Modeling total microcystin production by *Microcystis aeruginosa* using multiple regression

Marianna Correia Aragão, Kelly Cristina dos Reis, Allan Clemente Souza, Maria Aparecida Melo Rocha and Jose Capelo Neto

ABSTRACT

Microcystis sp. is one of the most studied genus of cyanobacteria worldwide. Once it has been identified in raw water, frequent analyses of cell density and toxic metabolites (microcystins) are recommended at the water treatment plants. However, both analytical procedures are highly time-consuming and labor-intensive, allowing the potentially contaminated finished water to reach customers. The identification of easily measurable parameters related to toxin production, preferably by on-line equipment, would mitigate this issue and help water companies to improve water safety and decrease operating costs. However, these devices still have precision limitations and need efficient mathematical models for converting light signals into cyanobacteria densities or cyanotoxin concentrations. In this scenario, this research aimed to develop a mathematical correlation between microcystin production and cell age and density, chlorophyll-a, pheophytin and phycocyanin in a *Microcystis aeruginosa* culture using a multiple linear regression model. Despite the significant correlation ($p < 0.05$) found between all the variables and total microcystin, a simplified and precise model (Adjusted $R^2 = 0.824$) involving only phycocyanin and pheophytin concentrations was developed in order to provide an initial attempt to easily and cheaply predict microcystin concentration in raw water.

Key words | cyanotoxin, pheophytin, phycocyanin, predicting model

Marianna Correia Aragão
Kelly Cristina dos Reis
Allan Clemente Souza
Maria Aparecida Melo Rocha
Jose Capelo Neto (corresponding author)
Universidade Federal do Ceará,
Departamento de Engenharia Hidráulica e Ambiental,
Bloco 713, Campus do Pici,
Fortaleza, Ceará,
Brazil, 60440-970
E-mail: *zecapelo@hotmail.com*

INTRODUCTION

Cyanobacteria that produce toxic or taste and odor metabolites have become a widespread and critical problem in aquatic environments around the world (Saraf *et al.* 2018; Zhang *et al.* 2018). Intensive agricultural practices, the release of untreated domestic and industrial wastewater into water bodies, and climate change have boosted the growth rates of cyanobacteria biomass (Jacinavicius 2015). The annual global estimates of the socioeconomic impacts of cyanobacteria metabolites can reach billions of US dollars when considering water monitoring, treatment and analysis as well as adverse impacts on recreational use and fishing (Wiltsie *et al.* 2018).

One of the most studied species of cyanobacteria in lakes is *Microcystis aeruginosa* – MA (Chaffin *et al.* 2018). It can produce a group of toxic monocyclic heptapeptides called microcystins – MCs (Minasyan *et al.* 2018), the most common toxin found in natural waters (Walls *et al.* 2018). In addition, these cyanobacteria are problematic because it rapidly increases biomass on the water surface, limiting sunlight penetration, reducing dissolved oxygen concentration and making water treatment more laborious and costly (Ger *et al.* 2010). MCs can accumulate in the trophic network and cause harmful effects to mammalian health, especially to the liver, inhibiting essential enzymatic

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functions of the hepatic tissue (Yoshizawa *et al.* 1990; Massey *et al.* 2018; Mohamed *et al.* 2018). As a consequence, the World Health Organization proposed a maximum concentration of 1 μg L^{-1} in finished water (WHO 2003, 2011), limit adopted by Brazil and Uruguay. Countries such as Australia and Canada have adopted MCs limit concentration of 1.3 and 1.5 μg L^{-1}, respectively (Canada 2002; NHMRC & NRMMC 2011).

The release of MCs occurs preferentially due to cellular senescence, when membrane lysis occurs naturally, rather than continuous excretion (Zi *et al.* 2018). Lysis may also occur during the application of chemicals for the treatment or control of cyanobacteria, especially algaecides (Chorus & Bartran 1999). After cyanobacteria lysis, the intracellular contents are dissolved into water, making them available to organisms (Schmidt *et al.* 2013) and accumulating in the aquatic systems (Pereira *et al.* 2018). Dissolved MCs are more difficult and costly to remove in conventional water treatment plants (Van Apeldoorn *et al.* 2007) since extra treatment steps must be incorporated into the treatment train (Hamilton *et al.* 2013). In addition, to minimize risks, frequent analysis of MCs and cyanobacteria cell density is required, further increasing the costs and time consumed. Frequently, by the time analysis confirms the presence of toxins, the finished water has already been delivered to consumers.

In order to overcome the high costs and time required for cell count and cyanotoxin analysis, some attempts to develop predictive methods were carried out to evaluate how environmental variables are connected to the growth of toxic cyanobacteria and toxin production (Bortoli *et al.* 2014). Harris & Graham (2017) observed that pigments, iron and dissolved oxygen presented a high correlation with MCs concentration. Marion *et al.* (2012) developed a multivariate logistic regression model using real-time measurements of phycocyanin *in vivo* and Secchi depth to evaluate the risks of MCs concentration exceeding 4 μg L^{-1} in waters of a recreation pond. The final model reached high statistical significance as well as a good calibration, proving to be effective in identifying the risks involved with bathing. McQuaid *et al.* (2011) used *in vivo* analyses of phycocyanin in an attempt to evaluate the abundance of cyanobacteria and the associated MCs concentration in water sources. The results of this study demonstrated that there is a potential for the prediction of cyanobacteria biovolume and the concentration of MCs using phycocyanin probes.

Phycocyanin is a water-soluble and strongly fluorescent compound, making it viable to detect it with portable instruments such as probes (McQuaid *et al.* 2011). Probes based on phycocyanin fluorescence are applied for the rapid assessment of cyanobacteria in drinking water sources (Bastien *et al.* 2011; McQuaid *et al.* 2011), offering an alternative to monitoring cyanobacteria and hence cyanotoxins. However, most probes still have limitations when transforming pigment concentration signals into other cyanobacterial parameters, such as biomass and cell densities, and only perform accurately within very limited concentration ranges (Glibert *et al.* 2018). In this sense, the development of an efficient model that precisely correlates pigment concentration and cyanotoxins would be beneficial to the water industry.

Although it has been demonstrated that the concentration of MCs is related to the number and the growth phase of cells (McQuaid *et al.* 2011), studies comparing the productivity of metabolites at different stages of growth and with different MCs variants are still limited. Crettaz Minaglia *et al.* (2017), for example, mathematically modeled *M. aeruginosa* growth and microcystin-LR production in BG11 media at different temperatures. The authors established that there is a relationship between some environmental variables and one variant of MC. However, a mathematical model that estimates the concentration of total microcystin (McT) using easily measurable parameters, such as pigments, has not yet been found in the literature.

In this context, the main objective of this work was to evaluate the production of intra- and extracellular MCs throughout the life cycle of an MA culture under controlled conditions and to correlate McT concentration and cell age and density, concentrations of chlorophyll-a, phycocyanin and pheophytin in order to develop a mathematical model to estimate the concentration of McT using few parameters with relatively easy and rapid analysis.

MATERIALS AND METHODS

The suspension using an MA culture and an ASM1 medium was prepared in one batch and, after that, it was subdivided

into five individual flasks of 1 L each. Each flask was completely used for that week's analyses, so no changes in the initial volume occurred. The flasks were submitted to the same conditions. The *M. aeruginosa* strain (CIAR 03) was cultured in 1 L flasks in an ASM-1 medium (Gorham *et al.* 1964) using 12 : 12 h (L : D) photoperiod at 22 μ E m^{-2} s^{-1} (Digital Lux Tester YF-1065), pH 8, 25 °C (±1 °C) and under continuous aeration. In each of the subsequent five weeks (cell age – CA), one flask was used in full for analysis of the following parameters: MA cell density (Dens), chlorophyll-a (Chlo.a), phycocyanin (Phyc) and pheophytin (Pheo), called the explanatory variables, and Total Microscystin – McT, the response variable. The experiment was carried out in triplicate for 5 weeks, totaling 15 analysis. Aliquots of 1 mL were used for cell density analysis. For the determination of chlorophyll-a (Chlo.a) and pheophytin (Pheo), an aliquot of 100 mL was used; for phycocyanin (Phyc), an aliquot of 100 mL was used and for total microcystin – McT, an aliquot of 100 mL was used.

For microcystin analysis, the aliquot of 100 mL was filtered through 110 mm GF/C discs (Whatman, Maidstone, Kent, UK). The GF/C discs with the cell-bound or intracellular microcystin were freeze–thawed and placed in glass beakers containing 10 mL of methanol (100%) (J.T. Baker – Grade UV-HPLC) to extract the intracellular content for 1 h at room temperature (25 °C). The extract was removed and evaporated at 50 °C in a vacuum until dry. The residue was resuspended in 1 mL of methanol (80%) prior to analysis by high-performance liquid chromatography (HPLC). For the extracellular microcystin, the filtered samples (100 mL) were passed through C18 Cartridges (SupelClean LC-18 – SUPELCO 1 g and 3 mL) using a vacuum manifold system (Varian Analytical Instruments, San Fernando, CA, USA). The cartridges were conditioned before sample concentration using 10 mL of methanol (100%) (J.T. Baker – Grade UV-HPLC) followed by 10 mL of water. Samples were applied to the cartridges at a flow rate of 1 drop min^{-1}. After the sample was passed through, the cartridge was washed with 10 mL of 10, 20 and 30% v/v aqueous methanol in sequence. The air was drawn through the cartridge for about 10 min to minimize the amount of aqueous methanol eluted from the previous step. Then, the cartridge was eluted with 0.8 mL of methanol (80%). The analysis was performed using high-performance liquid chromatography (Agilent Technologies, model 1260 Infinity), with a reverse-phase column (Waters Symmetry C18, 5 μm, 2.1 × 150 mm) at 40 °C with a DAD detector set at wavelengths 238 and 222 nm. The mobile phases used were (i) 0.05% trifluoroacetic acid – TFA acid (Dynamic – Grade UV-HPLC) in water and (ii) 0.05% TFA in acetonitrile (J.T. Baker – Grade UV-HPLC) with a flow rate of 0.5 mL min^{-1} and a total run time of 35 min. Gradient elution was applied using the following program: linear increase from 25 to 100% B within 22 min, hold at 100% B for 2 min and subsequently decrease to 25% B within 11 min. A calibration curve for each variant (Mc-LR, Mc-LA and Mc-LY) was produced using standards provided by the Cyanosol Laboratory (UK). The equipment's limit of detection (LOD) and quantification (LQD) was 0.25 and 0.5 mg L^{-1}, respectively, but the method's limit of detection (LOD) and quantification (LQD) was based on those limits and the degree of sample concentration detailed previously. This method was adapted from Lawton *et al.* (1994).

Total microcystin concentration (McT) comprised of the sum of the Mc-LR, Mc-LA and Mc-LY concentrations (intra- and extracellular). For the cell quota calculation, the total intracellular microcystin concentration (sum of the intracellular Mc-LR, Mc-LA, and Mc-LY) was divided by the number of cells or biomass, in the same base volume.

After preserving MA samples with Lugol, cell density (Dens) at different growth stages was measured using a Sedgewick-rafter chamber and an inverted optical microscope with a magnification of 40× (Zeiss, Model Vert. A1; APHA 2012). For the determination of cell density, each unicellular organism was considered as an individual (Yoshida *et al.* 2006).

The spectrophotometric method (10200H), proposed in APHA (2012), was used for the determination of chlorophyll-a (Chlo.a) and pheophytin (Pheo). Samples were analyzed at wavelengths of 664, 665 and 750 nm, respectively. Phycocyanin (Phyc) was analyzed using the spectrophotometric method at 665 and 750 nm wavelengths proposed by Bennett & Bogorad (2011), with adaptations (Genesys 10-S, Thermo-Scientific, USA).

The hypothesis of data normality and homogeneity of variance was verified using the Shapiro–Wilk ($P_{S\text{-}W}$) test

and the Levene test. For comparisons between the analyzed values at each week after using the analysis of variance (ANOVA) test, the Tukey test was performed for samples with normal distribution, and pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni adjustment for samples without normal distribution. The Spearman correlation coefficient (R_s), as well as its intensity (weak, moderate and strong) and significance, was determined to verify the degree of association between all variables. For this study, it was considered a strong association when $|R_s| \geq 0.7$ and moderate when $0.7 > |R_s| \geq 0.5$ (Zar 1996).

The explanatory variables (CA, Dens, Chlo.a, Phyc and Pheo) that correlated significantly with the response variable (McT) were incorporated into the model using the Stepwise method. The regression model that contained easily determined explanatory variables was preferred among the models with the highest Adjusted R^2. The selected regression model was then evaluated by both normality and homoscedasticity of the residues (evaluated graphically – Figure 4) and coefficient significance. All the statistical tests were performed using a significance level of 5% ($\alpha = 5\%$) and the RStudio program (R Core Team 2018). Details of the data analyses mentioned are available in the Supplementary material.

RESULTS AND DISCUSSION

Until the fourth week of the experiment (28 days), there was a significant ($p < 0.05$) increase in cell density (Figure 1). However, no significant difference ($p > 0.05$) was observed between the fourth and fifth weeks indicating that the culture reached the stationary phase. There was no need for additional points after that since they would not improve the model precision. Bortoli *et al.* (2014) studied the growth of a MA strain isolated from the Billings Reservoir (Brazil) under different nutritional conditions and observed that the stationary phase was reached after 21 days, differing from our experiment probably because of the different experimental conditions and MA strain. According to Bittencourt-Oliveira *et al.* (2005), MA growth and microcystin production vary according to a biochemical activity regulator, which is different for each strain.

The MA strain cultivated produced Mc-LR, Mc-LA and Mc-LY which were detected in the chromatograms at 4.7, 16.1 and 19.7 min, respectively. The intracellular Mc-LR variant was predominant during the whole duration of the experiment (Figure 2). In the fourth week, intracellular Mc-LR concentration was 70 $\mu g\ L^{-1}$, while extracellular concentration was 1.25 $\mu g\ L^{-1}$, close to the concentrations

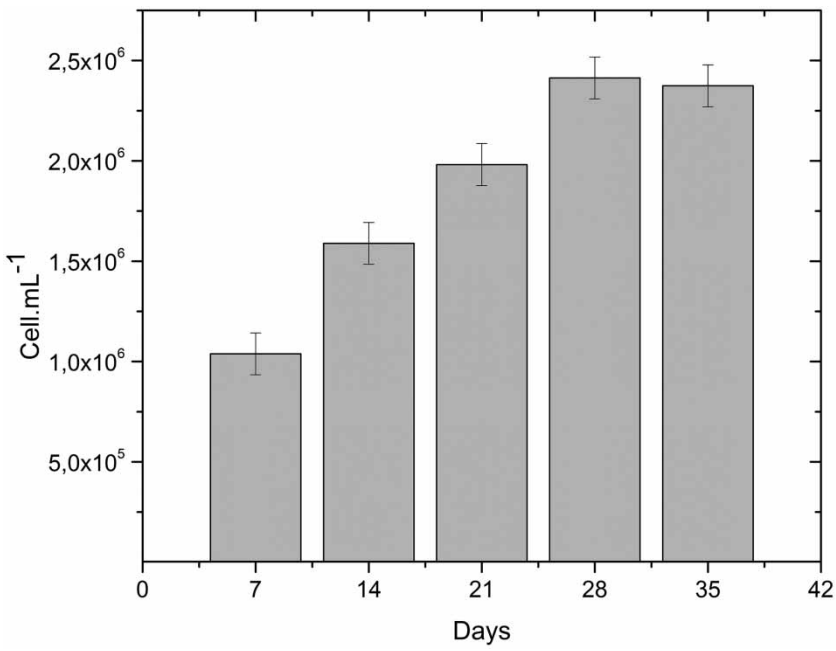

Figure 1 | MA cell density during the growth cycle under controlled conditions.

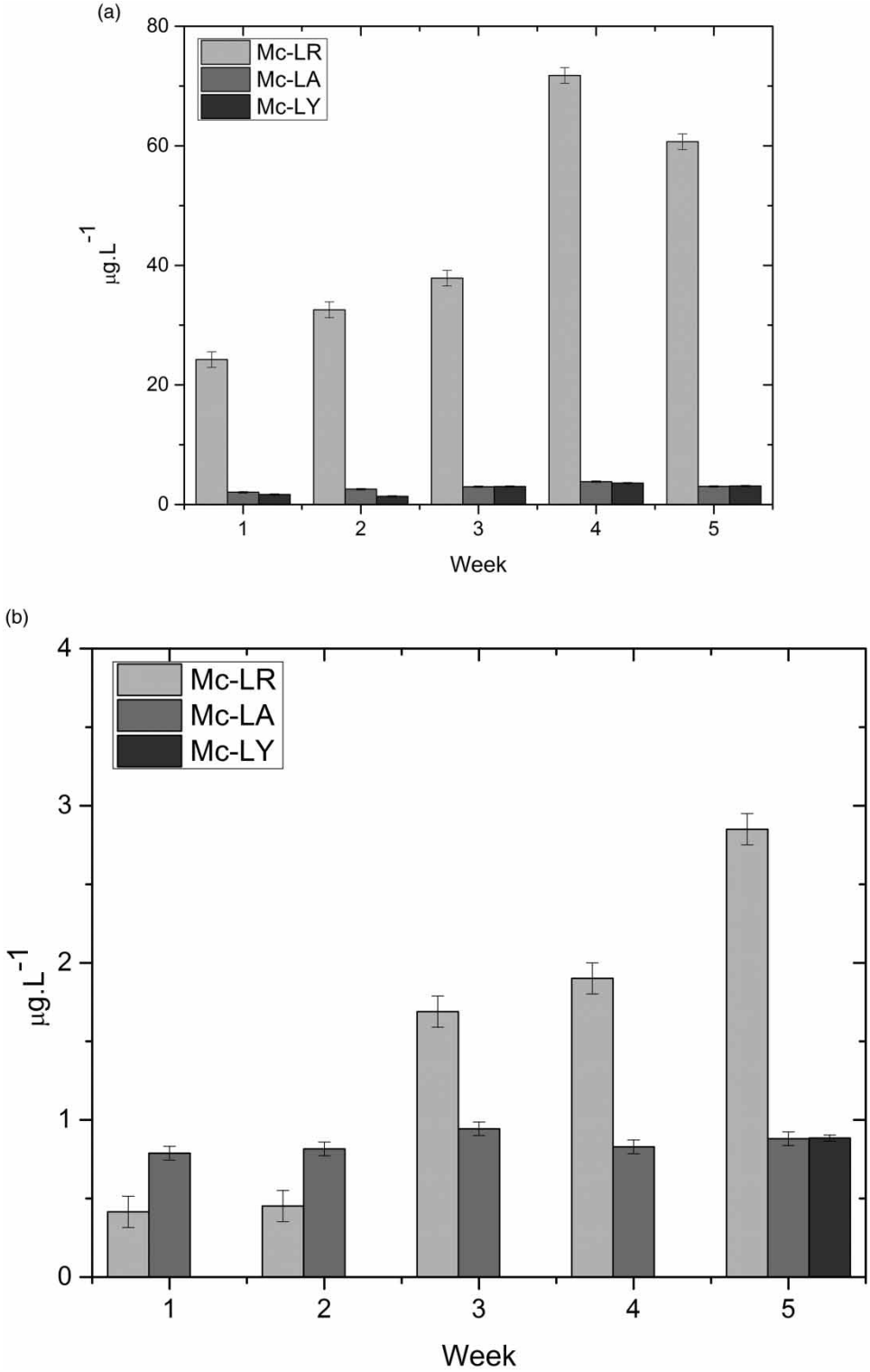

Figure 2 | Concentrations of intracellular (a) and extracellular (b) Mc-LR, Mc-LA and Mc-LY variants.

observed by Turner *et al.* (2018). On the other hand, Almuhtaram *et al.* (2018), studying a reservoir used for water supply, observed that intra- and extracellular MC concentrations were approximately 4 and 0.5 μg L^{-1}, respectively. This difference between intra- and extracellular proportions may be explained by the fact that extracellular MC modifies the intracellular MC synthesis (Schatz *et al.* 2007). The authors suggested that Microcystis cell lysis enhances MC concentration in the medium, inducing accumulation of *mcy*B (genetic subunits involved in the synthesis of MCs) and increasing the intracellular production of MC in the remaining intact cells. With the release of MCs and other oligopeptides, microgynin and micropeptin, the intact population responded by increasing the intracellular production of MC (Schatz *et al.* 2007), a feature more easily identified in axenic cultures.

In natural waters, MCs are preferentially found inside the MA cells (Westrick *et al.* 2010; Merel *et al.* 2013) and released in greater amounts during the cell senescence or death phase (Merel *et al.* 2013; Ye *et al.* 2017). This behavior may also be observed in MA cells submitted to controlled conditions (Neilan *et al.* 2013). The concentration of MC may be correlated with the cell growth rate, with the highest concentrations of MCs observed at the end of the exponential phase of growth. In the stationary phase, concentrations of MCs tend to stabilize and then to decrease in the cell senescence phase (Repka *et al.* 2004). This reduction of MC concentration can be explained by the reduced activity of enzymes complex NRPS/PKS, responsible for the biosynthesis of secondary metabolites (Nikolouli & Mossialos 2012).

According to Crettaz Minaglia *et al.* (2017), Mc-LR cell quota is an important parameter in determining the cellular physiology of MC production. The authors also observed average cell quota values (20 ± 10 fg $cell^{-1}$) during MA growth similar to the ones found in our study (20 ± 10 fg $cell^{-1}$).

The cell quotas, a relation between total intracellular MC concentration and cell density or biomass, showed no significant difference ($p > 0.05$) along all growth periods, suggesting that the cell quota was constant throughout this experiment (Table 1). Long *et al.* (2001) found that to be a common behavior during the growth phase of MA. This linear correlation between MC production and the cyanobacterial growth rate has also been reported by Orr & Jones (1998) and Bortoli *et al.* (2014). Puddick *et al.* (2019) evaluated 187 MC quotas in New Zealand and found that the highest value detected was 13 pg $cell^{-1}$ (equivalent to 13,000 fg $cell^{-1}$), much higher than the quota found in our study (32 fg $cell^{-1}$). This may be due to the fact that Microcystis strains found in natural environments have shown the capacity to produce higher cell quota than strains cultivated under controlled conditions (Wood *et al.* 2012).

Table 1 | Cell quota for intracellular microcystin throughout the experiment weeks

Time (day)	Cell quota (fg $cell^{-1}$) ± SD
7	$26.62 \pm 1.55 \times 10^{-03}$
14	$23.81 \pm 1.56 \times 10^{-03}$
21	$23.56 \pm 1.25 \times 10^{-03}$
28	$33.94 \pm 1.33 \times 10^{-03}$
35	$30.77 \pm 1.86 \times 10^{-03}$

SD, standard deviation.

Therefore, it is feasible to consider that the number of Microcystis cells is an indication of the concentration of MCs in both cultured and natural waters. It is important to remember, however, that the cellular quota can vary between different Microcystis taxa, environmental conditions and in different natural reservoirs (Salmaso *et al.* 2014).

The first line of Figure 3 shows that all explanatory variables were strongly correlated ($p < 0.001$) to the response variable (McT). In the left column, the scatter plots of the McT values versus the covariates (explanatory variables) are presented with their respective linear regression lines. The visual verification of the scatter plots can be used to estimate the McT values from each explanatory variable and to evaluate how closely the experimental results are to the modeled values. Looking at the linear regression adjustment lines (graphs below the main diagonal of Figure 3), it is noted that the explanatory variables alone do not explain the McT production adequately because the vertical distances between the points and the line are large. This justifies the search for a more elaborate model to estimate McT concentration. Thus, according to Figure 3, the most relevant variables are CA, Dens, Chlo.a, Phyc and Pheo with $R_s > 0.7$, indicating that these variables could be used to estimate McT.

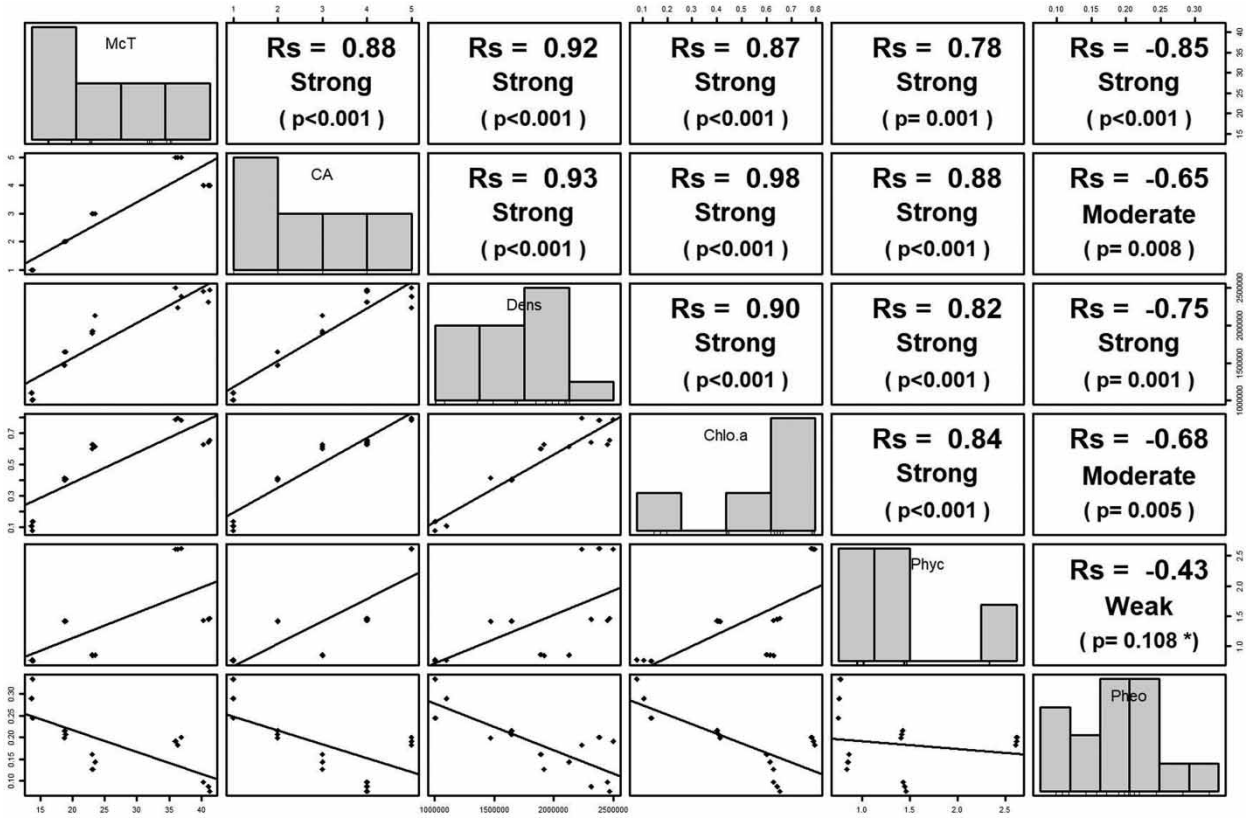

Figure 3 | Visualization matrix of cell age (CA) and density (Dens), chlorophyll-a (Chlo.a), phycocyanin (Phyc), pheophytin (Pheo) and total microcystin (McT).

In the search for a mathematical model capable of estimating McT concentration based on an explanatory variable or a set of them, a multiple regression of the data was performed. Multiple regression models were generated, with their respective correlation coefficient (Adjusted R^2), involving all possible combinations (Table 2).

Models containing only pigments presented Adjusted R^2 values lower than those containing Dens and CA (Table 2). Beversdorf *et al.* (2015, 2018) showed that there is a strong correlation between MA cell density and MCs production in isolated MA cultures, indicating that it is possible to estimate MCs concentration using the number cells. Pereira *et al.* (2018) demonstrated that increased cell density of MA induced an increase in chlorophyll and MCs.

On the other hand, although the model selected to describe the response variable (McT) should have high Adjusted R^2, for simplicity's sake, it is preferable that it requires the smallest number of variables and that these variables are easily analyzed. According to Table 2, the presence of a single variable in the mathematical model does not explain the production of McT satisfactorily. This can also be observed by the small Adjusted R^2 of single-variable models (Models 12, 19, 28, 30 and 31 – Table 2). Determining cell age (CA) and density (Dens) in environmental samples is not an easy task, and therefore, models based only on pigments were selected since they can be analyzed by simple multiparametric probes. On this basis, the models selected for determining McT were models 25 and 26 with an Adjusted $R^2 = 0.824$ and 0.814, respectively. The adjusted coefficient of determination, also called Adjusted R^2, is a criterion used for the selection of an optimal model with the largest Adjusted R^2 with a reduced set of explanatory variables. This choice is corroborated by studies that have shown a strong correlation between pigments and McT concentrations in environmental samples (Lee *et al.* 2000; Downing *et al.* 2005; Francy *et al.* 2016).

Table 3 shows the coefficients of the selected multiple regression models, as well as *p*-values and upper and

Table 2 | Multiple regression models between the response variable (McT) and the explanatory variables (CA, Dens, Chlo.a, Phyc and Pheo)

Model	Variables	Adjusted R^2	Model	Variables	Adjusted R^2
1	CA + Dens + Chlo.a + Phyc + Pheo	0.988	17	Dens + Chlo.a + Pheo	0.854
2	CA + Dens + Chlo.a + Pheo	0.986	18	CA + Chlo.a	0.842
3	CA + Chlo.a + Phyc + Pheo	0.985	**19**	**CA**	**0.832**
4	Dens + Chlo.a + Pheo	0.984	20	Dens + Pheo	0.841
5	CA + Dens + Chlo.a	0.935	21	Dens + Phyc + Pheo	0.848
6	CA + Dens + Chlo.a + Phyc	0.934	22	CA + Dens + Phyc	0.847
7	Dens + Chlo.a + Phyc + Pheo	0.899	23	CA + Phyc	0.838
8	Dens + Chlo.a + Phyc	0.881	24	CA + Dens + Phyc + Pheo	0.848
9	CA + Chlo.a + Phyc	0.880	***25***	***Phyc + Pheo***	***0.824***
10	CA + Pheo	0.870	***26***	***Chlo.a + Phyc + Pheo***	***0.814***
11	Dens + Chlo.a	0.866	27	Chlo.a + Pheo	0.693
12	**Dens**	**0.853**	**28**	**Chlo.a**	**0.674**
13	CA + Dens	0.859	29	Chlo.a + Phyc	0.661
14	CA + Phyc + Pheo	0.860	**30**	**Pheo**	**0.529**
15	CA + Dens + Pheo	0.860	**31**	**Phyc**	**0.387**
16	Dens + Phyc	0.849			

Table 3 | Coefficients of equations obtained from the correlation between parameters

				95% confidence interval	
Estimated parameter	**Related parameter**	**Coefficients**	***p*-Value**	**Inferior limit**	**Upper limit**
Model 25 ($R^2 = 0.849$/Adjusted $R^2 = 0.824$)					
McT	Intercept	64.99	1.00×10^{-5}	45.44	84.54
	Pheo	−194.65	9.01×10^{-5}	−268.29	−121.02
	Phyc	17.01	4.60×10^{-4}	9.23	24.79
Model 26 ($R^2 = 0.854$/Adjusted $R^2 = 0.814$)					
McT	Intercept	75.82	2.7×10^{-3}	32.46	119.17
	Chlo.a	−16.64	0.546	−80.08	44.79
	Pheo	−232.77	7×10^{-3}	−387.78	−77.76
	Phyc	20.70	0.012	5.36	36.04

lower limits for each estimated coefficient. Models 25 and 26 were then evaluated using the linear regression method to choose the most appropriate one. From Table 3, it is possible to observe that model 26 is not the most appropriate because Chlo.a *p*-value is higher (*p*-value = 0.546) than the adopted significance level ($\alpha = 5\%$) and the confidence interval for each coefficient goes through zero. On the other hand, model 25, which includes the concentration of Phyc and Pheo pigments, is a reasonable choice since its *p*-values are below the adopted significance level ($\alpha = 5\%$) and the confidence interval for each coefficient does not go through zero. Arranging the variables and their respective coefficients, Equation (1) is obtained.

Our results could be an improvement to the state-of-the-art since so far researches have suggested that monitoring chlorophyll could be used as a warning sign for the presence of toxins in water as the correlation between pigments and cyanobacterial cyanotoxins was observed (Wicks & Thiel

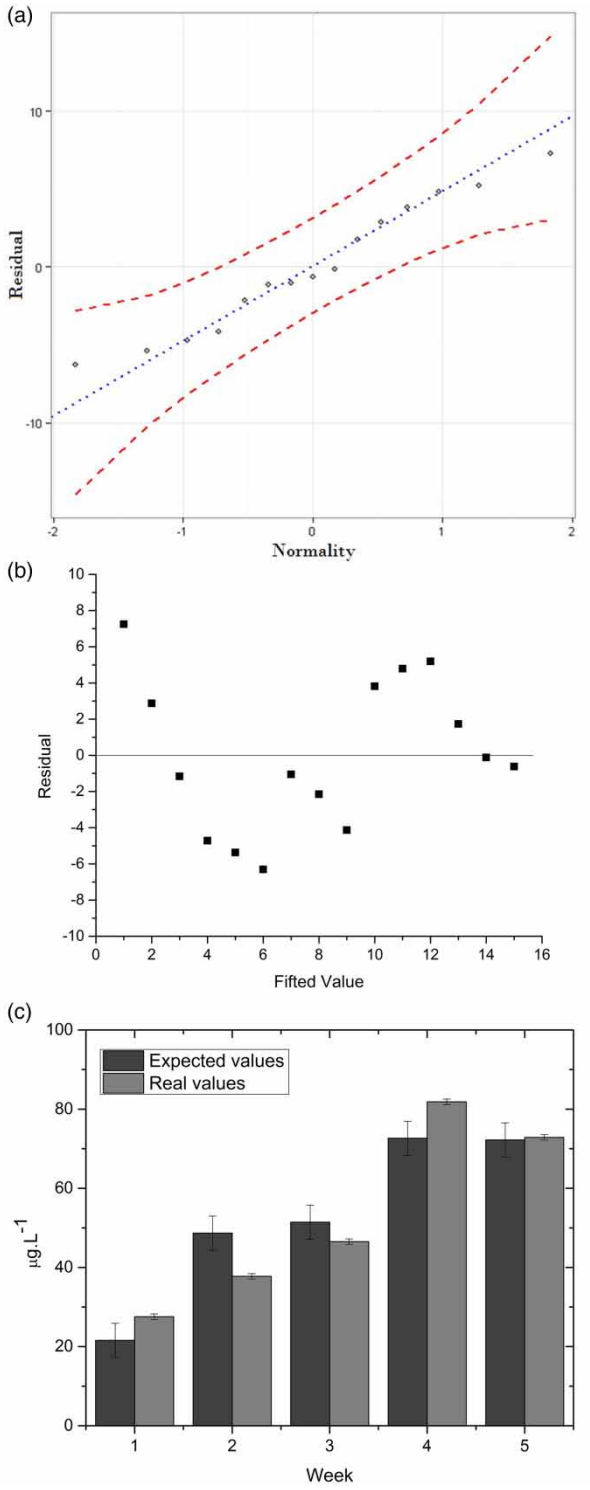

Figure 4 | (a) Normal probability for residues obtained from prediction results, (b) residual dispersion provided by the selected model and (c) comparison between the values predicted by the model and the actual values obtained experimentally with confidence intervals.

1990; Rinta-Kanto *et al.* 2009; Francy *et al.* 2015).

$$McT = -194.65Pheo + 17.01Phyc + 64.99 \quad (1)$$

Figure 4 shows the analysis of the residues obtained from the model selected (25) for the prediction of McT. According to Figure 4(a), the residues were found to be normally distributed, a required condition for the regression model validity (Mann 2010; Zar 2010). Figure 4(b) shows the distribution of the residues, indicating a good fit since the residues are randomly scattered around zero. This graph is important for verifying the assumption that residues are randomly distributed and have constant variance (Mann 2010; Zar 2010). Therefore, there are no indications of a violation of assumptions to certify the quality of the model according to Cook & Weisberg (1982).

A comparison between the actual values obtained in the experiment and those predicted by the model selected for the McT variable (Equation (1)) is presented in Figure 4(c). Based on this figure, it is possible to state that the evidence of normality and homoscedasticity of the residues was confirmed by adopting an Adjusted R^2 of 0.824. Thus, the multiple regression model proved to be valid and was able to explain 82.4% of the McT variable.

CONCLUSIONS

The multiple regression model was able to predict McT concentration in cultured MA using easily measured variables such as pheophytin and phycocyanin. Although the model containing only pigments presented an Adjusted R^2 value lower than the one with all the studied variables, it was considered a more practical solution and showed good precision.

Although differences between calculated and measured McT concentrations were less than 5%, demonstrating that the model can be applied for our cultured MA, its use for environmental samples still depends on further investigations. The combination of sensor technology and accurate mathematical modeling can be an important tool to alert for toxins in water and to the need for complementary treatments such as activated carbon application or, in extreme cases, the complete stop of the water distribution.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/aqua.2020.128.

REFERENCES

Almuhtaram, H., Cui, Y., Zamyadi, A. & Hofmann, R. 2018 Cyanotoxins and cyanobacteria cell accumulations in drinking water treatment plants with a low risk of bloom formation at the source. *Toxins* **10**, 430. https://doi.org/10.3390/toxins10110430.

APHA (American Public Health Association) 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association, Washington DC.

Bastien, C., Cardin, R., Veilleux, É., Deblois, C., Warren, A. & Laurion, I. 2011 Performance evaluation of phycocyanin probes for the monitoring of cyanobacteria. *J. Environ. Monit.* **13**, 110–118. https://doi.org/10.1039/C0EM00366B.

Bennett, A. & Bogorad, L. 2011 Complementary chromatic adaptation in a filamentous blue-green alga complementary in a filamentous blue-green alga. *J. Cell Biol.* **58**, 419–435.

Beversdorf, L. J., Chaston, S. D., Miller, T. R. & McMahon, K. D. 2015 Microcystin mcyA and mcyE gene abundances are not appropriate indicators of Microcystin concentrations in lakes. *PLoS One* **10** e0125353. https://doi.org/10.1371/journal.pone.0125353.

Beversdorf, L. J, Rude, K, Weirich, C. A, Bartlett, S. L, Seaman, M, Kozik, C, Biese, P, Gosz, T, Suha, M, Stempa, C, Shaw, C, Hedman, C, Piatt, J. J & Miller, T. R. 2018 Analysis of cyanobacterial metabolites in surface and raw drinking waters reveals more than microcystin *Water Res.* **140** 280–290. https://doi.org/10.1016/j.watres.2018.04.032.

Bittencourt-Oliveira, M. d. C., Kujbida, P., Cardozo, K. H. M., Carvalho, V. M., Moura, A. d. N., Colepicolo, P. & Pinto, E. 2005 A novel rhythm of microcystin biosynthesis is described in the cyanobacterium *Microcystis panniformis* Komárek *et al. Biochem. Biophys. Res. Commun.* **326**, 687–694. https://doi.org/10.1016/j.bbrc.2004.11.091.

Bortoli, S., Oliveira-Silva, D., Krügerd, T., Dörra, F. A., Colepicolo, P., Volmerb, D. A. & Pinto, E. 2014 Growth and microcystin production of a Brazilian *Microcystis aeruginosa* strain (LTPNA 02) under different nutrient conditions. *Br. J. Pharmacogn.* **24**, 389–398. https://doi.org/10.1016/j.bjp.2014.07.019.

Canada, H. 2002 *Guidelines for Canadian Drinking Water Quality: Supporting Documentation – Cyanobacterial Toxins – Microcystin-LR*. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch. Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/doc_supappui/index_e.html.

Chaffin, J. D., Davis, T. W., Smith, D. J., Baer, M. M. & Dick, G. J. 2018 Interactions between nitrogen form, loading rate, and light intensity on Microcystis and Planktothrix growth and microcystin production. *Harmful Algae* **73**, 84–97. https://doi.org/10.1016/j.hal.2018.02.001.

Chorus, I. & Bartran, J. 1999 *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*. WHO, Spon Press, London.

Cook, R. D. & Weisberg, S. 1982 *Residuals and Influence in Regression*. Chapman and Hall, New York.

Crettaz Minaglia, M. C., Aranda, J. O., Goñi, S., Sedan, D., Andrinolo, D. & Giannuzzi, L. 2017 Mathematical modeling of *Microcystis aeruginosa* growth and [D-leu1] microcystin-LR production in culture media at different temperatures. *Harmful Algae* **67**, 13–25. https://doi.org/10.1016/j.hal.2017.05.006.

Downing, T. G., Sember, C. S., Gehringer, M. M. & Leukes, W. 2005 Medium N:P ratios and specific growth rate comodulate microcystin and protein content in *Microcystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. *Microb. Ecol.* **49**, 468–473. https://doi.org/10.1007/s00248-004-0054-2.

Francy, D. C., Graham, J. L., Stelzer, E. A., Ecker, C. D., Brady, A. M. G., Struffolino, P. & Loftin, K. A. 2015 *Water Quality, Cyanobacteria, and Environmental Factors and Their Relations to Microcystin Concentrations for Use in Predictive Models at Ohio Lake Erie and Inland Lake Recreational Sites*, 2013–14. USGS. https://doi.org/10.3133/sir20155120.

Francy, D. S., Brady, A. M. G., Ecker, C. D., Graham, J. L., Stelzer, E. A., Struffolino, P., Dwyer, D. F. & Loftin, K. A. 2016 Estimating microcystin levels at recreational sites in western Lake Erie and Ohio. *Harmful Algae* **58**, 23–34. https://doi.org/10.1016/j.hal.2016.07.003.

Ger, K. A., Teh, S. J., Baxa, D. V., Lesmeister, S. & Goldman, C. R. 2010 The effects of dietary *Microcystis aeruginosa* and microcystin on the copepods of the upper San Francisco Estuary. *Freshwater Biol.* **55**, 1548–1559. https://doi.org/10.1111/j.1365-2427.2009.02367.x.

Glibert, P. M., Berdalet, E., Burford, M. A., Pitcher, G. C. & Zhou, M. 2018 *Global Ecology and Oceanography of Harmful Algal Blooms, Ecological Studies*. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-70069-4.

Gorham, P. R., McLachlan, J., Hammer, U. T. & Kim, W. K. 1964 Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de Bréb. *SIL Proceedings, 1922–2010*, Vol. **15**, pp. 796–804. https://doi.org/10.1080/03680770.1962.11895606.

Hamilton, D. P., Dietrich, D. R., Wood, S. & Puddick, J. 2013 Costs of harmful blooms of freshwater cyanobacteria. In: *Cyanobacteria: An Economic Perspective* (N. K. Sharma, A. K. Rai & L. J. Stal, eds). John Wiley & Sons, pp. 245–256. https://doi.org/10.1002/9781118402238.ch15.

Harris, T. D. & Graham, J. L. 2017 Predicting cyanobacterial abundance, microcystin, and geosmin in a eutrophic drinking-water reservoir using a 14-year dataset. *Lake Reserv. Manag.* **33**, 32–48. https://doi.org/10.1080/10402381.2016.1263694.

Jacinavicius, F. R. 2015 *Aspectos Morfológicos, Fisiológicos e bioquímicos e suas relações com produção de microsistinas em cepas de Microcystis aeruginosa (Cyanobacteria)*. Universidade de São Paulo, São Paulo.

Lawton, L. A., Edwards, C. & Codd, G. A. 1994 Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. *Analyst* **119**, 1525. https://doi.org/10.1039/an9941901525.

Lee, S. J., Jang, M.-H., Kim, H.-S., Yoon, B.-D. & Oh, H.-M. 2000 Variation of microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. *J. Appl. Microbiol.* **89**, 323–329. https://doi.org/10.1046/j.1365-2672.2000.01112.x.

Long, B. M., Jones, G. J. & Orr, P. T. 2001 Cellular Microcystin content in N-Limited *Microcystis aeruginosa* can be predicted from growth rate. *Appl. Environ. Microbiol.* **67**, 278–283. https://doi.org/10.1128/AEM.67.1.278–283.2001.

Mann, P. S. 2010 *Introductory Statistics*, 7th edn. John Wiley & Sons, Hoboken, NJ.

Marion, J. W., Lee, J., Wilkins, J. R., Lemeshow, S., Lee, C., Waletzko, E. J. & Buckley, T. J. 2012 In vivo phycocyanin fluorometry as a potential rapid screening tool for predicting elevated microcystin concentrations at eutrophic lakes. *Environ. Sci. Technol.* **46**, 4523–4531. https://doi.org/10.1021/es203962u.

Massey, I. Y., Yang, F., Ding, Z., Yang, S., Guo, J., Tezi, C., Al-Osman, M., Kamegni, R. B. & Zeng, W. 2018 Exposure routes and health effects of microcystins on animals and humans: a mini-review. *Toxicon* **151**, 156–162. https://doi.org/10.1016/j.toxicon.2018.07.010.

McQuaid, N., Zamyadi, A., Prévost, M., Bird, D. F. & Dorner, S. 2011 Use of in vivophycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinkingwater source. *J. Environ. Monit.* **13**, 455–463. https://doi.org/10.1039/C0EM00163E.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E. & Thomas, O. 2013 State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.* **59**, 303–327. https://doi.org/10.1016/j.envint.2013.06.013.

Minasyan, A., Christophoridis, C., Wilson, A. E., Zervou, S.-K., Kaloudis, T. & Hiskia, A. 2018 Diversity of cyanobacteria and the presence of cyanotoxins in the epilimnion of Lake Yerevan (Armenia). *Toxicon* **150**, 28–38. https://doi.org/10.1016/j.toxicon.2018.04.021.

Mohamed, Z. A., Bakr, A. & Soliman, H. A. 2018 Bioavailability of bound microcystins in mice orally fed with contaminated tilapia edible tissues: implications to human health. *Toxicon* **151**, 34–36. https://doi.org/10.1016/j.toxicon.2018.06.082.

Neilan, B. A., Pearson, L. A., Muenchhoff, J., Moffitt, M. C. & Dittmann, E. 2013 Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environ. Microbiol.* **15**, 1239–1253. https://doi.org/10.1111/j.1462-2920.2012.02729.x.

NHMRC & NRMMC 2011 *Australian Drinking Water Guidelines Paper 6. National Water Quality Management Strategy*. National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia, Canberra. Available from: http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh52_australian_drinking_water_guidelines_150413.pdf.

Nikolouli, K. & Mossialos, D. 2012 Bioactive compounds synthesized by non-ribosomal peptide synthetases and type-I polyketide synthases discovered through genome-mining and metagenomics. *Biotechnol. Lett.* **34**, 1393–1403. https://doi.org/10.1007/s10529-012-0919-2.

Orr, P. T. & Jones, G. J. 1998 Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanogr.* **43**, 1604–1614.

Pereira, A. L., Santos, C., Azevedo, J., Martins, T. P. & Castelo-branco, R. 2018 Effects of two toxic cyanobacterial crude extracts containing microcystin-LR and cylindrospermopsin on the growth and photosynthetic capacity of the microalga *Parachlorella kessleri*. *Algal Res.* **34**, 198–208. https://doi.org/10.1016/j.algal.2018.07.016.

Puddick, J., Thomson-Laing, G. & Wood, S. A. 2019 Microcystins in New Zealand: a review of occurrence, congener diversity and cell quotas. *N. Z. J. Bot.* **57**, 93–111. https://doi.org/10.1080/0028825X.2019.1573742.

Repka, S., Meyerhöfer, M., von Bröckel, K. & Sivonen, K. 2004 Associations of cyanobacterial toxin, nodularin, with environmental factors and zooplankton in the Baltic Sea. *Microb. Ecol.* **47**. https://doi.org/10.1007/s00248-003-2010-y.

Rinta-Kanto, J. M., Konopko, E. A., DeBruyn, J. M., Bourbonniere, R. A., Boyer, G. L. & Wilhelm, S. W. 2009 Lake Erie Microcystis: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. *Harmful Algae* **8**, 665–673. https://doi.org/10.1016/j.hal.2008.12.004.

Salmaso, N., Copetti, D., Cerasino, L., Shams, S., Capelli, C., Boscaini, A., Valsecchi, L., Pozzoni, F. & Guzzella, L. 2014 Variability of microcystin cell quota in metapopulations of *Planktothrix rubescens*: Causes and implications for water management. *Toxicon* **90**, 82–96. https://doi.org/10.1016/j.toxicon.2014.07.022.

Saraf, S. R., Frenkel, A., Harke, M. J., Jankowiak, J. G., Gobler, C. J. & McElroy, A. E. 2018 Effects of Microcystis on development of early life stage Japanese medaka (*Oryzias latipes*): comparative toxicity of natural blooms, cultured Microcystis and microcystin-LR. *Aquat. Toxicol.* **194**, 18–26. https://doi.org/10.1016/j.aquatox.2017.10.026.

Schatz, D., Keren, Y., Vardi, A., Sukenik, A., Carmeli, S., Börner, T., Dittmann, E. & Kaplan, A. 2007 Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. *Environ. Microbiol.* **9**, 965–970. https://doi.org/10.1111/j.1462-2920.2006.01218.x.

Schmidt, J. R., Shaskus, M., Estenik, J. F., Oesch, C., Khidekel, R. & Boyer, G. L. 2013 Variations in the microcystin content of different fish species collected from a eutrophic lake. *Toxins* **5**, 992–1009. https://doi.org/10.3390/toxins5050992.

Team, R. C. 2018 *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/.

Turner, A., Dhanji-Rapkova, M., O'Neill, A., Coates, L., Lewis, A. & Lewis, K. 2018 Analysis of microcystins in cyanobacterial blooms from freshwater bodies in England. *Toxins* **10**, 39. https://doi.org/10.3390/toxins10010039.

Van Apeldoorn, M. E., Van Egmond, H. P., Speijers, G. J. A. & Bakker, G. J. I. 2007 Toxins of cyanobacteria. *Mol. Nutr. Food Res.* **51**, 7–60. https://doi.org/10.1002/mnfr.200600185.

Walls, J. T., Wyatt, K. H., Doll, J. C., Rubenstein, E. M. & Rober, A. R. 2018 Hot and toxic: temperature regulates microcystin release from cyanobacteria. *Sci. Total Environ.* **610–611**, 786–795. https://doi.org/10.1016/j.scitotenv.2017.08.149.

Westrick, J. A., Szlag, D. C., Southwell, B. J. & Sinclair, J. 2010 A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment. *Anal. Bioanal. Chem.* **397**, 1705–1714. https://doi.org/10.1007/s00216-010-3709-5.

WHO 2003 *Cyanobacterial Toxins: Microcystin-LR in Drinking Water. Background Document for Preparation of WHO Guidelines for Drinking Water Quality*. World Health Organization, Geneva.

WHO 2011 *Guidelines for Drinking Water Quality*, 4th edn. World Health Organization, Geneva.

Wicks, R. J. & Thiel, P. G. 1990 Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. *Environ. Sci. Technol.* **24**, 1413–1418. https://doi.org/10.1021/es00079a017.

Wiltsie, D., Schnetzer, A., Green, J., Borgh, M. V. & Fensin, E. 2018 Algal blooms and cyanotoxins in Jordan Lake, North Carolina. *Toxins* **10**, 92–114. https://doi.org/10.3390/toxins10020092.

Wood, S. A., Dietrich, D. R., Cary, S. C. & Hamilton, D. P. 2012 Increasing Microcystis cell density enhances microcystin synthesis: a mesocosm study. *Inland Waters* **2**, 17–22. https://doi.org/10.5268/IW-2.1.424.

Ye, J., Du, Y., Wang, L., Qian, J., Chen, J., Wu, Q. & Hu, X. 2017 Toxin release of cyanobacterium *Microcystis aeruginosa* after exposure to typical tetracycline antibiotic contaminants. *Toxins* **9**, 53. https://doi.org/10.3390/toxins9020053.

Yoshida, T., Takashima, Y., Tomaru, Y., Takao, Y., Hiroishi, S. & Shirai, Y. 2006 Isolation and characterization of a cyanophage infecting the toxic cyanobacterium *Microcystis aeruginosa*. *Appl. Environ. Microbiol.* **72**, 1239–1247. https://doi.org/10.1128/AEM.72.2.1239.

Yoshizawa, S., Matsushima, R., Watanabe, M. F., Harada, K. ichi, Ichihara, A., Carmichael, W. W. & Fujiki, H., 1990 Inhibition of protein phosphatases by microcystis and nodularin associated with hepatotoxicity. *J. Cancer Res. Clin. Oncol.* **116**, 609–614. https://doi.org/10.1007/BF01637082.

Zar, J. H. 1996 *Biostatistical Analysis*. Prentice-Hall International Inc., Upper Saddle River, NJ, USA.

Zar, J. H. 2010 *Biostatistical Analysis*, 5th edn. Pearson Prentice Hall, Northern Illinois University, USA.

Zhang, M., Wang, X., Tao, J., Li, S., Hao, S., Zhu, X. & Hong, Y. 2018 PAHs would alter cyanobacterial blooms by affecting the microcystin production and physiological characteristics of microcystis aeruginosa. *Ecotoxicol. Environ. Saf.* **157**, 134–142. https://doi.org/10.1016/j.ecoenv.2018.03.052.

Zi, J., Pan, X., MacIsaac, H. J., Yang, J., Xu, R., Chen, S. & Chang, X. 2018 Cyanobacteria blooms induce embryonic heart failure in an endangered fish species. *Aquat. Toxicol.* **194**, 78–85. https://doi.org/10.1016/j.aquatox.2017.11.007.

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