



UvA-DARE (Digital Academic Repository)

Sensitivity to hydrogen peroxide of the bloom-forming cyanobacterium *Microcystis* PCC 7806 depends on nutrient availability

Sandrini, G.; Piel, T.; Xu, T.; White, E.; Qin, H.; Slot, P.C.; Huisman, J.; Visser, P.M.

DOI

[10.1016/j.hal.2020.101916](https://doi.org/10.1016/j.hal.2020.101916)

Publication date

2020

Document Version

Final published version

Published in

Harmful Algae

License

CC BY

[Link to publication](#)

Citation for published version (APA):

Sandrini, G., Piel, T., Xu, T., White, E., Qin, H., Slot, P. C., Huisman, J., & Visser, P. M. (2020). Sensitivity to hydrogen peroxide of the bloom-forming cyanobacterium *Microcystis* PCC 7806 depends on nutrient availability. *Harmful Algae*, 99, Article 101916. <https://doi.org/10.1016/j.hal.2020.101916>

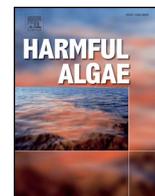
General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)



Sensitivity to hydrogen peroxide of the bloom-forming cyanobacterium *Microcystis* PCC 7806 depends on nutrient availability

Giovanni Sandrini^{a,#}, Tim Piel^{a,#}, Tianshuo Xu^a, Emily White^a, Hongjie Qin^{a,b}, Pieter C. Slot^a, Jef Huisman^a, Petra M. Visser^{a,*}

^a Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands

^b Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing, China

ARTICLE INFO

Keywords:

Nutrient limitation
Gene expression
Harmful algal blooms
Hydrogen peroxide
Microcystins
Microcystis
Photosynthetic yield

ABSTRACT

Application of low concentrations of hydrogen peroxide (H_2O_2) is a relatively new and promising method to selectively suppress harmful cyanobacterial blooms, while minimizing effects on eukaryotic organisms. However, it is still unknown how nutrient limitation affects the sensitivity of cyanobacteria to H_2O_2 . In this study, we compare effects of H_2O_2 on the microcystin-producing cyanobacterium *Microcystis* PCC 7806 under light-limited but nutrient-replete conditions, nitrogen (N) limitation and phosphorus (P) limitation. *Microcystis* was first grown in chemostats to acclimate to these different experimental conditions, and subsequently transferred to batch cultures where they were treated with a range of H_2O_2 concentrations ($0\text{--}10\text{ mg L}^{-1}$) while exposed to high light ($100\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$) or low light ($15\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$). Our results show that, at low light, N- and P-limited *Microcystis* were less sensitive to H_2O_2 than light-limited but nutrient-replete *Microcystis*. A significantly higher expression of the genes encoding for anti-oxidative stress enzymes (2-cys-peroxiredoxin, thioredoxin A and type II peroxiredoxin) was observed prior to and after the H_2O_2 treatment for both N- and P-limited *Microcystis*, which may explain their increased resistance against H_2O_2 . At high light, *Microcystis* was more sensitive to H_2O_2 than at low light, and differences in the decline of the photosynthetic yield between nutrient-replete and nutrient-limited *Microcystis* exposed to H_2O_2 were less pronounced. Leakage of microcystin was stronger and faster from nutrient-replete than from N- and P-limited *Microcystis*. Overall, this study provides insight in the sensitivity of harmful cyanobacteria to H_2O_2 under various environmental conditions.

1. Introduction

Harmful cyanobacterial blooms threaten the water quality of drinking water reservoirs and recreational waters, and can cause severe ecological and economic damage (Qin et al., 2010; Michalak et al., 2013; Huisman et al., 2018). Freshwater cyanobacteria can produce a variety of toxins which may lead to poisonings in birds and mammals, including humans (Carmichael, 2001; Codd et al., 2005). One of the most ubiquitous cyanotoxins is the hepatotoxin microcystin (MC), which is produced in many variants by a wide range of bloom-forming cyanobacteria (Meriluoto et al., 2017). Many cyanobacterial species grow well in nutrient-rich waters and high temperatures, and the expansion of cyanobacterial blooms in lakes and reservoirs across the globe is therefore commonly attributed to eutrophication and climate change (Paerl and Huisman, 2008; O'Neil et al., 2012; Visser et al., 2016a; Ho et al., 2019). Biomass production of

cyanobacterial blooms is often limited by phosphorus (P) and/or nitrogen (N) (Paerl, 2009) or, in highly eutrophied conditions, by light (Huisman et al., 1999; Verspagen et al., 2014).

Reduction of nutrient inputs into lakes is widely considered to be the most sustainable method to suppress cyanobacterial blooms (Fastner et al., 2016) and therefore preferred by water managers. However, effective nutrient reduction is often not feasible in a short time span. For example, nutrients often enter the water body via non-point sources or are internally released from the sediment, which hampers nutrient reduction efforts (Hamilton et al. 2016). Consequently, there is an increased interest in other methods to prevent, control or mitigate cyanobacterial blooms (Guo et al., 2007; Bullerjahn et al., 2016).

Various methods to suppress cyanobacterial blooms have been developed over the last decades, each with their own advantages and

* Corresponding author.

E-mail address: P.M.Visser@uva.nl (P.M. Visser).

These authors have contributed equally to this work.

disadvantages (Ibelings et al., 2016; Matthijs et al., 2016). Examples of these methods are the use of phosphate-adsorptive materials (Douglas et al., 2016), artificial mixing of the water column (Visser et al., 2016b), biomaniipulation with fish or mussels (Triest et al., 2016), use of water hyacinths (Qin et al., 2016), or the addition of chemical compounds such as copper sulfate or hydrogen peroxide (H_2O_2) (Jančula and Maršálek, 2011; Matthijs et al., 2016). In comparison to other chemical methods, the use of H_2O_2 to suppress cyanobacterial blooms has several advantages: H_2O_2 can selectively suppress cyanobacteria, it degrades rapidly to water and oxygen without leaving chemical traces in the environment, and it comes at a low economic cost compared to many other methods (Matthijs et al., 2016).

Application of low concentrations of $2-10\text{ mg L}^{-1}$ H_2O_2 generates sufficient oxidative stress to suppress cyanobacterial blooms, while eukaryotic algae such as green algae and diatoms are much less affected (Barroin and Feuillade, 1986; Drábková et al. 2007a; Matthijs et al., 2012; Weenink et al., 2015). This difference in sensitivity to oxidative stress between cyanobacteria and eukaryotic algae is thought to originate from differences in photosynthesis when cells are exposed to excess light energy. While chloroplasts of eukaryotic organisms produce H_2O_2 in the Mehler reaction, cyanobacteria use a different ‘Mehler-like’ reaction in which the flavodiiron proteins Flv1 and Flv3 produce water directly without H_2O_2 formation (Helman et al., 2003; Allahverdiyeva et al., 2015). Consequently, cyanobacteria do not have to deal with similar levels of intracellular H_2O_2 as eukaryotic organisms. Indeed, many cyanobacteria, including *Microcystis*, lack major antioxidant enzymes such as catalases which are able to rapidly degrade large quantities of H_2O_2 (Bernroither et al., 2009; Dietz et al., 2011; Schuurmans et al., 2018), although they still possess peroxidases to degrade low levels of H_2O_2 . These

physiological differences may explain why cyanobacteria have a lower defense mechanism against H_2O_2 compared to eukaryotic organisms and as a result are more sensitive to H_2O_2 .

For effective suppression of cyanobacteria, it is important to know which environmental factors affect the sensitivity of the cyanobacteria to H_2O_2 . Several studies have shown that high light strongly increases the H_2O_2 sensitivity of cyanobacterial cells (e.g. Drábková et al., 2007b; Mikula et al., 2012; Piel et al., 2020). This is likely caused by direct damage of H_2O_2 to photosystem II (PSII) and interference of H_2O_2 with the PSII repair mechanism (Nishiyama et al., 2006; Kojima et al., 2007; Blot et al., 2011; Wang et al., 2015), making cyanobacteria much more sensitive to photoinhibition. Whether nutrient availability also affects the sensitivity of cyanobacteria to H_2O_2 is not yet known. On the one hand, heterotrophic bacteria are often more resistant to oxidative stress under nutrient starvation (e.g., McDougald et al., 2002). On the other hand, nutrient limitation causes oxidative stress in phototrophic organisms including cyanobacteria, if they cannot process the excess light energy absorbed by photosynthesis (Kumar Saha et al., 2003; Schwarz and Forchhammer, 2005; Latifi et al., 2009). Therefore, one might expect that cyanobacteria will be more sensitive to H_2O_2 under nutrient-limited than under nutrient-replete conditions. Although cyanobacterial blooms often occur in lakes with high nitrogen (N) and phosphorus (P) loads, nutrients can temporarily limit the growth of cyanobacterial blooms (Davis et al., 2009; Xu et al., 2010). Hence, investigating effects of nutrient limitation on the H_2O_2 sensitivity of cyanobacteria is very relevant, not only for an improved fundamental understanding of the response of cyanobacteria to oxidative stress but also for practical applications such as the use of H_2O_2 in lake treatments.

In this study, we investigate how nutrient limitation affects oxidative stress in *Microcystis*, a harmful cyanobacterium that forms dense

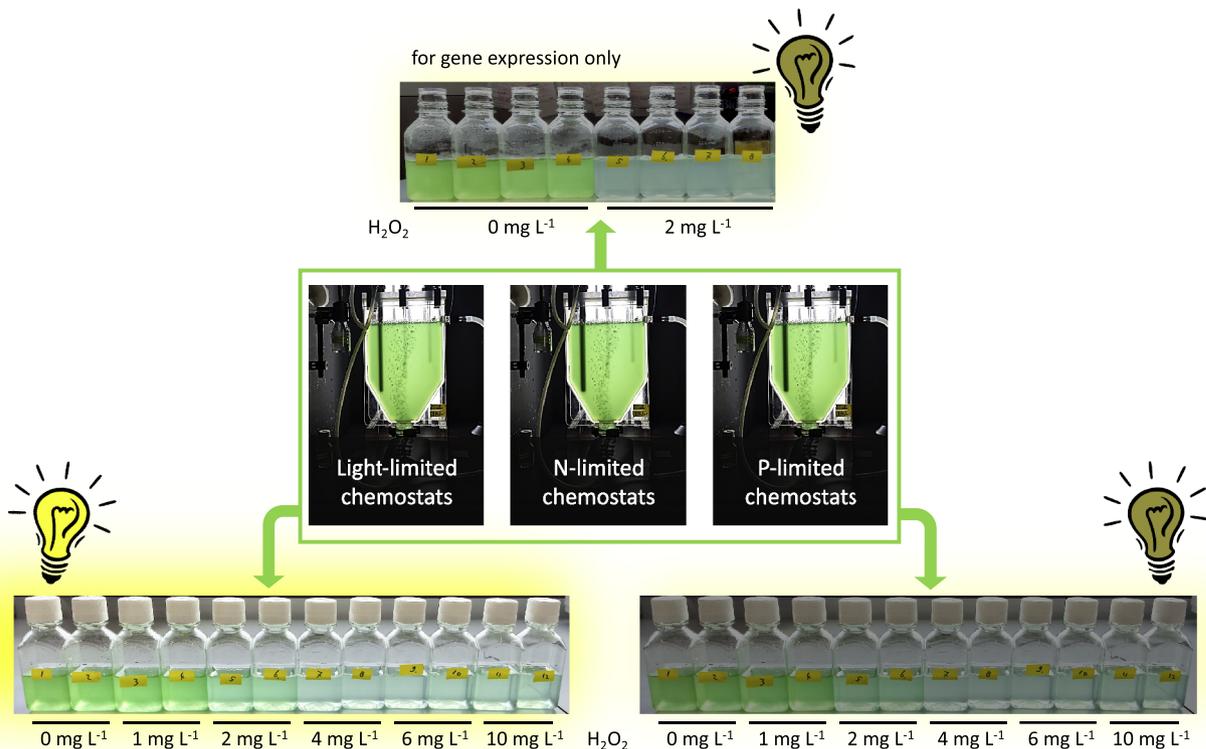


Figure 1. Overview of the experimental design. *Microcystis* was grown in chemostats under three different conditions: Light limitation, N limitation, and P limitation. Samples were transferred from the chemostats to batch cultures (bottles) in which the cells were treated with six different H_2O_2 concentrations (in the range of 0-10 mg L^{-1}). We ran two series of batch cultures, one exposed to high light ($100\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$) and the other to low light ($15\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$). In addition, a third series of batch cultures was run for gene expression analysis, in which the cells were treated with two H_2O_2 concentrations (0 and $2\ \text{mg L}^{-1}$) at low light only. The photos of the batch cultures were taken 24 h after H_2O_2 addition; the bleaching of the cultures can be clearly seen. Prior to H_2O_2 addition, the cell densities were similar in all batch cultures.

blooms in many eutrophic lakes across the globe (Harke et al., 2016; Huisman et al., 2018). In particular, we investigate the hypothesis that *Microcystis* grown under N or P limitation will be more sensitive to H₂O₂ than *Microcystis* grown under nutrient-replete conditions. For this purpose, *Microcystis aeruginosa* PCC 7806 (from now on referred to as *Microcystis*) was cultured in chemostats under light-limited but nutrient-replete, N-limited or P-limited conditions (Fig. 1). Samples of the chemostats were transferred to batch cultures where they were treated with a range of H₂O₂ concentrations and exposed to low or high light intensities. Sensitivity to H₂O₂ of these batch cultures was assessed by measuring the photosynthetic vitality, which is defined as the photosynthetic yield of a treated culture expressed as percentage of the photosynthetic yield of an untreated control culture (Matthijs et al., 2012; Piel et al., 2020). Furthermore, we measured intra- and extracellular MC concentrations as well as changes in the expression of genes involved in oxidative stress responses, photoprotection and microcystin production. The results of this study will show whether the effectiveness of H₂O₂ treatments of cyanobacterial blooms may depend on the nutrient status of lakes.

2. Materials and methods

2.1. Chemostat cultures

The axenic strain *Microcystis aeruginosa* PCC 7806 was kindly provided by the Pasteur Culture Collection (Paris, France). The *Microcystis* strain was cultured in chemostats optimized for phytoplankton growth (Huisman et al., 2002) to enable acclimation of the cells to stable growth conditions before the batch experiments with H₂O₂ were conducted (Fig. 1). All chemostats consisted of flat vessels with an optical path length of 5 cm and an effective working volume of ~1.8 L. Nutrient and light conditions were adjusted to obtain three different limiting factors: light, nitrogen (N) and phosphorus (P) limitation (Table 1). For each of these three limitations, *Microcystis* was grown in 4 chemostats to ensure enough culture material was available for the batch experiments. One of the chemostat cultures collapsed, and in the end we therefore had only 3 biological replicates (chemostats) for N limitation. All chemostats were supplied with BG-11 mineral medium (Rippka et al., 1979). Light-limited but nutrient-replete chemostats were supplied with full BG-11 medium containing 10 mmol L⁻¹ NaNO₃ and 230 μmol L⁻¹ K₂HPO₄, N-limited chemostats were supplied with a reduced NaNO₃ concentration of 0.40 mmol L⁻¹ and P-limited chemostats were supplied with a reduced K₂HPO₄ concentration of 17 μmol L⁻¹.

Light was supplied to the chemostats from one side by white fluorescent tubes (Philips Master TL-D 90 De Luxe 18 W/965, Philips Lighting, Eindhoven, The Netherlands). The incident light intensity was set by grey neutral density filters (LEE filters, Andover, UK). Light-limited chemostats were exposed to 15 μmol photons m⁻² s⁻¹ while N- and P-limited chemostats were exposed to 50 μmol photons m⁻² s⁻¹

Table 1

Experimental settings of the light-limited, N-limited and P-limited chemostats.

Parameters	Light-limited chemostats	N-limited chemostats	P-limited chemostats
I_{in} (μmol photons m ⁻² s ⁻¹) ^a	15.0 ± 0.1	50.2 ± 0.7	50.2 ± 0.6
CO ₂ (ppm)	400	400	400
Temperature (°C)	25	25	25
Dilution rate (h ⁻¹)	0.01	0.01	0.01
NaNO ₃ (μmol/L)	10000	400	10000
K ₂ HPO ₄ (μmol/L)	230	230	17

^a The values represent the mean ± SD of 3-4 replicate chemostats, where I_{in} was measured more than five times within a time span of several weeks during steady state of the chemostats.

(Table 1). While *Microcystis* in lakes can be exposed to much higher light intensities at the water surface, scum layer formation in lakes creates self-shading, lowering the light intensity experienced by the cells. The applied light intensities and nutrient concentrations of the mineral medium ensured that the biovolumes in steady state of the light-, N- and P-limited chemostats were similar, thereby avoiding effects caused by large differences in biovolume on the sensitivity of cells to H₂O₂.

The dilution rate of all chemostats was set at 0.01 h⁻¹, and hence at steady state the specific growth rate was identical for all applied conditions. The temperature in all chemostats was maintained at 25°C for optimal growth of *Microcystis*, using stainless steel cooling fingers connected to a water bath (Haake A28F/AC200; Thermo Fisher Scientific, Pittsburgh, PA, USA). This temperature is commonly found in surface waters during summer, when cyanobacteria proliferate in lakes (Verspagen et al., 2006; Diao et al., 2017). All chemostats were bubbled with CO₂-enriched air at a flow rate of 25 L h⁻¹. The CO₂ concentration in the pressurized air was adjusted to 400 ppm CO₂ as described before (Sandrini et al., 2015). The gas mixture was moistened with water at 25°C to suppress evaporation and led through a 0.20 μm Midisart 2000 filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany) to sterilize the air before entering the chemostats. The CO₂ concentration in the gas mixture was checked regularly with an Environmental Gas Monitor for CO₂ (EGM-4; PP Systems, Amesbury, MA, USA).

Incident irradiance (I_{in}) and the irradiance penetrating through the chemostat vessel (I_{out}) were measured almost every day with a LI-COR LI-250 quantum photometer (LI-COR Biosciences, Lincoln, NE, USA) at ten positions on the front and back surface of the chemostat vessels, respectively. The chemostats were sampled 2-3 times per week. Cell numbers, biovolumes and average cell size in samples were determined in triplicate using a Casy 1 TTC cell counter (Schärfe System GmbH, Reutlingen, Germany) with a 60 μm capillary. The pH of the samples was measured immediately after sample collection with a Lab 860 pH meter in combination with a BlueLine 28 Gel pH electrode (SCHOTT Instruments GmbH, Mainz, Germany). For the analysis of dissolved inorganic nutrients, samples were filtered over 47 mm GF/C glass filters (Whatman GmbH, Dassel, Germany). The filtrates were subsequently filtered over 0.2 μm pore size 25 mm Whatman polycarbonate membrane filters (GE Healthcare, Buckinghamshire, UK) and stored in plastic 15 mL tubes at -20°C until further analysis. Concentrations of dissolved nitrate, nitrite, ammonium and phosphate were measured using a San + + Automated Wet Chemistry Analyzer (Skalar Analytical B.V., Breda, The Netherlands).

Regular microscopical inspections of chemostat samples did not reveal contamination with other photosynthetic organisms. Although all chemostats were started with axenic cultures of *Microcystis*, we could not prevent contamination by heterotrophic bacteria during the chemostat experiments. The number of heterotrophic bacteria in the cultures remained low (< 5% of the total cell counts) until the end of the experiments.

2.2. H₂O₂ sensitivity tests in batch cultures

To investigate the H₂O₂ sensitivity of *Microcystis* acclimated to the three different limiting factors, samples of 200-300 mL were taken from each of the chemostats as soon as a steady state (i.e., a stable population density) was reached. Samples from replicate chemostats with the same limiting factor were mixed and subsequently sterile BG-11 mineral medium was added to reach 3 L stock cultures with a final *Microcystis* biovolume of 100 mm³ L⁻¹. We added full BG-11 medium for the light-limited stock culture, BG-11 with 10 mmol L⁻¹ NaCl instead of NaNO₃ and 230 μmol L⁻¹ K₂HPO₄ for the N-limited stock culture, and BG-11 with 10 mmol L⁻¹ NaNO₃ but no K₂HPO₄ for the P-limited stock culture. These stock cultures were used for batch culture experiments in which the cells were exposed to different H₂O₂ concentrations (Fig. 1).

The batch culture experiments were carried out in Nalgene plastic

bottles (Thermo Fisher Scientific, Waltham, MA, USA) of 250 mL without lids. Each bottle was filled with 200 mL of stock culture. The temperature was maintained at 25°C using stainless steel cooling fingers connected to a water bath and aeration of the batch cultures was provided with compressed air. H₂O₂ was added to the cultures to final concentrations of 0, 1, 2, 4, 6 and 10 mg L⁻¹. The batch cultures were run in duplicate for each H₂O₂ concentration, resulting in a total of 12 batch cultures per limiting factor. As controls, H₂O₂ degradation without *Microcystis* cells was investigated by adding a final concentration of 10 mg L⁻¹ H₂O₂ to BG-11 medium and to Milli-Q water in Nalgene bottles, also both in duplicate.

The H₂O₂ sensitivity of *Microcystis* was investigated during exposure of the batch cultures to a low light intensity of 15 μmol photons m⁻² s⁻¹, and two weeks later the whole experiment was repeated using a higher light intensity of 100 μmol photons m⁻² s⁻¹ (Fig. 1). These two light intensities are representative of the range of light levels commonly found in the upper 0.5-2 meters of turbid lakes dominated by cyanobacterial blooms (Huisman et al., 2004; Verspagen et al., 2006). Light was provided by the same fluorescent tubes as for the chemostats, using grey neutral density filters to adjust the light intensity. The time interval between the low light and high light experiments was two weeks to ensure recovery of the chemostats to steady state after sampling.

In order to analyze the photosynthetic vitality and H₂O₂ concentration, 5 mL samples were taken from each batch culture just before adding H₂O₂, immediately after addition, then every half-hour after addition of H₂O₂ for the first 4 hours, and after 24 hours. MC were analyzed (as described in section 2.6) in samples taken before the addition of H₂O₂ as well as 1, 2, 4 and 24 hours after H₂O₂ addition. In addition, for biovolume analysis with the Casy cell counter (as described in section 2.1), a 1 mL sample of each batch culture was taken just before and 24 h after H₂O₂ addition.

2.3. Photosynthetic vitality

The photosynthetic vitality was determined as described by Piel et al. (2020). *Microcystis* cells from the 5 mL samples were collected on 25 mm GF/C filters (Whatman GmbH, Dassel, Germany) by filtration on a Millipore 1225 Sampling Manifold (Merck KGaA, Darmstadt Germany). Immediately after filtration, the cells were dark adapted for 5 minutes by covering the filters with rubber stoppers on the Millipore filtration unit. Subsequently, the rubber stoppers covering the filters were exchanged with a rubber stopper connected to a Mini-PAM-fluorometer (Walz, Effeltrich, Germany), and the minimum fluorescence (F_0) and maximum fluorescence (F_m) were measured before and after a saturating light pulse, respectively. The maximum photosynthetic yield of dark adapted cells (F_v/F_m , also known as the maximum quantum yield of PSII electron transport) was calculated according to Maxwell and Johnson (2000):

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

Photosynthetic vitality is defined as the photosynthetic yield of the H₂O₂-treated samples as percentage of the photosynthetic yield of the untreated control samples.

2.4. Dose-response models

Photosynthetic vitality at 4 hours after addition of H₂O₂ was described as a function PV of the added H₂O₂ concentration according to a logistic dose-response model (Piel et al., 2020):

$$PV = \frac{C}{1 + e^{B(\log_{10}[H_2O_2] - \log_{10}(A))}} \quad (2)$$

where A is the estimated EC₅₀ value (i.e. the H₂O₂ concentration at which PV is 50%), B is the exponential slope of the dose-response model, and C is the photosynthetic vitality of the control samples that were not exposed to H₂O₂ (i.e., C was set to 100%). The parameters A

and B were estimated by fitting the dose-response model to the photosynthetic vitality data using nonlinear least-squares regression.

2.5. Hydrogen peroxide measurements

H₂O₂ concentrations were measured in filtrates of the 5 mL samples. Subsamples of 100 μL from these filtrates were immediately transferred to a 96-well microtiter plate and mixed with 100 μL of 2 mmol L⁻¹ *p*-nitrophenylboronic acid reagent (Merck KGaA, Darmstadt, Germany), according to Lu et al. (2011). After reacting with H₂O₂, the formation of the color reagent *p*-nitrophenolate at room temperature was complete in 30 to 45 min, and the color remained stable for several hours. For the quantification of the reagent, absorption of *p*-nitrophenolate was measured at its absorption peak (405 nm) using a SPECTROstar Nano plate reader (BMG Labtech GmbH, Ortenberg, Germany). A 33% (w/w) stock solution (VWR, Amsterdam, The Netherlands) was used to make a calibration curve (0.01-10 mg L⁻¹ H₂O₂) that was included on each microtiter plate. For each filtrate, the H₂O₂ concentration was measured in quadruplicate. Pilot experiments showed that losses of H₂O₂ by the filtration procedure were <1%.

2.6. Microcystin analysis

To assess the intracellular unbound MC content and the extracellular MC in the water phase, cells on the filters and the filtrates were stored at -20°C and subsequently freeze-dried. MC were extracted with 75% MeOH and analyzed with HPLC according to Van de Waal et al. (2011), using a Shimadzu LC-20AD HPLC system with a SPD-M20A photodiode array detector (Shimadzu, Kyoto, Japan). The two MC variants, [Asp³]MC-LR and MC-LR (Tonk et al., 2009), produced by *Microcystis* PCC 7806 were summed as separation of both peaks was not possible. The sum of the two variants is hereafter referred to as MC. Each sample was analyzed in duplicate. A heatmap to summarize the toxin data was made with R (version 3.6.2) using the *heatmap.2* function of the package *gplots* (version 3.0.3) (www.R-project.org).

2.7. Gene expression analysis

Gene expression was analyzed with reverse transcription quantitative PCR (RT-qPCR), applied to a separate series of batch cultures using the same experimental design. A separate series of batch experiments was necessary, because (1) the volume of the batch cultures was too small to obtain sufficient material for both RT-qPCR and the previous measurements, and (2) it was not possible to process the RNA samples quickly enough when many other measurements needed to be done at the same time. In the gene expression experiments, batch cultures were treated with 0 mg L⁻¹ and 2 mg L⁻¹ H₂O₂ at a low light intensity of 15 μmol photons m⁻² s⁻¹. The batch cultures were run in quadruplicate for each H₂O₂ concentration, resulting in a total of 8 batch cultures per limiting factor. From each batch culture, 40 mL samples for gene expression analysis were taken just before and 2 h after the addition of H₂O₂. The 40 mL samples were centrifuged immediately in a pre-cooled Rotanta 460R centrifuge (Hettich GmbH & Co. KG, Tuttlingen, Germany) at 4°C and 2380 g for 5 minutes. Subsequently, the medium was decanted and the cells were resuspended in 1 mL TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), frozen in liquid nitrogen, and stored in -80°C until further analysis.

We investigated the expression of a selected number of genes that had shown a strong response to H₂O₂ addition in the full transcriptome analysis of Schuurmans et al. (2018). Specifically, using primers designed in this study and previous studies (Sandrini et al., 2014, Sandrini et al., 2016a, Sandrini et al., 2016b) (Table S1), transcripts of the following genes were targeted with RT-qPCR: *2-cys prx* (peroxiredoxin), *trxA* (thioredoxin), *t2prx* (type-2 peroxiredoxin), *isiA* (iron stress-induced protein A), *flv2* (photoprotective flavodiiron-2 protein), *flv4* (photoprotective flavodiiron-4 protein), *mcvB* (microcystin

synthetase, operon *mcvABC*), *mcvE* (microcystin synthetase, operon *mcvDEFGHIJ*) and *16S rRNA* (reference gene).

RNA extraction and RT-qPCR were done as described previously (Sandrini et al., 2015). In short, RNA was extracted with TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) according to the supplier's instructions, using 0.5 mm BashingBeads (Zymo Research, Orange, CA, USA) to facilitate cell disruption. After the phase separation steps, the Direct-Zol™ RNA MiniPrep kit (Zymo Research, Orange, CA, USA) was used for RNA purification. The optional in-column DNase I digestion was included. RNA concentrations were quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and all RNA samples had A_{260}/A_{280} values above 1.7. Reverse transcription reactions were completed using the Superscript III enzyme (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the qPCR Maxima SYBR green master mix (Thermo Fisher Scientific, Waltham, MA, USA) was applied with our primers to the obtained cDNA samples to analyze PCR amplification in an ABI 7500 real-time PCR device (Applied Biosystems, Foster City, CA). Samples were analyzed in duplicate. The two-step cycling protocol was applied, with a denaturation temperature of 95°C (15 s) and a combined annealing/extension temperature of 60°C (60 s) during 40 cycles. Melting-curve analysis was performed on all samples to rule out non-specific PCR products. The passive reference dye ROX was used to correct for any well-to-well variation.

Relative changes in gene expression for all samples were calculated using the *16S rRNA* gene as reference gene and batch cultures that originated from the light-limited chemostats before addition of H_2O_2 ($t=0$ h) as reference sample. The LinRegPCR software tool version 2017.1 (Ramakers et al., 2003; Ruijter et al., 2009) was used for baseline correction, calculation of quantification cycle (C_q) values and calculation of the amplification efficiency (E) of each individual run using linear regression (Table S1). Amplification efficiencies of individual samples calculated with LinRegPCR were between 1.8 and 2.0. In addition, standard curves were made using 10-fold dilutions of cDNA samples. These standard curves were used to calculate amplification efficiencies for each of the applied primer sets. Amplification efficiencies of the primer sets calculated from standard curves were between 1.92 and 2.06 (Table S1). The relative changes in gene expression were calculated with the comparative cycle threshold (C_T) method (Livak and Schmittgen, 2001). Negative controls of the qPCR and negative controls of the reverse transcriptase reaction did not result in significant amplification. Two-way ANOVA tests were applied to identify effects of the limiting factor and of the addition of H_2O_2 ($n=4$ biological replicates per treatment). Post-hoc comparisons of the means were based on Tukey's HSD test, using a significance level α of 0.05. A heatmap to summarize the gene expression data was made with R (version 3.6.2) using the *heatmap.2* function of the package *gplots* (version 3.0.3) (www.R-project.org).

3. Results

3.1. Chemostats in steady state

At steady state, the light intensity I_{out} transmitted through the chemostat vessels was high in N- and P-limited chemostats, but significantly lower in light-limited chemostats (one-way ANOVA: $F_{2,8} = 43.54$, $p < 0.001$; Table 2). Dissolved inorganic nitrogen (DIN) concentrations differed between conditions, with high nitrate concentrations in P-limited and light-limited chemostats but significantly lower nitrate concentrations in N-limited chemostats (one-way ANOVA: $F_{2,8} = 6507$, $p < 0.001$; Table 2). Dissolved inorganic phosphorus (DIP) was high in light-limited and N-limited chemostats, but significantly lower in P-limited chemostats (one-way ANOVA: $F_{2,8} = 2224$, $p < 0.001$; Table 2). This confirms that the applied chemical composition of the mineral media indeed led to light-limited, N-limited and P-limited conditions.

N-limited *Microcystis* had a slightly but significantly larger cell diameter than light-limited and P-limited *Microcystis* (one-way ANOVA: $F_{2,8} = 7.338$, $p = 0.015$; Table 2). Extracellular MC concentrations (expressed per liter of culture) were significantly lower in N-limited chemostats (one-way ANOVA: $F_{2,7} = 74.3$, $p = 0.001$; Table 2). Intracellular MC contents (expressed per cell) also tended to be lower under N-limited conditions, but did not differ significantly between the three limitations (one-way ANOVA: $F_{2,7} = 2.40$, $p = 0.161$; Table 2).

3.2. Photosynthetic vitality

The nutrient and light conditions influenced the decline of the photosynthetic vitality after H_2O_2 addition. The photosynthetic vitality declined faster in batch cultures exposed to high light than in those exposed to low light (Fig. 2). At low light, *Microcystis* pre-cultured under light-limited but nutrient-replete conditions was more sensitive to H_2O_2 concentrations ≥ 2 mg L⁻¹ than N- and P-limited *Microcystis* (Fig. 2B-E). At high light, *Microcystis* was more sensitive to H_2O_2 than at low light, but the difference in H_2O_2 sensitivity between the three limiting factors was less pronounced (Fig. 2A-E). The decline in photosynthetic vitality was accompanied by strong bleaching of the cultures (see Fig. 1).

Dose-response models of the photosynthetic vitality after 4 h as function of the H_2O_2 concentration confirmed that nutrient limitation affected the sensitivity of *Microcystis* to H_2O_2 (Fig. 3). At low light, the estimated EC_{50} values ranged from 2.0 mg L⁻¹ H_2O_2 for light-limited *Microcystis* to 2.9 and 3.0 mg L⁻¹ H_2O_2 for N-limited and P-limited *Microcystis*, respectively (Fig. 3A; Table S2). At high light, the estimated EC_{50} was ~ 1.1 mg L⁻¹ H_2O_2 for all three limitations (Fig. 3B; Table S2).

3.3. Hydrogen peroxide degradation

H_2O_2 in the batch cultures disappeared within 1-1.5 h after addition of 1 mg L⁻¹ H_2O_2 (Fig. 4A), while H_2O_2 degradation took longer after addition of higher H_2O_2 concentrations (Fig. 4B). At 4, 6 and 10 mg L⁻¹ H_2O_2 , degradation of H_2O_2 was faster for N-limited *Microcystis* than for light-limited and P-limited *Microcystis* (Fig. 4C,D,E). Furthermore, in N-limited cultures, H_2O_2 degradation was faster in low light than in high light. As control, H_2O_2 degradation was tested in Milli-Q water and BG-11 mineral medium without cells (Fig. S1). No H_2O_2 degradation was observed in Milli-Q water, while the H_2O_2 concentration in BG-11 mineral medium decreased only slightly, from 10 to 9.7 mg L⁻¹ after four hours and to 8.8 mg L⁻¹ after 24 hours.

3.4. Biovolume changes

The biomass development of *Microcystis* (expressed as the 24-hour change in total biovolume) differed between the experimental conditions (Fig. 5). In the control treatment (no H_2O_2 addition) and after addition of only 1 mg L⁻¹ H_2O_2 the light-limited *Microcystis* population showed growth, whereas N-limited and P-limited *Microcystis* showed very little or no change in total biovolume. At low light, the biovolume of light-limited *Microcystis* declined after addition of ≥ 2 mg L⁻¹ H_2O_2 (Fig. 5A), but a higher H_2O_2 dosage of ≥ 4 mg L⁻¹ H_2O_2 was required to suppress the biovolume of N- and P-limited *Microcystis* (Fig. 5C,E). At high light, the biovolume declined for all three limiting factors after addition of ≥ 2 mg L⁻¹ H_2O_2 (Fig. 5B,D,F). Cell counts reported in Fig. S2 showed less pronounced differences between the treatments than the total biovolumes, indicating that the cells affected by H_2O_2 shrank in size.

3.5. Intra- and extracellular microcystins

Intracellular MC of light-limited *Microcystis* decreased two to four hours after addition of ≥ 2 mg L⁻¹ H_2O_2 (Fig. 6A,B). In contrast, intracellular MC in N- and P-limited *Microcystis* decreased only after more

Table 2
Steady state values of *Microcystis* cultures grown in light-limited, N-limited and P-limited chemostats.

Parameters	Light-limited chemostats	N-limited chemostats	P-limited chemostats
I_{out} ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	1.6 \pm 0.2 ^a	15.8 \pm 3.2 ^b	13.0 \pm 2.4 ^b
Biovolume (mm^3/L)	322 \pm 21 ^{a,b}	303 \pm 40 ^a	385 \pm 43 ^b
Cell counts (million cells/mL)	12.3 \pm 0.9 ^b	9.0 \pm 0.4 ^a	13.1 \pm 2.3 ^b
Mean cell diameter (μm)	3.61 \pm 0.01 ^a	3.87 \pm 0.13 ^b	3.68 \pm 0.1 ^a
pH	8.9 \pm 0.1 ^b	8.4 \pm 0.1 ^a	8.6 \pm 0.2 ^{a,b}
NO_3^- ($\mu\text{mol/L}$)	3567 \pm 76 ^b	2.2 \pm 1.7 ^a	9989 \pm 178 ^c
NO_2^- ($\mu\text{mol/L}$)	1.6 \pm 0.1 ^b	0.1 \pm 0.0 ^a	1.7 \pm 0.7 ^b
NH_4^+ ($\mu\text{mol/L}$)	5.5 \pm 0.4 ^c	3.6 \pm 1.1 ^b	1.6 \pm 0.9 ^a
DIP ($\mu\text{mol/L}$)	157.4 \pm 1.7 ^b	164.6 \pm 7.4 ^b	0.3 \pm 0.07 ^a
Extracellular MC-LR ($\mu\text{g/L}$)	89 \pm 12 ^b	39 \pm 8.0 ^a	131 \pm 4.0 ^c
Intracellular MC-LR (fg/cell)	48.1 \pm 7.4 ^a	36.6 \pm 8.2 ^a	42.2 \pm 4.0 ^a

The values represent the averages \pm SD of 3-4 replicate chemostats (4 for light- and P-limited chemostats and 3 for N-limited chemostats). Light, cell counts, and pH were measured on five different days within a time span of several weeks during steady state of the chemostat. Nutrients were measured once during steady state. MC concentrations were determined on three different days during steady state. Values with different superscript letters differ significantly from each other ($p < 0.05$), as tested by a one-way ANOVA followed by post-hoc comparison of the means using Tukey's HSD.

than four hours after addition of $\geq 4 \text{ mg L}^{-1} \text{ H}_2\text{O}_2$ (Fig. 6C-F). Parallel to the decrease in intracellular MC, extracellular MC increased. However, the extracellular MC concentration never exceeded 79% of the intracellular MC concentration at the beginning of the experiment.

3.6. Gene expression of selected genes

H_2O_2 addition and the imposed nutrient or light limitation both affected expression of the investigated *Microcystis* genes (Fig. 7; see Tables S3 and S4 for the underlying statistics).

Prior to H_2O_2 addition, three genes encoding for anti-oxidant enzymes, *2-cys-prx* (2-cys-peroxiredoxin), *trxA* (thioredoxin A) and *t2prx* (type II peroxiredoxin), were expressed significantly higher in N- and P-limited *Microcystis* than in light-limited *Microcystis* (Fig. 7; Tables S3 and S4). Expression of *trxA* and *t2prx* strongly increased after addition of H_2O_2 in all cultures, with higher expression levels in N- and P-limited *Microcystis* than in light-limited *Microcystis*.

Expression of the genes *flv2* and *flv4*, encoding for photoprotective flavodiiron proteins, was slightly but significantly higher prior to H_2O_2 addition for P-limited *Microcystis* (in case of *flv2*) or for both N- and P-limited *Microcystis* (in case of *flv4*) than for light-limited *Microcystis* (Fig. 7; Tables S3 and S4). Addition of H_2O_2 slightly reduced the expression of *flv2* and *flv4* in all cultures. Conversely, expression of the iron stress-induced gene *isiA* was lower prior to H_2O_2 addition in N- and P-limited *Microcystis* than in light-limited *Microcystis*. Addition of H_2O_2 increased the expression of *isiA* to similar levels as in light-limited *Microcystis* prior to H_2O_2 addition.

Expression of MC synthetase genes *mcyB* and *mcyE* prior to H_2O_2 addition was significantly higher in the N- and P-limited cultures than in light-limited *Microcystis*. Addition of H_2O_2 resulted in a reduction in expression levels of *mcyB* and *mcyE* in all cultures (Fig. 7; Tables S3 and S4).

4. Discussion

4.1. *Microcystis* is more resistant to H_2O_2 under nutrient limitation and low light intensity

This study aimed to investigate whether nutrient limitation of cyanobacteria is a factor that needs to be considered when treating cyanobacterial blooms in lakes with H_2O_2 . Since nutrient limitation is known to induce oxidative stress in cyanobacteria (Kumar Saha et al., 2003; Schwarz and Forchhammer, 2005; Latifi et al., 2009), we hypothesized that *Microcystis* grown under N or P limitation will be more sensitive to H_2O_2 than *Microcystis* grown under replete conditions. In contrast to this hypothesis, our results indicate an increased resistance to H_2O_2 of N- and P-limited *Microcystis* compared to light-limited but

nutrient-replete *Microcystis*. This difference in H_2O_2 sensitivity can most likely be explained by the observed higher expression of genes encoding for anti-oxidative stress enzymes in N- and P-limited *Microcystis* than in light-limited *Microcystis* prior to H_2O_2 treatment (Fig. 7). Consequently, H_2O_2 degradation by *Microcystis* was faster under N limitation than under light-limited but nutrient-replete conditions (Fig. 4). However, despite being similarly resistant to H_2O_2 as N-limited *Microcystis*, P-limited *Microcystis* showed a slower H_2O_2 degradation rate quite similar to that of light-limited *Microcystis*.

Our photosynthetic vitality measurements confirm previous findings that H_2O_2 suppresses cyanobacteria more effectively at high light than at low light (Piel et al., 2020). H_2O_2 directly damages the D1 protein of PSII (Wang et al., 2015) and also suppresses the repair mechanism of PSII (Nishiyama et al., 2006; Kojima et al., 2007; Blot et al., 2011), which likely explains why H_2O_2 causes greater damage to cyanobacteria at high light intensities (Nishiyama et al., 2006; Piel et al., 2020). Consequently, sensitivity differences between light-limited and nutrient-limited *Microcystis* were minor when exposed to high oxidative stress induced by H_2O_2 at high light intensity, whereas these sensitivity differences were much more pronounced at low light intensity (Figs. 2 and 3).

4.2. Anti-oxidative stress genes are higher expressed in nutrient-limited *Microcystis*

Genome sequencing has revealed that many cyanobacteria lack catalase but do contain other anti-ROS enzymes such as peroxiredoxins (Bernroither et al., 2009; Dietz et al., 2011). This also applies to sequenced genomes of *Microcystis*, including *Microcystis* NIES-843 (Kaneko et al., 2007; Bernroither et al., 2009), *Microcystis* PCC 7005 (Sandrini et al., 2014) and *Microcystis* PCC 7806 (Frangoul et al., 2008; Schuurmans et al., 2018) and the transcriptome of *Microcystis* LE-3 (Harke and Gobler, 2013). Schuurmans et al (2018) investigated the full transcriptome of *Microcystis* PCC 7806, and found that of all anti-ROS genes, the peroxiredoxin genes *2-cys-prx* and *t2prx* and thioredoxin gene *trxA* gave the strongest response to H_2O_2 addition. For this reason, we investigated the expression of these three anti-ROS genes in our study.

Our results show that gene expression of *2-cys-prx*, *trxA* and *t2prx* differed between nutrient-limited and light-limited *Microcystis*. Prior to H_2O_2 addition, all three genes were significantly higher expressed in N- and P-limited *Microcystis* than in light-limited *Microcystis*. This observation is consistent with previous studies, which have shown that preconditioning of cyanobacteria to N- or P-starvation results in increased oxidative stress and prompts an elevated expression of anti-oxidative stress genes (Kumar Saha et al., 2003; Schwarz and Forchhammer, 2005; Latifi et al., 2009). Specifically, in line with our

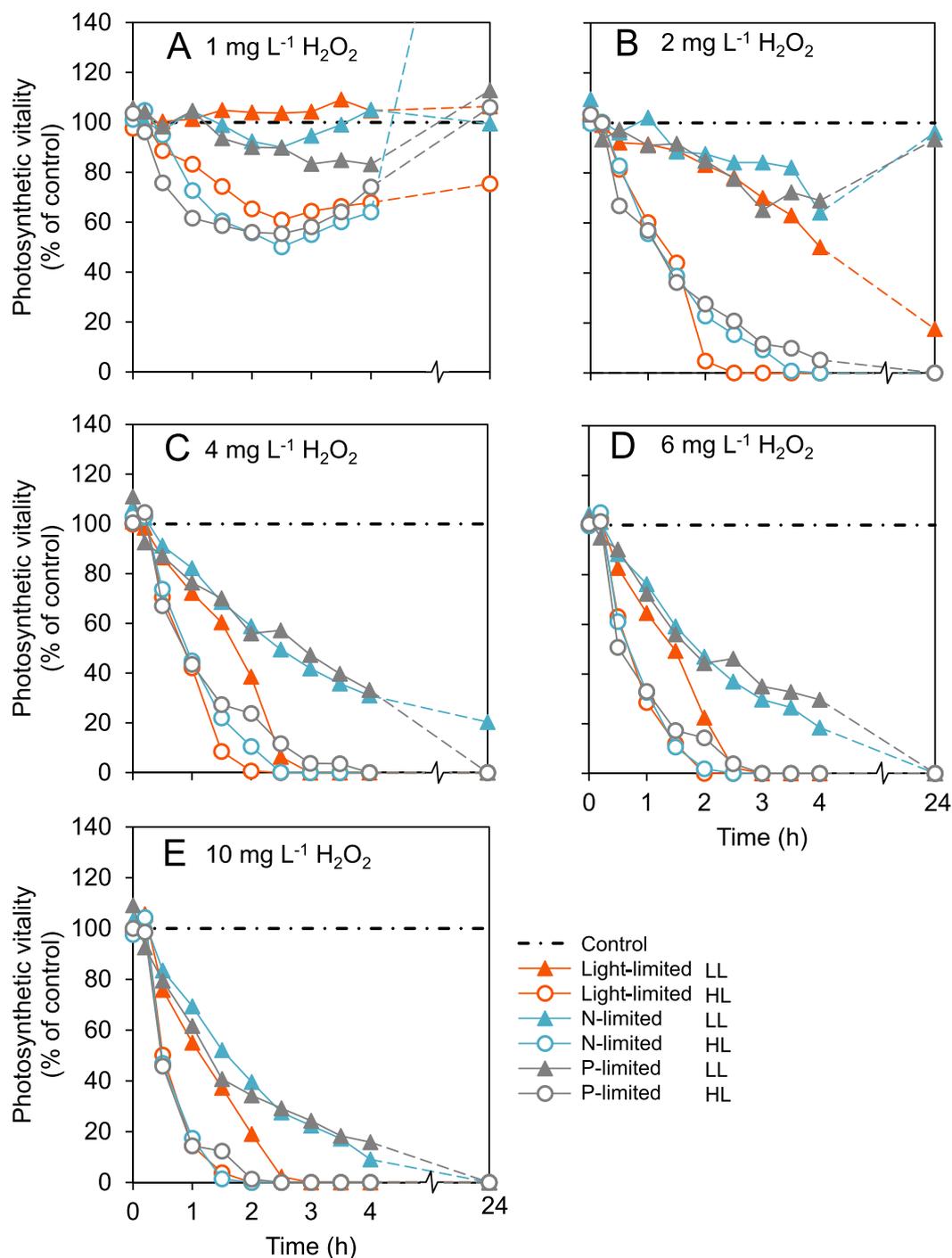


Figure 2. Photosynthetic vitality of *Microcystis* PCC 7806 under N-limited, P-limited and light-limited conditions after addition of different H_2O_2 concentrations. (A) 1 mg L⁻¹ H_2O_2 , (B) 2 mg L⁻¹ H_2O_2 , (C) 4 mg L⁻¹ H_2O_2 , (D) 6 mg L⁻¹ H_2O_2 , (E) 10 mg L⁻¹ H_2O_2 . The photosynthetic vitality is defined as the photosynthetic yield of a H_2O_2 -treated sample as percentage of the photosynthetic yield of the control (without H_2O_2). Triangles indicate batch cultures exposed to low light (LL, 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), while open circles indicate cultures exposed to high light (HL, 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Each data point represents the average of two biological replicates. The black dashed lines indicate the photosynthetic vitality of the controls. The photosynthetic vitality 24 h after addition of 1 mg L⁻¹ H_2O_2 to N-limited *Microcystis* at high light is 272 % and lies outside the boundaries of the graph.

findings, N limitation also induced an increased expression of *2-cys-prx* in *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 (Stork et al., 2005). Our results show that expression of *2-cys-prx*, *trxA* and *t2prx* in N-limited *Microcystis* and of *trxA* and *t2prx* in P-limited *Microcystis* increased even further during H_2O_2 exposure, to levels well above those in light-limited *Microcystis*. In a similar experiment,

Schuermans et al. (2018) measured higher expression levels of *2-cys-prx*, *trxA* and *t2prx* in a non-toxic mutant strain of *Microcystis* PCC 7806 than in the wildtype, and showed that the non-toxic mutant was better prepared to oxidative stress and hence more resistant to H_2O_2 than the wildtype. Along the same lines, the observed higher expression levels of anti-oxidative stress genes prior to H_2O_2 addition implies that nutrient-

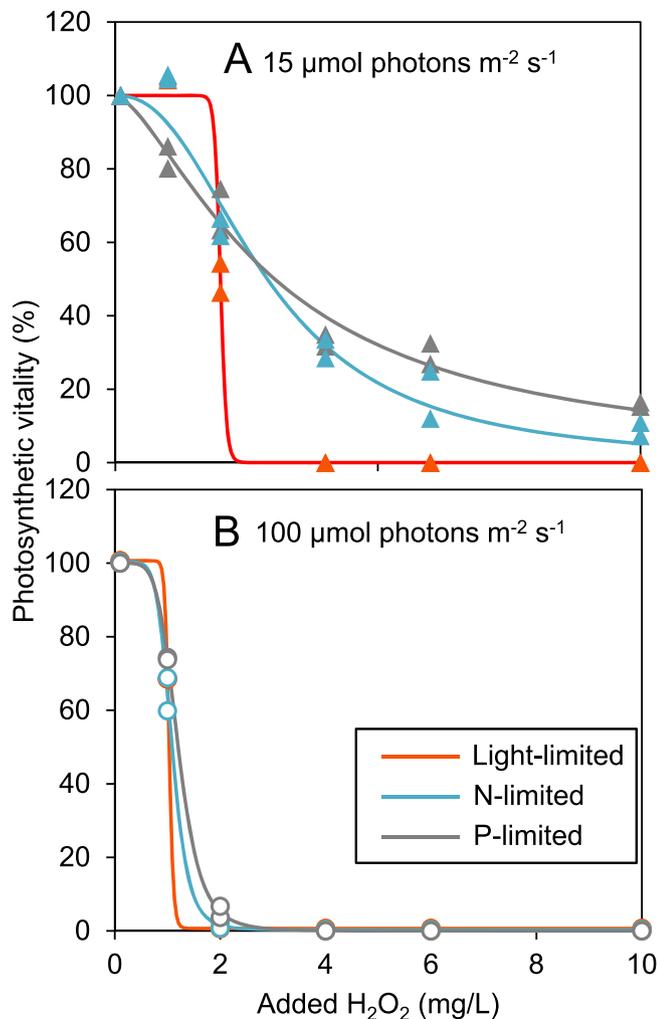


Figure 3. Dose-response models of *Microcystis* PCC 7806 under N-limited, P-limited and light-limited conditions. Dose-response models show the photosynthetic vitality after 4 h as function of the H_2O_2 concentration, for *Microcystis* cells exposed to (A) low light ($15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and (B) high light ($100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Symbols represent the data of two independent biological replicates per H_2O_2 concentration. Colored lines represent the dose-response models fitted to these data with nonlinear regression ($n = 12$ data points per regression; see Table S2 for the statistics).

limited *Microcystis* are better prepared for high oxidative stress, which is likely responsible for their higher resistance to H_2O_2 in comparison to light-limited *Microcystis*.

Upregulation of anti-oxidative stress genes in N- and P-limited *Microcystis* is more likely caused by oxidative stress due to nutrient limitation, rather than by preconditioning of these cultures to a higher light intensity. Nutrient limitation has been shown to increase internal ROS formation in cyanobacteria by affecting photosynthesis and respiration, forcing a response of anti-ROS enzymes (Kumar Saha et al., 2003; Schwarz and Forchhammer, 2005). Drábková et al. (2007b) found that preconditioning of *Microcystis* PCC 7806 to higher light intensities did not influence the effectiveness of H_2O_2 to suppress cyanobacteria. In addition, Mikula et al. (2012) and Lürling et al. (2014) preconditioned nutrient-replete *Microcystis* to light intensities of $140 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively, and found a comparable H_2O_2 sensitivity as for the light-limited *Microcystis* of our study which was supplied with only $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Expression of photoprotective genes was also investigated in our study, since they may contribute to the defense of phototrophic

organisms against oxidative stress. Flavodiiron proteins encoded by the genes *flv2* and *flv4* are known to play an important role in the photoprotection of cyanobacteria, by dissipating excess light energy at PSII (Zhang et al., 2012; Bersanini et al., 2014). While expression of *flv2* in N-limited *Microcystis* and *flv2* and *flv4* in P-limited *Microcystis* was slightly higher in comparison to light-limited *Microcystis* prior to H_2O_2 addition, both genes were downregulated after H_2O_2 addition. Furthermore, the iron stress-induced gene, *isiA*, which can also contribute to the protection of cyanobacteria from high light stress by dissipating excess energy (Yeremenko et al., 2004; Havaux et al., 2005), did not show a consistent response to H_2O_2 exposure. These results indicate that the photoprotective proteins Flv2, Flv4 and IsiA do not have an active involvement in the defense of cyanobacteria against H_2O_2 .

4.3. Microcystin dynamics

Our results show that expression of the MC synthetase genes *mcyB* and *mcyE* was strongly downregulated after H_2O_2 treatment of *Microcystis*. This appears to contradict the previously suggested hypothesis that MC protects *Microcystis* against oxidative stress when cells are exposed to H_2O_2 (Dziallas and Grossart, 2011; Zilliges et al., 2011). We note that the studies of Dziallas and Grossart (2011) and Zilliges et al. (2011) used a very low H_2O_2 dosage representative of natural H_2O_2 concentrations in lakes ($0.001\text{--}0.050 \text{ mg L}^{-1}$; Cooper and Lean, 1989; Häkkinen et al., 2004; Cory et al., 2017). Their H_2O_2 concentrations were much lower than the $2\text{--}10 \text{ mg L}^{-1}$ of H_2O_2 required to suppress photosynthesis and biomass development of bloom-forming cyanobacteria in the laboratory experiments of, e.g., Drábková et al. (2007a), Schuurmans et al. (2018), Piel et al. (2020) and this study and in the lake treatments of Matthijs et al. (2012, 2016). In fact, contrary to Zilliges et al. (2011), Schuurmans et al. (2018) showed that MC-producing strains of *Microcystis* were more sensitive to H_2O_2 than non-MC-producing strains. They hypothesized that MC interferes with the activity of redox-sensitive proteins including peroxiredoxins and thioredoxin and thereby prevents these anti-ROS enzymes from effectively protecting the cells against H_2O_2 . Similar to Schuurmans et al. (2018), in our study *mcy* genes were strongly downregulated whereas genes encoding peroxiredoxin and thioredoxin were strongly upregulated after H_2O_2 addition, supporting the view that MC does not offer protection to *Microcystis* when exposed to high levels of oxidative stress.

During H_2O_2 treatment of *Microcystis*, intracellular MC leaked out of the cells resulting in a subsequent increase of extracellular MC. Oxidative stress has been shown to damage cell membrane integrity of *Microcystis*, allowing MC and other cellular contents to exudate from the cells (Ross et al., 2006; Mikula et al., 2012; Wang et al., 2015; Piel et al., 2020). Quantitatively, however, the decrease of intracellular MC exceeded the increase of extracellular MC concentration, such that the total MC (intracellular MC + extracellular MC) declined after H_2O_2 addition. This might be attributed to MC degradation, but it is more likely that MC was binding to the thiol groups of redox-sensitive proteins under oxidative conditions (Zilliges et al., 2011) and consequently became undetectable with standard extraction techniques (Meissner et al., 2013). Schuurmans et al. (2018) found that MC bound to proteins likely get converted back to detectable “unbound” MC after several days, consistent with other observations that thiol-conjugation of MC is reversible and hence free MC can be slowly released from the protein-bound fraction (Miles et al., 2016). Extracellular MC concentrations were highest 24 hours after the treatment, with a strong tendency to be higher in samples that were treated with a lower H_2O_2 concentration. A possible explanation for this observation is that higher H_2O_2 concentrations may cause more extensive binding of MC to proteins and hamper the subsequent release of MC from the proteins.

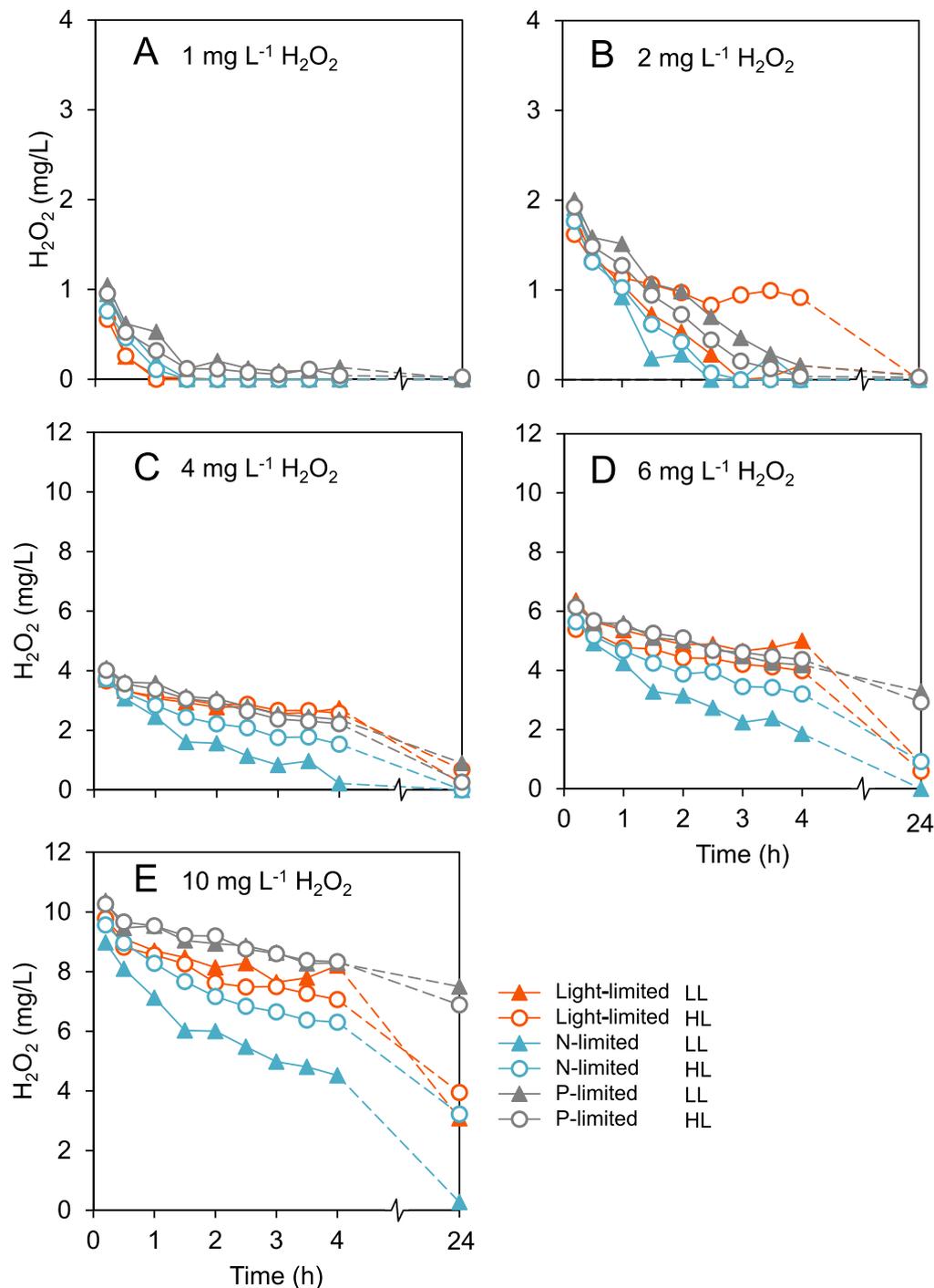


Figure 4. Hydrogen peroxide degradation of *Microcystis* PCC 7806 under N-limited, P-limited and light-limited conditions after addition of different H_2O_2 concentrations. (A) 1 $mg\ L^{-1}$ H_2O_2 , (B) 2 $mg\ L^{-1}$ H_2O_2 , (C) 4 $mg\ L^{-1}$ H_2O_2 , (D) 6 $mg\ L^{-1}$ H_2O_2 , (E) 10 $mg\ L^{-1}$ H_2O_2 . Triangles indicate batch cultures exposed to low light (LL, 15 $\mu mol\ photons\ m^{-2}\ s^{-1}$), while open circles indicate cultures exposed to high light (HL, 100 $\mu mol\ photons\ m^{-2}\ s^{-1}$). Each data point represents the average of two biological replicates. Please note that panels (A) and (B) use a different scale for the y-axis than panels (C)–(E).

4.4. Implications for lake treatments

Our results show that, at low light intensities, N- and P-limited *Microcystis* were more resistant to H_2O_2 than light-limited *Microcystis*, probably due to a higher expression of anti-oxidative stress genes prior and also after H_2O_2 addition. At higher light intensities, suppression of cyanobacteria by H_2O_2 was more effective (Piel et al., 2020), and differences in H_2O_2 sensitivity between nutrient-replete and nutrient-limited *Microcystis* were negligible. These results indicate that, during cloudy days or in very turbid lakes, H_2O_2 resistance of *Microcystis* will

be more pronounced in nutrient-limited than in nutrient-saturated waters. Furthermore, shaded *Microcystis* cells in layers within or below dense surface scums may be less affected by H_2O_2 than cells dwelling in full sunlight at or near the surface, especially if available nutrients have been depleted by the scum layer. In our experience, deliberate mixing of the upper meters of the water column during the H_2O_2 treatment of lakes not only disperses the added H_2O_2 but also disperses the scum layer, and thereby enhances H_2O_2 exposure and brings shaded cells from deeper layers below the scums into the sunlit surface. We conclude that for the treatment of nutrient-limited lakes, H_2O_2 concentrations

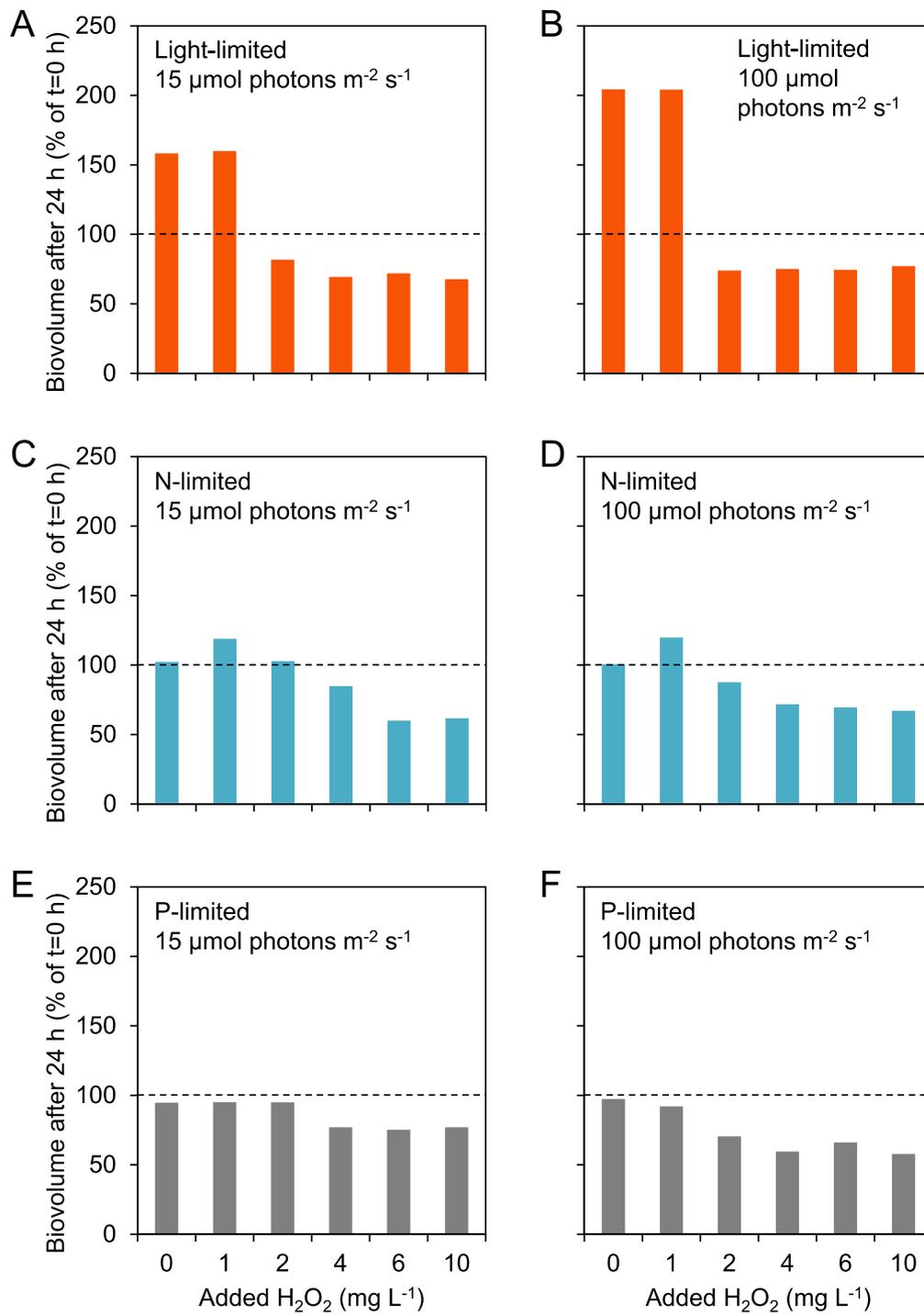


Figure 5. Relative biovolumes of *Microcystis* PCC 7806 under light-limited, N-limited and P-limited conditions after addition of different H₂O₂ concentrations. (A,B) Light-limited *Microcystis* exposed to (A) low light and (B) high light. (C,D) N-limited *Microcystis* exposed to (C) low light and (D) high light. (E,F) P-limited *Microcystis* exposed to (E) low light and (F) high light. The biovolumes after 24 h are expressed as percentage of biovolume at the start of the experiment (t = 0). Dashed black lines indicate the biovolumes of the control cultures at the start of the experiment. The bars represent the average of two biological replicates.

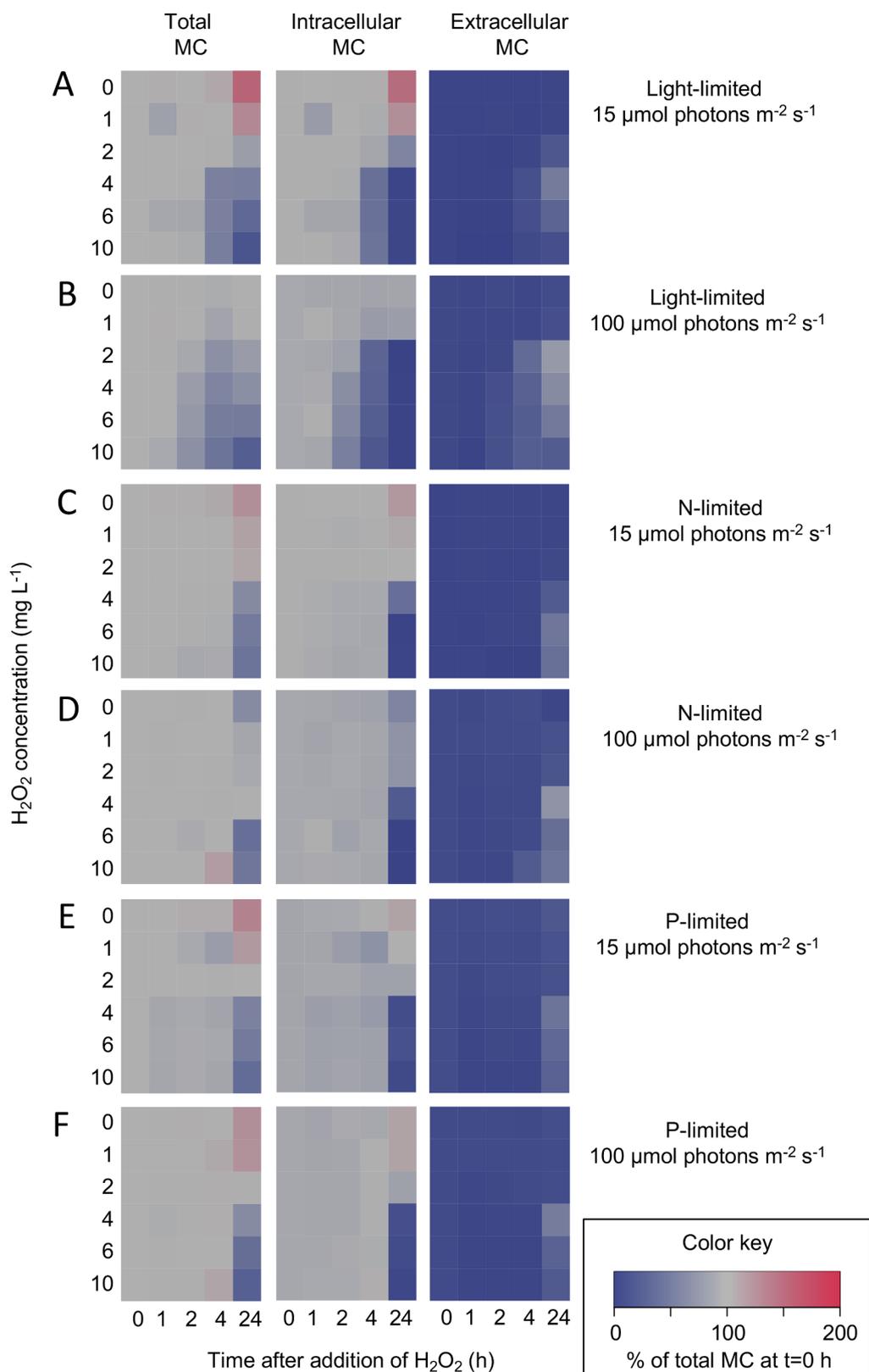


Figure 6. MC concentrations of *Microcystis* PCC 7806 under light-limited, N-limited and P-limited conditions after addition of different H₂O₂ concentrations. The heatmaps show the amounts of total MC, intracellular MC, and extracellular MC of (A,B) light-limited *Microcystis*, at (A) low light intensity and (B) high light intensity. (C,D) N-limited *Microcystis*, at (C) low light intensity and (D) high light intensity. (E,F) P-limited *Microcystis* at (E) low light and (F) high light intensity. The color key indicates the relative amount of MC as percentage of total MC at the start of the experiment (t = 0 h). Different rows in the heatmaps show different H₂O₂ concentrations used in the batch cultures, while columns in the heatmaps indicate different time points. The values represent the average of two biological replicates.

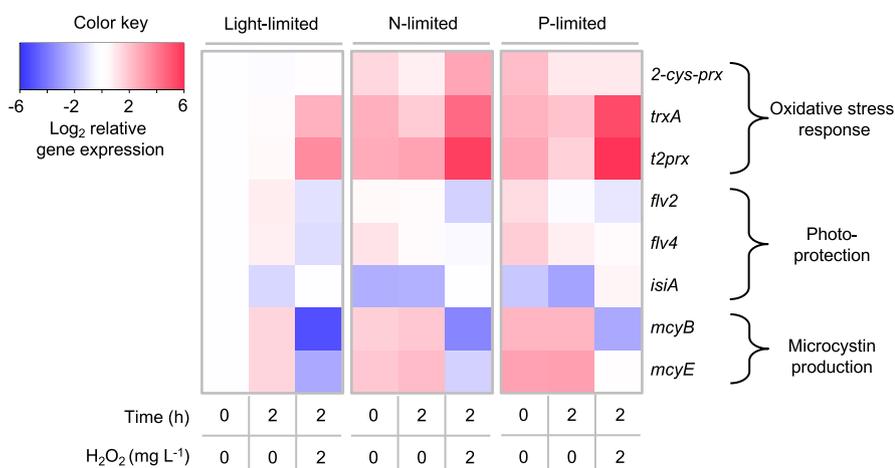


Figure 7. Changes in relative gene expression of selected genes of *Microcystis* PCC 7806 under light-limited, N-limited and P-limited conditions in response to H_2O_2 addition. The color key indicates the \log_2 relative changes in gene expression, where blue indicates strong downregulation, white indicates no change in expression, and red indicates strong upregulation. All gene expression values are relative to the start of the experiment, just prior to H_2O_2 addition ($t=0$ h), of light-limited *Microcystis*. The investigated genes are shown on the right. The three columns for each limiting factor in the heatmap show gene expression prior to H_2O_2 addition ($t=0$ h) and at 2 h after addition of 0 mg L^{-1} (control) and 2 mg L^{-1} H_2O_2 , respectively. Four biological replicates were used ($n=4$). The actual \log_2 relative gene expression values and underlying statistics are given in Tables S3 and S4.

may need to be adjusted during cloudy weather and in turbid waters. H_2O_2 treatments of cyanobacterial blooms are thus expected to be most effective in hypertrophic waters during sunny days, which coincides with the growth conditions that are usually considered to be ideal for cyanobacterial bloom development.

Author contributions

GS, TP, PMV and JH designed the experiments. GS, TP, TX, EW and HQ performed the lab experiments, assisted by PCS. GS and TP analyzed the data. GS, TP, PMV and JH wrote the manuscript. All authors have taken part in the manuscript revisions and agreed with the scientific content.

Funding

This research was funded by the Dutch Research Council (NWO), grant number NWO/TTW 14005

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are most grateful to our late colleague Dr. Hans C.P. Matthijs for initiating this research. We thank Arian van Huis for assistance with the lab experiments, and the reviewers for their helpful and constructive comments on the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.hal.2020.101916](https://doi.org/10.1016/j.hal.2020.101916).

References

- Allahverdiyeva, Y., Isojärvi, J., Zhang, P., Aro, E.-M., 2015. Cyanobacterial oxygenic photosynthesis is protected by flavodiiron proteins. *Life* 5, 716–743.
- Barroin, G., Feuillade, M., 1986. Hydrogen peroxide as a potential algicide for *Oscillatoria rubescens* D.C. *Water Res* 20, 619–623.
- Bernroither, M., Zamocky, M., Furtmüller, P.G., Peschek, G.A., Obinger, C., 2009. Occurrence, phylogeny, structure, and function of catalases and peroxidases in

- cyanobacteria. *J. Exp. Bot.* 60, 423–440.
- Bersanini, L., Battchikova, N., Jokel, M., Rehman, A., Vass, I., Allahverdiyeva, Y., Aro, E.-M., 2014. Flavodiiron protein Flv2/Flv4-related photoprotective mechanism dissipates excitation pressure of PSII in cooperation with phycobilisomes in cyanobacteria. *Plant Physiol* 164, 805–818.
- Blot, N., Mella-Flores, D., Six, C., Le Corguillé, G., Boutte, C., Peyrat, A., Monnier, A., Ratin, M., Gourvil, P., Campbell, D.A., Garczarek, L., 2011. Light history influences the response of the marine cyanobacterium *Synechococcus* sp. WH7803 to oxidative stress. *Plant Physiol* 156, 1934–1954.
- Bullerjahn, G.S., McKay, R.M., Davis, T.W., Baker, D.B., Boyer, G.L., D'Anglada, L.V., Doucette, G.J., Ho, J.C., Irwin, E.G., Kling, C.L., Kudela, R.M., Kurmayer, R., Michalak, A.M., Ortiz, J.D., Otten, T.G., Paerl, H.W., Qin, B., Sohngen, B.L., Stumpf, R.P., Visser, P.M., Wilhelm, S.W., 2016. Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. *A Lake Erie case study. Harmful Algae* 54, 223–238.
- Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABS". *Human Ecol. Risk Assess.* 7, 1393–1407.
- Codd, G.A., Morrison, L.F., Metcalf, J.S., 2005. Cyanobacterial toxins: risk management for health protection. *Toxicol. Appl. Pharm.* 203, 264–272.
- Cooper, W.J., Lean, D.R.S., 1989. Hydrogen peroxide concentration in a northern lake: photochemical formation and diel variability. *Environ. Sci. Technol.* 23, 1425–1428.
- Cory, R.M., Davis, T.W., Dick, G.J., Johengen, T., Denef, V.J., Berry, M.A., Page, S.E., Watson, S.B., Yuhas, K., Kling, G.W., 2017. Corrigendum: Seasonal dynamics in dissolved organic matter, hydrogen peroxide, and cyanobacterial blooms in Lake Erie. *Front. Mar. Sci.* 4, 377.
- Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J., 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae* 8, 715–725.
- Diao, M., Sinnige, R., Kalbitz, K., Huisman, J., Muyzer, G., 2017. Succession of bacterial communities in a seasonally stratified lake with an anoxic and sulfidic hypolimnion. *Front. Microbiol.* 8, 2511.
- Dietz, K.J., 2011. Peroxiredoxins in plants and cyanobacteria. *Antioxid. Redox Sign.* 15, 1129–1159.
- Douglas, G.B., Hamilton, D.P., Robb, M.S., Pan, G., Spears, B.M., Lüring, M., 2016. Guiding principles for the development and application of solid-phase phosphorus adsorbents for freshwater ecosystems. *Aquat. Ecol.* 50, 385–405.
- Drábková, M., Admiraal, W., Maršálek, B., 2007a. Combined exposure to hydrogen peroxide and light selective effects on cyanobacteria, green algae, and diatoms. *Environ. Sci. Technol.* 41, 309–314.
- Drábková, M., Matthijs, H.C.P., Admiraal, W., Maršálek, B., 2007b. Selective effects of H_2O_2 on cyanobacterial photosynthesis. *Photosynthetica* 45, 363–369.
- Dziallas, C., Grossart, H.P., 2011. Increasing oxygen radicals and water temperature select for toxic *Microcystis* sp. *PLoS ONE* 6, e25569.
- Fastner, J., Abella, S., Litt, A., Morabito, G., Vörös, L., Pálffy, K., Straile, D., Kümmerlin, R., Matthews, D., Phillips, M.G., Chorus, I., 2016. Combating cyanobacterial proliferation by avoiding or treating inflows with high P load—experiences from eight case studies. *Aquat. Ecol.* 50, 367–383.
- Franguel, L., Quillardet, P., Castets, A.M., Humbert, J.F., Matthijs, H.C.P., Cortez, D., Tolonen, A., Zhang, C.C., Gribaldo, S., Kehr, J.C., Zilliges, Y., Ziemert, N., Becker, S., Talla, E., Latifi, A., Billault, A., Lepelletier, A., Dittmann, E., Bouchier, C., Tandeau de Marsac, N., 2008. Highly plastic genome of *Microcystis aeruginosa* PCC 7806, a ubiquitous toxic freshwater cyanobacterium. *BMC Genomics* 9, 274.
- Guo, L., 2007. Doing battle with the green monster of Taihu Lake. *Science* 317, 1166.
- Häkkinen, P.J., Anesio, A.M., Granéli, W., 2004. Hydrogen peroxide distribution, production, and decay in boreal lakes. *Can. J. Fish. Aquat. Sci.* 61, 1520–1527.
- Hamilton, D.P., Salmaso, N., Paerl, H.W., 2016. Mitigating harmful cyanobacterial blooms: strategies for control of nitrogen and phosphorus loads. *Aquat. Ecol.* 50, 351–366.
- 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., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl,

- H.W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae* 54, 4–20.
- Havaux, M., Guedeny, G., Hagemann, M., Yermenko, N., Matthijs, H.C.P., Jeanjean, R., 2005. The chlorophyll-binding protein IsiA is inducible by high light and protects the cyanobacterium *Synechocystis* PCC6803 from photooxidative stress. *FEBS Lett.* 579, 2289–2293.
- Helman, Y., Tchernov, D., Reinhold, L., Shibata, M., Ogawa, T., Schwarz, R., Ohad, I., Kaplan, A., 2003. Genes encoding A-type flavoproteins are essential for photo-reduction of O₂ in cyanobacteria. *Curr. Biol.* 13, 230–235.
- Ho, J.C., Michalak, A.M., Pahlevan, N., 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. *Nature* 574, 667–670.
- Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. *Nat. Rev. Microbiol.* 16, 471–483.
- Huisman, J., Jonker, R.R., Zonneveld, C., Weissing, F.J., 1999. Competition for light between phytoplankton species: experimental tests of mechanistic theory. *Ecology* 80, 211–222.
- Huisman, J., Matthijs, H.C.P., Visser, P.M., Balke, H., Sigon, C.A.M., Passarge, J., Weissing, F.J., Mur, L.R., 2002. Principles of the light-limited chemostat: theory and ecological applications. *Antonie van Leeuwenhoek* 81, 117–133.
- Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M.H., Sommeijer, B., 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology* 85, 2960–2970.
- Ibelings, B.W., Bormans, M., Fastner, J., Visser, P.M., 2016. CYANOCOST special issue on cyanobacterial blooms: synopsis—a critical review of the management options for their prevention, control and mitigation. *Aquat. Ecol.* 50, 595–605.
- Jančula, D., Maršálek, B., 2011. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. *Chemosphere* 85, 1415–1422.
- Kaneko, T., Nakajima, N., Okamoto, S., Suzuki, I., Tanabe, Y., Tamaoki, M., Nakamura, Y., Kasai, F., Watanabe, A., Kawashima, K., Kishida, Y., Ono, A., Shimizu, Y., Takahashi, C., Minami, C., Fujishiro, T., Kohara, M., Katoh, M., Nakazaki, N., Nakayama, S., Yamada, M., Tabata, S., Watanabe, M.M., 2007. Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. *DNA Res.* 14, 247–256.
- Kojima, K., Oshita, M., Nanjo, Y., Kasai, K., Tozawa, Y., Hayashi, H., Nishiyama, Y., 2007. Oxidation of elongation factor G inhibits the synthesis of the D1 protein of photosystem II. *Mol. Microbiol.* 65, 936–947.
- Kumar Saha, S., Uma, L., Subramanian, G., 2003. Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. *FEMS Microbiol. Ecol.* 45, 263–272.
- Latifi, A., Ruiz, M., Zhang, C.C., 2009. Oxidative stress in cyanobacteria. *FEMS Microbiol. Rev.* 33, 258–278.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods* 25, 402–408.
- Lu, C.-P., Lin, C.-T., Chang, C.-M., Wu, S.-H., Lo, L.-C., 2011. Nitrophenylboronic acids as highly chemoselective probes to detect hydrogen peroxide in foods and agricultural products. *J. Agr. Food. Chem.* 59, 11403–11406.
- Lüring, M., Meng, D., Faassen, E.J., 2014. Effects of hydrogen peroxide and ultrasound on biomass reduction and toxin release in the cyanobacterium, *Microcystis aeruginosa*. *Toxins* 6, 3260–3280.
- Matthijs, H.C.P., Jančula, D., Visser, P.M., Maršálek, B., 2016. Existing and emerging cyanocidal compounds: new perspectives for cyanobacterial bloom mitigation. *Aquat. Ecol.* 50, 443–460.
- Matthijs, H.C.P., Visser, P.M., Reeze, B., Meeuse, J., Slot, P.C., Wijn, G., Talens, R., Huisman, J., 2012. Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Res* 46, 1460–1472.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence: a practical guide. *J. Exp. Bot.* 51, 659–668.
- McDougald, D., Gong, L., Srinivasan, S., Hild, E., Thompson, L., Takayama, K., Rice, S.A., Kjelleberg, S., 2002. Defences against oxidative stress during starvation in bacteria. *Antonie van Leeuwenhoek* 81, 3–13.
- Meissner, S., Fastner, J., Dittmann, E., 2013. Microcystin production revisited: conjugate formation makes a major contribution. *Environ. Microbiol.* 15, 1810–1820.
- Meriluoto, J., Spoo, L., Codd, G.A., 2017. Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. John Wiley & Sons, Inc, Chichester, UK.
- Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., Chaffin, J.D., Cho, K., Confesor, R., Daloğlu, I., DePinto, J.V., Evans, M.A., Fahnenstiel, G.L., He, L., Ho, J.C., Jenkins, L., Johengen, T.H., Kuo, K.C., LaPorte, E., Liu, X., McWilliams, M.R., Moore, M.R., Posselt, D.J., Richard, R.P., Scavia, D., Steiner, A.L., Verhamme, E., Wright, D.M., Zagorski, M.A., 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proc. Natl. Acad. Sci. USA* 110, 6448–6452.
- Mikula, P., Zezulka, S., Jančula, D., Marsalek, B., 2012. Metabolic activity and membrane integrity changes in *Microcystis aeruginosa*—new findings on hydrogen peroxide toxicity in cyanobacteria. *Eur. J. Phycol.* 47, 195–206.
- Miles, C.O., Sandvik, M., Nonga, H.E., Ballot, A., Wilkins, A.L., Rise, F., Jaabaek, J.A.H., Loader, J.I., 2016. Conjugation of microcystins with thiols is reversible: Base-catalyzed deconjugation for chemical analysis. *Chem. Res. Toxicol.* 29, 860–870.
- Nishiyama, Y., Allakhverdiev, S.I., Murata, N., 2006. A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *BBA-Bioenergetics* 1757, 742–749.
- 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.
- Paerl, H.W., 2009. Controlling eutrophication along the freshwater–marine continuum: dual nutrient (N and P) reductions are essential. *Estuar. Coasts* 32, 593–601.
- Paerl, H.W., Huisman, J., 2008. Blooms like it hot. *Science* 320, 57–58.
- Piel, T., Sandrini, G., White, E., Xu, T., Schuurmans, J.M., Huisman, J., Visser, P.M., 2020. Suppressing cyanobacteria with hydrogen peroxide is more effective at high light intensities. *Toxins* 12, 18.
- Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H.W., Carmichael, W.W., 2010. A drinking water crisis in Lake Taihu, China: linkage to climatic variability and lake management. *Environ. Manage.* 45, 105–112.
- Qin, H., Zhang, Z., Liu, H., Li, D., Wen, X., Zhang, Y., Wang, Y., Yan, S., 2016. Fenced cultivation of water hyacinth for cyanobacterial bloom control. *Env. Sci. Poll. Res.* 23, 17742–17752.
- Ramakers, C., Ruijter, J.M., Lekanne Depeze, R.H., Moorman, A.F.M., 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci. Lett.* 339, 62–66.
- Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111, 1–61.
- Ross, C., Santiago-Vázquez, L., Paul, V., 2006. Toxin release in response to oxidative stress and programmed cell death in the cyanobacterium *Microcystis aeruginosa*. *Aquat. Toxicol.* 78, 66–73.
- Ruijter, J.M., Ramakers, C., Hoogaars, W.M.H., Karlen, Y., Bakker, O., van den Hoff, M.J.B., Moorman, A.F.M., 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res* 37, e45.
- Sandrini, G., Cunsolo, S., Schuurmans, J.M., Matthijs, H.C.P., Huisman, J., 2015. Changes in gene expression, cell physiology and toxicity of the harmful cyanobacterium *Microcystis aeruginosa* at elevated CO₂. *Front. Microbiol.* 6, 401.
- Sandrini, G., Ji, X., Verspagen, J.M.H., Tann, R.P., Slot, P.C., Luimstra, V.M., Schuurmans, J.M., Matthijs, H.C.P., Huisman, J., 2016a. Rapid adaptation of harmful cyanobacteria to rising CO₂. *Proc. Natl. Acad. Sci. USA* 113, 9315–9320.
- Sandrini, G., Matthijs, H.C.P., Verspagen, J.M.H., Muyzer, G., Huisman, J., 2014. Genetic diversity of inorganic carbon uptake systems causes variation in CO₂ response of the cyanobacterium *Microcystis*. *ISME J* 8, 589–600.
- Sandrini, G., Tann, R.P., Schuurmans, J.M., van Beusekom, S.A.M., Matthijs, H.C.P., Huisman, J., 2016b. Diel variation in gene expression of the CO₂-concentrating mechanism during a harmful cyanobacterial bloom. *Front. Microbiol.* 7, 551.
- Schuurmans, J.M., Brinkmann, B.W., Makower, A.K., Dittmann, E., Huisman, J., Matthijs, H.C.P., 2018. Microcystin interferes with defense against high oxidative stress in harmful cyanobacteria. *Harmful Algae* 78, 47–55.
- Schwarz, R., Forchhammer, K., 2005. Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. *Microbiol* 151, 2503–2514.
- Stork, T., Michel, K.P., Pistorius, E.K., Dietz, K.J., 2005. Bioinformatic analysis of the genomes of the cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 for the presence of peroxidoreductases and their transcript regulation under stress. *J. Exp. Bot.* 422, 3193–3206.
- Tonk, L., Welker, M., Huisman, J., Visser, P.M., 2009. Production of cyanopeptolins, anabaenopeptins, and microcystins by the harmful cyanobacteria *Anabaena* 90 and *Microcystis* PCC 7806. *Harmful Algae* 8, 219–224.
- Triest, L., Stiers, I., Van Onsem, S., 2016. Biomanipliation as a nature-based solution to reduce cyanobacterial blooms. *Aquat. Ecol.* 50, 461–483.
- Van de Waal, D.B., Verspagen, J.M.H., Finke, J.F., Vournazou, V., Immers, A.K., Kardinaal, W.E.A., Tonk, L., Becker, S., Van Donk, E., Visser, P.M., Huisman, J., 2011. Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO₂. *ISME J* 5, 1438–1450.
- Verspagen, J.M.H., Passarge, J., Jöhnk, K.D., Visser, P.M., Peperzak, L., Boers, P., Laanbroek, H.J., Huisman, J., 2006. Water management strategies against toxic *Microcystis* blooms in the Dutch delta. *Ecol. Appl* 16, 313–327.
- Verspagen, J.M.H., Van de Waal, D.B., Finke, J.F., Visser, P.M., Van Donk, E., Huisman, J., 2014. Rising CO₂ levels will intensify phytoplankton blooms in eutrophic and hypertrophic lakes. *PLoS One* 9, e104325.
- Visser, P.M., Ibelings, B.W., Bormans, M., Huisman, J., 2016a. Artificial mixing to control cyanobacterial blooms: a review. *Aquat. Ecol.* 50, 423–441.
- Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W., Paerl, H.W., Huisman, J., 2016b. How rising CO₂ and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* 54, 145–159.
- Wang, B., Wang, X., Hu, Y., Chang, M., Bi, Y., Hu, Z., 2015. The combined effects of UV-C radiation and H₂O₂ on *Microcystis aeruginosa*, a bloom-forming cyanobacterium. *Chemosphere* 141, 34–43.
- Weenink, E.F.J., Luimstra, V.M., Schuurmans, J.M., Van Herk, M.J., Visser, P.M., Matthijs, H.C.P., 2015. Combatting cyanobacteria with hydrogen peroxide: a laboratory study on the consequences for phytoplankton community and diversity. *Front. Microbiol.* 6, 714.
- Xu, H., Paerl, H.W., Qin, B., Zhu, G., Gao, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnol. Oceanogr.* 55, 420–432.
- Yermenko, N., Kouřil, R., Ihalainen, J.A., D’Haene, S., van Oosterwijk, N., Andrizhivskaya, E.G., Keegstra, W., Dekker, H.L., Hagemann, M., Boekema, E.J., Matthijs, H.C.P., Dekker, J.P., 2004. Supramolecular organization and dual function of the IsiA chlorophyll-binding protein in cyanobacteria. *Biochemistry* 43, 10308–10313.
- Zhang, P., Eisenhut, M., Brandt, A.-M., Carmel, D., Silén, H.M., Vass, I., Allahverdiyeva, Y., Salminen, T.A., Aro, E.M., 2012. Operon *ftv4-ftv2* provides cyanobacterial photosystem II with flexibility of electron transfer. *Plant Cell* 24, 1952–1971.
- Zilliges, Y., Kehr, J.-C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, M., Kaplan, A., Börner, T., Dittmann, E., 2011. The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. *PLoS One* 6, e17615.