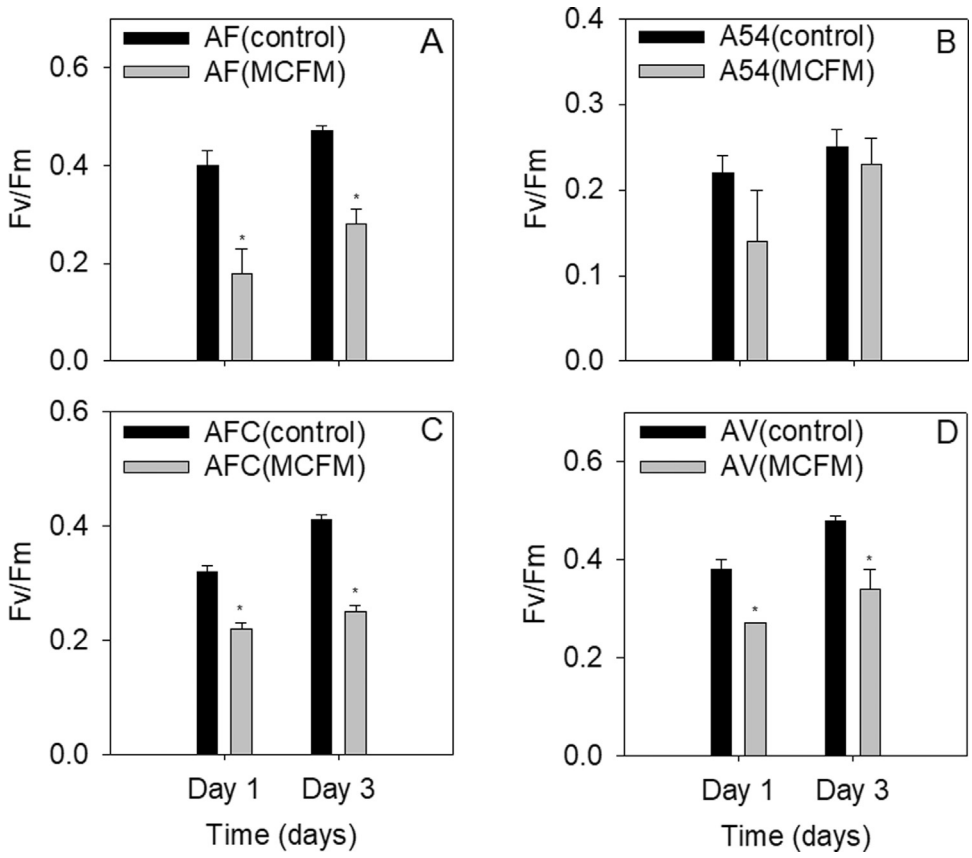

Fig. 3. Photosynthetic efficiency (Fv/Fm) of *Anabaena* sp. 54 (A54), *Anabaena flos-aquae* UTEX 1444 (AF), *A. flos-aquae* CPCC 64 (AFC), and *A. variabilis* UTEX B377 (AV) exposed to *Microcystis* LE 3 cell free medium (MCFM). Values with asterisk are significantly different ($p < 0.05$) from the control per time. Error bars represent standard deviation for $n = 3$.

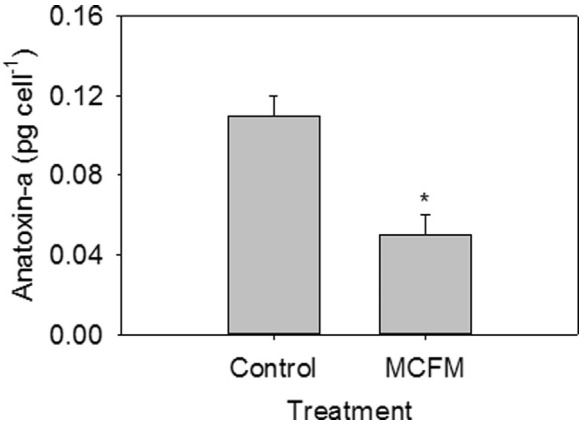

Fig. 4. Anatoxin-a content of *Anabaena* sp. 54 (A54) during cultivation with *Microcystis* LE 3 cell free medium of (MCFM). Bar with asterisk is significantly different from the control ($p < 0.05$). Error bars represent standard deviation for $n = 3$.

levels by *Microcystis* LE 3 exposure under low P conditions (Fig. 7). Exposure to *Microcystis* LE 3 also significantly ($p < 0.05$) increased GST enzyme activity in *A. variabilis* UTEXB 377 under high nutrient and low P conditions (Table 1).

4. Discussion

The present study revealed that different strains of *Microcystis* and *Anabaena* can have reciprocal growth inhibitory and stimulatory effects on each other. While *Microcystis* strongly suppressed *Anabaena* under nutrient replete conditions, precise growth effects were dependent on strain/species and were influenced by nutrient conditions. Furthermore, the interaction between cyanobacteria strains in combination with changing nutrient conditions altered the concentrations of cyanotoxins such as microcystins and anatoxin-a, antioxidant enzyme activities, and N_2-fixation by *Anabaena*. These findings provide important new insight regarding the ecology and toxicity of harmful cyanobacterial blooms.

4.1. Growth, pigment content, photosynthetic efficiency of photosystem II and GST activity

The *Microcystis* strains generally dominated *Anabaena* strains during this study. These results agree with those of previous studies that *Microcystis* spp. are capable of inhibiting or stimulating the growth of cyanobacteria and microalgae (De Nobel et al., 1998; Dunker et al., 2013; Zhang et al., 2014; Bittencourt-Oliveira et al., 2015). The mixed-cultivation of the six cyanobacterial strains was performed under nutrient-replete and optimum pH conditions, suggesting that the inhibition was likely due to allelopathic interactions between the strains. This hypothesis was affirmed by the ability of *Microcystis* LE 3 cell-free medium to significantly inhibit the growth of all *Anabaena* strains. The presence of *Microcystis* LE 3 also promoted the activity of GST in *A. variabilis* UTEX B377, an enzyme that plays important detoxification and biotransformation roles in plants exposed to bioactive secondary metabolites (Bártova et al., 2011) and a finding further supporting the hypothesis that allelochemicals mediated the interactions between these two cyanobacteria. Growth inhibition of the *Anabaena* spp. was likely caused, in part, by the significant decline in photosynthetic efficiency of photosystem II, which influenced

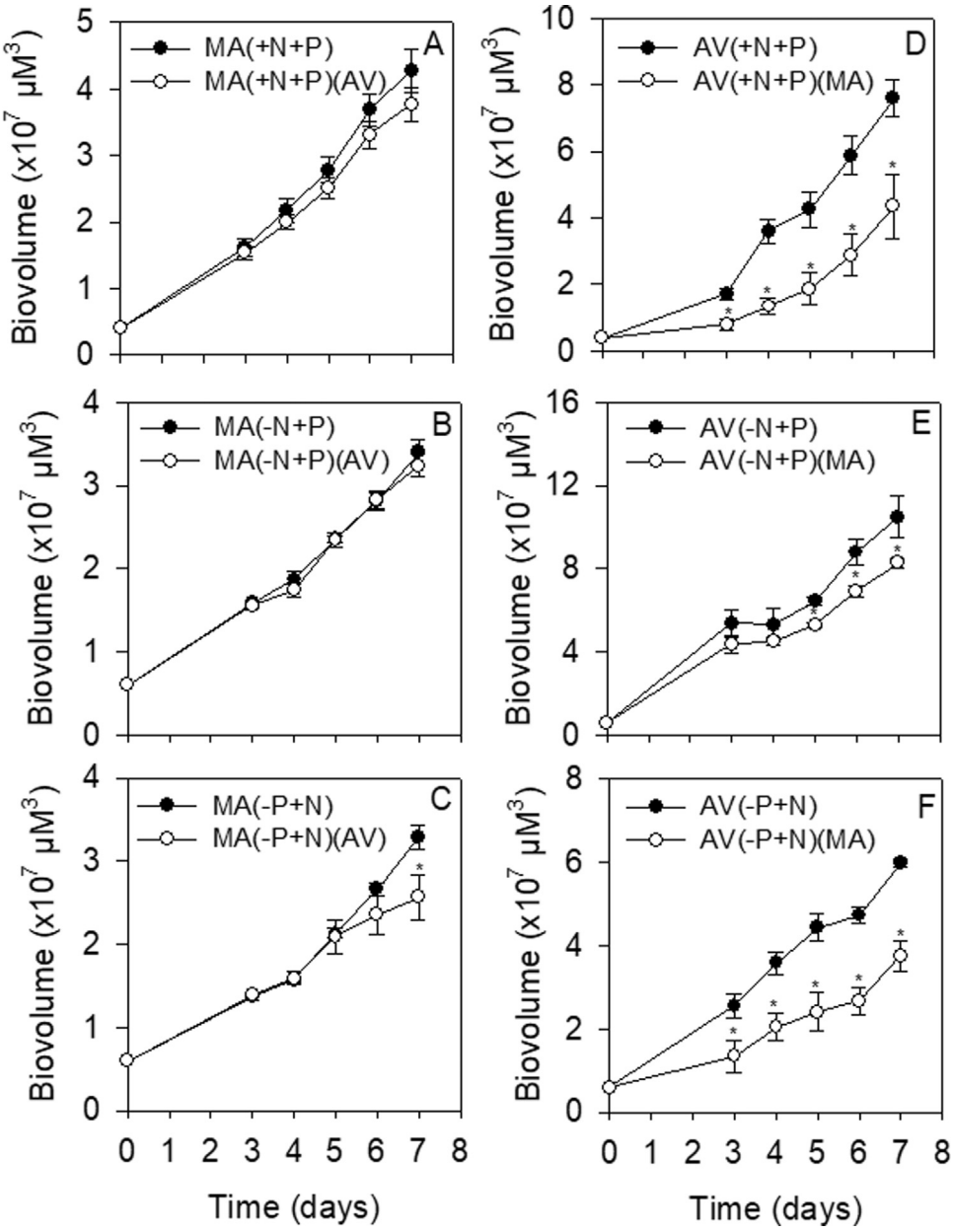

Fig. 5. Changes in biovolume of *Microcystis* LE 3 (MA) (a–c) and *Anabaena variabilis* UTEX B377 (AV) (d–f) during co-cultivation with partitioned chambers under high nutrient (+N + P), low nitrogen (−N + P) and low phosphorus (−P + N) conditions. Co-culture biovolumes with asterisk are significantly different ($p < 0.05$) from those of the respective monocultures of the strains. Error bars represent standard deviation for $n = 3$.

Table 1
Chlorophyll *a* content (pg cell^{-1}) of *Microcystis* LE 3 (MA) and *Anabaena variabilis* UTEX B377 (AV), and glutathione S-transferase (GST) activity (nkat 10^{-6} cells) of *A. variabilis* UTEX B377 during co-cultivation using partitioned chambers under high nutrient, low nitrogen, and low phosphorus conditions. Values are mean plus or minus standard deviation for $n = 3$, and those with asterisks are significantly different from the control.

Parameters	Strain	High nutrient	Low nitrogen	Low phosphorus
Chlorophyll *a*	MA (control)	1.40 + 0.01	1.10 + 0.03	1.20 + 0.03
	MA (co-culture)	1.18 + 0.05*	1.13 + 0.02	1.03 + 0.03*
	AV (control)	2.23 + 0.20	1.94 + 0.05	2.53 + 0.12
	AV(co-culture)	2.25 + 0.03	1.79 + 0.00*	2.56 + 0.02
GST activity	AV (control)	12.14 + 1.80	4.09 + 0.38	7.00 + 0.22
	AV(co-culture)	18.64 + 2.36*	5.03 + 0.19	12.43 + 1.29*

macromolecular allocations and biomass production (Larkum et al., 2003). Sukenik et al. (2002) reported that *Microcystis*-free media inhibited photosynthesis by abolishing internal carbonic anhydrase activity in the dinoflagellate *Peridinium gatunense*. The inhibition of *Anabaena* strains was likely caused by secondary metabolites released by *Microcystis* LE 3 into the growth medium and is in agreement with evidence that *Microcystis* spp. release bioactive substances into their environment to influence the

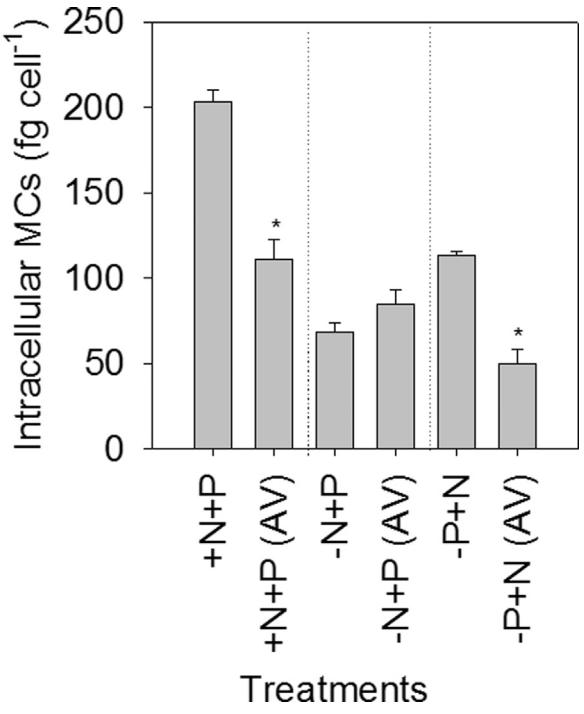

Fig. 6. Intracellular microcystins content of *Microcystis* LE 3 during co-cultivation with *Anabaena variabilis* UTEX B377 (AV) with partitioned chambers under high nutrient (+N + P), low nitrogen (−N + P) and low phosphorus (−P + N) conditions. Co-culture microcystins concentrations with asterisk are significantly different ($p < 0.05$) from those of the respective monocultures of *Microcystis* LE 3. Error bars represent standard deviation for $n = 3$.

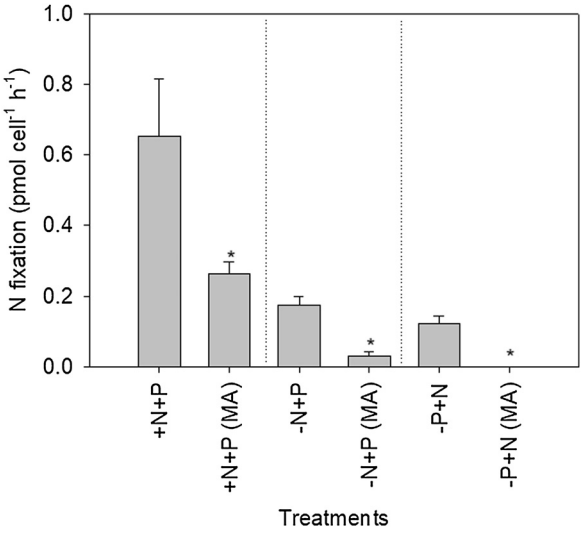

Fig. 7. Nitrogen fixation by *A. variabilis* UTEX B377 during co-cultivation with *Microcystis* LE 3 (MA) using partitioned chambers under high nutrient (+N + P), low nitrogen (−N + P) and low phosphorus (−P + N) conditions. Co-culture N_2-fixation values with asterisk are significantly different from those of the respective monocultures of *A. variabilis* UTEX B377. Error bars represent standard deviation for $n = 3$.

growth of competing phytoplankton (Johnson et al., 2009; Hiltunen et al., 2012; Barreiro and Hairston, 2013b).

Nutrient limitation strongly influenced allelopathic interactions between *Microcystis* and *Anabaena*. The *A. variabilis* UTEX B377 strain appeared to be at a disadvantage under high nutrient and low P conditions when it experienced the greatest inhibitions in biomass when exposed to *Microcystis* LE 3, while the reverse occurred under low N condition. This is an indication that the allelopathic effect of *Microcystis* is highest under high N and low P conditions and implies that nutrient variation influences phytoplankton interactions by stimulating or inhibiting the production of secondary metabolites, and/or making cyanobacteria more vulnerable to allelochemicals. This finding complicates the relationship between nutrients and community dynamics by creating direct bottom-up effects (Barreiro and Hairston, 2013a). While Harke et al. (2016) observed that *Microcystis* dominated the cyanobacterial community (including *Anabaena* populations) of Lake Erie under low phosphorus conditions by up-regulating phosphorus scavenging genes (*pstSCAB*), this may have also been assisted by the production of allelochemicals. Interestingly, GST activity was elevated in *A. variabilis* UTEX B377 cultures exposed to *Microcystis* LE 3 under all conditions except low nitrogen. Thus, *A. variabilis* appeared to be less inhibited by *Microcystis* under low N conditions, which suggests the lowered allelopathic effect of the latter and/or the higher tolerance of the former was likely related to the ability of *A. variabilis* to fix nitrogen under low N conditions.

4.2. Toxin production

The production of MC has been linked to various physiological processes such as cell division (Kurmayer, 2011), colony formation and homeostasis (Gan et al., 2012), and N (Harke and Gobler, 2013; Harke et al., 2016) and P (Kuniyoshi et al., 2013) availability. The higher concentration of MC detected in *Microcystis* LE 3 under high N concentration was likely due to the higher biosynthesis of the toxin under these conditions (Harke and Gobler, 2013; Harke et al., 2016). Conversely, nutrient limitation may account for the lower concentrations of the toxin in *Microcystis* LE 3 under low N and low P conditions, as the synthesis of MCs can be proportional to growth rate (Briand et al., 2012). The present results further revealed that competitive pressure from *A. variabilis* UTEX B377 resulted in lower MC concentrations in *Microcystis* LE 3 under low P and high nutrient conditions. In agreement with the results of the present study, Chen et al. (Chen et al., 2016) observed that *Microcystis* displayed down-regulation of MC synthetase genes and lower MC concentration when co-cultured with *A. flos-aquae*. Since MC-LR at moderate concentrations (25 $\mu g\,L^{-1}$) seems to have no significant effect on the growth of *A. variabilis* UTEX B377 (unpublished data), MC is likely not an allelochemical. The results of the present results differ from those of Li and Li (2012), which demonstrated that MC producing *Microcystis* responded to competition with *Anabaena* by increasing the production of the toxin. Overall, the results of the present study and those reported by other authors show that interspecific interactions and nutrients can play a central role in regulating the MC content of *Microcystis* spp.

While a number of studies have shown that physicochemical conditions influence ATX-A production by cyanobacteria (Rapala et al., 1993; Gagnon and Pick, 2012; Gagnon and Pick, 2012; Heath et al., 2014), the effect of biological factors on the production of the toxin by cyanobacteria has not been previously investigated. This study demonstrates, for the first time, that allelopathic interactions influence the level of ATX-A toxin in *Anabaena* spp., as the cell-free medium of *Microcystis* LE 3 caused a more than 50% reduction in ATX-A content of *Anabaena* sp. 54. The reduction of ATX-A concentration in *Anabaena* sp. 54 by allelochemicals from *Microcystis* is because it inhibited phytoplankton growth (Chia et al., 2016), and increased oxidative stress in phytoplankton species (Chia et al., 2015, 2016) and aquatic macrophytes (Mitrovic et al., 2004; Ha and Pflugmacher, 2013a,b). Therefore, the ability of *Microcystis* to inhibit the production of this molecule may provide it a competitive advantage over *Anabaena* sp. 54. Regardless, given the severe potency of this neurotoxin (Zimba et al., 2006), the finding that ATX-A levels in *Anabaena* can be suppressed by

Microcystis has broad and important implications for both ecological succession and human health.

4.3. Nitrogen fixation

N_2-fixation is a fundamental biogeochemical process in marine, brackish and freshwater ecosystems (Herrero et al., 2001). The highest N_2-fixation rates by *A. variabilis* UTEXB377 occurred under high nutrient conditions, which suggest that the cyanobacterium did not use the high nitrate concentration but rather used N_2-fixation to meet cellular demands (Herrero et al., 2001; Popa et al., 2007). Nitrogenase activity is often high when environmental N concentrations or forms do not meet cellular N demands (Postgate, 1998; Moisander et al., 2012). The lowest N_2-fixation rates occurred under low P conditions, a finding consistent with prior studies of diazotrophic cyanobacteria grown under limiting conditions in culture (De Nobel et al., 1998; Boussiba and Gibson, 1991; Layzell et al., 1985) and Lake Erie (Harke et al., 2016). The ability of *Microcystis* LE 3 to inhibit N_2-fixation by *A. variabilis* UTEX B377 may be related to the oxidative stress caused by allelochemicals from *Microcystis* LE 3 that induce higher catalase, peroxidase, and superoxide dismutase activities of *A. variabilis* UTEXB377 (unpublished data), thereby indicating increased reactive oxygen species (ROS) production (Yu, 1994). Such increases in ROS have been shown to be involved in the rapid reduction of nitrogenase activity due to the alteration of protein structure and the inhibition of nitrogenase synthesis in diazotrophic cyanobacteria (Berman-Frank et al., 2003). Furthermore, the biosynthesis of nitrogenase is extremely sensitive to enhanced O_2 levels (Gallon, 1992; Berntzon et al., 2013). Therefore, the ROS and O_2 produced by *A. variabilis* UTEXB377 in the presence of *Microcystis* and/or directly produced by *Microcystis* likely contributed to the suppression of N_2-fixation.

Filamentous, nitrogen-fixing cyanobacteria such as *Anabaena* spp. strongly influence nutrient cycling (Herrero et al., 2001). During this study, *Microcystis* and changing nutrient conditions disrupted the N_2-fixation capability of *Anabaena* strains: a finding with important implications on the nature and availability of N in aquatic ecosystems. While low N in the environment will lead to the dominance of *Anabaena* over *Microcystis*, the reverse will occur under high nutrients and low P conditions. In part, this explains the well-defined successional processes that have been recorded between *Anabaena* and *Microcystis* populations in aquatic ecosystems such as Lake Biwa Japan (Nalewajko and Murphy, 2001), Lake Okeechobee Florida (Havens et al., 2003), Lake Taihu China (Liu et al., 2011), Lake Erie (Harke et al., 2016), and the Baltic Sea (Andersson et al., 2015). In these environments, the general trend has been the replacement of *Anabaena* and other diazotrophs by *Microcystis* under low P and high N conditions, and replacement of *Microcystis* by *Anabaena* under low N and high P conditions. This study demonstrates that in addition to the physiological effects of exogenous N and P variation, direct interference via allelopathy by *Anabaena* and *Microcystis* will contribute towards the success of this particular non-diazotrophic cyanobacterium in aquatic ecosystems.

5. Conclusions

The results of the present study revealed significant allelopathic inhibition of *Anabaena* strains during co-cultivation with *Microcystis* spp. and these allelopathic effects were verified using cell free medium. Changing nutrient conditions altered the allelopathic interactions between *Microcystis* LE3 and *A. variabilis* UTEXB377 with *A. variabilis* UTEXB377 most sensitive to the presence of *Microcystis* LE3 under low P and high nutrient conditions but conditions favoring *Anabaena* under low N. Allelochemicals from *Microcystis* LE3 induced lower ATX-A content and N_2-fixation in *A. variabilis* UTEXB377 whereas alleloc

hemicals from *Anabaena* led to lower microcystin levels in *Microcystis*. From the results of the present study, it evident that low N will lead to the dominance of *Anabaena* over *Microcystis*, while the reverse will occur under high nutrients and low P conditions with such alternation between genera partly controlled by allelopathic interactions between the species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.hal.2018.03.002.

References

Andersson, A., Hoglander, H., Karlsson, C., Huseby, S., 2015. Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea. Estuar. Coast Shelf S. 164, 161–171.

Bártova, K., Hilscherova, K., Babica, P., Marsalek, B., Bláha, L., 2011. Effects of microcystin and complex cyanobacterial samples on the growth and oxidative stress parameters in green alga *Pseudokirchneriella subcapitata* and comparison with the model oxidative stressor herbicide paraquat. Environ. Toxicol. 26, 641–648.

Barreiro, A., Hairston Jr., N.G., 2013a. The influence of resource limitation on the allelopathic effect of *Chlamydomonas reinhardtii* on other unicellular freshwater planktonic organisms. J. Plankton Res. 35, 1339–1344.

Barreiro, A., Hairston Jr., N.G., 2013b. Indirect bottom-up control of consumer-resource dynamics: resource-driven algal quality alters grazer numerical response. Limnol. Oceanogr. 58, 827–838.

Berman-Frank, I., Lundgren, P., Falkowski, P., 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res. Microbiol. 154, 157–164.

Berntzon, L., Erasmie, S., Celepli, N., Eriksson, J., Rasmussen, U., Bergman, B., 2013. BMAA inhibits nitrogen fixation in the cyanobacterium *Nostoc* sp. PCC 7120. Mar. Drugs 11, 3091–3108.

Bittencourt-Oliveira, M.C., Chia, M.A., Oliveira, H.S.B., Cordeiro-Araújo, M.K., Molica, R.J.R., Dias, C.T.S., 2015. Allelopathic interactions between microcystin-producing and non-microcystin-producing cyanobacteria and green microalgae: implications for microcystins production. J. Appl. Phycol. 27, 275–284.

Boussiba, S., Gibson, J., 1991. Ammonia translocation in cyanobacteria. FEMS Microbiol. Rev. 88, 1–14.

Briand, E., Bormans, M., Quiblier, C., Salencon, M.J., Humbert, J.F., 2012. Evidence of the cost of the production of microcystins by *Microcystis aeruginosa* under differing light and nitrate environmental conditions. PLoS One 7, e29981.

Brittain, S.M., Wang, J., Babcock-Jackson, L., Carmichael, W.W., Rinehart, K.L., Culver, D.A., 2000. Isolation and characterization of microcystins: cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. J. Great Lakes Res. 26, 241–249.

Campos, P., Azevedo, J., Campos, A., Loureiro, S., Vasconcelos, V., 2013. Absence of negative allelopathic effects of cylindrospermopsin and microcystin-LR on selected marine and freshwater phytoplankton species. Hydrobiologia 705, 27–42.

Chen, R., Li, F., Liu, J., Zheng, H., Shen, F., Xue, Y., Liu, C., 2016. The combined effects of *Dolichospermum flos-aquae* light, and temperature on microcystin production by *Microcystis aeruginosa*. Chin. J. Oceanol. Limn. 34, 1173–1182.

Chia, A.M., Cordeiro-Araújo, M.K., Bittencourt-Oliveira, M.C., 2015. Growth and antioxidant response of *Microcystis aeruginosa* (Cyanobacteria) exposed to anatoxin-a. Harmful Algae 49, 135–146.

Chia, A.M., Cordeiro-Araújo, M.K., Lorenzi, A.S., Bittencourt-Oliveira, M.C., 2016. Does anatoxin-a influence the physiology of *Microcystis aeruginosa* and *Acutodesmus acuminatus* under different light and nitrogen conditions? Environ. Sci. Pollut. Res. 23, 23092–23102.

Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol-Prog. Ser. 210, 223–253.

De Nobel, W.T., Huisman, J., Snoep, J.L., Mur, L.R., 1997. Competition for phosphorus between the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. FEMS Microbiol. Ecol. 24, 259–267.

De Nobel, W.T., Matthus, H.C.P., Von Elert, E., Mur, L.R., 1998. Comparison of the light-limited growth of the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. New Phytol. 138, 579–587.

Dell'Aversano, C., Hess, P., Quilliam, M.A., 2005. Hydrophilic interaction liquid chromatography-mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins. J. Chromatogr. A 1081, 190–201.

Dunker, S., Jakob, T., Wilhelm, C., 2013. Contrasting effects of the cyanobacterium Microcystis aeruginosa on the growth and physiology of two green algae, Oocystis marsonii and Scenedesmus obliquus, revealed by flow cytometry. Freshw. Biol. 58, 1573–1587.
Figueredo, C.C., Giani, A., Bird, D.F., 2007. Does allelopathy contribute to *Cylindrospermopsis raciborskii* (cyanobacteria) bloom occurrence and geographic expansion? J. Phycol 43, 256–265.
Flett, R.J., Hamilton, R.D., Campbell, N.E.R., 1976. Aquatic acetylene-reduction techniques: solutions to several problems. Can. J. Microbiol. 22, 43–51.
Flores, E., Wolk, C.P., 1986. Production by filamentous, nitrogen fixing cyanobacteria of a bacteriocin and of other antibiotics that kill related strains. Arch. Microbiol. 145, 215–219.
Francis, G., 1878. Poisonous Australian lake. Nature 18, 11–12.
Gagnon, A., Pick, F.R., 2012. Effect of nitrogen on cellular production and release of the neurotoxin anatoxin-a in a nitrogen fixing cyanobacterium. Front. Microbiol. 3, 211.
Gallon, J.R., 1992. Reconciling the incompatible: N_2 fixation and O_2. New Phytol. 122, 571–609.
Gan, N., Xiao, Y., Zhu, L., Wu, Z., Liu, J., Hu, C., Song, L., 2012. The role of microcystins in maintaining colonies of bloom-forming *Microcystis* spp. Environ. Microbiol. 14, 730–742.
Gobler, C.J., Burkholder, J.M., Davis, T.W., Harke, M.J., Johengen, T., Stow, C.A., Van de Waal, D.B., 2016. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. Harmful Algae 54, 87–97.
Gomes, A.M.A., Azevedo, S.M.F.O., Lurling, M., 2015. Temperature effect on exploitation and interference competition among *Microcystis aeruginosa, Planktothrix agardhii*, and *Cyclotella meneghiniana*. Sci. World J. (Article ID: 834197).
Graneli, E., Weberg, M., Salomon, P., 2009. Harmful algal blooms of allelopathic microalgal species: the role of eutrophication. Harmful Algae 8, 94–102.
Ha, M.H., Pflugmacher, S., 2013a. Time-dependent alterations in growth: photosynthetic pigments and enzymatic defense systems of submerged *Ceratophyllum demersum* during exposure to the cyanobacterial neurotoxin anatoxin-a. Aquat. Toxicol. 138–139, 26–34.
Ha, M.H., Pflugmacher, S., 2013b. Phytotoxic effects of the cyanobacterial neurotoxin anatoxin-a: morphological, physiological and biochemical responses in aquatic macrophyte, *Ceratophyllum demersum*. Toxicon 70, 1–8.
Harke, M.J., Gobler, C.J., 2013. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. PLoS One 8, e69834.
Harke, M.J., Davis, T.W., Watson, S.B., Gobler, C.J., 2016. Nutrient-controlled niche differentiation of Western Lake Erie Cyanobacterial populations revealed via metatranscriptomic surveys. Environ. Sci. Technol. 50, 604–615.
Harke, M.J., Jankowiak, J.G., Morrell, B.K., Gobler, C.J., 2017. Transcriptomic responses in the bloom-forming cyanobacterium *Microcystis* induced during exposure to zooplankton. Appl. Environ. Microb (AE M-02832).
Havens, K.E., James, R.T., East, T.L., Smith, V.H., 2003. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environ. Pollut. 122, 379–390.
Heath, M.W., Wood, S.A., Barbieri, R.F., Young, R.G., Ryan, K.G., 2014. Effects of nitrogen and phosphorus on anatoxin-a homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a production by *Phormidium autumnale*. Toxicon 92, 179–185.
Herrero, A., Muro-Pastor, A.M., Flores, E., 2001. Nitrogen control in cyanobacteria. J. Bacteriol. 183, 411–425.
Hillebrand, H., Durselen, C.D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35, 403–424.
Hiltunen, T.J., Barreiro, A., Hairston Jr., N.G., 2012. Mixotrophy and the toxicity of *Ochromonas* in a pelagic food web. Freshw. Biol. 57, 2262–2271.
James, K.B., Crowley, J., Hamilton, B., Lehane, M., Skulberg, O., Furey, A., 2005. Anatoxins and degradation products, determined using hybrid quadrupole time-of-flight and quadrupole ion-trap mass spectrometry: forensic investigations of cyanobacterial neurotoxin poisoning. Rapid Commun. Mass Sp. 19, 1167–1175.
Jensen, B.B., Cox, R.P., 1983. Direct measurements of steady-state kinetics of cyanobacterial N_2 uptake by membrane-leak mass spectrometry and comparisons between nitrogen fixation and acetylene reduction. Appl. Environ. Microb. 45, 1331–1337.
Jochimsen, E.M., Carmichael, W.W., An, J., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., Antunes, M.B., de Melo Filho, D.A., Lyra, T.M., Barret, V.S.T., Azevedo, S.M., Jarvis, W.R., 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. New Engl. J. Med. 338, 873–878.
Johnson, P.R., Pavia, H., Toh, G., 2009. Formation of harmful algal blooms cannot be explained by allelopathic interactions. Proc. Natl. Acad. Sci. U. S. A. 106, 11177–11182.
Keating, K.I., 1978. Blue-green-algal inhibition of diatom growth – transition from mesotrophic to eutrophic community structure. Science 199, 971–973.
Komárek, J., Zapomelova, E., 2007. Planktic morphospecies of the cyanobacterial genus Anabaena = subg. Dolichospermum-1. part: coiled types. Fottea 7, 1–31.
Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Putz, S., Metcalf, J.S., Codd, G.A., Pflugmacher, S., 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiol. Ecol. 43, 141–148.
Kuiper-Goodman, T., Falconer, I., Fitzgerald, J., 1999. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management. Spoon, London, pp. 113–153.
Kuniyoshi, T.M., Gonzalez, A., Lopez-Gomollon, S., Valladares, A., Bes, M.T., Fillat, M. F., Peleato, M.L., 2013. 2-oxoglutarate enhances NtcA binding activity to promoter regions of the microcystin synthesis gene cluster. FEBS Lett. 584, 3921–3926.
Kurmayer, R., 2011. The toxic cyanobacterium *Nostoc* sp. strain 152 produces highest amounts of microcystin and nostophycin under stress conditions. J. Phycol. 47, 200–207.
Larkum, A.W.D., Douglas, S.E., Raven, J.A., 2003. Photosynthesis in Algae. Kluwer, Dordrecht Netherlands, pp. 479.
Layzell, D.B., Turpin, D.H., Elrifi, I.R., 1985. Effect of N source on the steady state growth and N assimilation of P limited *Anabaena flos-aquae*. Plant Physiol. 78, 739–745.
Leão, P.N., Vasconcelos, M.T.S.D., Vasconcelos, V.M., 2009. Allelopathic activity of cyanobacteria on green microalgae at low cell densities. Eur. J. Phycol. 44, 347–355.
Leflaive, J., Ten-Hage, L., 2007. Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshw. Biol. 52, 199–214.
Legrand, C., Rengefors, K., Fistarol, G.O., Granéli, E., 2003. Allelopathy in phytoplankton – biochemical, ecological and evolutionary aspects. Phycologia 42, 406–419.
Li, Y., Li, D., 2012. Competition between toxic *Microcystis aeruginosa* and nontoxic *Microcystis wesenbergii* with *Anabaena* PCC7120. J. Appl. Phycol. 24, 69–78.
Li, X., Dreher, T.W., Li, R., 2016. An overview of diversity, occurrence, genetics and toxin production of bloom-forming *Dolichospermum* (*Anabaena*) species. Harmful Algae 54, 54–68.
Liu, X., Lu, X., Chen, Y., 2011. The effects of temperature and nutrient ratios on *Microcystis* blooms in Lake Taihu, China: an 11-year investigation. Harmful Algae 10, 337–343.
Ma, Z., Fang, T., Thring, R.W., Li, Y., Yu, H., Zhou, Q., Zhao, M., 2015. Toxic and non-toxic strains of *Microcystis aeruginosa* induce temperature dependent allelopathy toward growth and photosynthesis of *Chlorella vulgaris*. Harmful Algae 48, 21–29.
Meriluoto, J., Codd, G.A., 2005. TOXIC: Cyanobacterial Monitoring and Cyanotoxin Analysis. Abo Akademi University Press (ISBN 951-765- 259-3).
Mitrovic, S.M., Pflugmacher, S., James, K.J., Furey, A., 2004. Anatoxin-a elicits an increase in peroxidase and glutathione S-transferase activity in aquatic plants. Aquat. Toxicol. 68, 185–192.
Moisander, P.H., Cheshire, L.A., Braddy, J., Calandrino, E.S., Hoffman, M., Piehler, M.F., Paerl, H.W., 2012. Facultative diazotrophy increases *Cylindrospermopsis raciborskii* competitiveness under fluctuating nitrogen availability. FEMS Microbiol. Ecol. 79, 800–811.
Nalewajko, C., Murphy, T.P., 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan? An experimental approach. Limnology 2, 45–48.
Nubel, U., Garcia-Pichel, F., Muyzer, G., 1997. Primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microb. 63, 3327–3332.
O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.
Olson, R.J., Zettler, E.R., Chisholm, S.W., 1991. Advances in oceanography through flow cytometry. In: Dusenberry, J.A., Demers, S. (Eds.), Particle Analysis in Oceanography, vol 27. NATO ASI Ser., Ser. G., pp. 351–399.
Paerl, H.W., Gardner, W.S., Havens, K.E., Joyner, A.R., McCarthy, M.J., Newell, S.E., Qin, B., Scott, J.T., 2016. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae 54, 213–222.
Paerl, H., 2008. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. Cyanobacterial harmful algal blooms. Adv. Exp. Med. Biol. 619, 217–237.
Parkhill, J.P., Maillet, G., Cullen, J.J., 2001. Florescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. J. Phycol. 37, 517–529.
Popa, R., Weber, P.K., Pett-Ridge, J., Finzi, J.A., Fallon, S.J., Hutcheon, I.D., Nealson, K.H., Capone, D.G., 2007. Carbon and nitrogen fixation and metabolite exchange in and between individual cells of *Anabaena oscillarioides*. ISME J. 1, 354–360.
Postgate, J.R., 1998. Nitrogen Fixation. Cambridge University Press, Cambridge, UK.
Puschner, B., Hoff, B., Tor, E.R., 2008. Diagnosis of anatoxin-a poisoning in dogs from North America. J. Vet. Diagn. Invest. 20, 89–92.
Rajaniemi, P., Hrouzek, P., Kastovská, K., Willame, R., Rantala, A., Hoffmann, L., Komárek, J., Sivonen, K., 2005. Phylogenetic and morphological evaluation of genera *Anabaena, aphanizomenon, trichormus* and *Nostoc* (Nostocales, cyanobacteria). Int. J. Syst. Evol. Micr. 5, 11–26.
Rantala, A., Fewer, D.P., Hisbergues, M., Rouhiainen, L., Vaitomaa, J., Borner, T., Sivonen, K., 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. Proc. Natl. Acad. Sci. 101, 568–573.
Rapala, J., Sivonen, K., Luukkainen, R., Niemela, S.I., 1993. Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena*-strains –a laboratory study. J. Appl. Phycol. 5, 581–591.
Ren, Y., Yang, M., Chen, M., Zhu, Q., Zhou, L., Qin, W., Wang, T., 2017. Microcystin-LR promotes epithelial-mesenchymal transition in colorectal cancer cells through PI3-K/AKT and SMAD2. Toxicol. Lett. 265, 53–60.
Reynolds, C.S., 2006. The Ecology of Phytoplankton. Cambridge University Press, Cambridge, UK.
Rice, E.L., 1984. Allelopathy, 2nd ed. Academic Press, New York, USA.

Rzymski, P., Poniedzialek, B., Kokocinski, M., Jurczk, T., Lipski, D., Wiktorowicz, K., 2014. Interspecific allelopathy in cyanobacteria: cylindrospermopsin and *Cylindrospermopsis raciborskii* effect on the growth and metabolism of *Microcystis aeruginosa*. Harmful Algae 35, 1–8.

Schatz, D., Keren, Y., Vardi, A., Sukenik, A., Carmeli, S., Borner, T., Dittmann, E., Kaplan, A., 2007. Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. Environ. Microbiol. 9, 965–970.

Sivonen, K., Jones, G., 1999. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management. In: Chorus, I., Bartram, J. (Eds.), Spoon, London, pp. 41–111.

Sukenik, A., Eshkol, R., Livne, A., Hadas, O., Rom, M., Tchernov, D., Vardi, A., Kaplan, A., 2002. Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp. (cyanobacteria): a novel allelopathic mechanism. Limnol. Oceanogr. 47, 1656–1663.

Urbach, E., Vergin, K.L., Young, L., Morse, A., Larson, G.L., Giovannoni, S.J., 2001. Unusual bacterioplankton community structure in the ultra-oligotrophic Crater Lake. Limnol. Oceanogr. 46, 5557–5572.

Van Der Grinten, E., Janssen, A., De Mutsert, K., Barranguet, C., Admiraal, W., 2005. Temperature- and light-dependent performance of the cyanobacterium *Leptolyngbya foveolarum* and the diatom *Nitzschia perminuta* in mixed biofilms. Hydrobiologia 548, 267–278.

Vardi, A., Schatz, D., Beeri, K., Motro, U., Sukenik, A., Levine, A., Kaplan, A., 2002. Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Curr. Biol. 12, 1767–1772.

Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74, 139–162.

Zhang, X.W., Fu, J., Song, S., Zhang, P., Yang, X.H., Zhang, L.R., Luo, Y., Liu, C.H., Zhu, H. L., 2014. Interspecific competition between *Microcystis aeruginosa* and *Anabaena flos-aquae* from Taihu Lake, China. Z. Naturforsch. 69C, 53–60.

Zimba, P.S., Camus, A., Allen, E.H., Burkholder, J.M., 2006. Co-occurrence of white shrimp, *Penaeus vannemei*, mortalities and microcystin toxin in a southeastern USA shrimp facility. Aquaculture 261, 1048–1055.