

Comment: An alternative interpretation of the relationship between TN:TP and microcystins in Canadian lakes

J. Thad Scott, Mark J. McCarthy, Timothy G. Otten, Morgan M. Steffen, Bryant C. Baker, Erin M. Grantz, Steven W. Wilhelm, and Hans W. Paerl

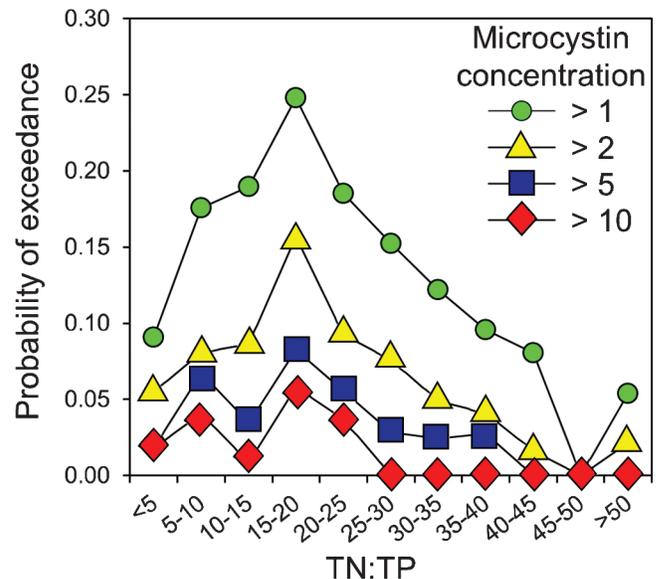
Introduction

Landscape-scale patterns of the cyanobacterial toxin, microcystin, in Canadian lakes were recently analyzed by Orihel et al. (2012). The analysis of this comprehensive dataset was important for linking accelerated eutrophication and water quality issues affecting human health. The primary conclusion by Orihel et al. (2012) was that high microcystin concentrations occur only at low total nitrogen to total phosphorus (TN:TP) ratios in nutrient-rich lakes. Using these same data, however, we show that high microcystin concentrations across Canadian lakes of all trophic states are more likely to occur at intermediate TN:TP, where the relative availability of N and P are more closely balanced with phytoplankton nutrient demand. Additionally, in the most nutrient-rich lakes, high microcystin concentrations were not related to TN:TP.

Orihel et al. (2012) hypothesized that “the presence of microcystins in lakes should theoretically be higher under low N:P ratios if cyanobacteria dominate under conditions of relative N deficiency.” To test this hypothesis, the authors used arbitrary TN:TP categories of <20, 20–40, 40–60, and >60 (all ratios presented by mass) based on the presumption that lakes with TN:TP < 30 would be dominated by N₂-fixing cyanobacteria (Smith 1983). However, more recent landscape-scale studies have shown that phytoplankton N limitation generally occurs at TN:TP < 9, and P limitation occurs at TN:TP > 22.5 (Guildford and Hecky 2000). Therefore, the TN:TP categories used by Orihel et al. (2012) did not include values at which phytoplankton N limitation was likely, even though their prediction of cyanobacterial dominance was predicated on the idea that N₂-fixing cyanobacteria can overcome N limitation. Our reanalysis of these same data illustrate that when TN:TP categories are expanded to encompass the full range over which both N and P deficiency are probable (i.e., <5, >50, and all increments of 5 in between), elevated microcystin concentrations are most frequently observed when the TN:TP ratio is between 15 and 20 (Fig. 1), which is the range for balanced phytoplankton growth identified by Guildford and Hecky (2000).

Orihel et al. (2012) developed a regression tree model using the TREES module in SYSTAT 13 (Systat Software Inc., Chicago, Illinois) to quantify the relationship between microcystin concentrations and TN and TP concentrations and the TN:TP ratio. We were able to recreate their analysis identically using the MVPART library in R 2.9.2, but only after removing one outlier with a microcystin concentration >500 µg·L⁻¹, which we assume was also done but not mentioned by Orihel et al. (2012). We also used nonparametric changepoint analysis in R 2.9.2 to calculate the

Fig. 1. Probability of exceeding various microcystin concentrations in Canadian lakes based on an expansion of TN:TP categories beyond what was shown by Orihel et al. (2012). The expanded TN:TP categories represent a more complete range from potentially N- to P-limiting conditions for phytoplankton based on the thresholds reported by Guildford and Hecky (2000). Data in this graph were from all lakes as shown in fig. 2c from Orihel et al. (2012).



statistical significance of the modeled thresholds, which is only possible with bootstrapping (Qian et al. 2003; King and Richardson 2003). The results of our regression tree analysis showed that TN was the strongest predictor of microcystin across all lakes (Fig. 2). Most lakes had low TN (<2600 µg·L⁻¹) and microcystins, while many lakes with TN exceeding 2600 µg·L⁻¹ had high microcystins (Fig. 2A). These high TN lakes had higher microcystin concentrations when TN:TP < 23 and lower microcystin concentrations when TN:TP > 23 (Fig. 2B). In lakes with TN > 2600 µg·L⁻¹ and TN:TP < 23, mean microcystin concentration was highest when TP < 219 µg·L⁻¹ and lower when TP > 219 µg·L⁻¹ (Fig. 2C). Thus, the regression tree shows that the highest microcystin concentrations across all Canadian lakes sampled occurred at TN:TP ratios between 12 and 23 because TN:TP was bound on the lower end by

Received 13 November 2012. Accepted 10 May 2013.

Paper handled by Associate Editor Ralph Smith.

J.T. Scott, B.C. Baker, and E.M. Grantz. Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, Arkansas, USA.

M.J. McCarthy. Marine Science Institute, The University of Texas at Austin, Port Aransas, Texas, USA.

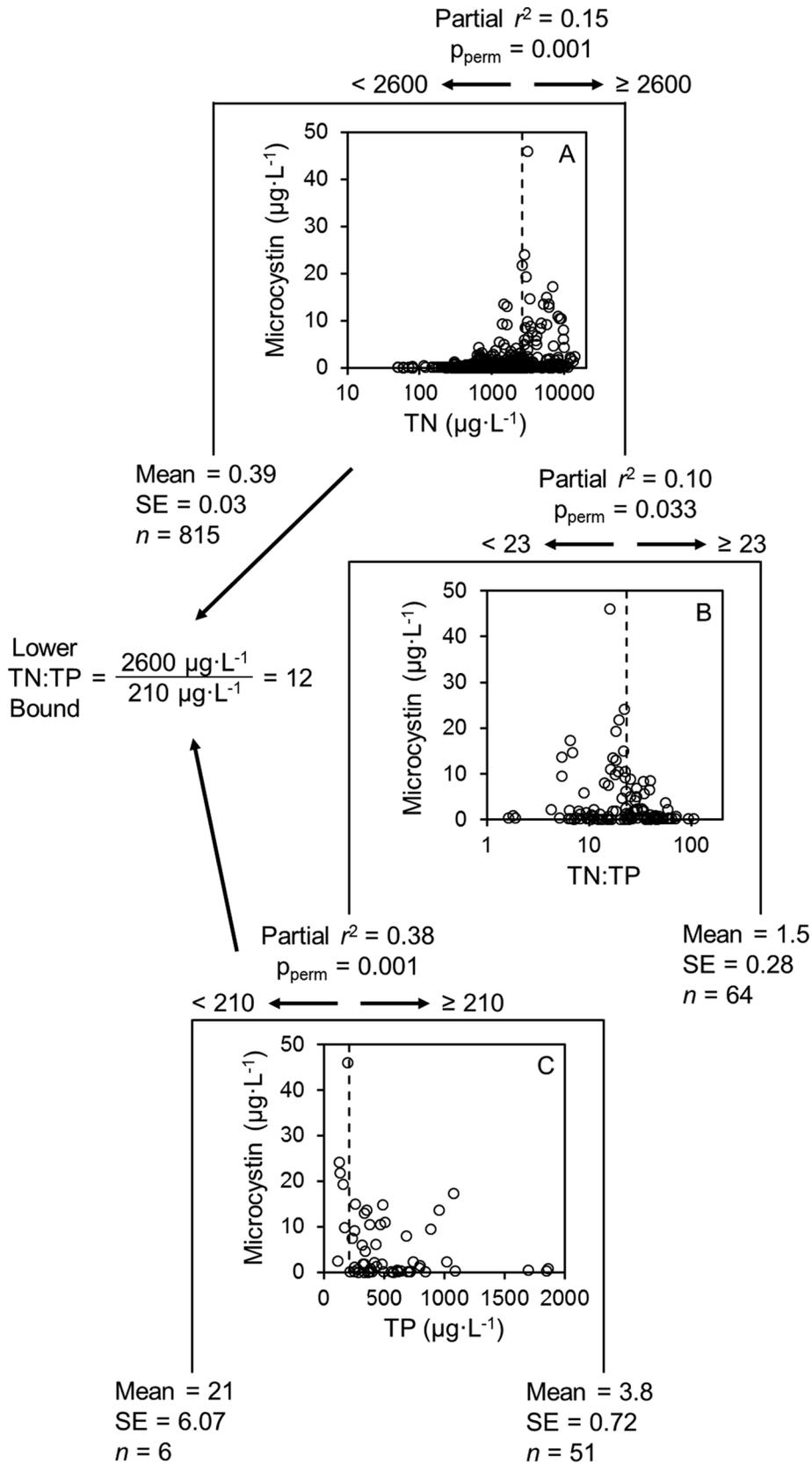
T.G. Otten. Institute of Marine Sciences, University of North Carolina, Morehead City, North Carolina, USA; Department of Microbiology, Oregon State University, 220 Nash Hall, Corvallis, Oregon, USA.

M.M. Steffen and S.W. Wilhelm. Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA.

H.W. Paerl. Institute of Marine Sciences, University of North Carolina, Morehead City, North Carolina, USA.

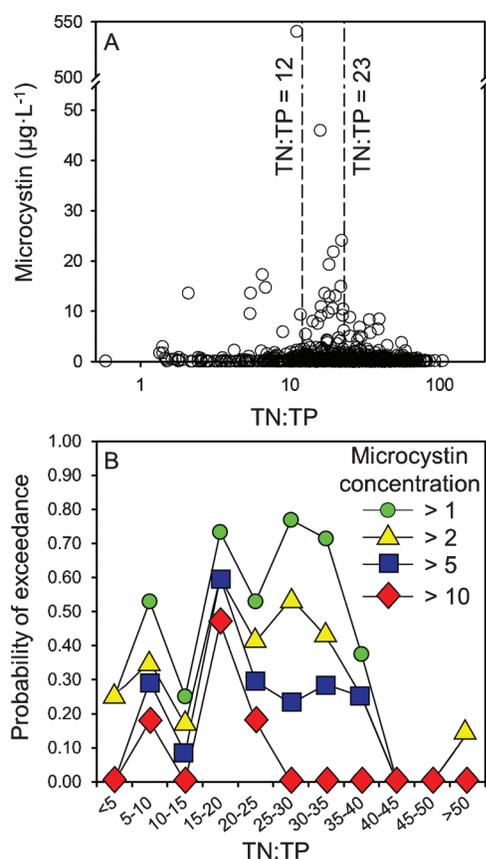
Corresponding author: J. Thad Scott (e-mail: jts004@uark.edu).

Fig. 2. Results of the regression tree analysis we conducted using the MVPART library R 2.9.2. Results were identical to those obtained by Orihel et al. (2012) and show that (A) TN is the strongest predictor of microcystin in the entire dataset; (B) at TN > 2600 µg·L⁻¹, microcystin was higher when TN:TP < 23; and (C) when TN > 2600 µg·L⁻¹ and TN:TP < 23, microcystin concentrations were highest when TP < 210 µg·L⁻¹. The combination of TN > 2600 µg·L⁻¹ and TP < 210 µg·L⁻¹ yields a lower bound on the TN:TP ratio of 12.



Can. J. Fish. Aquat. Sci. Downloaded from www.nrcresearchpress.com by 174.106.151.253 on 02/18/15
 For personal use only.

Fig. 3. (A) Relationship between TN:TP ratio and microcystin concentrations across all Canadian lakes sampled, along with the upper and lower bounds in TN:TP identified in the regression tree analysis. (B) Probability of exceeding various microcystin concentrations in nitrogen-rich Canadian lakes (TN > 2600 $\mu\text{g}\cdot\text{L}^{-1}$).



TN concentrations exceeding 2600 $\mu\text{g}\cdot\text{L}^{-1}$ and TP concentrations below 219 $\mu\text{g}\cdot\text{L}^{-1}$ (2600/219 = 12). TN:TP was bound on the upper end by a value of 23 identified in the regression tree analysis (Fig. 2).

Graphing these upper and lower TN:TP bounds with all microcystin data confirms that microcystin was highest at intermediate TN:TP across all lakes (Fig. 3A). Mean microcystin concentrations across all lakes were $0.82 \pm 0.18 \mu\text{g}\cdot\text{L}^{-1}$ and $0.41 \pm 0.04 \mu\text{g}\cdot\text{L}^{-1}$ (mean \pm SE) when TN:TP was <12 or >23 , respectively. When TN:TP was between 12 and 23, mean microcystin concentration across all lakes was $1.5 \pm 0.27 \mu\text{g}\cdot\text{L}^{-1}$. These findings again support the idea that microcystin concentrations across all lakes were highest at intermediate TN:TP, which represents balanced-growth conditions for phytoplankton (Guildford and Hecky 2000). However, it is important to note that these data include all lakes, not just nutrient-rich lakes. Therefore, we also recalculated the probability distributions across the various TN:TP categories using data from lakes with TN > 2600 $\mu\text{g}\cdot\text{L}^{-1}$. We selected the 2600 $\mu\text{g}\cdot\text{L}^{-1}$ TN concentration to define nutrient-rich lakes because this threshold was the strongest predictor of microcystin concentrations across all lakes in the regression tree model. As such, the probability of exceeding various microcystin concentrations in these nitrogen-rich lakes was not correlated with the TN:TP ratio because microcystin was likely to be elevated at low, high, and intermediate TN:TP (Fig. 3B).

We interpret the hierarchical nature of these results to suggest that the highest microcystin concentrations in Canadian lakes coincided with the highest cyanobacterial biomass, and cyanobacterial biomass was highest in lakes with balanced (TN:TP of 9–22.5; Guildford and Hecky 2000) and elevated nutrient concen-

trations (Downing et al. 2001). This pattern has been shown for *Microcystis* cultures (Watanabe and Oishi 1985; Lee et al. 2000; Oh et al. 2000) as well as at ecosystem scales and across trophic gradients (Giani et al. 2005; Gobler et al. 2007; Rinta-Kanto et al. 2009; Wilhelm et al. 2011; Dolman et al. 2012; Otten et al. 2012).

Although Orihel et al. (2012) do not claim that TN:TP ratios control microcystin biosynthesis, it is worth considering the mechanistic triggers that result in microcystin production. Total nutrient concentrations and TN:TP ratios likely reflect total phytoplankton biomass more than ambient nutrient availability patterns (Lewis and Wurtsbaugh 2008). Microcystin is synthesized by diverse cyanobacterial genera, including N_2 -fixers, but also by non- N_2 -fixers (*Microcystis* spp.), which suggests that low TN:TP should not favor microcystin-producing cyanobacteria. Therefore, we believe that microcystin concentrations and TN:TP ratios within this dataset are not likely to be causally linked. Lakes with high nutrient concentrations often have low TN:TP, mirroring many anthropogenic nutrient sources (Downing and McCauley 1992).

We are not suggesting that cellular nutrient status is unimportant to microcystin production. On the biomolecular level, the microcystin synthetase (*mcy*) promoter is controlled by cell redox state and specifically the global nitrogen regulator NtcA; as such, *mcy* expression should be highest under N-limitation (specifically, NH_4^+ ; Ginn et al. 2010). Additionally, within this promoter lies a ferric uptake regulator (FurA) binding motif, implying a potential role for Fe control of *mcy* as well (Neilan et al. 2013). The broad distribution of *mcy* operons across cyanobacterial genera (Rantala et al. 2004) further suggests that phytoplankton communities in most water bodies include members capable of toxin production. Therefore, as a lake's trophic state increases, so does phytoplankton biomass and potential microcystin concentration (Kurmayer and Kutzenberger 2003; Rinta-Kanto et al. 2009; Otten et al. 2012).

Although landscape-scale studies such as Orihel et al. (2012) are informative, understanding the mechanisms that trigger and modulate toxin production may provide clues to its environmental control (Paerl and Millie 1996). Using only TN:TP ratios to predict the likelihood of elevated microcystin is too simplistic, as we have shown in this paper, because in doing so one ignores important N and P fluxes into and out of biological pools. Indeed, recent studies have revealed that dissolved N can play a primary role in driving cyanobacterial biomass (Ahn et al. 2011; Paerl et al. 2011; Dolman et al. 2012) and may even enhance the growth of toxic *Microcystis* strains (Davis et al. 2010). The drawdown of reduced N (i.e., NH_4^+ and urea), even at high nitrate concentrations (Yan et al. 2004), has been associated with toxin production in *Microcystis* (Ginn et al. 2010). Thus, N forms can regulate toxin production directly at the biochemical level and indirectly via biological succession of species within the system (Wilhelm et al. 2003). Determining the benefits of microcystin production within or among cells is crucial to clarify the functional role(s) of microcystin for cyanobacteria, which will also improve the effectiveness of water quality management strategies. Finally, it is important to remember that nutrient ratios may be as much a result of phytoplankton growth and accumulation as they are a driver. As such, it is difficult to infer fluxes into phytoplankton from pool data gathered once the biomass has accumulated. Rather, drivers of bloom events are likely best determined prior to bloom occurrence, and the ongoing development of predictive capabilities for the modeling of biomass accumulation in lake systems will hopefully serve as a guide for sampling on more appropriate time scales.

Acknowledgements

We thank Diane Orihel, David Bird, Michael Brylinsky, Huirong Chen, Derek Donald, Dorothy Huang, Alessandra Giani, David Kinniburgh, Hedy Kling, Brian Kotak, Peter Leavitt, Charlene Nielsen, Sharon Reedyk, Rebecca Rooney, Sue Watson, Ron Zurawell, and Rolf Vinebrooke for sharing their data. We also

thank three anonymous reviewers whose comments improved a previous version of this manuscript.

References

- Ahn, C.-Y., Oh, H.-M., and Park, Y.-S. 2011. Evaluation of environmental factors on cyanobacterial bloom in eutrophic reservoir using artificial neural networks. *J. Phycol.* **47**: 495–504. doi:10.1111/j.1529-8817.2011.00990.x.
- Davis, T.W., Harke, M.J., Marcoval, M.A., Goleski, J., Orano-Dawson, C., Berry, D.L., and Gobler, C.J. 2010. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. *Aquat. Microb. Ecol.* **61**: 149–162. doi:10.3354/ame01445.
- Dolman, A.M., Rucker, J., Pick, F.R., Fastner, J., Rohrlack, T., Mischke, U., and Wiedner, C. 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. *Plos One*, **7**: 1–14. doi:10.1371/journal.pone.0038757.
- Downing, J.A., and McCauley, E. 1992. The nitrogen:phosphorus relationship in lakes. *Limnol. Oceanogr.* **37**: 936–945. doi:10.4319/lo.1992.37.5.0936.
- Downing, J.A., Watson, S.B., and McCauley, E. 2001. Predicting Cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* **58**(10): 1905–1908. doi:10.1139/f01-143.
- Giani, A., Bird, D.F., Prairie, Y.T., and Lawrence, J.F. 2005. Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. *Can. J. Fish. Aquat. Sci.* **62**(9): 2100–2109. doi:10.1139/f05-124.
- Ginn, H.P., Pearson, L.A., and Neilan, B.A. 2010. NtcA from *Microcystis aeruginosa* PCC 7806 is autoregulatory and binds to the microcystin promoter. *Appl. Environ. Microbiol.* **76**: 4362–4368. doi:10.1128/AEM.01862-09. PMID: 20453121.
- Gobler, C.J., Davis, T.W., Coyne, K.J., and Boyer, G.L. 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. *Harmful Algae*, **6**: 119–133. doi:10.1016/j.hal.2006.08.003.
- Guildford, S.J., and Hecky, R.E. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: is there a common relationship? *Limnol. Oceanogr.* **45**: 1213–1223. doi:10.4319/lo.2000.45.6.1213.
- King, R.S., and Richardson, C.J. 2003. Integrating bioassessment and ecological risk assessment: An approach to developing numerical water-quality criteria. *Environ. Manage.* **31**: 795–809. doi:10.1007/s00267-002-0036-4. PMID: 14565699.
- Kurmayer, R., and Kutzenberger, T. 2003. Application of real-time PCR for quantification of microcystin genotypes in a population of the toxic cyanobacterium *Microcystis* sp. *Appl. Environ. Microbiol.* **69**: 6723–6730. doi:10.1128/AEM.69.11.6723-6730.2003. PMID:14602633.
- Lee, S.J., Jang, M.-H., Kim, H.-S., Yoon, B.-D., and Oh, H.-M. 2000. Variation of microcystin content of *Microcystis aeruginosa* relative to medium N:P and growth stage. *J. Appl. Microbiol.* **89**: 323–329. doi:10.1046/j.1365-2672.2000.01112.x. PMID:10971766.
- Lewis, W.M., Jr., and Wurtsbaugh, W.A. 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *Int. Rev. Hydrobiol.* **93**: 446–465. doi:10.1002/iroh.200811065.
- Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., and Dittmann, E. 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. *Environ. Microbiol.* **15**: 1239–1253. doi:10.1111/j.1462-2920.2012.02729.x. PMID:22429476.
- Oh, H.-M., Lee, S.J., Jang, M.-H., and Yoon, B.-D. 2000. Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. *Appl. Environ. Microbiol.* **66**: 176–179. doi:10.1128/AEM.66.1.176-179.2000. PMID:10618220.
- Orihel, D.M., Bird, D.F., Brylinsky, M., Chen, H., Donald, D.B., Huang, D.Y., Giani, A., Kinniburgh, D., Kling, H., Kotak, B.G., Leavitt, P.R., Nielsen, C.C., Reedyk, S., Rooney, R.C., Watson, S.B., Zurawell, R.W., and Vinebrooke, R.D. 2012. High microcystin concentrations occur only at low nitrogen-to-phosphorus ratios in nutrient-rich Canadian lakes. *Can. J. Fish. Aquat. Sci.* **69**(9): 1457–1462. doi:10.1139/f2012-088.
- Otten, T.G., Xu, H., Qin, B., Zhu, G., and Paerl, H.W. 2012. Spatiotemporal patterns and ecophysiology of toxigenic *Microcystis* blooms in Lake Taihu, China: implications for water quality management. *Environ. Sci. Technol.* **46**: 3480–3488. doi:10.1021/es2041288. PMID:22324444.
- Paerl, H.W., and Millie, D.F. 1996. Physiological ecology of toxic aquatic cyanobacteria. *Phycologia*, **35**: 160–167. doi:10.2216/i0031-8884-35-6S-160.1.
- Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., and Gardner, W.S. 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. *Water Res.* **45**: 1973–1983. doi:10.1016/j.watres.2010.09.018. PMID:20934736.
- Qian, S.S., King, R.S., and Richardson, C.J. 2003. Two statistical methods for the detection of environmental thresholds. *Ecol. Modell.* **166**: 87–97. doi:10.1016/S0304-3800(03)00097-8.
- Rantala, A., Fewer, D.P., Hisbergues, M., Rouhiainen, L., Vaitomaa, J., Börner, T., and Sivonen, K. 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **101**: 568–573. doi:10.1073/pnas.0304489101. PMID:14701903.
- Rinta-Kanto, J.M., Konopko, E.A., DeBruyn, J.M., Bourbonniere, R.A., Boyer, G.L., and 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. doi:10.1016/j.hal.2008.12.004.
- Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science*, **221**: 669–671. doi:10.1126/science.221.4611.669. PMID:17787737.
- Watanabe, M.F., and Oishi, S. 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Appl. Environ. Microbiol.* **49**: 1342–1344. PMID:3923932.
- Wilhelm, S.W., DeBruyn, J.M., Gillor, O., Twiss, M.R., Livingston, K., Bourbonniere, R.A., Pickell, L.D., Trick, C.G., Dean, A.L., and McKay, R.M.L. 2003. Effect of phosphorus amendments on present day plankton communities in pelagic Lake Erie. *Aquat. Microb. Ecol.* **32**: 275–285. doi:10.3354/ame032275.
- Wilhelm, S.W., Farnsley, S.E., LeClerc, G.R., Layton, A.C., Satchwell, M.F., DeBruyn, J.M., Boyer, G.L., Zhu, G., and Paerl, H.W. 2011. The relationships between nutrients, cyanobacterial toxin and the microbial community in Lake Tai (Taihu), China. *Harmful Algae*, **10**: 207–215. doi:10.1016/j.hal.2010.10.001.
- Yan, H., Pan, G., Zou, H., Song, L., and Zhang, M. 2004. Effects of nitrogen forms on the production of cyanobacterial toxin microcystin-LR by an isolated *Microcystis aeruginosa*. *J. Environ. Sci. Health Part A Toxic-Hazard. Subst. Environ. Eng.* **39**: 2993–3003. doi:10.1081/ESE-200034799.