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DISCUSSION

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

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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 N_2-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 N_2-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 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

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. 2*c* from Orihel et al. (2012).

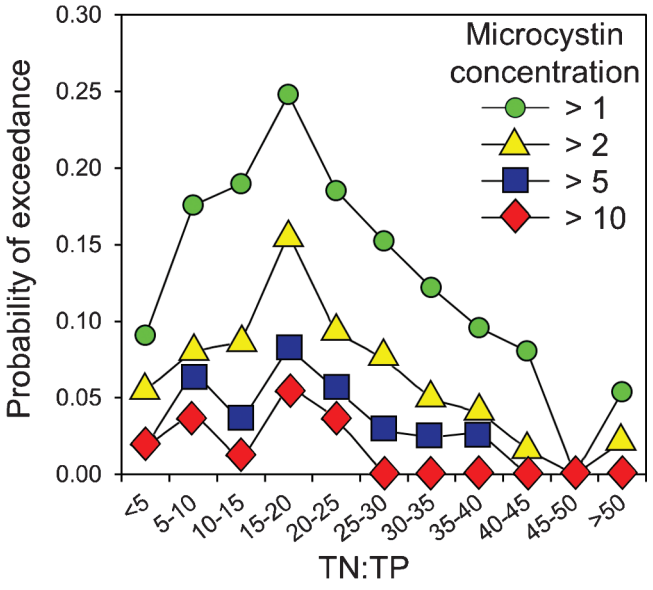

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Fig. 2. Results of the regression tree analysis we conducted using the MVPART library R 2.9.2. Results were identical to those obtained by Orihel et al. (2012) and show that (A) TN is the strongest predictor of microcystin in the entire dataset; (B) 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 < 219 μg·L^{-1}. The combination of TN > 2600 μg·L^{-1} and TP < 219 μg·L^{-1} yields a lower bound on the TN:TP ratio of 12.

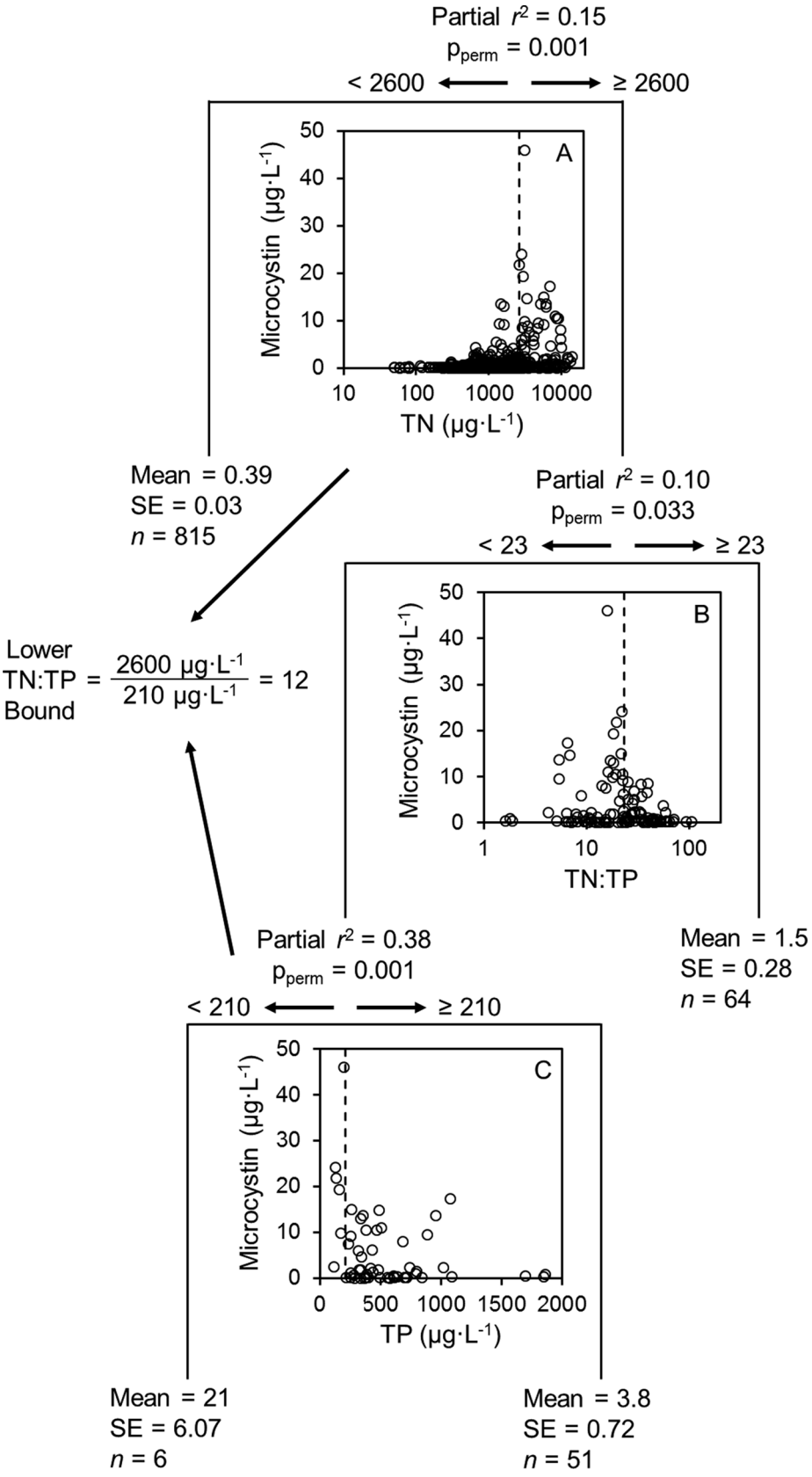
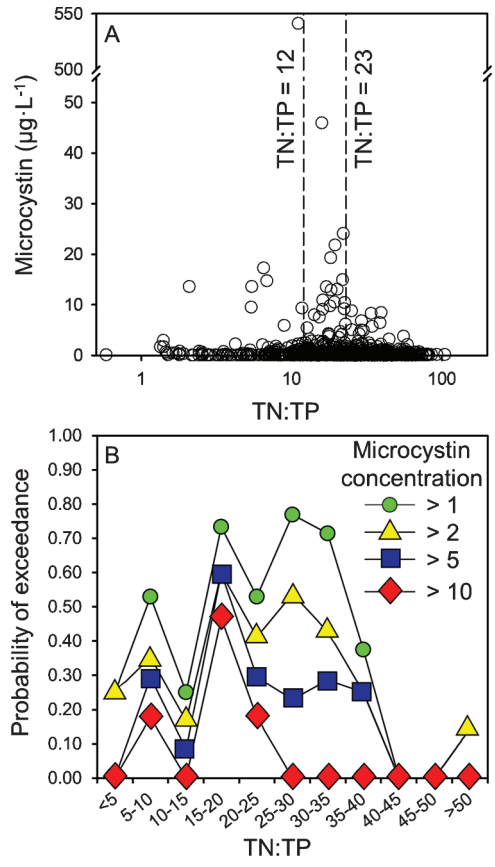


Fig. 3. (A) Relationship between TN:TP ratio and microcystin concentrations across all Canadian lakes sampled, along with the upper and lower bounds in TN:TP identified in the regression tree analysis. (B) Probability of exceeding various microcystin concentrations in nitrogen-rich Canadian lakes (TN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



TN concentrations exceeding 2600 μg·L^{-1} and TP concentrations below 219 μg·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 ± 0.18 μg·L^{-1} and 0.41 ± 0.04 μg·L^{-1} (mean ± 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 ± 0.27 μg·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 μg·L^{-1}. We selected the 2600 μg·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 concentrations (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.

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