Factors affecting local, regional, and global scale cyanobacterial dominance and secondary metabolite occurrence

By

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Factors affecting local, regional, and global scale cyanobacterial dominance and secondary metabolite occurrence

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## Abstract

Cyanobacteria are photosynthetic bacteria that serve as a primary producer in aquatic ecosystems. Cyanobacterial harmful algal blooms (CyanoHABs) are a major cause of water quality degradation in rivers, lakes, and estuaries worldwide because they disrupt food-webs and cause substantial changes in pH and dissolved oxygen. Additionally, many cyanobacterial species are capable of producing a suite of potent toxins and other secondary metabolites that cause taste and odor problems in drinking water supplies. The primary goal of my dissertation is to better understand the factors controlling CyanoHABs and their associated secondary metabolites at local, regional, and global scales.

I examined the factors that control local-scale CyanoHABs and the cyanobacterial metabolites microcystin and geosmin by comparing 12 linear and non-linear regression modeling techniques using a continuous 14 year dataset collected from Cheney Reservoir, Kansas. In Chapter 2, I explored the factors that control regional-scale cyanobacterial abundance, microcystin, geosmin, and 2-methylisoborneol concentrations in 4 Midwestern US reservoirs. Then, I used a meta-analysis to evaluate the relation of persistent organic pollutants, which include herbicides, pesticides, pharmaceutical, personal care products, and industrial chemicals, to CyanoHABs on a global scale in Chapter 3. Overall, the three chapters indicate that cyanobacterial blooms and their associated metabolites are driven by numerous factors at different scales.

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# Table of Contents

# List of Tables

# List of Figures

# List of Appendices

# Introduction

Cyanobacteria, also known as blue-green algae, are gram-negative bacteria that perform oxygenic photosynthesis (Paerl and Otten 2013a). Along with eukaryotic algae, cyanobacteria have a vital role as primary producers in aquatic ecosystems. Cyanobacterial harmful algal blooms (CyanoHABs), however, degrade water quality in rivers, lakes, and estuaries worldwide by disrupting food-webs and causing significant changes in dissolved oxygen and pH (Paerl 2014). In addition, many cyanobacterial taxa produce a diverse suite of potent cyanotoxins (Codd et al. 1999), which include nitrogen- rich toxins like microcystin, anatoxin-a, and saxitoxin, as well as other carbon-rich cellular metabolites that create taste and odor problems in drinking water supplies (Graham et al. 2010). CyanoHABs can pose significant human and animal health hazards, impair fisheries, drinking water, and irrigation supplies, and result in substantial economic damage (Dodds et al. 2009; Sharma et al. 2013).

Current research demonstrates that the abundance of cyanobacteria is strongly influenced by eutrophication, which is caused by the over-supply of two key nutrients, phosphorus (P) and nitrogen (N) to surface waters (Paerl and Otten 2013a). In particular, the absolute water column concentrations of total nitrogen (TN) and total phosphorus (TP) are critically important determinants of cyanobacterial biomass (Downing et al. 2001). Nitrogen: phosphorus (N:P) stoichiometry also effects cyanobacterial dominance, in particular cyanobacterial growth and toxin occurrence are favored when the N:P ratio is low (Smith 1983; Graham et al. 2004; Orihel et al. 2012; Harris et al. 2014). Other environmental factors also potentially influence nuisance cyanobacterial growth, including changes in food web structure (Elser 1999; Ekvall et al. 2014),

increased surface water temperature, longer water residence/stratification times, and nitrogen form (Blomqvist et al. 1994; Paerl et al. 2011; Schindler 2012; Paerl and Otten 2013a).

Factors promoting blooms of cyanobacteria that produce metabolites are currently not well understood. In general, the synthesis of metabolites by primary producers in terrestrial, aquatic, and arid desert ecosystems depends on the relative proportions of carbon and nutrients (Downing et al. 2005; Reich et al. 2006; Van de Waal et al. 2009; Van de Waal et al. 2014; Downing et al. 2015). The regulation of carbon- and nitrogen-rich toxic metabolites in phytoplankton depends on water column nutrient proportions (Granéli and Flynn 2006; Van de Waal et al. 2014; Beversdorf et al. 2015). Specifically, nitrogen limitation caused a decrease in the synthesis of nitrogen-rich toxins (including microcystin), and production of these toxins increased with cellular N:P ratios. Synthesis of carbon-rich toxins increased under both nitrogen and phosphorus limitation, when cellular carbon availability is high relative to nitrogen and phosphorus (Granéli, Johansson & Panosso 1998; Granéli & Flynn 2006; Van de Waal et al. 2014). Thus, the syntheses of metabolites that contain nitrogen seems favored when nitrogen availability is relatively high, while the syntheses of carbon-rich metabolites are favored when the availability of carbon is relatively high.

Concentrations of cyanobacterial metabolites in the water column depend on (i) the abundance of metabolite-producing strains, and (ii) the amount of metabolites produced by the cells (cellular quota). In concurrence with cyanobacterial abundance, the former is considered to depend on the TN:TP ratio for metabolite (i.e., microcystin) occurrence, with high (>20 ug/L) metabolite events being most probable when the TN:TP ratio is <50 (Graham et al. 2004; Orihel et al. 2012; Harris et al. 2014). Recent studies, however, have questioned whether moderate

TN:TP ratios (~25) favor nitrogen-rich metabolite occurrence more than low (<25) TN:TP ratios (Scott et al. 2013). Other factors suggested to influence metabolite synthesis in cyanobacteria, including light level, temperature, nitrogen form, and the presence of persistent organic pollutants (i.e., organo-phosphates; Blomqvist et al. 1994; Sun et al. 2013; Paerl and Otten 2013b; Paerl 2014).

My dissertation explores factors that affect local-, regional-, and global-scale CyanoHAB frequency and intensity, and examines factors that can be used to predict cyanobacterial dominance and associated metabolite concentration and occurrence. Specifically, in Chapter 1 I examine factors affecting cyanobacteria and secondary metabolites at a local-scale by comparing 12 unique linear and non-linear regression modeling techniques to predict cyanobacterial abundance and microcystin and geosmin using 14 years of physiochemical water-quality data collected from Cheney Reservoir, Kansas. The most important factors for predicting cyanobacterial abundance, microcystin, and geosmin were reservoir elevation, water temperature, and water column silica concentration, respectively.

In Chapter 2, I examined the regional spatial scale effects of TN: TP ratios and inorganic nitrogen form on cyanobacterial abundance, microcystin, geosmin, and 2-methylisoborneol (MIB) in four Midwestern USA reservoirs. I also examined the relationship between water column concentrations of chemically oxidized ($NO_3$) and reduced ($NH_3$) nitrogen, the novel $NO_3$:$NH_3$ ratio, cyanobacterial biovolume, and associated secondary metabolites. Results showed that the cyanobacterial secondary metabolites geosmin, MIB, and microcystin primarily occurred when the TN:TP ratio was <30:1 (by mass), and that relative cyanobacterial biovolume was inversely related to the $NO_3$:$NH_3$ ratio. Additionally, cyanobacteria had higher metabolite

concentrations per unit biovolume when $NO_3$:$NH_3$ ratios were <5 compared to when $NO_3$:$NH_3$ ratios were >5.

In Chapter 3, I explored whether persistent organic pollutants (POPs), such as herbicides, pesticides, pharmaceuticals, personal care products, and industrial chemicals, have an effect on global-scale cyanobacterial dominance. I used a meta-analysis to show that POP stressors may be significantly aggravating nutrient-driven harmful cyanobacterial blooms by suppressing the growth of competing phytoplankton, and/or by indirectly or directly stimulating cyanobacterial growth. Overall, the three chapters indicate that cyanobacterial blooms and their associated metabolites are driven by numerous factors at different scales.

# Chapter 1*

Predicting cyanobacterial abundance, microcystin, and geosmin in a eutrophic drinking-water reservoir using a 14 year dataset

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**Abstract**

Cyanobacterial blooms degrade water quality in drinking water supply reservoirs by producing toxic and taste-and-odor causing secondary metabolites, which ultimately cause public health concerns and lead to increased treatment costs for water utilities. There have been numerous attempts to create models that predict cyanobacteria and their secondary metabolites. Most studies have used linear models to predict cyanobacterial related events; however, linear models are limited by assumptions about the data and have had limited success as predictive tools. Thus, lake and reservoir managers need improved modeling techniques that can accurately predict large bloom events that have the highest impact on recreational activities and drinking-water treatment processes. In this study, I compared 12 unique linear and non-linear regression modeling techniques to predict cyanobacterial abundance and the cyanobacterial secondary metabolites microcystin and geosmin using 14 years of physiochemical water-quality data collected from Cheney Reservoir, Kansas. Support vector machine (SVM), random forest (RF), boosted tree (BT), and cubist modeling techniques were the most predictive of the compared modeling approaches. SVM, RF, and BT modeling techniques were able to successfully predict cyanobacterial abundance, microcystin, and geosmin concentrations less than 60,000 cells/mL, 2.5 µg/L, and 20 ng/L, respectively. Only cubist modeling predicted maxima concentrations of cyanobacteria and geosmin; no modeling technique was able to predict maxima microcystin concentrations. Because maxima concentrations are a primary concern for lake and reservoir managers, cubist modeling may help predict the largest and most noxious concentrations of cyanobacteria and their secondary metabolites.

## Introduction

Cyanobacteria are photosynthetic bacteria capable of forming large-scale harmful algal blooms (CyanoHABs) in aquatic systems. CyanoHABs are likely increasing in frequency, duration, intensity, and geographical extent worldwide (Paerl 2014; Otten & Paerl 2015). The apparent global increase in CyanoHABs has been linked to a multitude of factors including increased water temperature, longer periods of water stratification, and nutrient (N & P) and persistent organic pollutant loading (Paerl & Huisman 2008; Paerl & Otten 2013; Harris & Smith 2016).

CyanoHABs pose a serious problem for water users due to their ability to produce a suite of potent neuro- and hepatotoxins (cyanotoxins, e.g., microcystin; Otten & Paerl 2015). Cyanotoxins pose a health concern to water users due to their ability to adversely affect human health. Direct contact with blooms may cause asthma and skin irritations, whereas ingestion may cause vomiting, muscle weakness, and in rare cases death (Chorus & Bartram 1999; Otten & Paerl 2015). Additionally, CyanoHABs are the primary producers of metabolites that cause taste-and-odor (e.g., geosmin and 2- methylisoborneol) events in drinking water supply reservoirs (Jüttner & Watson 2007). Taste-and-odor metabolites impart unpalatable tastes and earthy and/or musty odors to raw drinking water supplies, which ultimately lead to increases in customer complaints to the water utilities that supply the tainted finished drinking water (Dietrich 2006). In response, water utilities must use expensive advanced treatment options (e.g., activated carbon and/or ozone) to remove cyanotoxins and taste-and-odor compounds (Dunlap et al. 2015). Thus, CyanoHABs are an increasingly expensive problem for recreational concessionaires and drinking water utilities.

Given the health hazards and taste-and-odor problems that CyanoHABs cause, there have been numerous attempts to create models that predict cyanobacteria bloom formation and their noxious secondary metabolite production. Most studies have used linear models to predict cyanobacteria and their secondary metabolites (e.g., Smith et al. 2002; Dzialowski et al. 2009; Beaulieu et al. 2014, and references therein). Recent studies have used more extensive, non-linear modeling techniques. These include neural networks (Nnet, Recknagel et al. 2006; Ahn et al. 2011; Parinet et al. 2013; Millie et al. 2014), support vector machines (SVM, Xie et al. 2012), and random forest (RF, Jacoby et al. 2015) modeling techniques, among others (e.g., mixed effect modeling, Taranu et al. 2012). Although these non-linear modeling techniques have been successful at predicting cyanobacteria and their secondary metabolites in the lakes and reservoirs studied, there are relatively few studies investigating if other non-linear modeling techniques (partial least squares, boosted tree (BT), multivariate adaptive regression splines (MARS), and cubist) can accurately predict cyanobacteria and their secondary metabolites.

Several studies have developed predictive models for cyanobacteria and their secondary metabolites in Cheney Reservoir, KS. Smith et al. (2002) were the first to create predictive models for Cheney Reservoir; using linear models, they showed that chlorophyll-*a* (Chl-*a*) was related to total phosphorus (TP), and that geosmin concentrations were related to Chl-*a*. However, only a small (n=6) amount of data were collected. Christensen et al. (2006) used a slightly larger dataset and found that the cyanobacterium *Anabaena* (currently *Dolichospermum*) and geosmin (n=16 and 18, respectively) were related to turbidity and specific conductance through ordinary least-squares linear regression modeling. Although these models initially were effective at predicting cyanobacterial related events in Cheney Reservoir, these models were not

robust over time due to the relatively small amount of data collected. In contrast to earlier studies on Cheney reservoir that found relations between environmental variables, cyanobacteria, and their secondary metabolites, more recent studies were either unable to develop significant linear regression models (Dzialowski et al. 2009) or linear regression models that explained more than 46% of the variation within the collected data (Stone et al. 2013). Given that linear models have performed poorly at predicting cyanobacteria and their secondary metabolites in Cheney Reservoir and many other lakes and reservoirs worldwide, studies investigating non-linear modeling techniques are needed to more accurately predict cyanobacteria and their secondary metabolites.

In this study, I developed and compared 12 unique linear and non-linear regression modeling techniques to predict cyanobacterial abundance and the cyanobacterial secondary metabolites microcystin and geosmin using 14 years of physiochemical water-quality data collected from Cheney Reservoir. The primary study objectives were to (1) examine the temporal trends related to cyanobacterial blooms, (2) develop and compare modeling techniques, and (3) build the best predictive models for cyanobacterial abundance, microcystin, and geosmin occurrence. The three best modeling techniques for each response variable were chosen by lowest root mean square error (RMSE) and investigated further by comparing observed and predicted values. Additionally, the most important predictor variables of each modeling technique were examined to better understand the underlying factors that cause CyanoHABs in Cheney Reservoir.

## Methods

### *Study Site*

Cheney Reservoir (W 97°50′16.11″, N 37°45′32.99″) is a large (surface area = 31 $km^2$), shallow (average depth= 6.1 m), eutrophic (average TP = 100 µg/L) impoundment located in south-central Kansas (Stone et al. 2013). The reservoir rarely thermally stratifies due to persistent winds and shallow depths. Cheney Reservoir serves as a water supply for the city of Wichita, Kansas; the reservoir supplies between 51 to 69 percent of the city's municipal water supply (Ziegler et al. 2010). The reservoir has had cyanobacteria caused taste-and-odor and toxin events since 1990 (Smith et al. 2002; Christensen et al. 2006), which have resulted in recreational advisories and increased water treatment costs.

*Sample collection and data analysis*

Since April 2001, the U.S. Geological Survey (USGS) has routinely collected discrete water-quality samples at Cheney reservoir near the dam (USGS station 07144790). All sample collection and analyses were conducted using USGS protocols as described in Stone et al. (2013). Briefly, samples were collected at bi-weekly or monthly intervals from May 2001 – June 2015. Samples were collected at the surface (0.5 m) with a Kemmerer sampler from May 2001 – July 2004; vertical integrated photic zone samples were collected from August 2004 – June 2015. No significant differences existed between surface and vertical integrated photic zone samples (Stone et al. 2013). With the exception of geosmin, microcystin, and phytoplankton, samples were analyzed by the U.S. Geological Survey National Water Quality Laboratory and the Wichita Municipal Water and Wastewater Laboratory as per Stone et al. 2013. Geosmin was analyzed using gas chromatography-mass spectrometry (GC-MS) by Engineering Performance Solutions (Zimmerman et al. 2002). Microcystin was analyzed via the congener independent enzyme-linked immunosorbent assays (ELISA) by the USGS Organic Geochemistry Research

Laboratory. Phytoplankton analyses were conducted by BSA Environmental Services, Inc. using membrane-filtered slides (McNabb 1960); a minimum of 400 natural units were counted per sample.

More than 100 physiochemical water-quality variables were measured at least once between April 2001 and June 2015 on Cheney Reservoir. All water-quality data are available through the USGS National Water Information System at *http://dx.doi.org/10.5066/F7P55KJN* and in Graham and Harris (2016). To avoid collinearity between potential explanatory variables, all explanatory variables with correlation coefficients greater than |0.75| were removed from further analyses as per Kuhn & Johnson (2013). Additionally, any potential explanatory variable with >5% of the observations missing was removed from further analyses. Response and explanatory data with concentrations less than the analytical limit of detection were substituted with a value half of the limit of detection (see Harris et al. (2016) Supplemental Table 1). Because past studies (Christensen et al. 2006; Stone et al. 2013) on Cheney reservoir have noted seasonality as a strong explanatory variable, all models used Fourier transformed variables (i.e., sin and cos) as potential explanatory variables (Helsel & Hirsch 2002); this left 24 potential explanatory variables for the models (Table 1). Additionally, because I wanted to include the effects of antecedent weather conditions on physiochemical conditions at the sampling site, reservoir elevation was used as an explanatory variable and served as surrogate for extreme precipitation events. Data for cyanobacterial abundance, microcystin, and geosmin included 185, 176, and 185 observations, respectively.

*Statistical Analyses*

To examine temporal trends in cyanobacterial abundance, microcystin, and geosmin, all discrete samples were normalized to the standard deviation of each variable, x, using the formula:

$$(x - Avg)/Stdev \quad (1)$$

where $x$ = a single discrete sample of a response variable, $Avg$ = the average of a response variable, and $Stdev$ = the standard deviation of a response variable. One-way Analysis of Variance (ANOVA) was used to compare monthly means of normalized cyanobacterial abundance, microcystin, and geosmin. If an ANOVA had a significant result ($p<0.05$), post-hoc Tukey tests were used to distinguish differences among normalized monthly means.

Predictive models were run in combination using the train function in the caret package in R (version 3.2.2) as per Kuhn & Johnson (2013, see chapter 10). Data were split into training and test datasets using the createDataPartion function in R; 75% of the response variable data was used in training. The createDataPartion function selects 75% of the data at random; the set.seed function in R was used so that the random data selection was consistent throughout the modeling procedures. Data were centered and scaled using the center and scale functions in the caret package in R prior to predictive modeling (Kuhn & Johnson 2013). Each model used repeated (repeats=5) 10-fold cross-validation using the trainControl function in R, and was tuned as per Kuhn & Johnson (2013).

Twelve different predictive models were trained using the training dataset and were compared by root mean square error (RMSE) performance on the test dataset using the resamples function in the caret package in R. Models included linear and non-linear regression models

(Table 2); this suite of models was selected because they are commonly found (e.g., ordinary linear regression, neural networks, support vector machine, random forest) or are absent from the current literature (e.g., elastic net, cubist). For each response variable (i.e., cyanobacterial abundance, microcystin, and geosmin), the lowest 3 average RMSE predictive models (Supplemental Figure 1a-c Appendix A) were compared by examining predicted and observed response variable concentrations using temporal plots created with Sigmaplot (version 11.0). Additionally, predicted and observed values were extensively compared in bivariate plots by regressing observed values on predicted data using ordinary least squares linear regression. Results from all developed models are in Supplemental Tables 1-3 Appendix A.

*Variable importance*

Variable importance for each modeling technique and the varimp function from the caret package in R are explained in (Kuhn & Johnson 2013); scale was set to "TRUE" for each varimp function used. Briefly, regardless of the modeling technique, each variable importance score represents how relatively important each explanatory variable is in predicting the response variable; each variable importance score is scaled from 0 to 100 with 100 representing the most important predictor variable.

## Results

*Temporal patterns in cyanobacterial abundance, microcystin, and geosmin*

Cyanobacterial abundance ranged from 1 to 129,836 cells/mL (median = 1861, average = 7532 cells/mL). Cyanobacterial blooms were evident in late summer from 2002-2015. Normalized cyanobacterial abundance was highest in August, September, and October (Figure

1a). May, August, September, and October had standard deviations greater than 1. March and June had the lowest normalized means and standard deviations compared to other months. Despite the apparent seasonal pattern, normalized means were not significantly different among months (ANOVA; $p = 0.39$).

Microcystin ranged from 0.1 to 9.0 µg/L (median = 0.1, average = 0.37 µg/L). Similar to cyanobacterial abundance, microcystin was also constrained to late summer. However, the highest microcystin concentrations occurred in August, whereas the highest cyanobacterial abundance concentrations occurred in September and October. Normalized microcystin was greater than the mean in only 3 of the 12 months (i.e., July, August, and September; Figure 1b), and those months also had much higher (>0.2 normalized standard deviations) variability than other months. With the exception of September, the normalized microcystin mean for August was significantly (ANOVA; $p< 0.001$) higher than all other months, and also had the largest standard deviation. Thus, the highest and most variable microcystin concentrations seemed to precede the highest and most variable cyanobacterial abundances in Cheney Reservoir.

Geosmin ranged from 1 to 113 ng/L (median = 2.5, average = 6.3 ng/L). Normalized geosmin showed more inter- and intra-annual variation than cyanobacterial abundance or microcystin (Figure 1c). Although geosmin was highest in June and July, which temporally proceeded the highest microcystin and cyanobacterial abundance concentrations, geosmin also had smaller peak concentrations in February and November. June, July, and October had standard deviations greater than 1, indicating substantial inter-annual variation. Therefore, geosmin was highly variable throughout the year but seemed to have the highest concentrations before the highest microcystin concentrations (August) and cyanobacterial abundances

(September and October). Normalized means were not significantly different among months (ANOVA; $p = 0.24$).

*Predicting cyanobacterial abundance*

Support vector machine (SVM), random forest (RF), and boosted tree (BT) models had the 3 lowest RMSE for cyanobacterial abundance (Supplemental Figure 1a Appendix1; Figure 2a-c). The SVM model had the lowest RMSE of any of the cyanobacterial abundance models, and did the best (by RMSE) on cyanobacterial abundances less than 60,000 cells/mL (Figure 3a). For abundances greater than 60,000 cells/mL, RF and BT models slightly outperformed SVM (Figures 3b and c). The top 3 models by RMSE were unable to predict the highest cyanobacterial abundances in the dataset. Although the cubist model was outperformed by multiple models on cyanobacterial abundances less than 60,000 cells/mL, the cubist model outperformed all models on cyanobacterial abundances greater than 60,000 cells/mL and had the highest $R^2$ value between predicted and observed abundances of all cyanobacteria models (Figure 3d). Additionally, the cubist model had a slope of observed vs. predicted of 0.70, whereas other models had slopes of 0.44 or less, indicating the cubist model had a more robust fit on larger cyanobacterial abundances compared to the other modeling techniques.

SVM, RF, and BT models all identified reservoir elevation and chlorophyll-*a* as relatively important predictor variables for cyanobacterial abundance (Figure 4a-c). Orthophosphate/phosphorus species, iron, temperature, and time of year (i.e., sin) were also important predictor variables to the SVM, RF, and BT models. In contrast to the SVM, RF, and BT models, cubist modeling identified specific conductance as an important predictor variable (Figure 4d). The most important variables for BT indicated that reservoir elevation and

chlorophyll-*a* had the most impact on predictive BT modeling. Variable importance plots for SVM, RF, and cubist modeling techniques had more variables with scaled scores >40 compared to BT, indicating that BT model performance is influenced by a smaller number of predictors than the other modeling approaches.

*Predicting microcystin*

Similar to cyanobacterial abundance, the SVM model for microcystin had the lowest RMSE compared to the other models (Supplemental Figure 1b Appendix A; Figure 5a). Cubist and BT models also had relatively low RMSE for predicting microcystin (Figures 5b and c). The cubist model outperformed SVM and BT models on the two largest microcystin concentrations in the dataset and explained nearly double the variance compared to the SVM and BT models in bivariate plots of predicted and observed concentrations (Figure 6a-c). Yet, none of the top three models predicted microcystin concentrations greater than 6 µg/L. With the exception of one predicted microcystin concentration of 6.1 µg/L by neural network modeling (observed concentration = 7.3 µg/L; Supplemental Table 2 Appendix A), no other modeling technique predicted microcystin concentrations greater than 2.5 µg/L, irrespective of the observed concentration.

The SVM, cubist, and BT models showed that temperature and time of year (i.e., sin) were the most important variables for predicting microcystin concentrations (Figure 7a-c). Chlorophyll-*a*, iron, and dissolved oxygen also were important predictor variables for microcystin. Cubist and BT plots had fewer variables with scaled scores >40 compared to SVM, indicating that temperature, sin, and chlorophyll-*a* variables had a substantially larger impact on modeling efforts compared to other predictor variables.

*Predicting geosmin*

RF, SVM, and BT had the 3 lowest RMSE values for predicting geosmin (Supplemental Figure 1c Appendix A; Figure 8a-c). When observed and predicted geosmin concentrations were compared, the RF model outperformed the SVM and BT models by RMSE overall, and especially on geosmin concentrations larger than 20 ng/L (Figure 8a and 9a-c). However, similar to the cyanobacterial abundance and microcystin models, the cubist model outperformed all models on the highest concentrations in the dataset (i.e., geosmin concentrations greater than 20 ng/L; Figure 9d). Although the cubist model had a lower $R^2$ than the RF model, the slope of the cubist model was much closer to 1 (0.85 compared to 0.45, respectively), indicating a more robust fit when geosmin concentrations exceeded 20 ng/L. Additionally, the cubist model for geosmin was the only model developed to accurately predict the highest concentration of a response variable (Figure 9d). The cubist model indicated that when turbidity was > 22.2 FNU and silica was < 10.4 mg/L, maxima geosmin concentrations could be accurately predicted using chlorophyll-*a* as an explanatory variable (data not shown).

The RF model identified suspended sediment, nitrate plus nitrite, and time of year (i.e., cos) as the 3 most important predictor variables. The SVM and BT models also identified light (i.e., suspended sediment concentration), nitrogen species and/or ratios (i.e., total Kjeldahl nitrogen, $NO_3$:$NH_3$ ratio), and chlorophyll-*a* as important variables for geosmin prediction. In contrast to the other modeling techniques, the cubist model used substantially fewer explanatory variables and identified silica as the most important predictor variable (Figure 10a-d). Similar to microcystin variable importance scores, SVM, BT, and cubist plots had few variables with scaled scores >40, indicating that TKN, cos, and silica, respectively, had a substantial impact on

predicting geosmin whereas RF modeling used substantially more explanatory variables within the model.

**Discussion**

Overall, predictive models for cyanobacterial abundance, microcystin, and geosmin performed poorly at predicting maxima concentrations in Cheney Reservoir. With the exception of the geosmin cubist model, no model predicted the highest 3% (i.e., maxima) of cyanobacterial abundance, microcystin, or geosmin concentrations in the dataset. There are several probable reasons for the underestimation of maxima concentrations by developed models. The most important variables (i.e., reservoir elevation, nutrient concentrations, temperature; Figure 4a-d) for cyanobacterial prediction exhibited seasonal patterns throughout the study period, whereas maxima cyanobacterial abundances during 2002-2015 were not constrained to a specific season (Figure 1a). There were substantial intra- and inter-annual variations in maximum cyanobacterial abundance, timing, and dominant taxa during blooms. The underperformance of models for maxima cyanobacterial abundance likely indicates that models attempting to predict cyanobacteria using seasonally-changing variables could not differentiate bloom forming conditions between seasons and/or years because cyanobacterial blooms occurred across a wide range of environmental conditions.

Although microcystin exhibited a clear seasonal pattern (Figure 1b), no modeling technique predicted maxima concentrations. All modeling techniques recognized the seasonal pattern (i.e., by temperature and sin predictor variables; Figure 7a-c) and accurately predicted that microcystin concentrations would occur, but no modeling approach discerned substantial inter-annual differences in the magnitude of late summer (July through September) microcystin

concentrations. *Microcystis* is likely the dominant microcystin producer in Cheney Reservoir (Otten et al. 2016), and observed inter-annual variation in microcystin concentrations are likely linked to the overall abundance of *Microcystis*. Additionally, each bloom, including those dominated by *Microcystis*, is unique in (i) the percentage of cells capable of producing microcystin and (ii) the amount of microcystin produced per cell (i.e., cell quota; Pearson et al. 2016). Thus, although the maxima microcystin concentrations were confined to late summer, not all late summer cyanobacterial blooms were capable of producing microcystin, which in turn caused predictive models to underperform on maxima concentrations.

Similar to cyanobacterial abundance, geosmin exhibited substantial intra- and inter-annual variation (Figure 1c), which caused all modeling techniques except cubist to underestimate the highest concentrations observed in the dataset. In contrast to all other modeling approaches, cubist modeling recognized that when turbidity was relatively high (>22.2 NTU) and silica was relatively low (<10.43 mg/L of $SiO_2$), the highest concentrations were related to the chlorophyll-*a* concentration. The recognition of this pattern by cubist was likely due to its unique modeling structure. Similar to other tree-based modeling approaches, cubist models are constructed by creating a set of rules that split the data at terminal nodes; each of these nodes uses a linear equation to predict response variables. Cubist modeling differs from other approaches because it uses (i) a unique smoothing process for linear models created at each terminal node, (ii) a boosting-like procedure called committees, and (iii) finalized committees that are adjusted to increase prediction performance using a K nearest neighbors-like procedure (further details on cubist modeling construction and procedures can be found in Kuhn and Johnson 2013). Overall, these differences allowed the cubist modeling technique to be effective

at predicting maxima geosmin concentrations compared to other modeling attempts; however, the reason the cubist modeling technique could not accurately predict cyanobacterial abundance or microcystin concentration maxima is unknown. Therefore, although the cubist modeling technique should be attempted in a variety of systems before widespread implementation, it has the potential to improve regression modeling efforts aimed at predicting maxima cyanobacterial metabolites.

In the earliest attempt to model cyanobacteria or their metabolites, Smith et al. (2002) found that geosmin concentration was linearly related to water-column chlorophyll-*a* concentrations in a small (n=6) dataset, and explained 72% of the variation within the collected data. Christensen et al. (2006) was able develop a linear model to predict geosmin concentrations; log transformed geosmin was related to log transformed turbidity and specific conductance and explained 71% of the variation in the data. Although the studies cited above explained more than 70% of the variation in geosmin concentrations with linear models, Dzialowski et al. (2009) was unable to develop any significant regression models for geosmin in Cheney Reservoir. Additionally, Stone et al. (2013) was only able to explain 21% of the variation in geosmin concentrations with a linear model based on turbidity and pH; a linear model for microcystin was only able to explain 46% of the variation in the data for Cheney Reservoir. Otten et al. (2016) also attempted linear regression models for cyanobacterial secondary metabolites, and found that a model with 4 explanatory variables was only able to explain 51% of the variation in geosmin concentrations in 2013 and 2014. In contrast, a microcystin model was able to explain 82% of the variation; however, the y-intercept in the model was -17 µg/L indicating poor predictive power. This study also attempted linear

regression models and found results consistent with Dzialowski et al. (2009) and Stone et al. (2013). It should be noted that the $R^2$ of prior studies were likely overly optimistic because $R^2$ values were based on the computed fit model, whereas in my study computed $R^2$ values were only on the validation (test) dataset.

The range in predictive ability of, and explanatory variables in, developed models over time is likely because of several reasons. The earliest models developed (e.g., Smith et al. 2002; Christensen et al. 2006) used relatively small datasets, and data were primarily collected in spring and summer months. Because data were collected over a relatively small period of time and were constrained to relatively warm months, these datasets did not capture the full spectrum of intra- and inter-annual variability within the reservoir. Thus, these models were not robust over time, and also indicate the importance of using long-term datasets that capture multi-year variability within explanatory and response variables when developing predictive models. Second, climate change has caused environmental variability to increase over time throughout the Midwestern U.S., which also may affect model outcomes (Committee on Extreme Weather Events and Climate Change Attribution et al. 2016; discussed further below, but see http://www.ncdc.noaa.gov/extremes/cei/graph/wn/4/04-09 for data and graphics on increases in the frequency of extremes in 1-day precipitation for the Midwestern U.S. from 1910-2015). Thus, results of predictive modeling efforts have likely been so varied in Cheney Reservoir because (i) earlier models did not successfully capture the environmental variation occurring in the reservoir and (ii) environmental variability caused by climate change is likely increasing, and may pose challenges to developing predictive models.

*Effects of climate change on predictive models*

Predictive models rely on the recognition of pattern-based processes; however, these processes have been and will continue to be altered by climate change. As recently shown by the Committee on Extreme Weather Events and Climate Change Attribution et al. (2016), climate change will likely cause more frequent extreme heat and rainfall events, droughts, and severe storms, resulting in ever more extreme environmental variability. These climate change driven patterns have also been recognized to make existing models less predictive over time, which led Gail (2016) to term the upcoming era the "dark age" of predictive modeling. These extreme events have also been shown to stimulate cyanobacterial blooms in temperate, subtropical, and tropical regions of the world (Kosten et al. 2012; Jeppesen et al. 2015; Brasil et al. 2016). In Cheney Reservoir, several extreme weather events, possibly exacerbated by climate change, seemed to fuel cyanobacterial blooms and relatively high secondary metabolite concentrations while potentially reducing the predictive ability of models on maxima concentrations. For example, droughts from August 2006-April 2007 and August 2011-July 2013 caused extremes in reservoir elevation (Supplemental Figure 2 Appendix A) and reduced the mean depth of the reservoir by 1 and 2.5 meters, respectively. In both cases, lowered reservoir mean depth created conditions that resulted in the highest annual cyanobacterial abundances (*Microcystis* dominated) and microcystin concentrations observed in the reservoir (Figures 2a-c and 5a-c).

Extreme rainfall events have also led to cyanobacterial related events in Cheney Reservoir. Following a severe drought from 2011-July 2013, a heavy precipitation event in the watershed caused the 9th largest sediment inflow event in the history of the reservoir (Supplemental Figure 2 Appendix A; Stone et al. 2015). This large inflow event stimulated an *Anabaena* bloom that caused geosmin concentrations to exceed 50 ng/L (Otten et al. 2016). The

results are consistent with Reichwaldt & Ghadouani (2012), who hypothesized that heavy rainfall events may cause sharp increases in cyanobacterial metabolites. Although my predictive models recognized that reservoir elevation (cyanobacterial abundance and geosmin, Figures 4a-d and 10d), and temperature (microcystin, Figures 7a-c) were important explanatory variables for cyanobacteria and secondary metabolite prediction in Cheney reservoir, drought and extreme rainfall events likely caused predictive models to underestimate observed maxima cyanobacteria and secondary metabolite concentrations. If the frequency of extreme drought and inflow events continue to increase, I hypothesize that larger, more frequent cyanobacterial blooms will occur in the future. Consequently, pattern-reliant modeling approaches for cyanobacterial abundance, microcystin, and geosmin based on historical datasets may not be robust over time (Gail, 2016).

*Important explanatory variables for predicting cyanobacteria and cyanobacterial metabolites*

Explanatory variables identified as important in Cheney Reservoir were consistent with what has been shown to be predictive of cyanobacteria in other studies. Similar to Cheney Reservoir, Taranu et al. (2012) found that water column TP, water temperature, and seasonality (similar to the sin and cos predictor variables) were predictive of cyanobacterial blooms in polymictic Canadian lakes, indicating that these factors likely promote cyanobacterial blooms in well-mixed systems regardless of waterbody location (Figure 4a-d). Beaulieu et al. (2013) and Millie et al. (2014) found that water column nutrient concentrations (TN and TP), water temperature, and chlorophyll were important factors in predicting cyanobacteria in 1147 US lakes and Lake Erie, USA, respectively. In contrast, few studies aimed at predicting cyanobacterial blooms in the current literature identified reservoir elevation as an important predictor variable (but see Francy et al. 2016; Figure 4a-d). Although not examined here, water

column stability and spring phosphorus loads were also identified as an important explanatory variables for predicting cyanobacterial blooms (Wagner & Adrian 2009; Millie et al. 2014; Bertani et al. 2016). Although Cheney Reservoir rarely stratifies, water column stability could be evaluated in future studies as changing environmental conditions may change patterns of stratification in the reservoir. Additionally, including spring phosphorus loading in predictive models was shown to substantially increase forecast accuracy of summer cyanobacterial blooms in Lake Erie (Bertani et al. 2016). Because spring nutrients loads are increased by heavy rainfall events within the lake watershed, and large inflow events seem to stimulate cyanobacterial related events in Cheney Reservoir (Otten et al. 2016), future studies on Cheney Reservoir may improve predictive models by including spring nutrient loading. Therefore, variables like reservoir elevation, water column stability, and spring nutrient loading may be important variables to consider in addition to water column nutrient concentrations and water temperature for cyanobacterial prediction in drinking water reservoirs.

Water temperature, nutrients, and nutrient ratios have been shown to be predictive of microcystin and geosmin concentrations. For instance, multiple studies have found that elevated water temperatures are predictive of, and favor, elevated microcystin concentrations (Davis et al. 2009; Joung et al. 2011; Dziallas & Grossart 2011; Beaver et al. 2014). Additionally, Jacoby et al. (2015) and Dzialowski et al. (2009) found relatively low TN:TP ratios were predictive of microcystin and geosmin concentrations, respectively, in Northwestern and Midwestern USA reservoirs, respectively. In contrast to other studies in the literature, accurate prediction of maxima geosmin concentrations depended on silica and turbidity (i.e. light environment) in Cheney Reservoir (Figure 10a, b, and d). Although Christensen et al. (2006) showed that

turbidity was a strong predictor variable for geosmin concentrations in Cheney Reservoir, few other studies have indicated that silica or turbidity are important predictors for geosmin. Thus, while some factors (e.g., nutrients, chlorophyll-*a*, and water temperature) identified here seem to be predictive of cyanobacteria and their metabolites throughout North America, including Cheney Reservoir, other factors (e.g., reservoir elevation, suspended solid concentration/turbidity, and silica; Figures 4a-d, 7a-c, and 10a-d) may be specific to Cheney Reservoir.

Given that climate change likely will cause predictive models developed using historical datasets to underperform, further research using recent analytical and technological advances are needed to accurately predict cyanobacterial blooms. For example, the incorporation of analytical techniques such as quantitative polymerase chain reaction (qPCR) have been shown to significantly improve data inputs for cyanobacterial abundance and cyanobacterial secondary metabolite models (Francy et al. 2016; Otten et al. 2016). Advances in sensor technology could also improve modeling efforts. Specifically, sensors that can accurately measure cyanobacteria and other algal taxa in real-time will help reservoir managers better understand the factors that lead to cyanobacterial or other harmful algal blooms, and will allow for real-time prediction of events. Additionally, real-time measurement of nitrogen species and phosphorus concentrations will allow for (i) more frequent measurements of nutrient concentrations and (ii) the development of real-time cyanobacterial management, which will ultimately lessen the reliance on predictive modeling. Programs currently using discrete sampling to monitor cyanobacteria and their metabolites may need to consider continuous real-time monitoring using new advanced sensor technologies to provide public and private stakeholders more accurate predictions of

cyanobacterial related events. Therefore, although we may be entering a “dark age” (sensu Gail 2016) of predicting environmental biotic variables, emerging analytical and technological advances could be used to combat historical pattern-based information made erroneous by climate change.

## Figures and Figure legends

**Figure 1.** Normalized temporal trends of cyanobacterial abundance (a), microcystin (b), and geosmin (c) concentrations. Each datapoint represents the average normalized value of observations collected from a specific month from 2002 to 2015. Error bars represent standard deviations from the normalized mean.

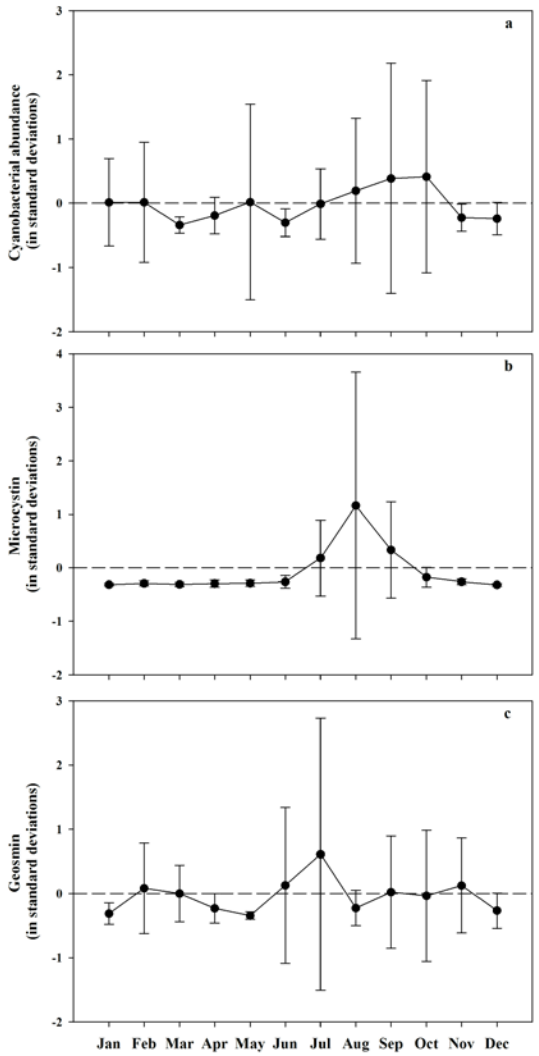


***Figure 1-1***

**Figure 2.** Observed (dots) and predicted (line) cyanobacterial abundance from 2002 to 2015 in Cheney Reservoir using Support Vector Machine (SVM;a), Random Forest (RF;b), and Boosted Tree (BT;c) modeling approaches.

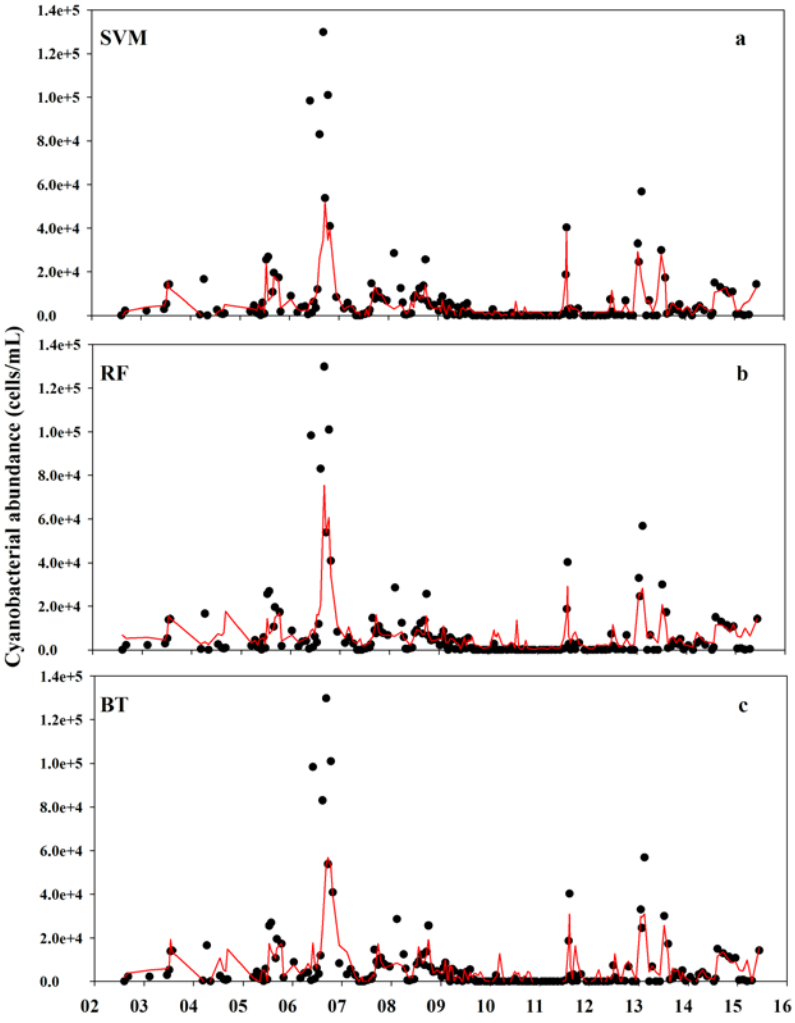


***Figure 1-2***

**Figure 3.** Observed compared to predicted cyanobacterial abundance using Support Vector Machine (SVM;a), Random Forest (RF;b), Boosted Tree (BT;c), and Cubist (d) modeling approaches. Solid line represents linear regression line, dashed lines represent 95% prediction intervals, and dotted line represents 1:1 line.

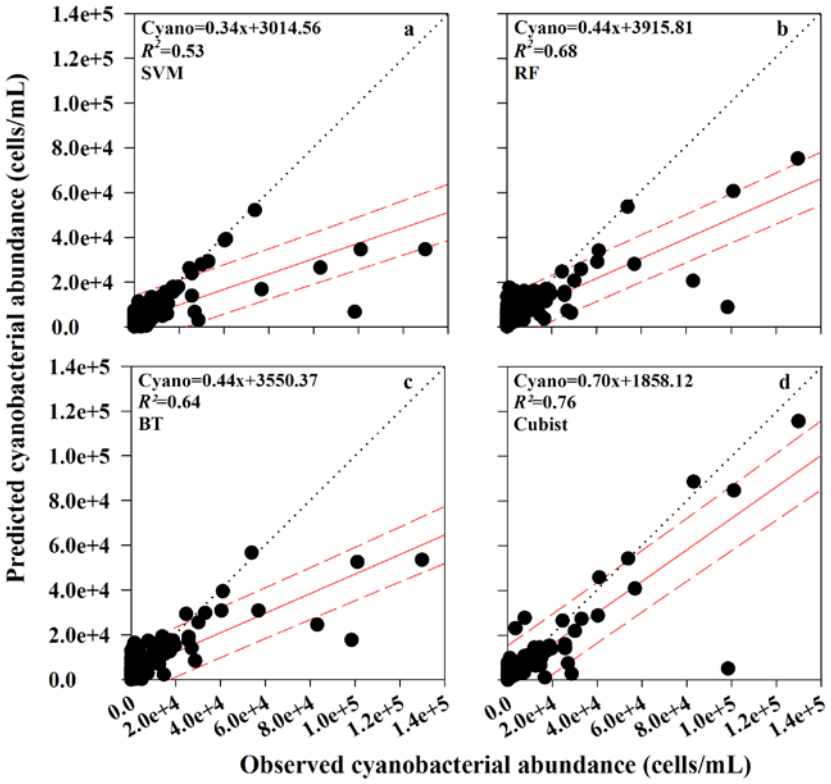


***Figure 1-3***

**Figure 4.** Top 20 most important variables for predicting cyanobacterial abundance for Support Vector Machine (SVM;a), Random Forest (RF;b), Boosted Tree (BT;c), and Cubist (d) modeling approaches.

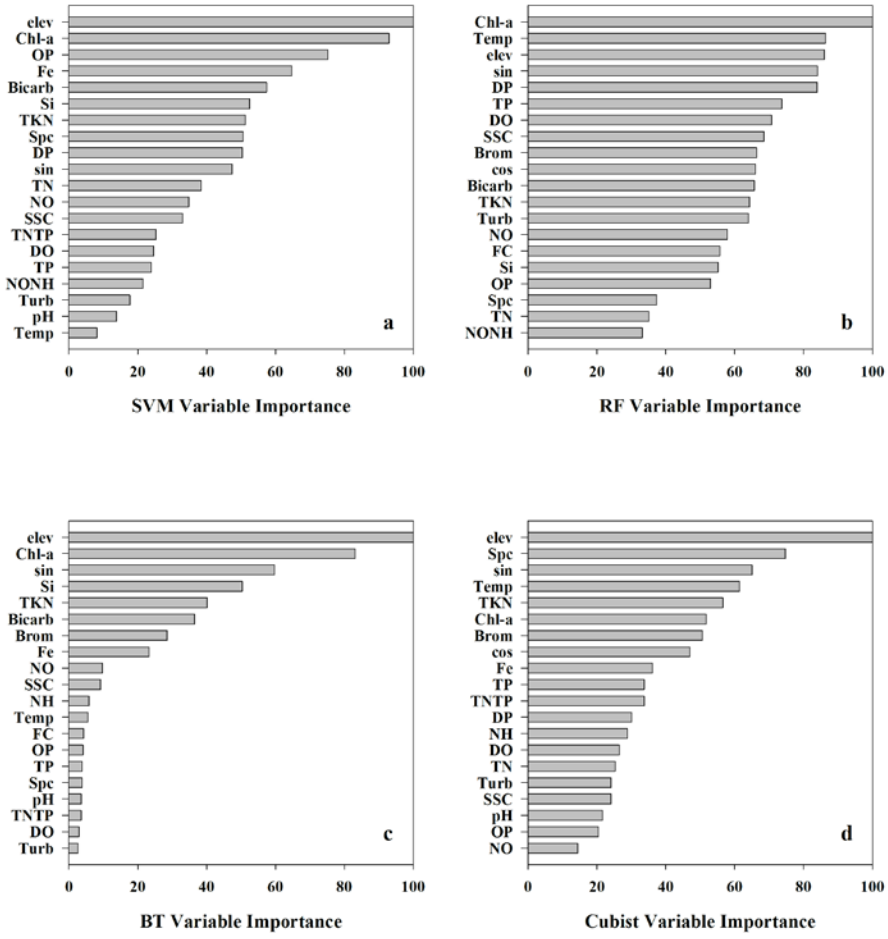


***Figure 1-4***

**Figure 5.** Observed (dots) and predicted (line) microcystin concentration from 2002 to 2015 in Cheney Reservoir using Support Vector Machine (SVM;a), Cubist (b), and Boosted Tree (BT;c) modeling approaches.

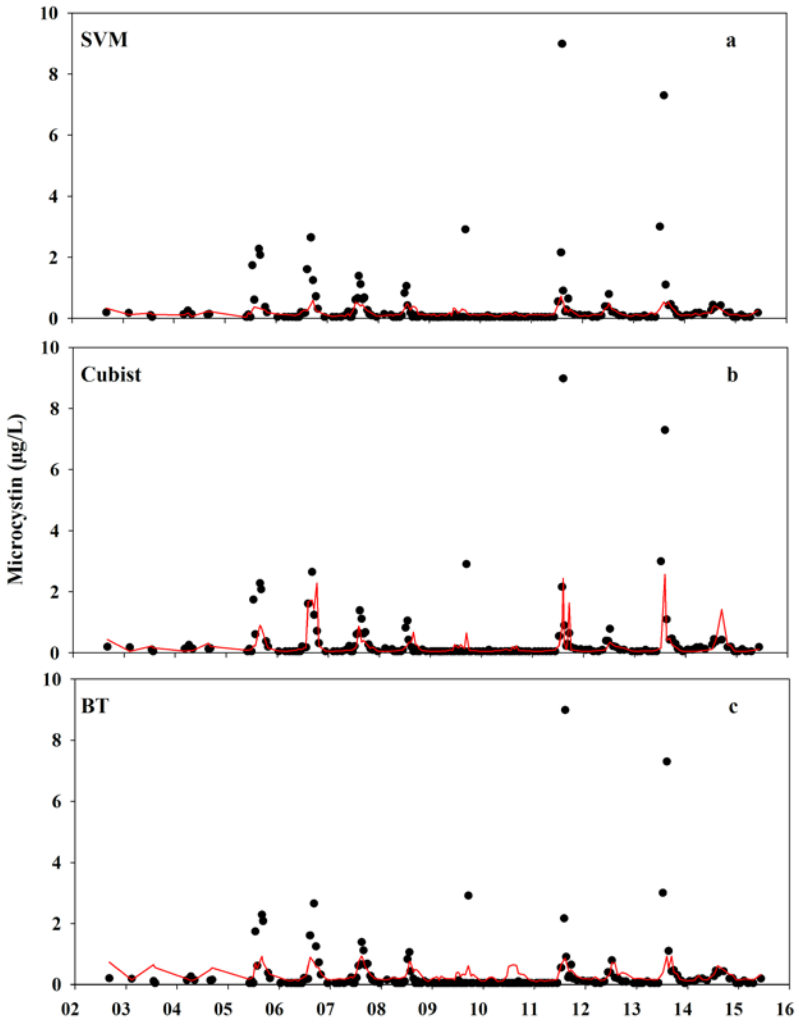


***Figure 1-5***

**Figure 6.** Observed compared to predicted microcystin concentration using Support Vector Machine (SVM;a), Cubist (b), and Boosted Tree (BT;c) modeling approaches. Solid line represents linear regression line, dashed lines represent 95% prediction intervals, and dotted line represents 1:1 line.

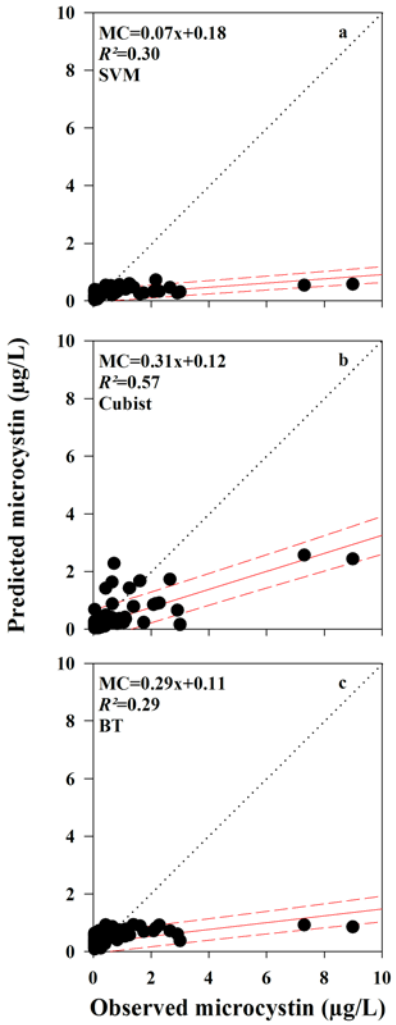


***Figure 1-6***

**Figure 7.** Top 20 (where applicable) most important variables for predicting microcystin concentration for Support Vector Machine (SVM;a), Cubist (b), and Boosted Tree (BT;c) modeling approaches.

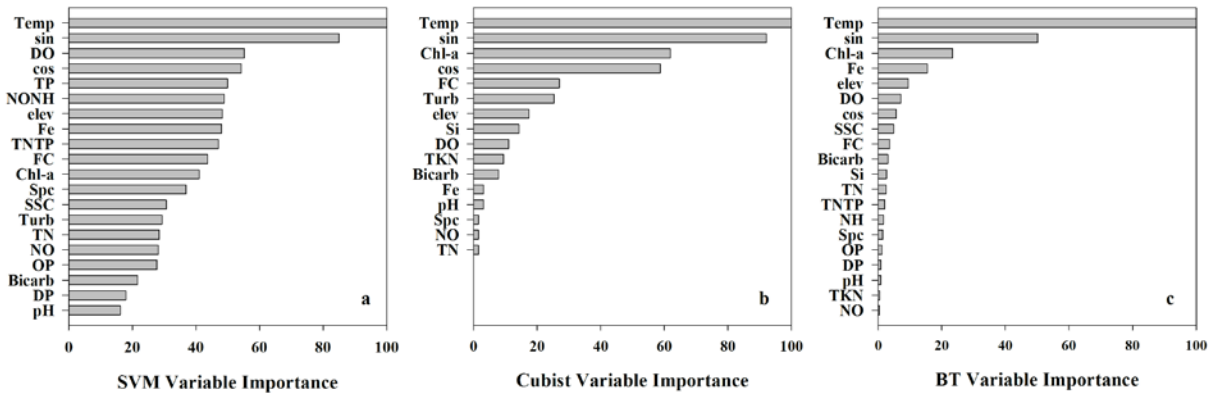


***Figure 1-7***

**Figure 8.** Observed (dots) and predicted (line) geosmin concentration from 2002 to 2015 in Cheney Reservoir using Random Forest (RF;a), Support Vector Machine (SVM;b), and Boosted Tree (BT;c) modeling approaches.

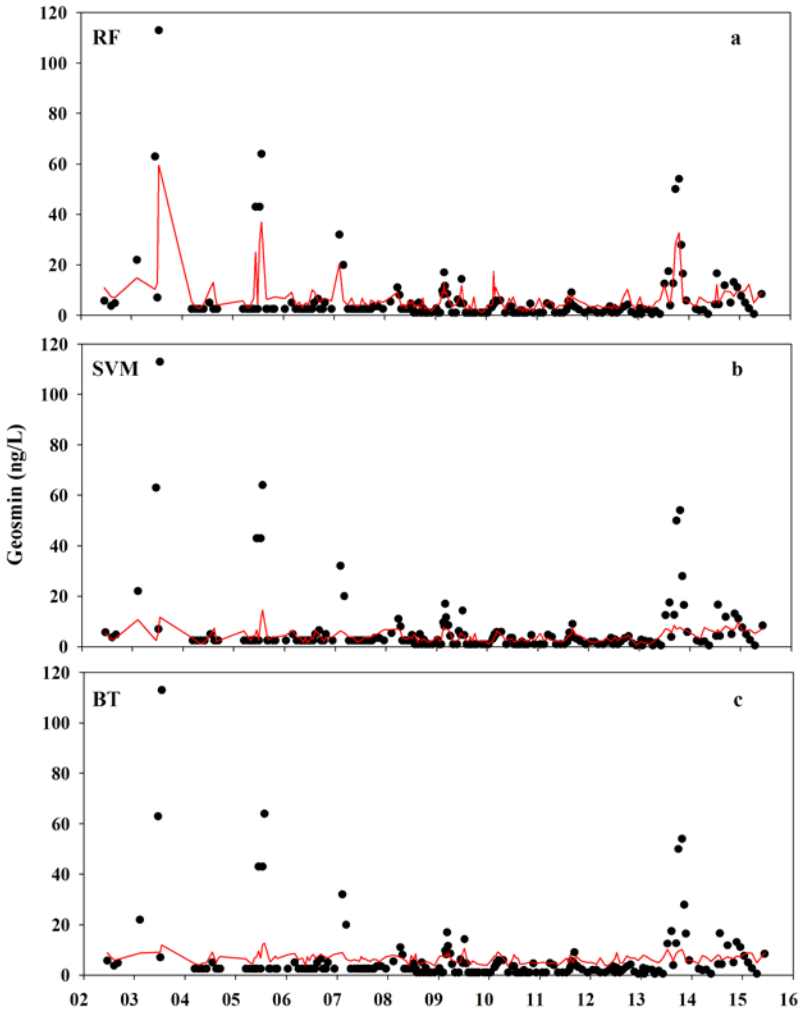


***Figure 1-8***

**Figure 9.** Observed compared to predicted geosmin concentration using Random Forest (RF;a), Support Vector Machine (SVM;b), Boosted Tree (BT;c), and Cubist (d) modeling approaches. Solid line represents linear regression line, dashed lines represent 95% prediction intervals, and dotted line represents 1:1 line.

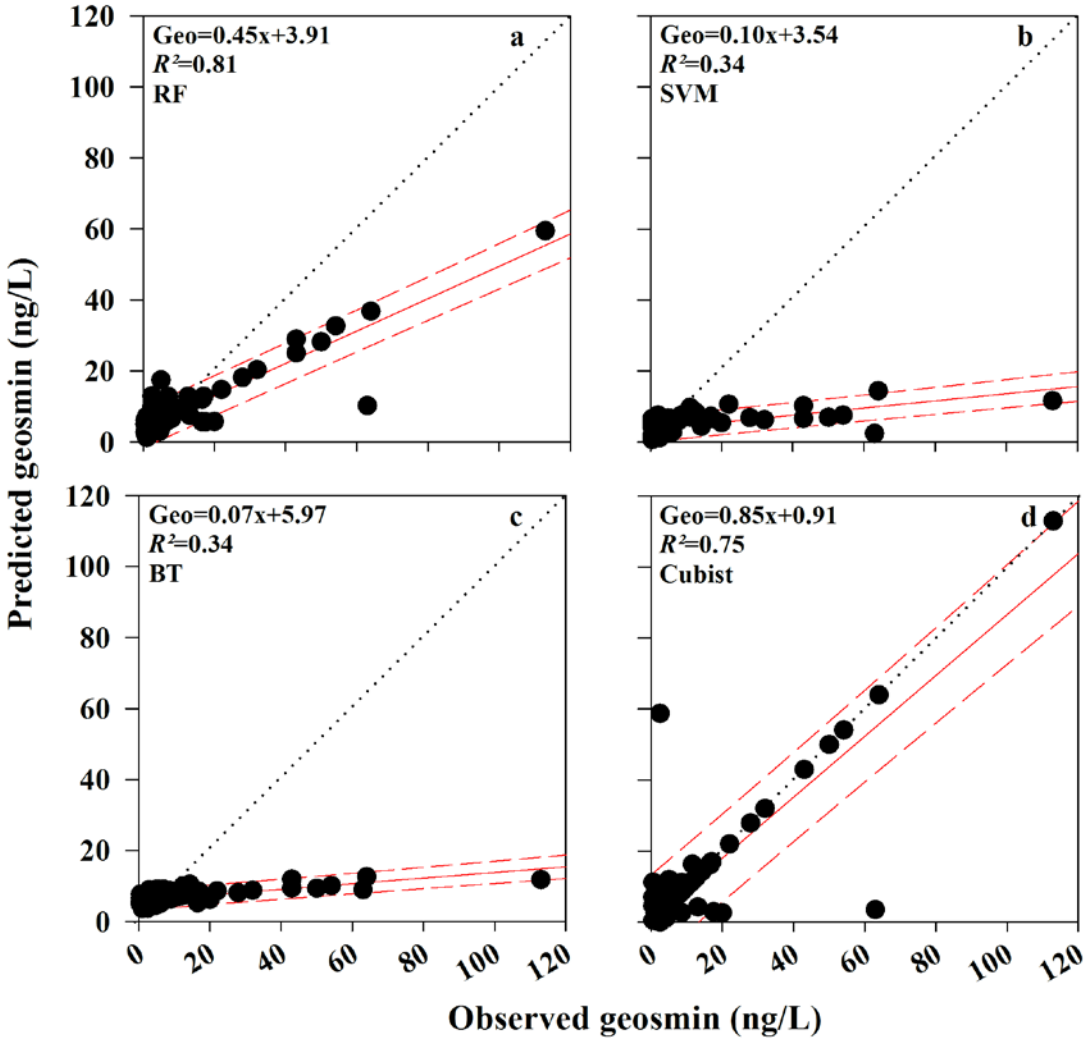


***Figure 1-9***

**Figure 10.** Top 20 (where applicable) most important variables for predicting geosmin concentration for Random Forest (RF;a), Support Vector Machine (SVM;b), Boosted Tree (BT;c), and Cubist (d ) modeling approaches.

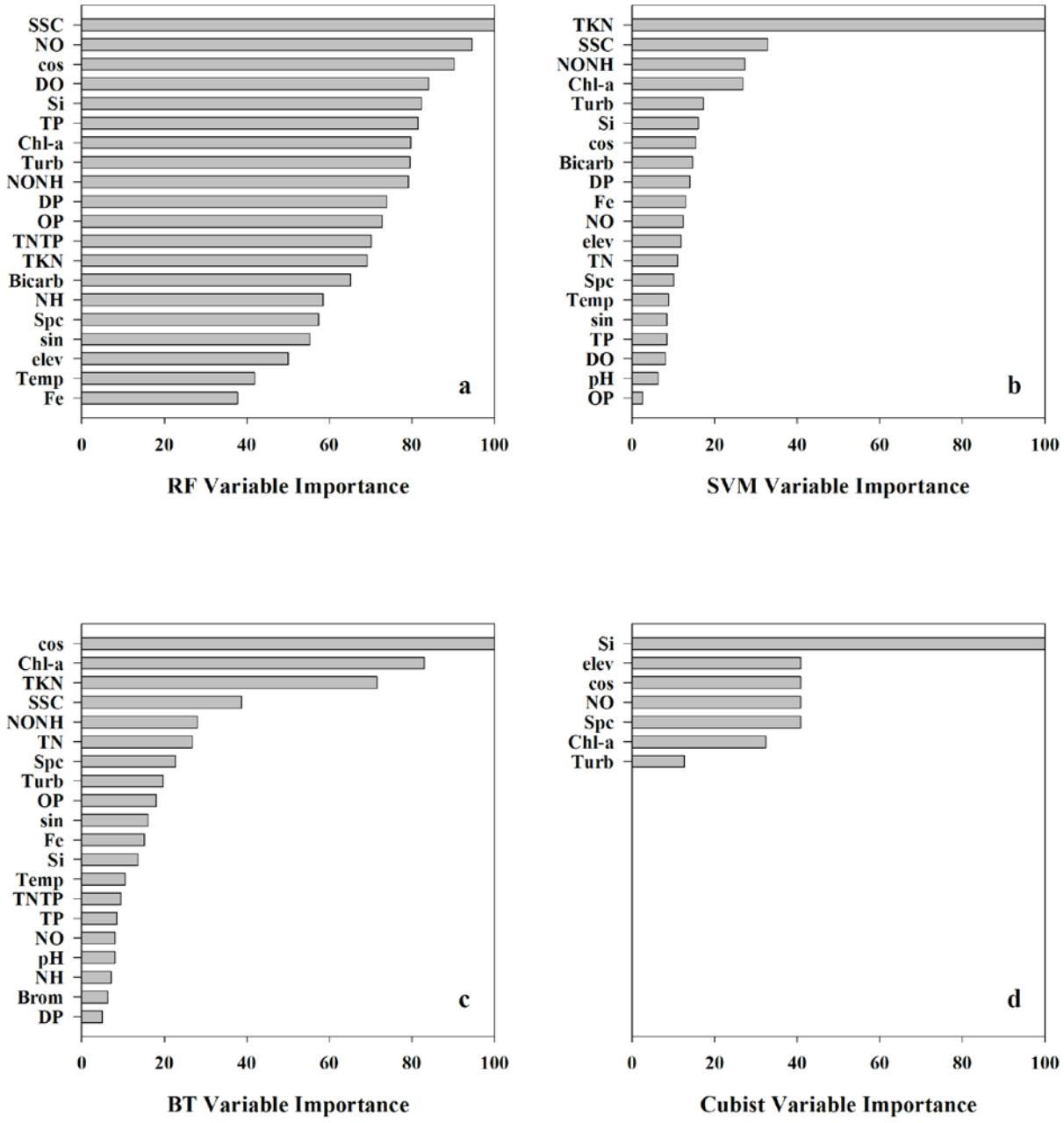


***Figure 1-10***

## Tables

**Table 1.** List of variables used in model development. With the exception of the three response variables (cyanobacterial abundance, microcystin, and geosmin), all variables were used as explanatory variables. P-code represents US Geological Survey parameter code for the explanatory variable. NGVD 1929= National Geodetic Vertical Datum of 1929.

| Variable | Abbreviation | Units | P-code |
|---|---|---|---|
| Fourier transformed date | sin | unitless | - |
| Fourier transformed date | cos | unitless | - |
| Dissolved oxygen | DO | mg/L | P00300 |
| Reservoir surface elevation above NGVD 1929 | elev | ft | P62614 |
| pH | pH | unitless | P00400 |
| Specific conductance | Spc | µS/cm | P00095 |
| Temperature | Temp | °C | P00010 |
| Turbidity | Turb | FNU | P63680 |
| Bicarbonate | Bicarb | mg/L | P29806 |
| Bromide | Brom | mg/L | P71870 |
| Silica | Si | mg/L as $SiO_2$ | P00956 |
| Total kjeldahl nitrogen | TKN | mg/L as N | P00625 |
| Ammonia | NH | mg/L as N | P00608 |
| Nitrate plus nitrite | NO | mg/L as N | P00631 |
| Orthophosphate | OP | mg/L as P | P00671 |
| Dissolved phosphorus | DP | mg/L as P | P00666 |
| Total Phosphorus | TP | mg/L as P | P00665 |
| Total Nitrogen | TN | mg/L as N | P00600 |
| Fecal coliforms | FC | colonies per 100 mL | P31625 |
| Chlorophyll a | Chl | µg/L | P70953 |
| Iron | Fe | µg/L | P01045 |
| Suspended sediment concentration | SSC | mg/L | P80154 |
| Total nitrogen to total phosphorus ratio | TNTP | unitless | - |
| Nitrate plus nitrite to ammonia ratio | NONH | unitless | - |
| Cyanobacterial abundance | Cyano | cells/mL | - |
| Microcystin | MC | µg/L | P65210 |
| Geosmin | Geo | ng/L | P51285 |

***Table 1-1***

**Table 2.** List of compared modeling approaches and their abbreviation (Abbrev.)

| Model | Abbrev. |
|---|---|
| Ordinary Linear Regression | Linear |
| Partial Least Squares | PLS |
| Elastic Net | Enet |
| Neural Networks | Nnet |
| Multivariate Adaptive Regression Splines | MARS |
| Support Vector Machines | SVM |
| Single Trees | CART |
| Bagged Trees | BagT |
| Boosted Trees | BT |
| Conditional Inference Trees | CI Tree |
| Random Forest | RF |
| Cubist | - |

***Table 1-2***

# Chapter 2*

Combined effects of nitrogen to phosphorus and nitrate to ammonia ratios on cyanobacterial metabolite concentrations in eutrophic Midwestern USA reservoirs

*Harris, T.D., Smith, V.H., Graham, J.L., Van de Waal, D.B., Tedesco, L.P., Clercin, N. 2016. Combined effects of nitrogen to phosphorus and nitrate to ammonia ratios on cyanobacterial metabolite concentrations in eutrophic Midwestern USA reservoirs. Inland Waters. 6:199-210.

**Abstract**

Recent studies have shown that the total nitrogen to total phosphorus (TN:TP) ratio and nitrogen oxidation state may have substantial effects on secondary metabolite (e.g., microcystins) production in cyanobacteria. I investigated the relationship between the water column TN:TP ratio and the cyanobacterial secondary metabolites geosmin, 2-methylisoborneol (MIB), and microcystin using multiple years of data from 4 reservoirs located in the Midwestern United States. I also examined the relationship between water column concentrations of chemically oxidized ($NO_3$) and reduced ($NH_3$) nitrogen, the $NO_3$:$NH_3$ ratio, cyanobacterial biovolume, and associated secondary metabolites. I found that the cyanobacterial secondary metabolites geosmin, MIB, and microcystin primarily occurred when the TN:TP ratio was <30:1 (by mass), likely due to higher cyanobacterial biovolumes at lower TN:TP ratios. I also found that relative cyanobacterial biovolume was inversely related to the $NO_3$:$NH_3$ ratio. Both $N_2$-and non-$N_2$-fixing cyanobacteria seemed to produce secondary metabolites and had higher concentrations per unit biovolume when $NO_3$:$NH_3$ ratios were relatively low. The data thus are consistent with the hypothesis that lower TN:TP ratios favor cyanobacterial dominance and also suggest that relatively low $NO_3$:$NH_3$ ratios provide conditions that may favor the production of cyanobacterial secondary metabolites. The data further suggest that increases in the absolute concentrations of TP or $NH_3$ (or both), causing decreases in TN:TP and $NO_3$:$NH_3$ ratios, respectively, may stimulate cyanobacteria having the metabolic ability to produce geosmin, MIB, or microcystins. Future studies should address how the $NO_3$:$NH_3$ ratio affects phytoplankton community structure and occurrence and production of cyanobacterial secondary metabolites.

## Introduction

Cyanobacterial blooms are increasing globally because of multiple factors, including nutrient enrichment, increased surface water temperature, persistent organic pollutant loading, and longer water residence and stratification times (Paerl et al. 2011, Schindler 2012, Paerl and Otten 2013, Harris and Smith 2016). In conjunction with increases in the frequency and intensity of cyanobacterial blooms, the occurrence of associated toxic and nuisance secondary metabolites also seems to be increasing worldwide (Winter et al. 2011). These cyanobacterial secondary metabolites include potent toxins (e.g., microcystins) capable of poisoning terrestrial and aquatic organisms (Stewart et al. 2008, Wood et al. 2010, Lurling and Faassen 2013) as well as compounds that cause taste and odor problems in drinking water supplies, such as geosmin and 2-methylisoborneol (MIB; Graham et al. 2010).

The concentrations of cyanobacterial secondary metabolites in the water column depend on (1) the abundance of secondary metabolite-producing species and strains, and (2) the quantity of secondary metabolites produced by the cells. In general, cyanobacterial abundance is considered to be in part dependent on the total nitrogen to total phosphorus (TN:TP) ratio, with dominance by cyanobacteria often greatest when the TN:TP ratio is low (<29:1 by mass; Smith 1983, Graham et al. 2004). In eutrophic systems where nutrients are not limiting, however, the TN:TP ratio is less predictive of cyanobacterial dominance (Paerl and Fulton 2006); thus, the absolute magnitude of nutrient supply rates and nutrient supply ratios are both important determinants of phytoplankton community structure.

Recent research also has shown that nitrogen forms (nitrate, ammonium, or urea) may influence the abundance of cyanobacteria capable of producing nitrogen-rich microcystins (for

simplicity, all microcystin structural variants will be referred to here as microcystin; Finlay et al. 2010, Donald et al. 2011, Monchamp et al. 2014, Beversdorf et al. 2015). Whether the oxidation state of nitrogen has an effect on carbon-rich cyanobacterial secondary metabolites such as geosmin and MIB has rarely been examined in empirical or experimental studies. Because the chemical form of inorganic nitrogen (i.e., oxidized nitrogen [$NO_3$] vs. reduced nitrogen [$NH_3$]) has different cellular energetic costs and assimilation rates for different phytoplankton taxa (Flores and Herrero 2005), the proportion of oxidized to reduced nitrogen (the $NO_3$:$NH_3$ ratio) may cause differences in phytoplankton community structure (Riegman et al. 1992, Hallegraeff 1993, Riegman 1995, McCarthy et al. 2009, Glibert et al. 2016). Such cellular preferences for nitrogen oxidation state thus may in turn influence the abundance of cyanobacteria capable of producing toxic and nuisance secondary metabolites.

The synthesis of secondary metabolites by primary producers in terrestrial, aquatic, and arid desert ecosystems has been consistently shown to depend on the relative proportions of carbon and nutrients (Downing et al. 2005, Reich et al. 2006, Van de Waal et al. 2009, 2014, Downing et al. 2015). The regulation of carbon- and nitrogen-rich toxic secondary metabolites in phytoplankton was shown to depend on water column nutrient proportions (Granéli and Flynn 2006, Van de Waal et al. 2014, Beversdorf et al. 2015). More specifically, nitrogen limitation caused a decrease in the synthesis of nitrogen-rich toxins (including microcystin), and the production of these toxins was observed to increase with increases in intracellular N:P ratios. Synthesis of carbon-rich toxins increased under both nitrogen and phosphorus limitation and showed a V-shaped pattern in response to cellular N:P ratios (Granéli et al. 1998, Granéli and Flynn 2006, Van de Waal et al. 2014). Thus, the synthesis of secondary metabolites that contain

nitrogen seems to be favored when nitrogen availability is relatively high, whereas the synthesis of carbon-rich secondary metabolites is favored when the availability of nitrogen is relatively low (i.e., when carbon availability is high). Little is known, however, about the impact of chemical nitrogen forms on the synthesis of nitrogen- and carbon-rich secondary metabolites, particularly in cyanobacteria.

The ratio of oxidized nitrogen (nitrate and nitrite) to reduced nitrogen (ammonia and ammonium) has important implications for the growth rate, mineral composition, and production of carbon-rich organic compounds in photosynthetic organisms (Warncke and Barber 1973, Johnson et al. 1984, Praveen et al. 2011). For example, the nitrate to ammonium ratio ($NO_3$:$NH_4$) plays a role in the formation of oxalic acid in terrestrial plants, with decreasing $NO_3$:$NH_4$ ratios causing decreases in oxalic acid concentrations (Palaniswamy et al. 2004, Zhang et al. 2005, Fontana et al. 2006). Furthermore, increases in the $NO_3$:$NH_4$ ratio caused increases in shikonin and betacyanins, whereas decreases in the $NO_3$:$NH_4$ ratio caused increases in berberine and ubiquinone, indicating that the synthesis of metabolites in terrestrial plant taxa may be modulated by the oxidized:reduced nitrogen ratio (Fujita et al. 1981, Nakagawa et al. 1984, Ramachandra Rao and Ravishankar 2002).

The synthesis of metabolites by phytoplankton, including cyanobacteria, also has been shown to be affected by the oxidized:reduced nitrogen ratio. In marine environments, for example, the $NO_3$:$NH_4$ ratio affected the formation of algal colonies and the production of alkenones and may have altered the species composition of nuisance algal blooms (Riegman et al. 1992, Harada et al. 2003). Additionally, a study by Leong et al. (2004) showed that experimental additions of $NH_4$ (thereby decreasing the $NO_3$:$NH_4$ ratio) resulted in higher cellular

quotas of the nitrogen-rich saxitoxin than additions of nitrate in cultures of the marine dinoflagellate *Alexandrium tamarense*. Furthermore, studies of freshwater phytoplankton have shown that cyanobacteria have low competitiveness for nitrate and high competitiveness for ammonium relative to other taxa (Blomqvist et al. 1994, Hyenstrand et al. 1998, McCarthy et al. 2009). To my knowledge, however, no study has explored whether the $NO_3$:$NH_3$ ratio plays a role in the occurrence or magnitude of secondary metabolites produced by cyanobacteria in freshwater ecosystems.

I investigated how total nutrients (TN, TP, TN:TP) and nitrogen speciation ($NO_3$, $NH_3$, and the $NO_3$:$NH_3$ ratio) affected the occurrence and abundance of cyanobacteria as well as the concentrations of a toxin (microcystin) and 2 nuisance cyanobacterial secondary metabolites (geosmin and MIB) using a multi-year dataset from 4 eutrophic reservoirs in the Midwestern United States. Additionally, I examined the composition of $N_2$-fixing (all potential $N_2$-fixing cyanobacteria referred to as $N_2$-fixers hereafter) and non-$N_2$-fixing cyanobacteria relative to nitrogen speciation and cyanobacterial secondary metabolites. Specifically, I tested the hypothesis that relatively low $NO_3$:$NH_3$ ratios favored relatively high metabolite concentrations per $N_2$- and non-$N_2$-fixer biovolume compared to relatively high $NO_3$:$NH_3$ ratios.

## Methods

### *Study sites*

Data from 4 Midwestern United States reservoirs were used because monitoring studies of each reservoir provided long-term (>2 years) data on geosmin, MIB, and microcystin. Cheney Reservoir (97°50′16.11″W, 37°45′32.99″N; surface area [A] = 31 $km^2$, average depth [Z] = 6.1

m) is located 40 km west of Wichita, Kansas. It rarely thermally stratifies due to persistent winds averaging 19 km/h and a relatively shallow depth. The 1501 $km^2$ watershed is dominated (>95%) by agricultural use (Stone et al. 2013). Eagle Creek Reservoir (86°18′13.07″W, 39°51′09.84″N; A = 5.0 $km^2$; Z = 4.2 m), Geist Reservoir (85°57′47.22″W, 35°56′16.84″N; A = 7.5 $km^2$; Z = 3.2 m), and Morse Reservoir (86°2′17.22″W, 40°6′16.84″N; A = 6.0 $km^2$; Z = 4.7 m) are located within 30 km of Indianapolis, Indiana, and have watersheds of 420 $km^2$, 554 $km^2$, and 567 $km^2$, respectively; land use in each watershed is primarily (60%, 77%, and 60%, respectively) agricultural (Song et al. 2012). All 4 reservoirs are eutrophic to hypereutrophic, with mean TP concentrations ranging from 0.06 to 0.14 mg/L and mean TN concentrations ranging from 0.9 to 4.1 mg/L (Song et al. 2012, Stone et al. 2013).

*Data collection and laboratory analyses*

Water samples were collected from a single site near the Cheney Reservoir dam at monthly or twice per month intervals from May 2001 to December 2012. From May 2001 through July 2004, discrete water samples were collected at 0.5 m depth using a 1L Kemmerer sampler. From August 2004 through December 2012, integrated photic zone samples were collected using a check-valve bailer. The photic zone was defined as the vertical area through the water column to a depth where light is ~1% of that at the surface. Integrated photic zone samples were collected in Eagle Creek, Geist, and Morse reservoirs from a single site near the dam at monthly or twice per month intervals during April through November 2008–2010. Samples were collected using a Van Dorn sampler throughout the photic zone, which was estimated using the empirical formula $Z_{photic} = 2.7 \times Z_S$, where $Z_{photic}$ = the depth of the photic zone and $Z_S$ = Secchi depth (Tedesco and Clercin 2011).

To determine concentrations of TN, TP, $NO_3$-N, and $NH_3$-N ($NO_3$-N and $NH_3$-N referred to as $NO_3$ and $NH_3$, respectively, hereafter) water samples were analyzed following Environmental Protection Agency (O'Dell 1993, Pfaff 1993) or Standard Methods (APHA 2005; see Supplemental Table 1 in Appendix C for specific methods used) for all reservoirs. All nutrient ratios were calculated by mass. Additionally, samples were analyzed for geosmin, MIB, microcystin, and phytoplankton identification, enumeration, and biovolume. To determine geosmin and MIB concentrations, solid phase microextraction gas chromatography/mass spectrometry was used following methods of Zimmerman et al. (2002) and APHA (2005) Method 6040D. Congener-independent total microcystin concentration was analyzed via Abraxis enzyme-linked immunosorbent assays (ELISA) using manufacturer methods (Abraxis LLC, Pennsylvania, USA). There were multiple limits of detection for geosmin and MIB, ranging from 1 to 5 ng/L, because analytical techniques improved during the course of this study. All values below the limit of detection were set to half the limit of detection for statistical analyses (Manly 2008); values for the limits of detection and the number of samples set to half the limit of detection for geosmin, MIB, microcystin, $NO_3$, and $NH_3$ were documented (Supplemental Table 1 Appendix C). Phytoplankton from Cheney Reservoir were identified and enumerated using a compound microscope and membrane-filtered slides (McNabb 1960). As per Lund et al. (1958), a minimum of 400 natural units were counted from each sample, providing accuracy within 90% confidence limits. Membrane-filtered slides were counted at 630× and 400× magnification to ensure complete species reporting. Biovolume calculations were based on Hillebrand et al. (1999); further information is provided in Beaver et al. (2014). Phytoplankton from Eagle Creek, Geist, and Morse reservoirs were enumerated using a Nageotte counting chamber (50 µL), which is efficient at mitigating the flotation of gas-vacuolated cyanobacteria (Brient et al. 2008).

Identification of the algal taxa was carried out at 200× and 400× magnification. As reported for Cheney Reservoir, a minimum of 400 natural units were counted for each sample to fall within the 90% confidence interval. Biovolume was determined using the methods of Hillebrand et al. (1999) and Sun and Liu (2003).

*Data and statistical analyses*

To determine if low $NO_3$:$NH_3$ ratios favored metabolite-producing cyanobacteria, box plots were constructed where metabolite per biovolume data above and below the median $NO_3$:$NH_3$ ratio (5) were compared. The median $NO_3$:$NH_3$ ratio value was used as a threshold so that compared datasets had a similar (within 5 data points) number of data points. Statistically significant differences ($p < 0.05$) between data above and below the median of $NO_3$:$NH_3$ ratios were determined using nonparametric Wilcoxson rank-sum tests. Multivariate relations between water quality parameters (TN, TP, TN:TP, $NO_3$, $NH_3$, $NO_3$:$NH_3$), cyanobacteria, and cyanobacterial secondary metabolites were determined using principal components analysis (PCA) as described by Kuhn and Johnson (2013). Because changes in TN:TP or $NO_3$:$NH_3$ ratios should affect phytoplankton community composition, they were used as explanatory variables in analyses of relative cyanobacteria biovolume; by contrast, TN, TP, $NO_3$, and $NH_3$ were used as explanatory variables in analyses of absolute cyanobacteria biovolume. Cyanobacteria were classified as $N_2$- or non-$N_2$-fixers as per Paerl and Fulton (2006) and Tomitani et al. (2006; see Supplemental Table 2 in Appendix Cfor a list of all observed cyanobacteria that were categorized as $N_2$-fixers). Metabolites were normalized to biovolume by dividing the metabolite concentration by $N_2$-fixer and non-$N_2$-fixer biovolumes. All statistics were run using R 3.0.1 (R Core Team 2014).

## Results

TN, TP, and the TN:TP ratio ranged from 0.16 to 7.96 mg/L (average: 1.68 mg/L, *n* = 292), 0.014 to 1.77 mg/L (average: 0.103 mg/L, *n* = 289), and <1 to 223 (average: 26, *n* = 289), respectively, in the 4 reservoirs throughout the study period. $NO_3$ and $NH_3$ ranged from limits of detection to 5.60 and 0.49 mg/L, respectively (averages: 0.68 and 0.07, *n* = 292 and 293, respectively); the $NO_3$:$NH_3$ ratio ranged from 0.02 to 560.3 (average: 28.43, *n* = 289). Concentrations of geosmin, MIB, and microcystin ranged from limits of detection to 133 ng/L (average: 10.8 ng/L, *n* = 298), 224 (average: 4.6 ng/L, *n* = 298), and 9.0 µg/L (average: 0.45 µg/L, *n* = 283), respectively.

Relative cyanobacterial biovolume temporally increased as $NO_3$:$NH_3$ ratios decreased in all 4 study reservoirs (Figure 1a–d). A similar trend occurred when relative cyanobacterial biovolume was compared to TN:TP (Supplemental Figure 1 Appendix C). With the exception of one occurrence of a high (>50 ng/L) MIB concentration, the highest secondary metabolite concentrations occurred when TN:TP (Figure 2a–c) and $NO_3$:$NH_3$ ratios were relatively low (i.e., generally <30; Figure 2d–f). Geosmin, MIB, microcystin, (Figure 3a–c) and absolute cyanobacterial biovolume (Supplemental Figure 2 Appendix C), but not relative cyanobacterial biovolume (Figure 3d), seemed to have a nonlinear association with the TN:TP and $NO_3$:$NH_3$ ratios (Figure 3e–h).

Microcystin, geosmin, and MIB per $N_2$- and non-$N_2$-fixer biovolume were significantly higher when $NO_3$:$NH_3$ ratios were less than the median ($NO_3$:$NH_3$ = 5) compared to when $NO_3$:$NH_3$ ratios were greater than the median (Figure 4). A similar statistically significant result

occurred when the average $NO_3$:$NH_3$ ratio (28.5) was used as a threshold instead of the median (data not shown).

The PCA analysis clearly defined patterns between nutrient concentrations and ratios and secondary metabolites. In PCA biplots, variables with strong positive correlations are close together, whereas strong negative correlations are shown in opposite directions in ordination space. The PCA analysis indicated that the TN:TP and $NO_3$:$NH_3$ ratios were closely related (Figure 5a), primarily due to strong correlations between TN and $NO_3$, and TP and $NH_3$ (Figure 5b). Relative and absolute cyanobacterial biovolume variables were clustered with cyanobacterial secondary metabolites when both absolute nutrient concentrations and nutrient ratios were examined (Figure 5a and b). Relatively high amounts of secondary metabolite per $N_2$-fixer and non-$N_2$-fixer cyanobacterial biovolume were negatively correlated with TN:TP and $NO_3$:$NH_3$ ratios; however, secondary metabolite per $N_2$-fixer biovolume formed a distinctly different cluster than secondary metabolite per non-$N_2$-fixer biovolume (Figure 5c).

A similar pattern formed when absolute nutrient concentrations were examined instead of nutrient ratios, but showed that high concentrations of secondary metabolite per non-$N_2$-fixer biovolume were highly correlated with $NH_3$, and both secondary metabolite per $N_2$- and non-$N_2$-fixer biovolume were comparably correlated with TP (Figure 5d). Additionally, the absolute concentrations of TP and $NH_3$ were positively correlated whereas TN and $NO_3$ were negatively correlated with both secondary metabolite per $N_2$-fixer and non- $N_2$-fixer biovolume. The highest secondary metabolite concentrations per $N_2$-fixer biovolume occurred when $NO_3$ and $NH_3$ concentrations were near their limit of detection (and at low $NO_3$:$NH_3$ ratios). The highest secondary metabolite concentrations per non-$N_2$-fixer biovolume were found when $NO_3$ was near

its limit of detection, but while $NH_3$ was analytically measureable. Thus, in general, the secondary metabolites studied here had the highest occurrence and concentration at relatively low TN:TP and $NO_3$:$NH_3$ ratios, with TP and $NH_3$ seeming to influence secondary metabolites more than TN or $NO_3$, especially for non-$N_2$-fixers.

**Discussion**

The empirical data showed that both cyanobacterial biovolume and the concentrations of 3 common cyanobacterial secondary metabolites were highest when TN:TP ratios were relatively low (Figure 2a–c and 3a–c). This finding suggests that the concentrations of cyanobacterial secondary metabolites likely increased as TN:TP ratio decreased because cyanobacteria also increased as the TN:TP ratio decreased (Supplemental Figures 1 and 2 Appendix C; Smith 1983) in the 4 systems studied here. This trend resembles those observed in other experimental and empirical studies (Graham et al. 2004, Orihel et al. 2012, Harris et al. 2014). When TN:TP ratios were low enough to favor dominance by cyanobacteria, relatively low $NO_3$:$NH_3$ ratios (primarily caused by increases in $NH_3$; Figure 5d) generally seemed to (1) favor cyanobacteria, and (2) favor the growth of cyanobacterial strains capable of secondary metabolite synthesis, and/or increase synthesis of secondary metabolites by capable cyanobacteria (Figure 4), especially for non-$N_2$-fixing cyanobacteria (Figure 5c–d). Specifically, $N_2$-fixing cyanobacteria seemed to produce the monitored secondary metabolites when absolute concentrations of both $NO_3$ and $NH_3$ were relatively low, suggesting that $N_2$-fixing cyanobacteria are favored when the availability of dissolved inorganic nitrogen is low. Non-$N_2$-fixing cyanobacteria seemed to be favored and produced secondary metabolites when $NO_3$ was low and $NH_3$ was still measurable, indicating that reduced nitrogen forms possibly influence non-$N_2$-fixing cyanobacteria,

especially those that can produce the investigated secondary metabolites, more than oxidized forms (Finlay et al. 2010, Donald et al. 2011). I thus hypothesize that when the TN:TP ratio is low enough to favor cyanobacteria, the presence of bio-available reduced nitrogen forms results in low $NO_3$:$NH_3$ ratios that seem to favor cyanobacterial species or strains that can produce the 3 toxic or taste-and-odor secondary metabolites studied here.

Resource competition between cyanobacteria and eukaryotic phytoplankton for chemically oxidized and reduced nitrogen forms may be why relative cyanobacterial biovolume increased as the $NO_3$:$NH_3$ ratio decreased. Cyanobacteria, especially non-$N_2$-fixing taxa, are superior competitors for reduced nitrogen forms compared to eukaryotic algae (Blomqvist et al. 1994, Hyenstrand et al. 1998, McCarthy et al. 2009) and possibly other bacteria (McCarthy et al. 2013). For example, Blomqvist et al. (1994) showed that non-$N_2$-fixing cyanobacteria were favored when $NO_3$ was depleted but $NH_4$ was simultaneously supplied or recycled at a sufficient rate for phytoplankton growth. Jacoby et al. (2000) and Chaffin et al. (2011) also found these conditions promoted non-$N_2$-fixing (*Microcystis* sp.) cyanobacterial blooms in Northwestern US lakes and Lake Erie, respectively. Moreover, McCarthy et al. (2009) showed that as the $NO_3$:$NH_4$ ratio decreased, diatom abundance decreased while cyanobacterial abundance increased. The inverse relation between the $NO_3$:$NH_4$ ratio and cyanobacterial abundance was likely because nitrate/nitrite reductase is used more efficiently by eukaryotes than prokaryotes, whereas prokaryotes assimilate reduced nitrogen forms more efficiently than eukaryotes (Blomqvist et al. 1994, Donald et al. 2011). This relationship may also explain why cyanobacteria contributed the highest proportion to the overall phytoplankton community when $NO_3$:$NH_3$ ratios were low in the study reservoirs (Figure 1a–d). Decreases in the $NO_3$:$NH_3$ ratio

may therefore be an indicator of cyanobacterial proliferation, especially non-$N_2$-fixing taxa, in eutrophic systems.

Cyanobacteria producing the nitrogen-rich secondary metabolite microcystin seemed to be favored at relatively low $NO_3$:$NH_3$ ratios (Figure 3g and 4), possibly because of differences in cellular energetic costs and assimilation time associated with oxidized and reduced nitrogen forms. Chemically, reduced nitrogen forms are preferred by cyanobacteria because they do not require the use (or energetic cost) of nitrate or nitrite reductase (Flores and Herrero 2005); therefore, the assimilation time for reduced nitrogen forms is less than that for oxidized forms (Dortch 1990). Thus, increases in water column concentrations of reduced nitrogen forms likely cause cellular nitrogen to build up more efficiently than when oxidized nitrogen forms are the predominant source of bioavailable nitrogen. This, in turn, may favor the synthesis of nitrogen-rich microcystins, and thereby perhaps also the cyanobacterial strains capable of producing these compounds.

Cyanobacteria producing geosmin and MIB also seemed to be favored at relatively low $NO_3$:$NH_3$ ratios (Figure 3e, 3f, and 4). A decrease in cellular energetics when switching from oxidized to reduced nitrogen forms may allow reallocation of energy from reducing oxidized nitrogen forms to building carbon-containing compounds, a possible explanation for low $NO_3$:$NH_3$ ratios favoring carbon-rich secondary metabolites. Alternatively, because secondary metabolite concentrations were significantly larger at relatively low $NO_3$:$NH_3$ ratios (Figure 4), my data could suggest that secondary metabolite-producing cyanobacteria strains have an advantage over non-metabolite-producing cyanobacteria strains when $NO_3$:$NH_3$ ratios are relatively low. Thus, the proportion of secondary metabolite-producing strains may increase

within the phytoplankton community when $NO_3$:$NH_3$ ratios are relatively low, ultimately causing a larger concentration of secondary metabolites within the water column. Overall, my data suggests that changes in nitrogen oxidation state and/or absolute concentration may indicate conditions that favor the occurrence of carbon-rich secondary metabolites like geosmin and MIB.

Field studies have indicated that chemical nitrogen forms affect carbon- and nitrogen-rich secondary metabolite occurrence. For example, increases in nitrogen, specifically reduced nitrogen forms (i.e., $NH_3$), have been linked to increases in geosmin and MIB concentrations in reservoir and river systems (Lind and Katzif 1988, Uwins et al. 2007). Similarly, experimental mesocosm studies have shown increases in microcystin concentrations with the addition of reduced nitrogen forms (i.e., $NH_3$ and urea; Finlay et al. 2010, Donald et al. 2011, Bogard et al. 2012). Combined with this study, it thus seems that the chemical form of nitrogen could potentially play an important role in cyanobacterial secondary metabolite production and their occurrence in surface waters, either directly through effects on metabolic processes and/or indirectly by favoring secondary metabolite-producing strains.

Overall, this study shows that the $NO_3$:$NH_3$ ratio may potentially affect, or at least be indicative of, conditions that favor changes in the phytoplankton community structure and in the production and occurrence of secondary cyanobacterial metabolites in surface waters. Although this study cannot specifically address whether the $NO_3$:$NH_3$ ratio is causally linked to metabolite-producing cyanobacteria, relatively low $NO_3$:$NH_3$ ratios may be predictive of conditions that favor metabolite-producing cyanobacterial blooms (Figure 4); therefore, the $NO_3$:$NH_3$ ratio merits further research. Given that geosmin, MIB, and microcystin production also can be affected by multiple additional environmental variables, such as temperature, light,

and pH (Wu and Jüttner 1988, Rashash et al. 1995, Zhang et al. 2009), future studies need to address how other variables, combined with the $NO_3$:$NH_3$ ratio, affect cyanobacterial secondary metabolite production and occurrence.

Irrespective of other environmental factors, however, my data suggest that nitrogen forms and nutrient ratios can be linked to distinctive changes in secondary metabolite concentrations, and correlate to the amount of secondary metabolite per cyanobacterial biovolume. Nonetheless, my results must be further explored to confirm mechanistic relationships between TN:TP and $NO_3$:$NH_3$ ratios, cyanobacteria biovolumes, and secondary metabolite production. Additionally, future studies also will be needed to address whether other bacterial taxa capable of producing geosmin and MIB (e.g., actinomycetes) are affected by the $NO_3$:$NH_3$ ratio. Although these studies need to occur before the $NO_3$:$NH_3$ ratio can be used by water managers as a predictor variable for secondary metabolite-producing cyanobacterial blooms, the findings of this study have important implications.

I suggest that because the risk of observing high microcystin concentrations in the water column increases as the TN:TP ratio decreases (Orihel et al. 2012, Harris et al. 2014), simultaneous increases in chemically reduced nitrogen forms ($NH_3$ and urea) and phosphorus may cause similar TN:TP ratios but lower $NO_3$:$NH_3$ ratios; this cascade of events could in turn cause increases in secondary metabolite-producing cyanobacterial blooms in waterbodies in which the TN:TP ratio is low (i.e., typically already phosphorus rich systems; Bogard et al. 2012, Donald et al. 2013). I thus conclude that concurrent decreases in the TN:TP and $NO_3$:$NH_3$ ratios have the potential to create conditions that favor increases in toxic and taste-and-odor causing cyanobacterial blooms.

**Figures and Figure legends**

**Figure 1.** Temporal dynamics of relative cyanobacterial biovolume, relative cyanobacterial $N_2$-fixer biovolume, and the $NO_3$:$NH_3$ ratio (by mass) in the 4 study reservoirs. Numbers on the x-axis represent the last 2 digits of the year post 2000 for (a) Cheney, (b) Eagle Creek, (c) Geist, and (d) Morse.

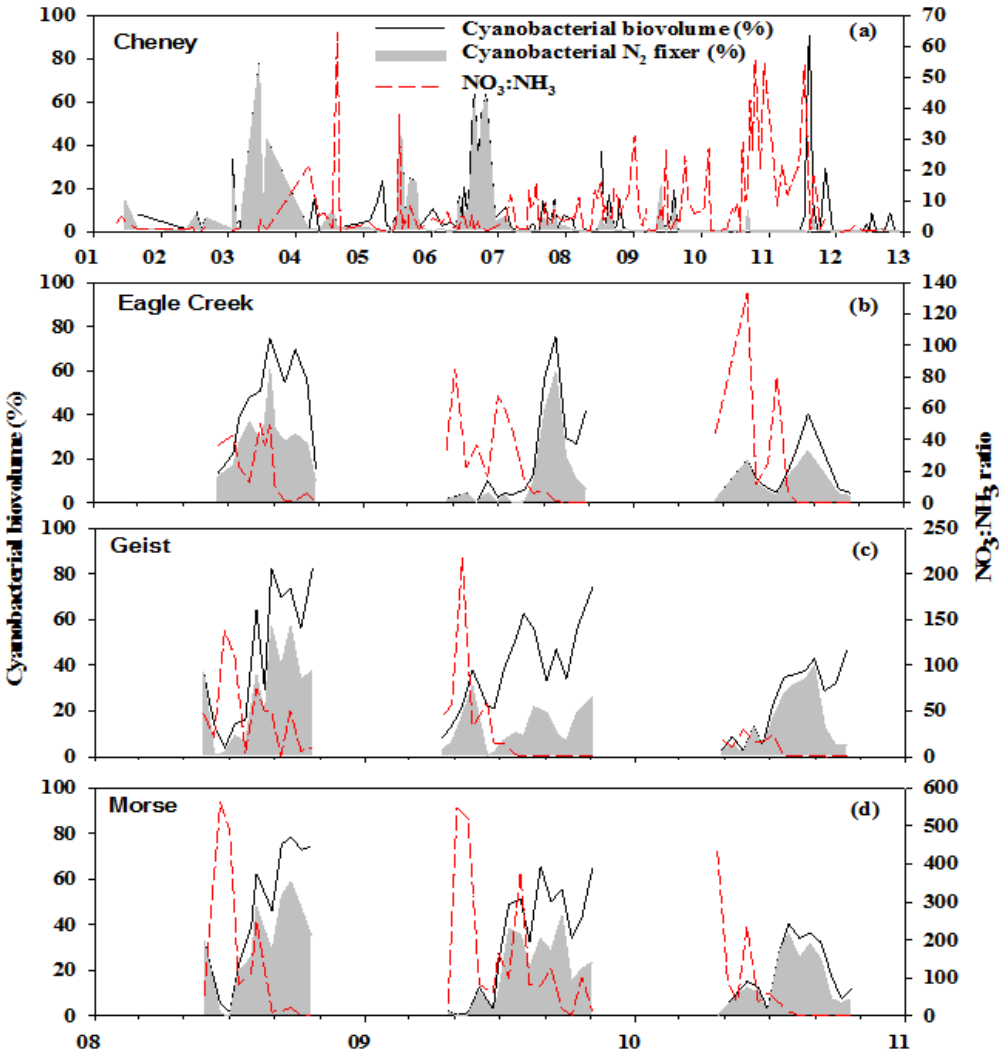


***Figure 2-1***

**Figure 2.** The relation between TN and TP (a-c) and $NO_3$ and $NH_3$ (d-f) with Geosmin (Geo), 2-methylisoborneol (MIB), and microcystin (MC) concentration categories denoted by colors. The solid black line represents a TN:TP ratio and $NO_3$:$NH_3$ ratio of 30 for a-c and d-f, respectively, which are used only for reference.

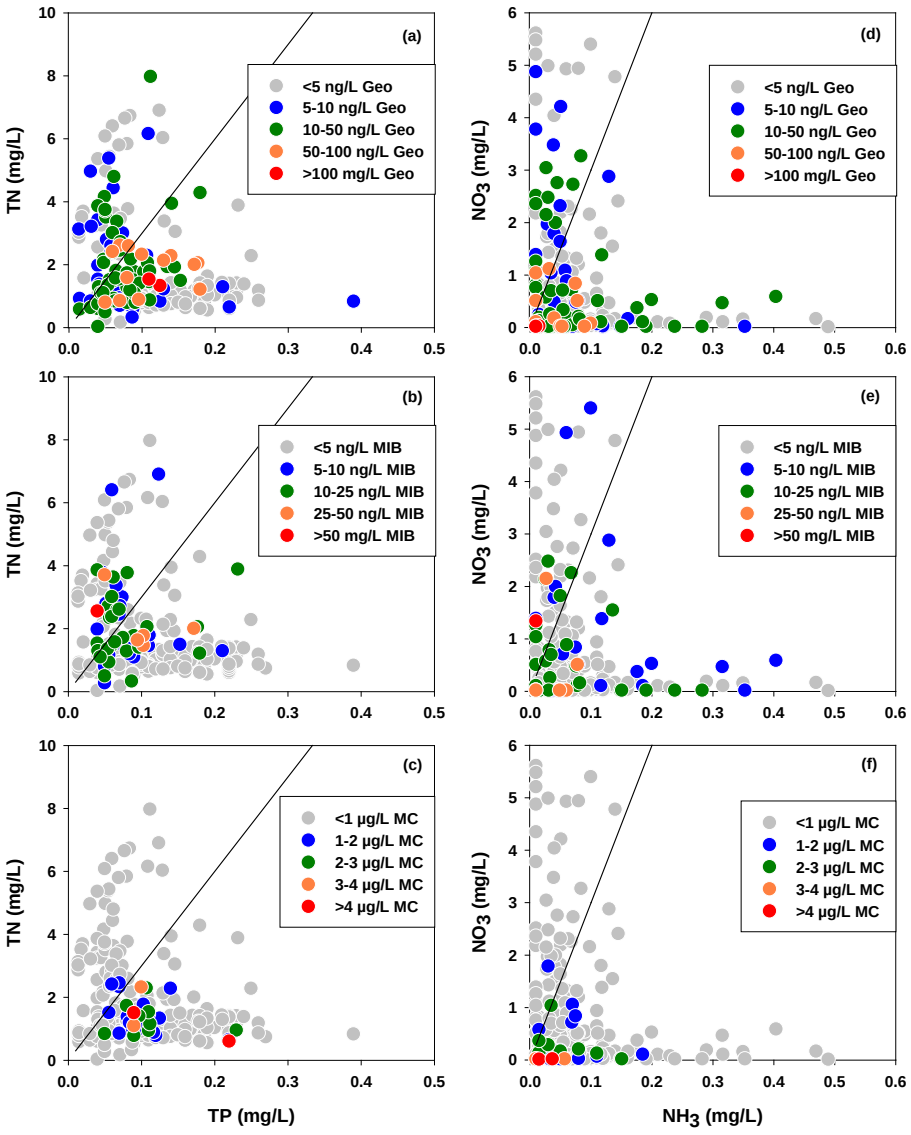


***Figure 2-2***

**Figure 3.** The relation between geosmin, 2- methylisoborneol (MIB), microcystin, and relative cyanobacterial biovolume (% Cyanobacteria) and the TN:TP (a-d) and $NO_3$:$NH_3$ ratios (e-h).

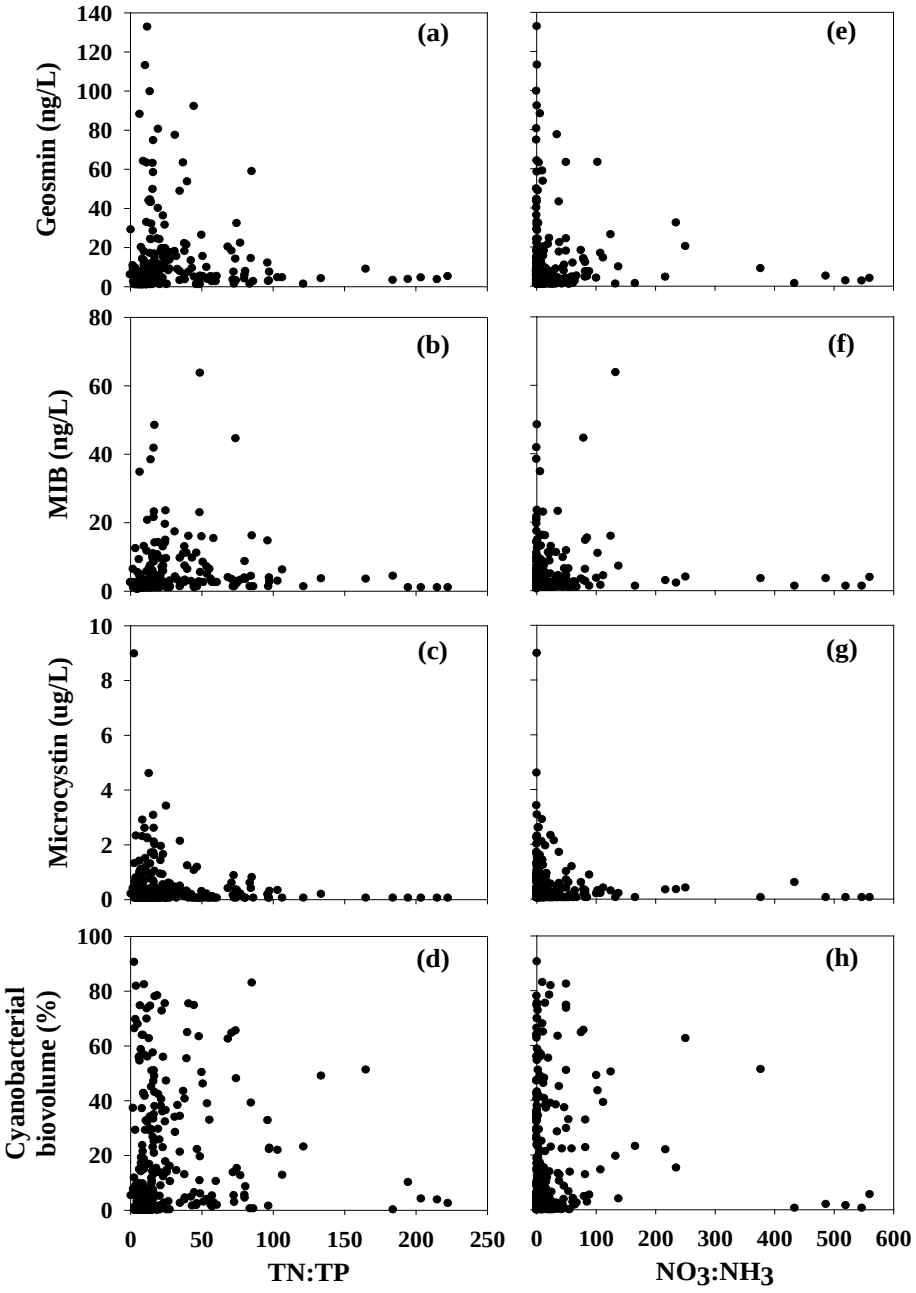


***Figure 2-3***

**Figure 4.** Cyanobacterial metabolites (microcystin, MC; geosmin, Geo; and 2-methylisoborneol, MIB) per $N_2$-fixer (/F) and non-$N_2$-fixer (/NF) biovolume greater than and less than, the median $NO_3$:$NH_3$ ratio of 5. All six metabolite per biovolume comparisons between $NO_3$:$NH_3$ ratios above and below 5 were significantly different (all *p*-values < 0.001).

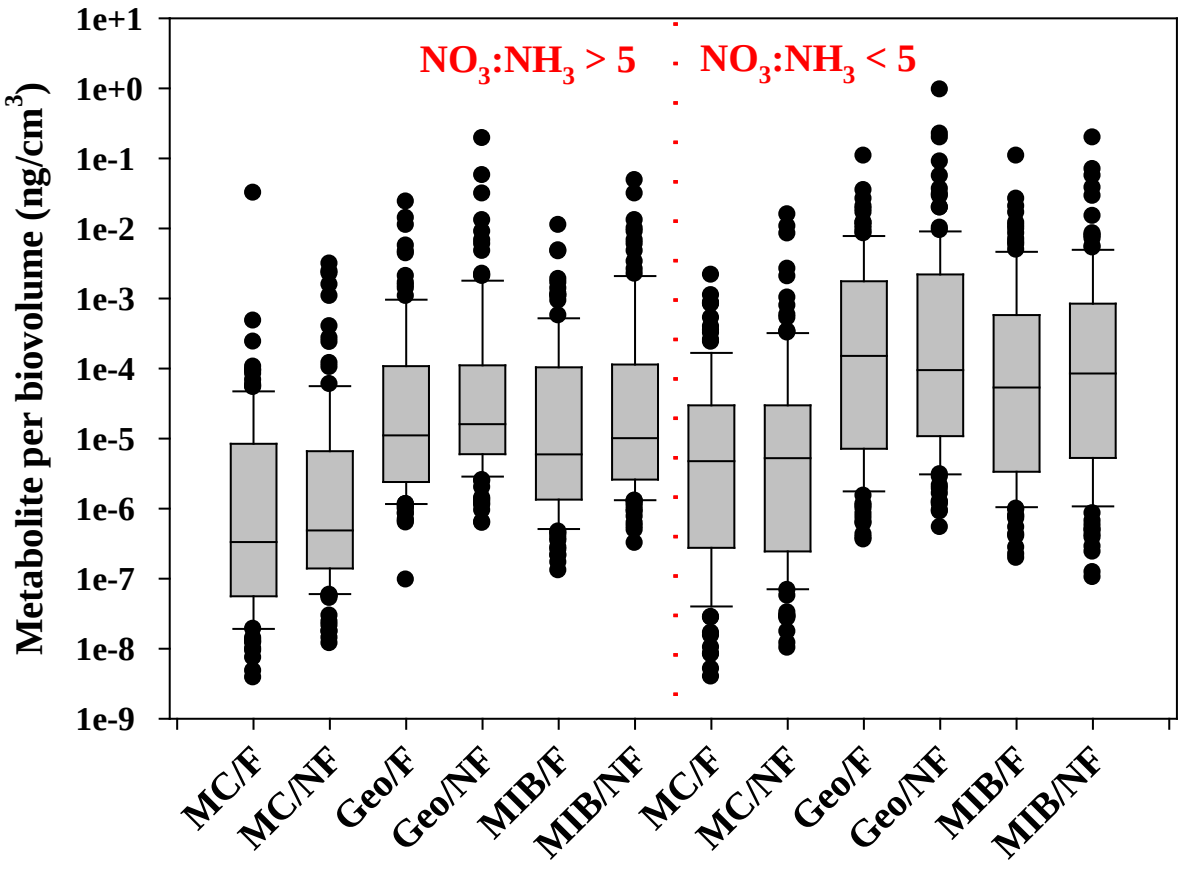


***Figure 2-4***

**Figure 5.** Principal component analysis (PCA) biplots for relative and absolute cyanobacterial biovolume (a and b, respectively), associated metabolites, and nutrient ratios, and for nutrient ratios and absolute nutrient concentrations (c and d, respectively) and metabolite per biovolume. Percentages in x and y axis labels represent the explained variation of each component. MC=microcystin, GEO=geosmin, MIB= 2-methylisoborneol, PF= percent $N_2$-fixers, PNF= percent non-$N_2$-fixers, TN= total nitrogen, TP= total phosphorus, CB= cyanobacterial biovolume, FIXB= $N_2$-fixer biovolume, NONFIXB= non- $N_2$-fixer biovolume, NO= nitrate, NH= ammonia, MC.F, GEO.F, MIB.F= metabolite per $N_2$-fixer biovolume, MC.NF, GEO.NF, MIB.NF= metabolite per non-$N_2$-fixer biovolume.

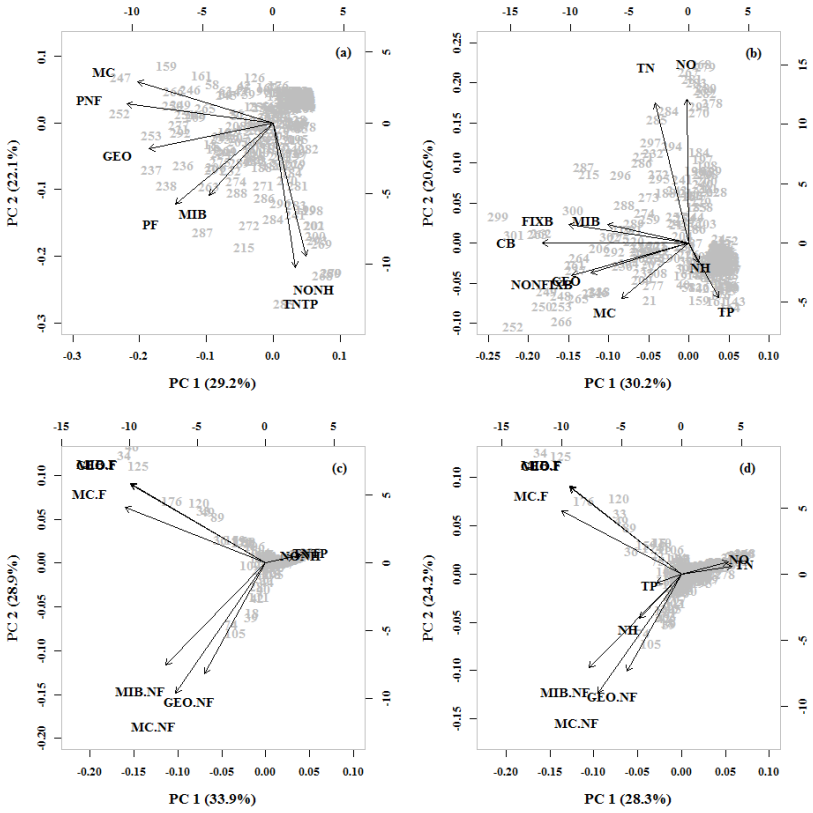


***Figure 2-5***

# Chapter 3*

Do persistent organic pollutants stimulate cyanobacterial blooms?

*Harris, T.D., Smith, V.H. 2016. Do persistent organic pollutants stimulate cyanobacterial blooms? Inland Waters. 6:124-130.

**Abstract**

The use of persistent organic pollutants (POPs), such as herbicides, pesticides, pharmaceutical and personal care products (PCPPs), and polycyclic aromatic hydrocarbons (PAHs), has more than doubled since 1950. POPs find their way into aquatic ecosystems through agricultural and industrial runoff, wastewater treatment effluent discharge, and atmospheric deposition. Cyanobacterial harmful algal blooms, which can produce toxins potent enough to cause human death, have been increasing in intensity, frequency, and spatial scale throughout the same time period as accelerated POP usage. Here, I provide a meta-analysis and suggest that POP stressors may be significantly aggravating nutrient-driven harmful cyanobacterial blooms by suppressing the growth of competing phytoplankton, and/or by indirectly or directly stimulating cyanobacterial growth.

## Introduction

Cyanobacterial harmful algal blooms (CyanoHABs) are a major cause of water quality degradation in rivers, lakes, and estuaries worldwide. CyanoHABs can disrupt food-webs and cause significant changes in dissolved oxygen and pH (Paerl 2014). In addition, because many cyanobacterial taxa produce a diverse suite of potent and deadly cyanotoxins (Codd et al. 1999), as well as other cellular metabolites that create taste and odor problems in drinking water supplies (Graham et al. 2010), CyanoHABs can pose significant human and animal health hazards, impair fisheries, drinking water, and irrigation supplies, and result in substantial economic damage (Sharma et al. 2013).

Extensive research has demonstrated that the abundance and toxicity of cyanobacteria is strongly influenced by eutrophication, which is caused by the over-supply of two key nutrients, phosphorus (P) and nitrogen (N) to surface waters (Paerl and Otten 2013). Nitrogen: phosphorus stoichiometry also has significant effects on cyanobacterial dominance; in particular, cyanobacterial growth tends to be favored when the N:P ratio is low (Smith 1983; Orihel et al. 2012; Harris et al. 2014). Other environmental factors also can potentially influence nuisance cyanobacterial growth, including changes in food web structure (Elser 1999; Ekvall et al. 2014) and global warming (Paerl and Huisman 2008; Kosten et al. 2012).

A recent study showed that cyanobacterial blooms have significantly increased globally relative to other phytoplankton taxa since the 1950s, with relatively sharper increases in cyanobacteria biomass noted in low-nutrient alpine systems compared with nutrient-rich lowland systems (Taranu et al. 2015). The cause for this increase in relative cyanobacterial abundance is not yet fully understood, but I hypothesize here that anthropogenic inputs of organic stressors

may be a substantial (up to 10% of total biomass; Everaert et al. 2015) under-recognized direct and/or indirect contributor to cyanobacterial proliferation in the world's surface waters.

**Persistent Organic Pollutants**

For more than six decades, a diverse mix of Persistent Organic Pollutants (POPs) that include more than 400 different herbicides, pesticides, fungicides (Figure 1), and a diverse suite of polycyclic aromatic hydrocarbons (PAHs), pharmaceutical and personal care products (PPCPs), and polychlorinated biphenyls (PCBs) have been released at accelerating rates into receiving waters via wastewater effluents, agricultural and industrial runoff, and atmospheric deposition (Boyd et al. 2003; Lohmann et al. 2009; Dougherty et al. 2010). Some of these compounds degrade in only a few weeks, while others persist for years, decades, or centuries before being degraded by abiotic and/or biotic processes (US EPA 2014a). POPs can persist in the environment in gas, liquid, or solid phases, and can be transported thousands of miles from their sources by atmospheric wind currents and bodily fluid excretion from birds and other migrating animals (Evenset et al. 2007). As a result, POPs frequently co-occur and are environmentally detectable throughout the year in a large proportion of the world's surface waters (Stone et al. 2014), including in relatively pristine Arctic regions (Halsall 2004; Rigét et al. 2010) .

Many POPs are known to have potent biological effects, including the taxon-specific modification of population growth in phytoplankton communities. I performed a synthetic review of the ecotoxicology literature to test the hypothesis that the presence of environmentally-relevant concentrations of POPs in aquatic ecosystems directly or indirectly favors the growth cyanobacteria relative to other phytoplankton taxa (i.e the percent composition of cyanobacterial

abundance and/or biovolume was higher than eukaryotic taxa in the presence of POPs). Relevant publications from 1980-2015 were found by searching Google Scholar and Web of Science for key words, including "cyanobacteria" and the general chemical classes of POPs, as well as the common and formula names of specific compounds (Supplemental Table 1 Appendix D). References within relevant publications found via Google Scholar or Web of Science were then searched for additional relevant data. I only included experiments that directly evaluated the quantitative response of cyanobacteria and eukaryotic phytoplankton cell counts or biovolume to the presence of POPs. To prevent discrepancies between different publications, the relative taxon-specific responses (or the reported effective concentration ($EC_{50}$) values) for a given suite of cyanobacteria and phytoplankton were only evaluated within a given study. I identified a total of 107 studies that examined a total of 227 individual compounds; 133 of these compounds were distinctly different POPs, and included 39 herbicides, 26 pesticides, 11 fungicides, 39 PPCPs, 17 PAHs, and 1 PCB.

Frequency histograms were created to quantify the results of the analysis (Figure 2, Supplemental Table 2 Appendix D). Each histogram depicts three categories that refer to the response of cyanobacteria relative to eukaryotic algae when both were exposed to a specific class of POP. A given POP response was classified as *positive* if its presence allowed cyanobacteria to become inhibited less by $EC_{50}$ or become dominant by greater abundance in mixed cultures/field experiments relative to all other phytoplankton taxa tested for that specific compound, and was classified as *negative* if the quantitative response of cyanobacteria was depressed relative to all the other tested species. A POP was classified as *neutral* if the response of cyanobacteria was intermediate relative to the other taxa being tested. Within each of the 107 studies examined, an

evaluation of response *frequency (f)* was performed for each individual experiment performed. Thus, if a given study performed multiple experiments that examined the responses of cyanobacteria to multiple POPs, each individual experiment contributed a value of $f$=1 to one (and only one to avoid double counting) of the three different response categories. In cases where multiple studies investigated a single unique POP (e.g., glyphosate), the response observed in each individual study of this POP contributed a value of $f$=1.

**Effects of herbicides on cyanobacterial growth**

The 133 different compounds evaluated in the analysis of the POP literature can be broadly grouped into two categories, those that have a known mode of action against photosynthetic organisms (i.e., herbicides), and those that do not. Herbicides affect photosynthetic organisms through modes of action that include, but are not limited to: amino acid pathway inhibitors (e.g., glyphosate), photosynthesis inhibitors (e.g., atrazine), growth regulators (e.g., 2-4D), cell membrane disruptors (e.g., paraquat and diquat), and shoot and/or root inhibitors (e.g., alachlor).

Since the introduction of genetically modified glyphosate-resistant crops in 1996, glyphosate (i.e., Roundup®) usage in the United States has increased from less than 15 to more than 50 million kilograms of glyphosate per year, and it has become the most widely used herbicide in the world (Perez et al. 2011). Herbicides containing glyphosate generally inhibit cyanobacterial growth less than the growth of other phytoplankton because cyanobacteria are not sensitive to glyphosate (Forlani et al. 2008; Perez et al. 2011). In the glyphosate-cyanobacteria studies that I surveyed, all showed either a positive or neutral effect on cyanobacteria relative to other phytoplankton taxa (Figure 2). Furthermore, likely with the help of heterotrophic bacteria

(Saxton et al. 2011), some species of cyanobacteria can even use the phosphorus bound within the glyphosate molecule to support their growth (Bai et al. 2014), allowing these cyanobacteria to have a potential competitive advantage over eukaryotic algal species that cannot use this source of organic phosphorus.

Atrazine and metribuzin herbicides in environmentally relevant concentrations also have been found to favor cyanobacterial growth, at the cost of other algal species (Lürling and Roessink 2006; Pannard et al. 2009). For example, Pannard et al. (2009) demonstrated a high sensitivity of multispecies phytoplankton assemblages to long-term herbicide exposure, and observed significant effects of atrazine on algal community structure even at herbicide concentrations as low as 0.1 $\mu g\ L^{-1}$. They concluded that cyanobacteria were more tolerant to atrazine than other phytoplankton taxa, particularly under conditions of elevated nutrient supply. Other studies have found that cyanobacteria and/or diatoms are more tolerant to atrazine than chlorophyte phytoplankton taxa (DeLorenzo et al. 1999; Magnusson et al. 2012). This suggests that in systems that are cyanobacteria-chlorophyte co-dominated, the presence of herbicides like atrazine may have the potential to shift the system to cyanobacterial dominance. Additionally, although most relevant studies in the meta-analysis held nutrient concentrations and temperature constant when comparing cyanobacteria and eukaryotic phytoplankton taxa in the presence of POPs, Bérard et al. (1999) reported that inhibition of cyanobacterial growth by herbicides is reduced at elevated water temperatures, and I thus have concerns that global warming could potentially influence or modify interactions between eutrophication and POP stressors.

Metribuzin and other herbicides have also been shown to favor cyanobacteria relative to other phytoplankton taxa (Figure 2; Fairchild et al. 1998; Gustavson et al. 2003; Caquet et al.

2005). For example, in a lab-based competition experiment between a cyanobacteria and a chlorophyte alga, cyanobacteria became dominant in the presence of 100 μg $L^{-1}$ of metribuzin; in sharp contrast, the chlorophyte alga completely dominated cyanobacteria in mixed cultures that did not contain metribuzin (Lürling and Roessink 2006). This led Lürling and Roessink (2006) to conclude that herbicide-contaminated surface waters potentially may be "on the way to cyanobacterial blooms".

**Effects of non-herbicide POPs on cyanobacteria**

## *Pesticides and fungicides*

POPs that do not have a specific mode of action against photosynthetic organisms include pesticides, fungicides, PPCPs, PAHs, and PCBs. Although pesticides (i.e., insecticides) and fungicides have been found to favor cyanobacteria over other phytoplankton taxa in laboratory studies (Wendt-Rasch et al. 2003; Ma et al. 2008), other mesocosm and laboratory studies have shown mixed results (Figure 2; DeLorenzo et al. 1999; Leboulanger et al. 2011). In natural systems, heterotrophic bacteria, zooplankton, and fungi community composition may play a role in the success of cyanobacteria in the presence of POPs compared to other phytoplankton. For example, Saxton et al. (2011) showed that the heterotrophic bacterial community was instrumental in allowing cyanobacteria to use phosphorus that is bound within phosphorus-rich POPs, which can serve as a novel potential organic phosphorus source that cannot be utilized by other phytoplankton. This microbial interaction may explain why some studies have shown that organophosphorus pesticides stimulate cyanobacterial growth at environmentally-relevant pesticide concentrations (Sun et al. 2013). Thus, POP-specific changes associated within the

heterotrophic bacteria, zooplankton, and/or fungi communities could be one reason why mixed results were seen in the pesticide and fungicide frequency histogram (Figure 2). Given that the current literature shows an ambiguous response of cyanobacteria to organic pesticides and fungicides, more research is needed to determine whether cyanobacteria are directly and/or indirectly favored over other phytoplankton taxa in the presence of pesticide and fungicide POPs.

**Pharmaceutical and Personal Care Products (PPCPs), Polycyclic Aromatic Hydrocarbons (PAHs), and Polychlorinated Biphenyls (PCBs)**

PPCPs include pharmaceutical compounds like prescription antibiotics and anti-cancer drugs, as well as personal care products like over the counter antimicrobials, fragrances, and UV blockers (Bernot and Justice 2014). The analysis suggests that some of these POPs may favor the growth of cyanobacteria (Stoichev et al. 2011; Liu et al. 2012; Nietch et al. 2013; Brezovšek et al. 2014), while others, especially antibiotics, appear to favor other phytoplankton taxa over cyanobacteria (Figure 2; Ebert et al. 2011; Qian et al. 2012; González-Pleiter et al. 2013). However, a very intriguing trend is evident in the literature: recent studies show that cyanobacteria may be favored in the presence of antibiotics (Figure 3), contrasting past observations. A recent study even found that the presence of antibiotics caused increased production of the cyanobacterial hepatotoxin microcystin (Liu et al. 2015). This could reflect differences among the antibiotics used in these experiments, or differences in experimental methodology, but this observation could also potentially suggest that cyanobacteria may be becoming more tolerant or possibly antibiotic-resistant over ecological and evolutionary time (i.e., pollution-induced community tolerance; sensu Blanck and Wängberg 1988).

Similarly, a diverse set of other PPCPs has consistently been observed to favor cyanobacteria relative to eukaryotic phytoplankton taxa. For example, Drury et al. (2013) observed a 6-fold increase in the relative abundance of cyanobacteria in the presence of common antimicrobial agents like triclosan. Proia et al. (2013) have recently attributed substantial increases in cyanobacteria and decreases of other algal taxa in a Mediterranean river to the presence of ibuprofen and paracetamol in the water column. Moreover, because PPCP concentrations are in general 3-5 times higher in winter months compared to summer months due to the temperature dependence of their biological degradation (Vieno et al. 2005), winter-time relative cyanobacterial abundance may perhaps increase in surface waters experiencing high PPCP loading, especially in areas where nutrient and light conditions are already favorable for cyanobacterial growth.

PAHs and PCBs also have been observed to stimulate relative cyanobacterial abundance and/or biovolume in experimental laboratory communities. Indeed, of the seven studies examining these compounds that I identified in my literature survey, four showed that the presence of PAHs and PCBs had a positive effect on cyanobacteria relative to eukaryotic taxa (Figure 2). Although the manufacturing of PCBs was banned in the United States in 1979 (US EPA 2014b), substantial concentrations of legacy PCBs still remain in marine and freshwater sediments, and their presence could help to favor the growth and ecological success of cyanobacterial akinetes (resting cells) and/or viable sedimented cyanobacterial cells (Latour et al. 2004) over other phytoplankton taxa. Given that relatively few studies have investigated the relative effects of cyanobacterial abundance in the presence of PAHs and PCBs, additional

research is needed to fully understand the relative response of cyanobacteria to PAHs and PCBs, as well as other organic stressors such as polybrominated diphenyl ethers (PBDEs).

**Conclusions and future research directions**

Is our aquatic future thus likely to be increasingly blue-green (Elliott 2012)? Unfortunately, the extensive empirical evidence that I have provided here suggests that the answer to this question may be *yes*. I conclude that cyanobacteria are in general favored over other phytoplankton taxa when taxon-sensitive POPs are present because cyanobacteria (1) have a higher tolerance (less sensitive) to POPs than other taxa, and (2) in some cases have the ability to use nutrients bound within POPs to stimulate their growth, possibly in conjunction with POP biodegradation by heterotrophic bacteria. The results of the meta-analysis suggest that systems with relatively high POP loading will potentially have higher relative cyanobacterial abundance relative to systems experiencing relatively low POP loading, especially in areas where environmental conditions are already favorable for cyanobacteria bloom development.

Nonetheless, as shown by the survey of the current literature, there are some studies that have shown other phytoplankton taxa are favored over cyanobacteria in the presence of POPs (Figure 2), indicating that ambiguity exists concerning whether cyanobacteria are consistently favored relative to other phytoplankton taxa in the complex chemical mixtures that exist in the world's surface waters. Given that most studies present in the literature are laboratory based (Supplemental Table 2 Appendix D), I am hopeful that future field-based experimental and empirical studies will quantitatively elucidate whether relative cyanobacterial abundance is indeed higher in natural systems exposed to relatively high POP loading. At a minimum, I hope that future studies can answer important new questions that are raised by this study, including (1)

Do current POP mixtures released into surface waters promote more frequent CyanoHABs? (2) Will inputs of future POPs (e.g., Enlist Duo® herbicide) favor cyanobacteria over other phytoplankton taxa? (3) Do sedimented POPs cause cyanobacteria resting cells to outcompete the resting cells of eukaryotic phytoplankton? (4) Will/have winter-time CyanoHABs increase(d) because of increased POP loading? (5) Are cyanobacteria becoming more tolerant or even possibly resistant to common POPs like antibiotics and herbicides over ecological and evolutionary time? and (6) Can POPs cause changes within the consumer community, which in turn may lead to consumer-driven nutrient recycling stoichiometry that indirectly promotes CyanoHABs?

I conclude that selective pressures from multiple POPs, in combination with the previously recognized factors of nutrient enrichment and warmer temperatures, will favor increases in the frequency, intensity, and geographical extent of nuisance cyanobacterial blooms in the world's surface waters. Therefore, I hypothesize that aquatic ecosystems receiving significant inputs of POPs may exhibit a greater probability of experiencing CyanoHABs than nearby ecosystems that have a similar nutrient content, but low POP loading. Because the formation of cyanobacterial blooms is multifactorial in nature, I strongly suggest that future water quality management efforts must focus upon more than nutrient loading control alone to fully combat the expansion of undesirable CyanoHABs in the world's surface waters.

**Figures and Figure legends**

**Figure 1.** Total kilograms of active ingredient biocides (herbicides, pesticides, and fungicides) applied to the U.S. landscape from 1931–2007. Note: the data shown represent time trends derived from a merger of multiple published datasets (Donaldson et al. 2002; Aspelin 2003; Grube et al. 2011); these databases overlapped between 1964–2007.

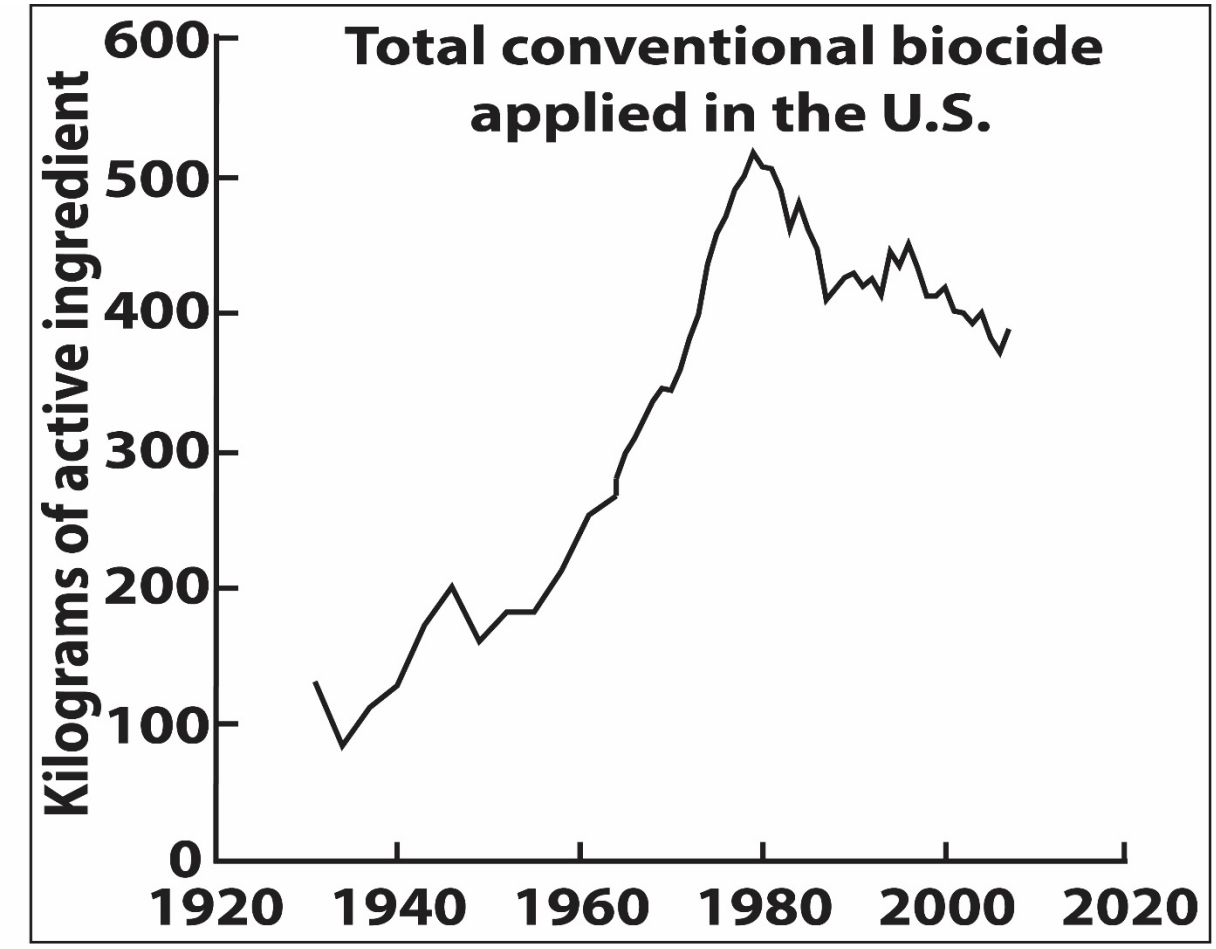


***Figure 3-1***

**Figure 2.** Histograms showing the response of cyanobacteria relative to eukaryotic phytoplankton taxa in the presence Persistent Organic Pollutants (POPs). The positive (green), neutral (brown), and negative (blue) categories refer to POPs that favored cyanobacteria, showed taxa more and less sensitive than cyanobacteria, and did not favor cyanobacteria relative to eukaryotic taxa, respectively, in multi-phytoplankton species experiments. The herbicide POP category includes glyphosate experiments; dashed red bars indicate the frequency of herbicide POP experiments without glyphosate. Numbers above histogram bars represent the number of POP experiments in each category. PPCPs = pharmaceutical and personal care products (including antibiotics); PAHs & PCBs = polycyclic aromatic hydrocarbons and polychlorinated biphenyls.

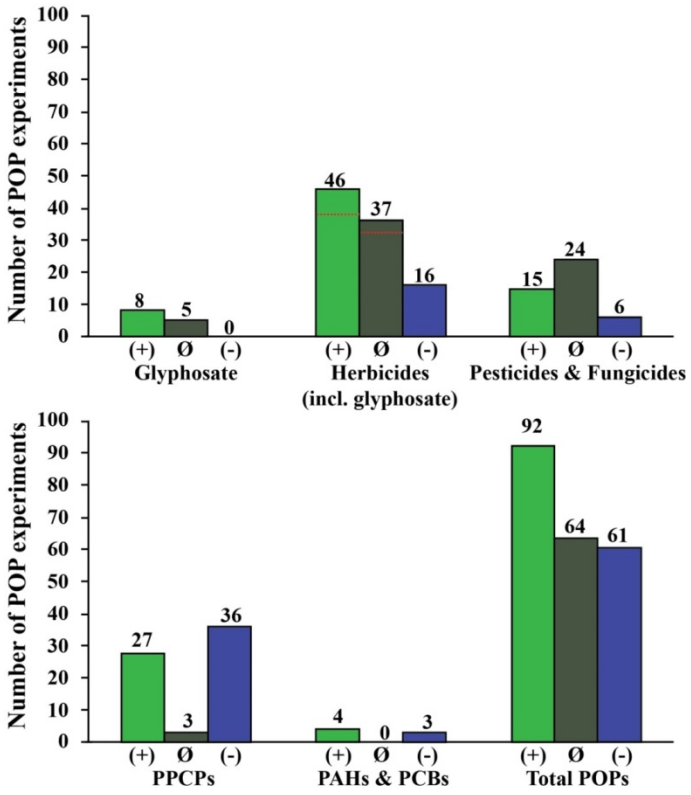


***Figure 3-2***

**Figure 3.** Percentage of experiments that compared cyanobacteria and eukaryotic phytoplankton in the presence of antibiotics by year, with positive (green), neutral (brown), and negative (blue) categories referring to antibiotics that favored cyanobacteria, showed taxa more and less sensitive than cyanobacteria, and did not favor cyanobacteria relative to eukaryotic taxa, respectively, in multi-phytoplankton species experiments. Numbers inside histogram bars represent the number of experiments comparing cyanobacteria and eukaryotic taxa in the presence of antibiotics each year.

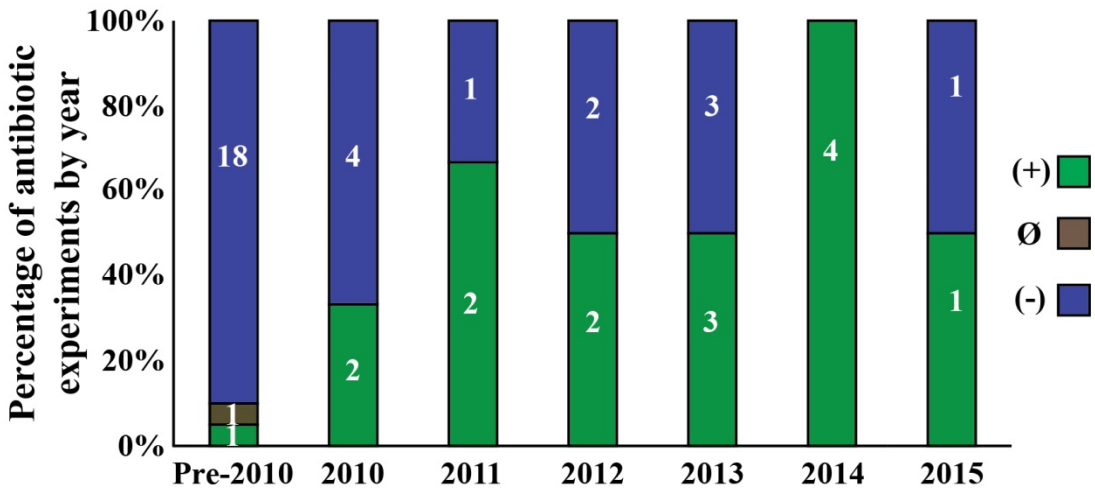


***Figure 3-3***

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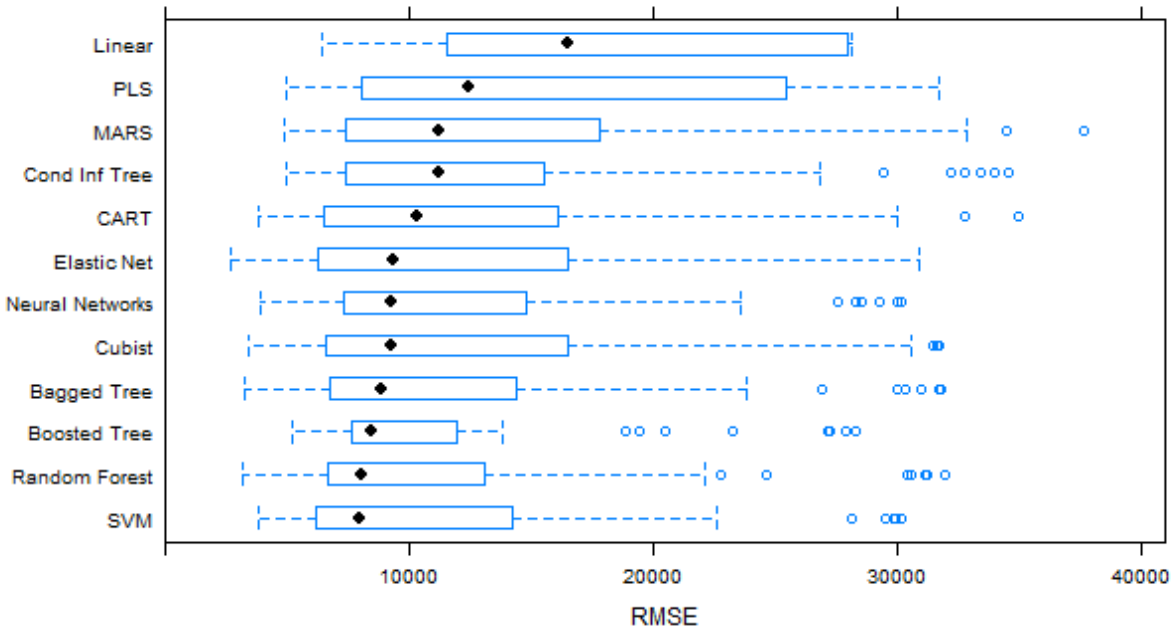
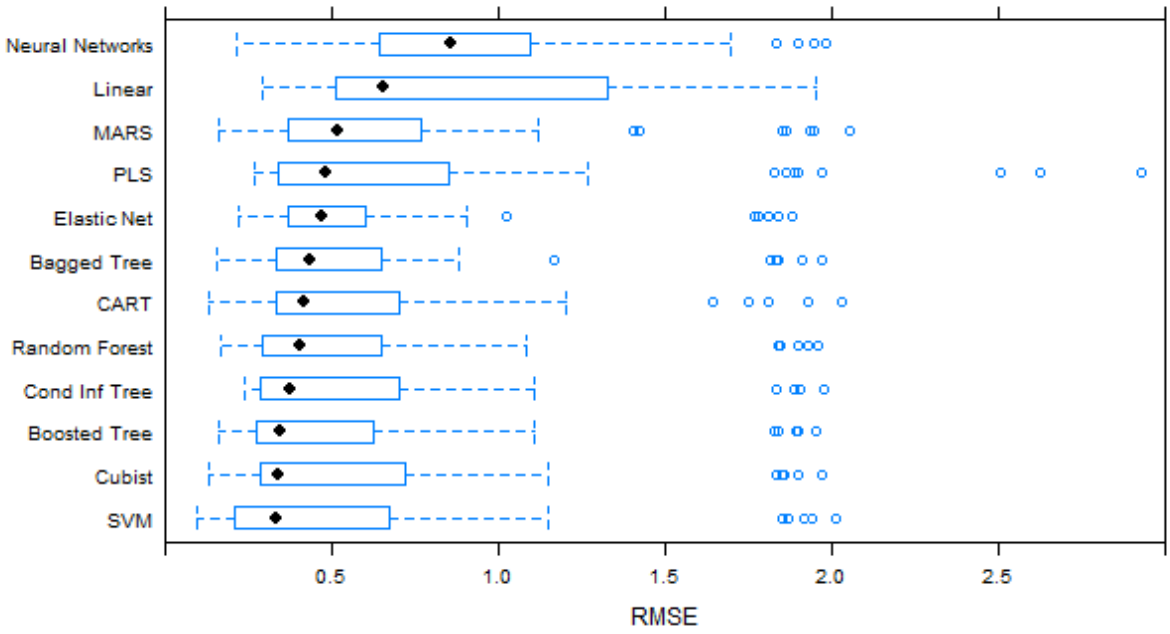
# Appendices

## Appendix A: Chapter 1 Supplemental Figures and Tables

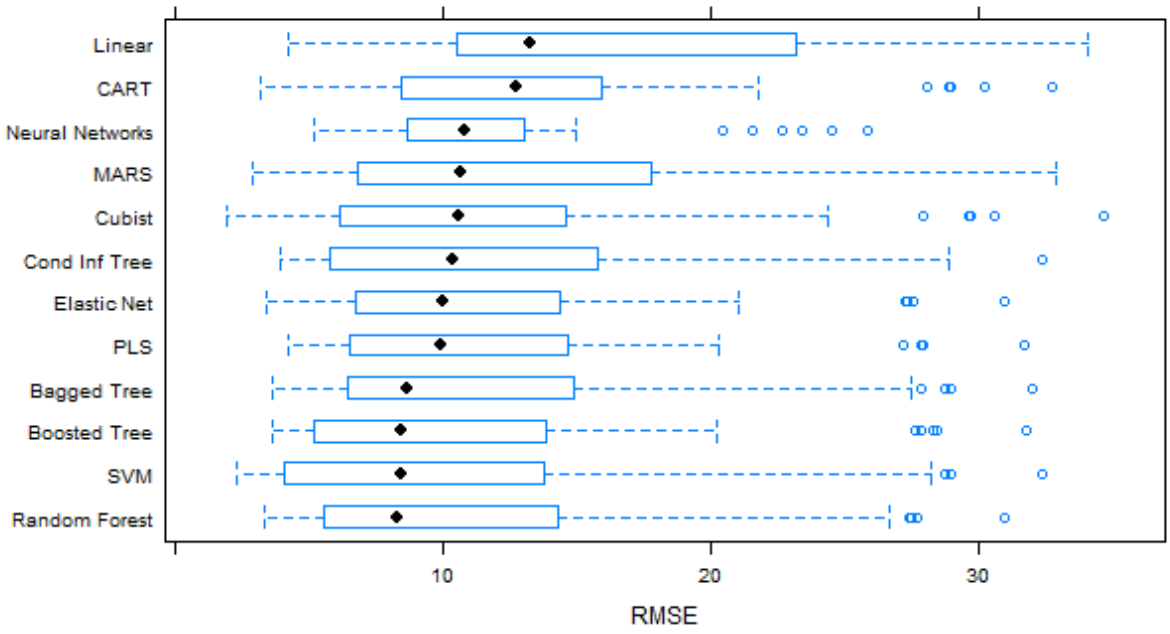
**Supplemental Figure 1.** Boxplots of the Root Mean Square Error (RMSE) values of 12 predictive modeling techniques for cyanobacterial abundance (a), microcystin (b), and geosmin (c) concentrations in Cheney Reservoir, KS. The models were trained using a training dataset comprised of 75% of the response variable data and models were computed on a test dataset comprised of the other 25% of the dataset. Each modeling technique used a repeated (repeats = 5) 10-fold cross validation to generate multiple RMSE values and ensure the most robust fit of the models. The models with the 3 lowest average RMSE values were the most robust, and thus were deemed the best predictive modeling techniques for the response variable. Black dots represent the average RMSE for each modeling technique.




Supplemental Figure 1a

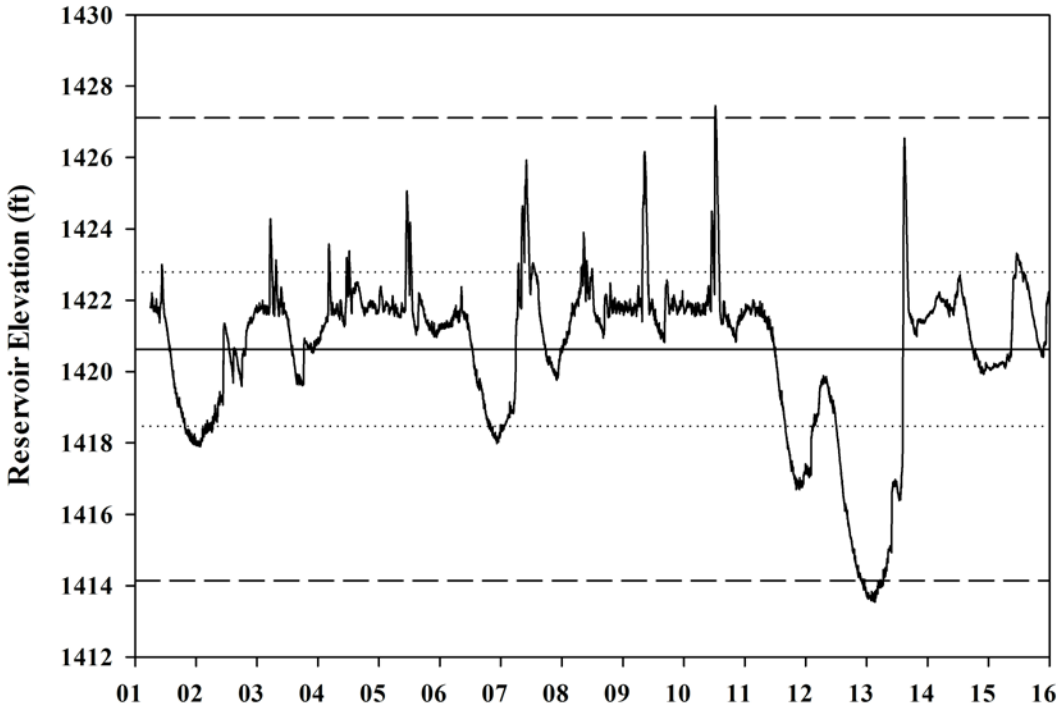



Supplemental Figure 1b




Supplemental Figure 1c

**Supplemental Figure 2.** Hourly measurements of reservoir elevation of Cheney Reservoir throughout the study period (2001-2015). Reservoir elevation is in feet above the National Geodetic Vertical Datum of 1929. Solid, dotted, and dashed lines represent the mean, 1 standard deviation, and 3 standard deviations from the mean, respectively. Drought events in 2006-2007 and 2011-2013 and corresponding large inflow events after droughts caused data to exceed 1 and/or 3 standard deviations from the mean, respectively, and thus represent extremes in reservoir elevation. Data from the USGS National Water Information System at *http://dx.doi .org/10.5066/F7P55KJN.*



**Supplemental Table 1.** Observed (Obs.) and predicted model outcomes for developed cyanobacterial abundance models. See Table 2 for model abbreviations. Cyanobacterial abundance is in cells/mL.

| Date | Obs. Cyano | Linear | PLS | Enet | MARS | SVM | Nnet | CART | CI Tree | BagT | RF | BT | Cubist |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8/7/2002 | 48 | 7842 | 11114 | 5118 | 7638 | -1491 | 2692 | 5118 | 7262 | 7335 | 6851 | 1845 | 3133 |
| 9/4/2002 | 2278 | 15716 | 10910 | 6596 | 7638 | 1827 | 6366 | 5118 | 7262 | 6167 | 5322 | 3786 | 5063 |
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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| 12/12/2013 | 248 | -7213 | 4559 | 3646 | 7638 | 1889 | 814 | 5118 | 7262 | 3361 | 2498 | 1483 | 1532 |
| 1/15/2014 | 2112 | -16026 | 766 | 2073 | 7638 | 4277 | -2912 | 5118 | 7262 | 2443 | 1941 | 441 | 1624 |
| 2/19/2014 | 1 | -16330 | 577 | 2199 | 7638 | 262 | -2689 | 5118 | 7262 | 2327 | 1342 | -1872 | 710 |
| 3/19/2014 | 3232 | -8755 | 13476 | 1236 | -13374 | 2232 | 5383 | 5118 | 7262 | 7278 | 8106 | 2900 | 2528 |
| 4/16/2014 | 4319 | -4999 | 9165 | 3867 | 7638 | 5392 | 4827 | 5118 | 7262 | 3037 | 5636 | 5236 | 4138 |
| 5/20/2014 | 2349 | -5602 | 8066 | 7185 | 7638 | 3460 | -1675 | 5118 | 7262 | 3441 | 3753 | 1815 | 2828 |
| 7/10/2014 | 35 | 454 | 6513 | 7642 | 7638 | 1985 | 7174 | 5118 | 7262 | 4977 | 3354 | 1000 | 4611 |
| 7/22/2014 | 1249 | 5541 | 8420 | 12216 | 7638 | 3090 | 6840 | 5118 | 7262 | 5506 | 3060 | 2865 | 5373 |
| 8/5/2014 | 15018 | 8476 | 10817 | 12777 | 7638 | 10505 | 5124 | 5118 | 7262 | 8885 | 11447 | 11541 | 9833 |
| 9/16/2014 | 12966 | 11706 | 12354 | 15467 | 7638 | 11334 | 11672 | 5118 | 7262 | 8982 | 11624 | 13581 | 9944 |
| 10/28/2014 | 11406 | 15933 | 11490 | 11188 | 7638 | 12710 | 17037 | 5118 | 7262 | 9797 | 10105 | 9884 | 11509 |
| 11/20/2014 | 10203 | 8943 | 14440 | 6974 | 7638 | 8959 | 10251 | 5118 | 7262 | 8234 | 8087 | 8641 | 7932 |
| 12/16/2014 | 10854 | 10976 | 12139 | 5672 | 7638 | 9220 | 2386 | 5118 | 7262 | 11080 | 12088 | 9225 | 9960 |
| 1/13/2015 | 645 | 4432 | 4105 | 3646 | 7638 | 2280 | 5989 | 5118 | 7262 | 7267 | 6405 | 5230 | 4979 |
| 2/10/2015 | 790 | 5624 | 13427 | 4564 | 7638 | 2430 | 7687 | 5118 | 7262 | 5972 | 5877 | 4794 | 3544 |
| 3/10/2015 | 91 | -3419 | 12030 | 3204 | 7638 | 5383 | 8207 | 5118 | 7262 | 6744 | 10048 | 9858 | 2945 |
| 4/15/2015 | 405 | 3546 | 8029 | 4468 | 7638 | 6506 | 3765 | 5118 | 7262 | 3711 | 6374 | -84 | 4461 |
| 6/9/2015 | 14313 | 5956 | 12163 | 6808 | 7638 | 12678 | 1848 | 5118 | 7262 | 11418 | 14388 | 15538 | 11130 |

**Supplemental Table 2.** Observed (Obs.) and predicted model outcomes for developed microcystin models. See Table 2 for model abbreviations. Microcystin concentrations are in µg/L.

| Date | Obs. MC | Linear | PLS | Enet | MARS | SVM | Nnet | CART | CI Tree | BagT | RF | BT | Cubist |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9/4/2002 | 0.2 | 0.77 | 0.84 | 0.57 | 0.40 | 0.34 | 0.67 | 0.22 | 0.33 | 0.97 | 0.64 | 0.73 | 0.44 |
| 2/10/2003 | 0.18 | 0.28 | -0.05 | 0.19 | 0.22 | 0.12 | 0.08 | 0.22 | 0.33 | 0.11 | 0.14 | 0.15 | 0.05 |
| 7/17/2003 | 0.11 | 0.62 | 0.67 | 0.83 | 0.24 | 0.17 | 0.25 | 1.04 | 0.33 | 0.49 | 0.50 | 0.65 | 0.22 |
| 7/28/2003 | 0.05 | 0.54 | 0.62 | 0.57 | 0.22 | 0.13 | 0.24 | 1.04 | 0.33 | 0.51 | 0.29 | 0.55 | 0.16 |
| 3/10/2004 | 0.14 | 0.04 | -0.09 | 0.14 | 0.22 | 0.11 | 0.35 | 0.22 | 0.33 | 0.10 | 0.25 | 0.18 | 0.05 |
| 4/8/2004 | 0.26 | -0.14 | -0.35 | -0.14 | 0.22 | 0.18 | 0.08 | 0.22 | 0.33 | 0.10 | 0.20 | 0.12 | 0.07 |
| 5/5/2004 | 0.14 | -0.33 | 0.05 | -0.20 | 0.22 | 0.06 | -0.01 | 0.22 | 0.33 | 0.10 | 0.17 | 0.15 | 0.07 |
| 8/27/2004 | 0.13 | 0.59 | 0.64 | 0.66 | 0.22 | 0.23 | 0.27 | 0.22 | 0.33 | 0.47 | 0.44 | 0.48 | 0.32 |
| 9/9/2004 | 0.15 | 0.75 | 0.64 | 0.68 | 0.22 | 0.23 | 0.36 | 0.22 | 0.33 | 0.53 | 0.46 | 0.54 | 0.21 |
| 6/1/2005 | 0.05 | 0.16 | 0.38 | -0.04 | 0.39 | 0.04 | -0.05 | 0.22 | 0.33 | 0.13 | 0.19 | 0.17 | 0.08 |
| 6/15/2005 | 0.13 | 0.92 | 0.43 | 0.46 | 0.38 | 0.19 | 0.79 | 0.22 | 0.33 | 0.18 | 0.28 | 0.20 | 0.10 |
| 6/29/2005 | 0.05 | 0.34 | 0.44 | 0.16 | 0.22 | 0.13 | 0.20 | 0.22 | 0.33 | 0.22 | 0.15 | 0.26 | 0.10 |
| 7/13/2005 | 1.74 | 0.59 | 0.47 | 0.83 | 0.22 | 0.28 | 0.34 | 1.04 | 0.33 | 0.75 | 0.70 | 0.70 | 0.23 |
| 7/27/2005 | 0.61 | 0.59 | 0.72 | 0.98 | 0.22 | 0.38 | 0.56 | 0.22 | 0.33 | 0.39 | 0.73 | 0.52 | 0.22 |
| 8/30/2005 | 2.28 | 0.86 | 0.66 | 0.96 | 0.22 | 0.33 | 1.97 | 1.04 | 0.33 | 1.32 | 1.42 | 0.92 | 0.90 |
| 9/7/2005 | 2.08 | 0.81 | 0.60 | 0.89 | 0.22 | 0.32 | 1.91 | 0.22 | 0.33 | 1.16 | 1.52 | 0.72 | 0.85 |
| 10/13/2005 | 0.38 | 0.54 | 0.46 | 0.56 | 0.22 | 0.28 | 0.94 | 0.22 | 0.33 | 0.54 | 0.38 | 0.42 | 0.33 |
| 10/27/2005 | 0.2 | 0.21 | 0.29 | 0.08 | 0.22 | 0.20 | 0.21 | 0.22 | 0.33 | 0.26 | 0.17 | 0.33 | 0.16 |
| 1/11/2006 | 0.05 | 0.47 | -0.17 | 0.09 | 0.22 | 0.14 | 0.47 | 0.22 | 0.33 | 0.10 | 0.10 | 0.21 | 0.06 |
| 3/1/2006 | 0.05 | 0.09 | -0.15 | -0.01 | 0.22 | 0.13 | 0.05 | 0.22 | 0.33 | 0.11 | 0.08 | 0.13 | 0.05 |
| 3/29/2006 | 0.05 | -0.23 | 0.08 | -0.07 | 0.22 | 0.12 | 0.06 | 0.22 | 0.33 | 0.14 | 0.17 | 0.12 | 0.05 |
| 4/25/2006 | 0.05 | -0.03 | 0.09 | -0.03 | 0.22 | 0.12 | 0.02 | 0.22 | 0.33 | 0.10 | 0.15 | 0.16 | 0.08 |
| 5/17/2006 | 0.05 | 0.30 | 0.26 | 0.11 | 0.22 | 0.09 | 0.00 | 0.22 | 0.33 | 0.14 | 0.20 | 0.12 | 0.08 |
| 5/31/2006 | 0.05 | 0.53 | 0.46 | 0.27 | 0.44 | 0.13 | 0.15 | 0.22 | 0.33 | 0.25 | 0.16 | 0.16 | 0.12 |
| 6/14/2006 | 0.05 | 0.09 | 0.51 | 0.10 | 0.48 | 0.13 | 0.03 | 0.22 | 0.33 | 0.29 | 0.16 | 0.20 | 0.11 |
| 6/28/2006 | 0.21 | 0.45 | 0.50 | 0.40 | 0.50 | 0.20 | 0.11 | 0.22 | 0.33 | 0.21 | 0.34 | 0.24 | 0.11 |
| 7/13/2006 | 0.17 | 0.56 | 0.69 | 0.49 | 0.57 | 0.26 | 0.19 | 0.22 | 0.33 | 0.27 | 0.38 | 0.27 | 0.16 |
| 7/26/2006 | 0.18 | 0.78 | 0.80 | 0.81 | 0.61 | 0.28 | 0.63 | 1.04 | 0.33 | 0.80 | 0.30 | 0.58 | 0.17 |
| 8/10/2006 | 1.61 | 0.89 | 0.67 | 1.04 | 0.66 | 0.25 | 1.19 | 1.04 | 0.33 | 1.21 | 0.85 | 0.89 | 1.68 |
| 9/6/2006 | 2.65 | 1.32 | 0.87 | 1.37 | 0.39 | 0.47 | 2.51 | 0.22 | 0.33 | 1.03 | 1.66 | 0.72 | 1.73 |
| 9/20/2006 | 1.25 | 1.15 | 0.68 | 1.16 | 0.32 | 0.60 | 1.26 | 0.22 | 0.33 | 0.68 | 0.97 | 0.57 | 1.42 |
| 10/11/2006 | 0.72 | 0.46 | 0.38 | 0.51 | 0.22 | 0.24 | 0.39 | 0.22 | 0.33 | 0.62 | 0.78 | 0.55 | 2.28 |
| 10/25/2006 | 0.32 | 0.19 | 0.17 | 0.18 | 0.22 | 0.24 | 0.19 | 0.22 | 0.33 | 0.27 | 0.38 | 0.36 | 0.23 |
| 12/12/2006 | 0.05 | -0.16 | 0.10 | -0.15 | 0.22 | 0.13 | 0.03 | 0.22 | 0.33 | 0.10 | 0.11 | 0.14 | 0.05 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2/7/2007 | 0.05 | -0.03 | 0.01 | 0.07 | 0.22 | 0.06 | 0.06 | 0.22 | 0.33 | 0.10 | 0.08 | 0.16 | 0.05 |
| 3/7/2007 | 0.05 | -0.06 | 0.15 | 0.21 | 0.22 | 0.08 | 0.08 | 0.22 | 0.33 | 0.11 | 0.17 | 0.19 | 0.06 |
| 4/9/2007 | 0.05 | -0.08 | 0.07 | -0.01 | 0.22 | 0.11 | 0.14 | 0.22 | 0.33 | 0.10 | 0.18 | 0.17 | 0.06 |
| 5/8/2007 | 0.1 | 0.25 | 0.25 | 0.04 | 0.22 | 0.06 | 0.14 | 0.22 | 0.33 | 0.10 | 0.19 | 0.20 | 0.08 |
| 5/31/2007 | 0.23 | 0.33 | -0.05 | -0.02 | 0.22 | 0.16 | 0.18 | 0.22 | 0.33 | 0.17 | 0.24 | 0.25 | 0.08 |
| 6/13/2007 | 0.05 | 0.23 | 0.18 | 0.04 | 0.22 | 0.08 | 0.04 | 0.22 | 0.33 | 0.18 | 0.11 | 0.21 | 0.09 |
| 6/25/2007 | 0.05 | 0.04 | 0.40 | 0.01 | 0.22 | 0.19 | -0.03 | 0.22 | 0.33 | 0.20 | 0.23 | 0.27 | 0.10 |
| 7/9/2007 | 0.22 | 0.21 | 0.47 | 0.35 | 0.22 | 0.30 | 0.28 | 0.22 | 0.33 | 0.25 | 0.24 | 0.40 | 0.15 |
| 7/23/2007 | 0.61 | 0.62 | 0.71 | 0.85 | 0.22 | 0.53 | 0.56 | 1.04 | 0.33 | 0.68 | 0.65 | 0.75 | 0.21 |
| 8/7/2007 | 0.66 | 0.68 | 0.82 | 0.83 | 0.22 | 0.50 | 0.27 | 1.04 | 0.33 | 0.96 | 0.66 | 0.87 | 0.88 |
| 8/15/2007 | 1.39 | 0.79 | 0.84 | 0.84 | 0.22 | 0.47 | 0.95 | 1.04 | 0.33 | 1.13 | 1.15 | 0.93 | 0.78 |
| 8/28/2007 | 1.12 | 0.84 | 0.68 | 0.92 | 0.22 | 0.41 | 0.69 | 1.04 | 0.33 | 1.11 | 0.80 | 0.78 | 0.35 |
| 9/12/2007 | 0.63 | 0.72 | 0.76 | 0.86 | 0.22 | 0.46 | 0.66 | 0.22 | 0.33 | 0.78 | 0.67 | 0.54 | 0.41 |
| 9/24/2007 | 0.68 | 0.31 | 0.66 | 0.50 | 0.22 | 0.36 | 0.21 | 0.22 | 0.33 | 0.51 | 0.54 | 0.50 | 0.34 |
| 10/15/2007 | 0.28 | 0.39 | 0.45 | 0.36 | 0.22 | 0.26 | 0.13 | 0.22 | 0.33 | 0.28 | 0.26 | 0.31 | 0.22 |
| 10/29/2007 | 0.15 | 0.26 | 0.39 | 0.11 | 0.22 | 0.23 | 0.11 | 0.22 | 0.33 | 0.17 | 0.21 | 0.26 | 0.15 |
| 11/13/2007 | 0.1 | 0.28 | 0.36 | 0.11 | 0.22 | 0.18 | 0.04 | 0.22 | 0.33 | 0.12 | 0.19 | 0.19 | 0.19 |
| 12/19/2007 | 0.05 | -0.19 | 0.13 | -0.12 | 0.22 | 0.08 | 0.05 | 0.22 | 0.33 | 0.12 | 0.08 | 0.17 | 0.05 |
| 2/11/2008 | 0.15 | -0.09 | 0.01 | 0.12 | 0.22 | 0.09 | 0.03 | 0.22 | 0.33 | 0.10 | 0.12 | 0.15 | 0.05 |
| 4/1/2008 | 0.12 | 0.07 | 0.26 | 0.08 | 0.22 | 0.12 | 0.08 | 0.22 | 0.33 | 0.10 | 0.11 | 0.23 | 0.06 |
| 4/16/2008 | 0.05 | -0.28 | 0.07 | -0.04 | 0.22 | 0.09 | 0.01 | 0.22 | 0.33 | 0.10 | 0.09 | 0.19 | 0.06 |
| 4/29/2008 | 0.05 | -0.29 | -0.21 | -0.08 | 0.22 | 0.12 | 0.05 | 0.22 | 0.33 | 0.10 | 0.08 | 0.19 | 0.06 |
| 5/13/2008 | 0.05 | 0.11 | -0.14 | 0.03 | 0.22 | 0.13 | 0.09 | 0.22 | 0.33 | 0.10 | 0.08 | 0.19 | 0.07 |
| 6/3/2008 | 0.05 | 0.39 | 0.23 | 0.15 | 0.29 | 0.13 | 0.00 | 0.22 | 0.33 | 0.14 | 0.10 | 0.22 | 0.09 |
| 6/18/2008 | 0.1 | 0.20 | 0.20 | 0.21 | 0.26 | 0.17 | 0.03 | 0.22 | 0.33 | 0.19 | 0.14 | 0.24 | 0.09 |
| 7/7/2008 | 0.83 | 0.39 | 0.58 | 0.46 | 0.23 | 0.32 | 0.41 | 0.22 | 0.33 | 0.38 | 0.63 | 0.40 | 0.20 |
| 7/21/2008 | 1.06 | 0.82 | 0.70 | 1.10 | 0.25 | 0.46 | 0.97 | 1.04 | 0.33 | 0.61 | 0.73 | 0.75 | 0.24 |
| 7/28/2008 | 0.43 | 0.71 | 0.79 | 1.03 | 0.26 | 0.49 | 0.62 | 1.04 | 0.33 | 0.66 | 0.58 | 0.75 | 0.34 |
| 8/18/2008 | 0.19 | 0.67 | 0.67 | 0.66 | 0.30 | 0.27 | 0.20 | 0.22 | 0.33 | 0.42 | 0.29 | 0.41 | 0.36 |
| 9/2/2008 | 0.05 | 0.88 | 0.80 | 0.74 | 0.33 | 0.40 | 0.78 | 0.22 | 0.33 | 0.40 | 0.60 | 0.49 | 0.68 |
| 9/17/2008 | 0.11 | 0.84 | 0.62 | 0.72 | 0.22 | 0.39 | 1.44 | 0.22 | 0.33 | 0.51 | 0.45 | 0.46 | 0.27 |
| 10/1/2008 | 0.05 | 1.15 | 0.60 | 0.73 | 0.22 | 0.33 | 0.65 | 0.22 | 0.33 | 0.41 | 0.29 | 0.38 | 0.25 |
| 10/15/2008 | 0.05 | 0.40 | 0.18 | 0.27 | 0.22 | 0.13 | 0.16 | 0.22 | 0.33 | 0.23 | 0.09 | 0.29 | 0.13 |
| 11/4/2008 | 0.11 | 0.43 | 0.10 | 0.07 | 0.22 | 0.15 | 0.17 | 0.22 | 0.33 | 0.15 | 0.10 | 0.18 | 0.07 |
| 12/2/2008 | 0.05 | 0.24 | 0.04 | 0.05 | 0.22 | 0.12 | 0.06 | 0.22 | 0.33 | 0.10 | 0.07 | 0.10 | 0.06 |
| 1/6/2009 | 0.05 | 0.29 | 0.00 | 0.17 | 0.22 | 0.12 | 0.23 | 0.22 | 0.33 | 0.12 | 0.29 | 0.20 | 0.05 |
| 1/20/2009 | 0.05 | 0.19 | -0.03 | 0.14 | 0.22 | 0.10 | 0.05 | 0.22 | 0.33 | 0.10 | 0.07 | 0.22 | 0.05 |
| 2/2/2009 | 0.05 | 0.74 | 0.03 | 0.14 | 0.22 | 0.14 | 0.95 | 0.22 | 0.33 | 0.10 | 0.14 | 0.25 | 0.05 |
| 2/18/2009 | 0.05 | 0.07 | 0.08 | 0.14 | 0.22 | 0.08 | 0.00 | 0.22 | 0.33 | 0.11 | 0.06 | 0.13 | 0.05 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2/25/2009 | 0.05 | 0.02 | 0.14 | 0.12 | 0.22 | 0.09 | 0.00 | 0.22 | 0.33 | 0.11 | 0.09 | 0.24 | 0.05 |
| 3/3/2009 | 0.05 | -0.02 | 0.26 | 0.13 | 0.22 | 0.09 | 0.05 | 0.22 | 0.33 | 0.11 | 0.10 | 0.26 | 0.05 |
| 3/9/2009 | 0.05 | 0.09 | 0.21 | 0.13 | 0.22 | 0.09 | -0.01 | 0.22 | 0.33 | 0.11 | 0.09 | 0.26 | 0.05 |
| 3/16/2009 | 0.05 | 0.15 | 0.19 | 0.11 | 0.22 | 0.12 | 0.03 | 0.22 | 0.33 | 0.10 | 0.07 | 0.22 | 0.05 |
| 3/25/2009 | 0.05 | 0.20 | 0.29 | 0.09 | 0.22 | 0.13 | 0.04 | 0.22 | 0.33 | 0.11 | 0.09 | 0.21 | 0.06 |
| 4/8/2009 | 0.05 | -0.27 | 0.18 | -0.04 | 0.22 | 0.12 | 0.07 | 0.22 | 0.33 | 0.11 | 0.15 | 0.19 | 0.05 |
| 4/29/2009 | 0.05 | -0.10 | -0.09 | -0.06 | 0.22 | 0.10 | 0.03 | 0.22 | 0.33 | 0.10 | 0.08 | 0.18 | 0.07 |
| 5/27/2009 | 0.05 | 0.25 | 0.20 | 0.13 | 0.22 | 0.19 | 0.26 | 0.22 | 0.33 | 0.12 | 0.15 | 0.19 | 0.10 |
| 6/9/2009 | 0.05 | -0.21 | 0.18 | -0.13 | 0.22 | 0.08 | 0.11 | 0.22 | 0.33 | 0.16 | 0.09 | 0.14 | 0.08 |
| 6/23/2009 | 0.05 | 0.40 | 0.53 | 0.77 | 0.22 | 0.34 | 0.08 | 0.22 | 0.33 | 0.25 | 0.37 | 0.39 | 0.27 |
| 7/7/2009 | 0.13 | 0.27 | 0.76 | 0.62 | 0.22 | 0.31 | 0.05 | 0.22 | 0.33 | 0.38 | 0.33 | 0.39 | 0.20 |
| 7/21/2009 | 0.05 | 0.11 | 0.59 | 0.28 | 0.22 | 0.17 | 0.15 | 0.22 | 0.33 | 0.38 | 0.19 | 0.26 | 0.17 |
| 8/5/2009 | 0.05 | 0.26 | 0.64 | 0.39 | 0.22 | 0.21 | -0.03 | 0.22 | 0.33 | 0.36 | 0.34 | 0.34 | 0.27 |
| 8/24/2009 | 0.05 | 0.41 | 0.65 | 0.52 | 0.22 | 0.31 | 0.16 | 0.22 | 0.33 | 0.40 | 0.22 | 0.31 | 0.17 |
| 9/2/2009 | 0.05 | 0.45 | 0.67 | 0.55 | 0.22 | 0.28 | 0.35 | 0.22 | 0.33 | 0.31 | 0.23 | 0.42 | 0.14 |
| 9/16/2009 | 2.91 | 0.72 | 0.58 | 0.48 | 0.22 | 0.27 | 2.10 | 0.22 | 0.33 | 0.79 | 1.59 | 0.61 | 0.65 |
| 10/5/2009 | 0.05 | 0.05 | 0.14 | 0.27 | 0.22 | 0.13 | 0.03 | 0.22 | 0.33 | 0.35 | 0.15 | 0.27 | 0.08 |
| 10/19/2009 | 0.05 | -0.18 | 0.04 | -0.09 | 0.22 | 0.11 | 0.00 | 0.22 | 0.33 | 0.24 | 0.13 | 0.33 | 0.06 |
| 11/23/2009 | 0.05 | 0.35 | 0.09 | -0.01 | 0.22 | 0.13 | 0.09 | 0.22 | 0.33 | 0.14 | 0.07 | 0.19 | 0.07 |
| 12/16/2009 | 0.05 | -0.05 | -0.07 | -0.06 | 0.22 | 0.10 | 0.05 | 0.22 | 0.33 | 0.10 | 0.12 | 0.11 | 0.05 |
| 1/12/2010 | 0.05 | -0.03 | -0.23 | 0.05 | 0.22 | 0.13 | 0.05 | 0.22 | 0.33 | 0.10 | 0.13 | 0.11 | 0.05 |
| 2/10/2010 | 0.05 | 0.55 | 0.15 | 0.09 | 0.22 | 0.13 | 0.11 | 0.22 | 0.33 | 0.10 | 0.20 | 0.21 | 0.05 |
| 2/17/2010 | 0.05 | 1.07 | 0.42 | -0.02 | 0.22 | 0.16 | 1.02 | 0.22 | 0.33 | 0.10 | 0.23 | 0.23 | 0.05 |
| 2/24/2010 | 0.1 | 0.53 | 0.26 | 0.10 | 0.22 | 0.14 | 0.12 | 0.22 | 0.33 | 0.10 | 0.17 | 0.21 | 0.05 |
| 3/3/2010 | 0.05 | 0.39 | 0.20 | -0.11 | 0.22 | 0.13 | 0.07 | 0.22 | 0.33 | 0.10 | 0.19 | 0.24 | 0.05 |
| 3/10/2010 | 0.05 | -0.20 | 0.12 | -0.03 | 0.22 | 0.11 | 0.03 | 0.22 | 0.33 | 0.14 | 0.31 | 0.23 | 0.05 |
| 3/22/2010 | 0.05 | -0.23 | 0.19 | -0.10 | 0.22 | 0.13 | 0.06 | 0.22 | 0.33 | 0.10 | 0.20 | 0.20 | 0.05 |
| 4/12/2010 | 0.05 | -0.26 | 0.07 | -0.19 | 0.22 | 0.07 | -0.09 | 0.22 | 0.33 | 0.10 | 0.08 | 0.09 | 0.07 |
| 5/18/2010 | 0.05 | -0.10 | 0.01 | -0.13 | 0.22 | 0.08 | 0.03 | 0.22 | 0.33 | 0.10 | 0.12 | 0.11 | 0.08 |
| 6/17/2010 | 0.05 | 1.09 | 0.48 | 0.69 | 0.22 | 0.15 | 0.39 | 0.22 | 0.33 | 0.19 | 0.33 | 0.27 | 0.11 |
| 6/30/2010 | 0.05 | -0.15 | 0.12 | 0.16 | 0.22 | 0.13 | 0.06 | 1.04 | 0.33 | 0.41 | 0.19 | 0.59 | 0.11 |
| 7/14/2010 | 0.05 | 0.65 | 0.31 | 0.42 | 0.22 | 0.13 | 0.57 | 1.04 | 0.33 | 0.54 | 0.37 | 0.61 | 0.09 |
| 7/29/2010 | 0.05 | 0.24 | 0.26 | 0.15 | 0.22 | 0.19 | 0.30 | 1.04 | 0.33 | 0.59 | 0.75 | 0.64 | 0.08 |
| 8/12/2010 | 0.05 | -0.01 | 0.39 | 0.22 | 0.22 | 0.13 | 0.03 | 1.04 | 0.33 | 0.84 | 0.19 | 0.64 | 0.20 |
| 8/26/2010 | 0.05 | 0.09 | 0.29 | 0.20 | 0.22 | 0.13 | 0.10 | 1.04 | 0.33 | 0.82 | 0.19 | 0.61 | 0.12 |
| 9/8/2010 | 0.1 | 0.15 | 0.33 | 0.17 | 0.22 | 0.18 | 0.10 | 0.22 | 0.33 | 0.27 | 0.19 | 0.35 | 0.23 |
| 9/22/2010 | 0.05 | 0.31 | 0.23 | 0.16 | 0.22 | 0.13 | 0.12 | 0.22 | 0.33 | 0.39 | 0.15 | 0.34 | 0.09 |
| 10/4/2010 | 0.05 | 0.19 | 0.02 | -0.24 | 0.22 | 0.09 | 0.59 | 0.22 | 0.33 | 0.35 | 0.18 | 0.33 | 0.07 |
| 10/18/2010 | 0.05 | 0.30 | -0.11 | -0.09 | 0.22 | 0.12 | 0.08 | 0.22 | 0.33 | 0.20 | 0.13 | 0.32 | 0.07 |
| 11/15/2010 | 0.05 | -0.01 | -0.21 | -0.30 | 0.22 | 0.06 | 0.20 | 0.22 | 0.33 | 0.14 | 0.14 | 0.19 | 0.06 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12/6/2010 | 0.05 | -0.34 | -0.38 | -0.42 | 0.22 | 0.13 | 0.06 | 0.22 | 0.33 | 0.13 | 0.11 | 0.13 | 0.06 |
| 1/18/2011 | 0.05 | 0.52 | -0.62 | 0.05 | 0.22 | 0.13 | 0.17 | 0.22 | 0.33 | 0.12 | 0.15 | 0.09 | 0.05 |
| 2/14/2011 | 0.05 | -0.38 | -0.42 | -0.09 | 0.22 | 0.12 | 0.54 | 0.22 | 0.33 | 0.11 | 0.26 | 0.14 | 0.05 |
| 3/14/2011 | 0.05 | -0.53 | -0.25 | 0.15 | 0.22 | 0.13 | 0.06 | 0.22 | 0.33 | 0.10 | 0.09 | 0.10 | 0.05 |
| 4/11/2011 | 0.05 | -0.35 | -0.42 | -0.14 | 0.22 | 0.10 | 0.12 | 0.22 | 0.33 | 0.10 | 0.10 | 0.13 | 0.07 |
| 5/11/2011 | 0.05 | -0.22 | -0.07 | -0.18 | 0.22 | 0.13 | 0.06 | 0.22 | 0.33 | 0.11 | 0.08 | 0.13 | 0.08 |
| 6/13/2011 | 0.05 | 0.32 | 0.01 | 0.17 | 0.33 | 0.13 | 0.12 | 0.22 | 0.33 | 0.11 | 0.10 | 0.15 | 0.10 |
| 7/11/2011 | 0.55 | 0.64 | 0.30 | 0.52 | 0.58 | 0.44 | 0.47 | 1.04 | 0.33 | 0.75 | 0.42 | 0.65 | 0.24 |
| 8/1/2011 | 2.16 | 0.87 | 0.85 | 1.33 | 0.74 | 0.73 | 1.95 | 1.04 | 0.33 | 1.02 | 1.55 | 0.82 | 0.88 |
| 8/8/2011 | 8.985 | 0.83 | 0.94 | 1.82 | 0.59 | 0.58 | 2.48 | 1.04 | 0.33 | 1.23 | 1.55 | 0.86 | 2.44 |
| 8/15/2011 | 0.905 | 0.54 | 0.80 | 0.40 | 0.48 | 0.57 | 0.79 | 1.04 | 0.33 | 1.12 | 0.80 | 0.75 | 0.36 |
| 8/30/2011 | 0.225 | 0.16 | 0.41 | 0.33 | 0.65 | 0.20 | 0.14 | 0.22 | 0.33 | 0.73 | 0.49 | 0.50 | 0.11 |
| 9/6/2011 | 0.295 | 0.62 | 0.70 | 0.79 | 0.56 | 0.33 | 0.26 | 0.22 | 0.33 | 0.32 | 0.35 | 0.42 | 0.28 |
| 9/20/2011 | 0.65 | 0.36 | 0.51 | 2.37 | 0.30 | 0.20 | 0.08 | 0.22 | 0.33 | 0.51 | 0.82 | 0.49 | 1.63 |
| 10/4/2011 | 0.19 | 0.53 | 0.64 | 0.53 | 0.23 | 0.30 | 0.29 | 0.22 | 0.33 | 0.35 | 0.34 | 0.40 | 0.10 |
| 11/1/2011 | 0.14 | 0.28 | 0.44 | 0.13 | 0.22 | 0.19 | 0.13 | 0.22 | 0.33 | 0.17 | 0.17 | 0.26 | 0.08 |
| 12/12/2011 | 0.12 | -0.10 | 0.04 | -0.38 | 0.22 | 0.07 | 0.24 | 0.22 | 0.33 | 0.11 | 0.24 | 0.18 | 0.05 |
| 1/18/2012 | 0.1 | -0.18 | -0.07 | -0.08 | 0.22 | 0.10 | 0.10 | 0.22 | 0.33 | 0.14 | 0.12 | 0.18 | 0.05 |
| 2/14/2012 | 0.11 | -0.37 | -0.11 | 0.15 | 0.22 | 0.07 | 0.03 | 0.22 | 0.33 | 0.11 | 0.14 | 0.17 | 0.05 |
| 3/12/2012 | 0.05 | 0.05 | 0.27 | 0.40 | 0.22 | 0.13 | 0.06 | 0.22 | 0.33 | 0.13 | 0.28 | 0.24 | 0.06 |
| 4/16/2012 | 0.05 | 0.34 | -0.11 | 0.05 | 0.22 | 0.13 | 0.11 | 0.22 | 0.33 | 0.10 | 0.08 | 0.13 | 0.08 |
| 5/16/2012 | 0.1 | 0.78 | 0.34 | 0.19 | 0.50 | 0.12 | 0.09 | 0.22 | 0.33 | 0.10 | 0.17 | 0.15 | 0.09 |
| 6/11/2012 | 0.4 | 0.51 | 0.49 | 0.23 | 0.99 | 0.32 | 0.32 | 0.22 | 0.33 | 0.22 | 0.44 | 0.27 | 0.11 |
| 6/27/2012 | 0.39 | 0.60 | 0.63 | 0.38 | 1.26 | 0.35 | 0.23 | 0.22 | 0.33 | 0.40 | 0.37 | 0.36 | 0.27 |
| 7/9/2012 | 0.79 | 1.06 | 0.87 | 1.13 | 1.78 | 0.50 | 1.04 | 1.04 | 0.33 | 0.93 | 0.83 | 0.71 | 0.35 |
| 7/31/2012 | 0.22 | 0.79 | 0.85 | 0.79 | 1.91 | 0.30 | 0.36 | 1.04 | 0.33 | 0.91 | 0.87 | 0.70 | 0.26 |
| 8/21/2012 | 0.19 | 0.84 | 0.73 | 0.83 | 1.25 | 0.27 | 0.41 | 0.22 | 0.33 | 0.51 | 0.52 | 0.30 | 0.21 |
| 9/19/2012 | 0.1 | 0.88 | 0.67 | 0.88 | 0.68 | 0.18 | 0.30 | 0.22 | 0.33 | 0.64 | 0.23 | 0.39 | 0.18 |
| 10/16/2012 | 0.1 | 0.51 | 0.52 | 0.40 | 0.22 | 0.13 | 0.20 | 0.22 | 0.33 | 0.51 | 0.30 | 0.35 | 0.15 |
| 12/11/2012 | 0.05 | 0.12 | 0.30 | 0.04 | 0.22 | 0.07 | 0.13 | 0.22 | 0.33 | 0.44 | 0.21 | 0.19 | 0.06 |
| 1/15/2013 | 0.05 | -0.01 | 0.20 | 0.06 | 0.22 | 0.13 | 0.07 | 0.22 | 0.33 | 0.42 | 0.35 | 0.19 | 0.05 |
| 1/23/2013 | 0.05 | 0.00 | 0.19 | 0.10 | 0.22 | 0.13 | 0.05 | 0.22 | 0.33 | 0.42 | 0.28 | 0.19 | 0.05 |
| 2/12/2013 | 0.05 | -0.36 | 0.26 | 0.08 | 0.22 | 0.10 | 0.13 | 0.22 | 0.33 | 0.42 | 0.24 | 0.19 | 0.05 |
| 3/19/2013 | 0.1 | -0.52 | 0.24 | -0.08 | 0.22 | 0.10 | 0.04 | 0.22 | 0.33 | 0.42 | 0.44 | 0.15 | 0.05 |
| 4/9/2013 | 0.05 | -0.26 | 0.28 | -0.09 | 0.22 | 0.11 | -0.42 | 0.22 | 0.33 | 0.42 | 0.29 | 0.17 | 0.06 |
| 5/7/2013 | 0.05 | -5.69 | -0.66 | -0.14 | 0.22 | 0.20 | 2.44 | 0.22 | 0.33 | 0.41 | 0.30 | 0.14 | 0.06 |
| 6/6/2013 | 0.05 | 0.05 | 0.34 | 0.08 | 0.83 | 0.13 | 0.04 | 0.22 | 0.33 | 0.42 | 0.39 | 0.17 | 0.08 |
| 7/8/2013 | 3 | 0.93 | 0.72 | 1.43 | 1.55 | 0.32 | 2.15 | 0.22 | 0.33 | 0.64 | 0.74 | 0.38 | 0.16 |
| 8/6/2013 | 7.3 | 1.74 | 0.91 | 1.74 | 1.71 | 0.54 | 6.14 | 1.04 | 0.33 | 1.54 | 3.61 | 0.93 | 2.57 |
| 8/19/2013 | 1.1 | 1.42 | 0.79 | 0.88 | 0.22 | 0.44 | 1.14 | 0.22 | 0.33 | 0.56 | 0.85 | 0.53 | 0.41 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9/9/2013 | 0.43 | 1.00 | 0.94 | 1.51 | 0.22 | 0.55 | 0.74 | 1.04 | 0.33 | 1.07 | 0.95 | 0.93 | 0.48 |
| 9/25/2013 | 0.47 | 0.76 | 0.61 | 0.97 | 0.22 | 0.40 | 0.76 | 0.22 | 0.33 | 0.37 | 0.37 | 0.40 | 0.27 |
| 10/21/2013 | 0.3 | 0.27 | 0.41 | 0.35 | 0.22 | 0.28 | 0.25 | 0.22 | 0.33 | 0.24 | 0.26 | 0.33 | 0.19 |
| 11/6/2013 | 0.14 | 0.31 | 0.24 | 0.23 | 0.22 | 0.19 | 0.22 | 0.22 | 0.33 | 0.13 | 0.17 | 0.18 | 0.15 |
| 11/18/2013 | 0.12 | 0.33 | 0.17 | 0.13 | 0.22 | 0.13 | 0.18 | 0.22 | 0.33 | 0.13 | 0.14 | 0.16 | 0.09 |
| 12/12/2013 | 0.05 | -0.08 | -0.08 | -0.03 | 0.22 | 0.10 | 0.17 | 0.22 | 0.33 | 0.10 | 0.18 | 0.13 | 0.05 |
| 1/15/2014 | 0.11 | 0.07 | -0.15 | 0.11 | 0.22 | 0.11 | 0.09 | 0.22 | 0.33 | 0.10 | 0.13 | 0.12 | 0.05 |
| 2/19/2014 | 0.11 | -0.16 | -0.25 | 0.22 | 0.22 | 0.11 | 0.07 | 0.22 | 0.33 | 0.10 | 0.15 | 0.13 | 0.05 |
| 3/19/2014 | 0.18 | 0.00 | 0.20 | -0.07 | 0.22 | 0.17 | 0.09 | 0.22 | 0.33 | 0.10 | 0.26 | 0.29 | 0.05 |
| 4/16/2014 | 0.19 | -0.36 | 0.19 | -0.05 | 0.22 | 0.11 | 0.02 | 0.22 | 0.33 | 0.11 | 0.20 | 0.16 | 0.06 |
| 5/20/2014 | 0.13 | 0.11 | 0.36 | 0.14 | 0.22 | 0.18 | 0.13 | 0.22 | 0.33 | 0.11 | 0.15 | 0.15 | 0.08 |
| 7/10/2014 | 0.27 | 0.68 | 0.60 | 0.62 | 0.55 | 0.19 | 0.12 | 0.22 | 0.33 | 0.32 | 0.26 | 0.33 | 0.11 |
| 7/22/2014 | 0.45 | 0.93 | 0.70 | 0.95 | 0.60 | 0.37 | 0.61 | 0.22 | 0.33 | 0.39 | 0.44 | 0.45 | 0.27 |
| 8/5/2014 | 0.37 | 1.10 | 0.83 | 1.13 | 0.70 | 0.41 | 1.08 | 0.22 | 0.33 | 0.64 | 0.46 | 0.61 | 0.41 |
| 9/16/2014 | 0.43 | 1.13 | 0.64 | 1.08 | 0.39 | 0.30 | 1.89 | 0.22 | 0.33 | 0.54 | 0.39 | 0.48 | 1.42 |
| 10/28/2014 | 0.19 | 0.69 | 0.43 | 0.54 | 0.22 | 0.18 | 0.35 | 0.22 | 0.33 | 0.19 | 0.24 | 0.33 | 0.27 |
| 11/20/2014 | 0.2 | 0.38 | 0.41 | 0.15 | 0.22 | 0.12 | 0.14 | 0.22 | 0.33 | 0.17 | 0.15 | 0.31 | 0.24 |
| 12/16/2014 | 0.05 | 0.06 | -0.04 | 0.05 | 0.22 | 0.13 | 0.03 | 0.22 | 0.33 | 0.11 | 0.12 | 0.14 | 0.06 |
| 1/13/2015 | 0.05 | 0.17 | 0.03 | 0.23 | 0.22 | 0.13 | 0.06 | 0.22 | 0.33 | 0.11 | 0.20 | 0.15 | 0.05 |
| 2/10/2015 | 0.12 | 0.28 | 0.20 | 0.22 | 0.22 | 0.08 | 0.04 | 0.22 | 0.33 | 0.11 | 0.10 | 0.18 | 0.05 |
| 3/10/2015 | 0.05 | -0.01 | 0.20 | 0.17 | 0.22 | 0.09 | 0.05 | 0.22 | 0.33 | 0.11 | 0.09 | 0.18 | 0.05 |
| 4/15/2015 | 0.05 | 0.19 | 0.14 | 0.06 | 0.22 | 0.11 | 0.04 | 0.22 | 0.33 | 0.11 | 0.08 | 0.14 | 0.07 |
| 6/9/2015 | 0.19 | 1.24 | 0.77 | 0.54 | 1.08 | 0.26 | 0.48 | 0.22 | 0.33 | 0.22 | 0.48 | 0.32 | 0.12 |

**Supplemental Table 3.** Observed (Obs.) and predicted model outcomes for developed geosmin models. See Table 2 for model abbreviations. Geosmin is in ng/L.

| Date | Obs. Geo | Linear | PLS | Enet | MARS | SVM | Nnet | CART | CI Tree | BagT | RF | BT | Cubist |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6/19/2002 | 5.7 | 15.2 | 7.7 | 6.9 | 4.9 | 5.2 | 5.3 | 5.6 | 5.6 | 10.2 | 10.9 | 8.7 | 5.7 |
| 8/7/2002 | 3.7 | 3.0 | 8.6 | 5.4 | 4.9 | 2.7 | 4.1 | 5.6 | 5.6 | 4.3 | 7.2 | 5.8 | 4.8 |
| 9/4/2002 | 4.8 | 5.9 | 8.5 | 4.6 | 4.9 | 3.5 | 3.6 | 5.6 | 5.6 | 3.4 | 6.9 | 6.1 | 4.8 |
| 2/10/2003 | 22 | 6.6 | 5.9 | 1.4 | 4.9 | 10.7 | 13.0 | 20.6 | 5.6 | 14.6 | 14.7 | 8.6 | 22.0 |
| 6/20/2003 | 63 | -3.6 | 8.7 | 2.5 | 4.9 | 2.5 | 3.9 | 20.6 | 5.6 | 13.3 | 10.2 | 9.0 | 3.5 |
| 7/7/2003 | 7 | 11.1 | 9.7 | 8.0 | 4.9 | 5.7 | 6.3 | 5.6 | 5.6 | 14.1 | 12.8 | 9.1 | 7.0 |
| 7/17/2003 | 113 | 44.5 | 17.8 | 29.8 | 81.2 | 11.6 | 109.6 | 5.6 | 18.6 | 23.5 | 59.5 | 11.8 | 113.0 |
| 3/10/2004 | 2.5 | 2.8 | 5.3 | 2.4 | 4.9 | 3.4 | 8.1 | 5.6 | 5.6 | 4.3 | 5.3 | 4.5 | 2.0 |
| 4/8/2004 | 2.5 | -0.4 | 3.8 | 4.1 | 4.9 | 2.1 | 1.9 | 5.6 | 5.6 | 2.5 | 3.3 | 3.9 | 2.5 |
| 5/5/2004 | 2.5 | 2.6 | 5.3 | 2.4 | 4.9 | 1.2 | 1.9 | 5.6 | 5.6 | 2.5 | 2.7 | 4.7 | 2.5 |
| 6/3/2004 | 2.5 | -2.5 | 6.9 | 4.5 | 4.9 | 1.8 | 2.2 | 5.6 | 5.6 | 2.9 | 4.3 | 4.8 | 2.5 |
| 7/15/2004 | 5 | 10.8 | 11.9 | 5.8 | 4.9 | 4.7 | 4.3 | 5.6 | 5.6 | 10.8 | 9.8 | 9.0 | 5.0 |
| 8/12/2004 | 2.5 | -9.6 | 4.9 | 4.5 | 4.9 | 7.3 | 1.7 | 5.6 | 5.6 | 4.8 | 13.0 | 4.8 | 58.8 |
| 8/27/2004 | 2.5 | 11.8 | 11.0 | 7.7 | 4.9 | 2.1 | 0.7 | 5.6 | 5.6 | 4.3 | 7.1 | 6.7 | 0.0 |
| 9/9/2004 | 2.5 | 4.4 | 10.3 | 6.9 | 4.9 | 2.4 | 2.8 | 5.6 | 5.6 | 8.1 | 4.0 | 7.3 | 2.5 |
| 3/16/2005 | 2.5 | 8.1 | 9.9 | 6.3 | 4.9 | 6.2 | 9.3 | 5.6 | 5.6 | 5.3 | 5.7 | 6.4 | 2.5 |
| 4/13/2005 | 2.5 | 4.3 | 7.8 | 3.4 | 4.9 | 3.8 | 2.9 | 5.6 | 5.6 | 4.3 | 3.2 | 5.0 | 2.5 |
| 5/4/2005 | 2.5 | 9.4 | 7.0 | 3.8 | 4.9 | 2.3 | 2.4 | 5.6 | 5.6 | 3.7 | 3.0 | 4.5 | 2.5 |
| 5/16/2005 | 2.5 | 4.8 | 8.9 | 5.9 | 4.9 | 4.3 | 2.7 | 5.6 | 5.6 | 5.1 | 4.9 | 6.7 | 2.5 |
| 6/1/2005 | 2.5 | 8.5 | 8.9 | 7.1 | 4.9 | 4.0 | 3.6 | 5.6 | 5.6 | 4.2 | 6.7 | 7.3 | 2.5 |
| 6/15/2005 | 43 | 17.1 | 10.8 | 8.5 | 4.9 | 6.7 | 35.2 | 5.6 | 5.6 | 15.9 | 25.0 | 9.5 | 43.0 |
| 6/29/2005 | 2.5 | 6.8 | 6.3 | 4.6 | 4.9 | 2.6 | 2.9 | 5.6 | 5.6 | 4.9 | 4.5 | 6.7 | 2.5 |
| 7/13/2005 | 43 | 17.6 | 11.1 | 15.9 | 12.1 | 10.2 | 43.1 | 20.6 | 18.6 | 20.1 | 29.0 | 11.9 | 43.0 |
| 7/27/2005 | 64 | 34.8 | 13.0 | 23.1 | 4.9 | 14.5 | 64.0 | 20.6 | 5.6 | 21.2 | 36.8 | 12.7 | 64.0 |
| 8/30/2005 | 2.5 | 17.2 | 11.2 | 10.2 | 4.9 | 3.8 | 3.5 | 5.6 | 5.6 | 5.8 | 6.8 | 6.3 | 2.5 |
| 9/7/2005 | 2.5 | 7.7 | 10.2 | 6.1 | 4.9 | 2.8 | 3.4 | 5.6 | 5.6 | 6.2 | 6.2 | 5.2 | 2.5 |
| 10/13/2005 | 2.5 | 13.2 | 10.7 | 7.2 | 4.9 | 3.8 | 3.5 | 5.6 | 5.6 | 4.2 | 6.9 | 7.4 | 2.5 |
| 10/27/2005 | 2.5 | 13.5 | 9.0 | 6.9 | 4.9 | 3.8 | 1.2 | 5.6 | 5.6 | 4.2 | 7.2 | 6.5 | 2.5 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1/11/2006 | 2.5 | 5.7 | 8.0 | 3.7 | 4.9 | 4.5 | 5.7 | 5.6 | 5.6 | 4.3 | 6.6 | 8.0 | 6.1 |
| 3/1/2006 | 5 | 9.2 | 8.1 | 4.1 | 4.9 | 6.3 | 5.7 | 20.6 | 5.6 | 14.1 | 9.1 | 8.5 | 5.0 |
| 3/29/2006 | 2.5 | 8.0 | 7.7 | 4.2 | 4.9 | 3.8 | 3.1 | 5.6 | 5.6 | 3.7 | 3.8 | 6.0 | 2.5 |
| 4/25/2006 | 2.5 | 5.5 | 9.0 | 6.8 | 9.2 | 3.4 | 2.1 | 5.6 | 5.6 | 3.4 | 5.2 | 6.7 | 2.5 |
| 5/17/2006 | 2.5 | -1.1 | 8.1 | 3.2 | 4.9 | 1.7 | 2.4 | 5.6 | 5.6 | 3.2 | 2.9 | 3.8 | 2.5 |
| 5/31/2006 | 2.5 | 5.6 | 11.2 | 8.4 | 4.9 | 3.7 | 2.5 | 5.6 | 5.6 | 4.5 | 4.3 | 7.2 | 2.5 |
| 6/14/2006 | 2.5 | 0.2 | 8.9 | 7.4 | 4.9 | 1.5 | 2.8 | 5.6 | 5.6 | 6.9 | 4.9 | 5.6 | 2.5 |
| 6/28/2006 | 2.5 | -2.3 | 9.8 | 7.6 | 4.9 | 2.4 | 3.1 | 5.6 | 5.6 | 4.4 | 3.8 | 7.4 | 2.5 |
| 7/13/2006 | 2.5 | 8.0 | 11.1 | 7.6 | 4.9 | 3.6 | 2.3 | 5.6 | 5.6 | 8.2 | 7.1 | 8.1 | 2.5 |
| 7/26/2006 | 2.5 | 1.8 | 11.1 | 5.9 | 4.9 | 3.1 | 2.7 | 5.6 | 5.6 | 9.6 | 10.0 | 6.9 | 1.9 |
| 8/10/2006 | 5 | 20.4 | 15.8 | 21.5 | 29.6 | 6.3 | 4.3 | 5.6 | 18.6 | 15.0 | 9.3 | 7.1 | 5.0 |
| 9/6/2006 | 6.5 | 7.1 | 13.7 | 12.9 | 10.2 | 5.2 | 5.9 | 5.6 | 18.6 | 7.4 | 5.9 | 8.3 | 6.5 |
| 9/20/2006 | 2.5 | -9.5 | 5.3 | 6.2 | 11.6 | 3.8 | 2.2 | 5.6 | 5.6 | 5.5 | 5.1 | 7.6 | 2.5 |
| 10/11/2006 | 2.5 | -8.3 | 8.3 | 7.6 | 4.9 | 2.1 | -1.2 | 5.6 | 5.6 | 4.1 | 6.4 | 7.0 | 3.3 |
| 10/25/2006 | 5 | -8.4 | 5.4 | 4.6 | 4.9 | 3.7 | 2.0 | 5.6 | 5.6 | 4.7 | 6.7 | 6.8 | 5.0 |
| 12/12/2006 | 2.5 | 9.0 | 9.2 | 12.9 | 8.8 | 3.8 | 2.9 | 5.6 | 18.6 | 7.6 | 5.6 | 8.2 | 2.5 |
| 2/7/2007 | 32 | 5.0 | 6.2 | 4.8 | 4.9 | 6.3 | 13.4 | 20.6 | 5.6 | 13.3 | 20.3 | 8.8 | 32.0 |
| 3/7/2007 | 20 | 11.4 | 6.3 | 9.0 | 4.9 | 5.4 | 7.5 | 5.6 | 5.6 | 3.3 | 5.8 | 6.3 | 2.6 |
| 4/9/2007 | 2.5 | 8.5 | 8.4 | 8.7 | 4.9 | 3.8 | 4.6 | 5.6 | 5.6 | 3.4 | 4.0 | 5.6 | 2.5 |
| 5/8/2007 | 2.5 | 4.7 | 8.9 | 6.1 | 4.9 | 3.9 | 5.8 | 5.6 | 5.6 | 6.1 | 6.8 | 6.1 | 5.3 |
| 5/31/2007 | 2.5 | 3.7 | 7.9 | 8.9 | 11.8 | 2.4 | 4.2 | 5.6 | 5.6 | 5.9 | 3.7 | 5.6 | 2.5 |
| 6/13/2007 | 2.5 | 2.9 | 3.9 | 4.2 | 4.9 | 1.7 | 3.4 | 5.6 | 5.6 | 4.4 | 3.6 | 5.5 | 2.5 |
| 6/25/2007 | 2.5 | 3.9 | 4.9 | 4.1 | 4.9 | 2.2 | 3.1 | 5.6 | 5.6 | 4.2 | 3.5 | 7.3 | 2.5 |
| 7/9/2007 | 2.5 | 9.0 | 7.0 | 9.1 | 4.9 | 2.6 | 2.3 | 5.6 | 5.6 | 2.9 | 4.0 | 6.3 | 2.5 |
| 7/23/2007 | 2.5 | 10.6 | 9.4 | 9.1 | 4.9 | 3.4 | 3.8 | 5.6 | 5.6 | 7.2 | 6.7 | 6.4 | 0.7 |
| 8/7/2007 | 2.5 | 9.6 | 10.2 | 9.8 | 4.9 | 3.8 | 4.5 | 5.6 | 5.6 | 5.1 | 4.5 | 5.8 | 2.5 |
| 8/15/2007 | 2.5 | 13.0 | 11.0 | 8.3 | 4.9 | 3.8 | 4.9 | 5.6 | 5.6 | 4.5 | 4.8 | 5.8 | 2.5 |
| 8/28/2007 | 2.5 | 10.6 | 8.9 | 8.3 | 4.9 | 3.5 | 2.6 | 5.6 | 5.6 | 4.5 | 4.0 | 5.4 | 2.5 |
| 9/12/2007 | 2.5 | 10.8 | 9.9 | 7.9 | 4.9 | 3.8 | 5.0 | 5.6 | 5.6 | 6.5 | 3.8 | 6.1 | 2.5 |
| 9/24/2007 | 2.5 | 9.0 | 9.5 | 9.7 | 4.9 | 3.8 | 5.0 | 5.6 | 5.6 | 6.3 | 6.0 | 6.2 | 2.5 |
| 10/15/2007 | 3.5 | 7.2 | 7.5 | 7.1 | 4.9 | 4.8 | 5.0 | 5.6 | 5.6 | 4.2 | 5.3 | 6.0 | 3.5 |
| 10/29/2007 | 3.5 | 14.0 | 7.2 | 8.9 | 4.9 | 4.8 | 3.8 | 5.6 | 5.6 | 2.5 | 4.3 | 5.2 | 3.5 |
| 11/13/2007 | 3.5 | 10.8 | 7.8 | 7.0 | 4.9 | 5.5 | 11.2 | 5.6 | 5.6 | 3.8 | 5.7 | 5.8 | 4.3 |
| 12/19/2007 | 2.5 | 8.9 | 5.7 | 4.5 | 4.9 | 6.7 | 2.4 | 5.6 | 5.6 | 3.0 | 5.1 | 7.2 | 4.7 |
| 2/11/2008 | 5.4 | 5.5 | 7.3 | 5.4 | 4.9 | 6.7 | 7.8 | 20.6 | 5.6 | 13.5 | 7.0 | 7.8 | 5.4 |
| 4/1/2008 | 11 | 5.4 | 9.7 | 7.9 | 4.9 | 7.0 | 8.9 | 5.6 | 5.6 | 5.7 | 9.1 | 7.1 | 11.0 |
| 4/16/2008 | 8 | 14.3 | 8.8 | 9.7 | 4.9 | 6.1 | 5.2 | 5.6 | 5.6 | 4.8 | 6.6 | 6.7 | 8.0 |
| 4/29/2008 | 2.5 | 11.2 | 7.5 | 10.0 | 26.1 | 3.5 | 1.7 | 5.6 | 18.6 | 12.9 | 3.5 | 6.0 | 2.5 |
| 5/13/2008 | 2.5 | 12.3 | 8.9 | 10.3 | 29.6 | 3.1 | 2.5 | 5.6 | 18.6 | 15.4 | 4.4 | 5.6 | 2.5 |
| 6/3/2008 | 2.5 | 0.7 | 5.6 | 6.2 | 4.9 | 2.9 | 1.7 | 5.6 | 5.6 | 2.5 | 2.6 | 3.7 | 2.5 |

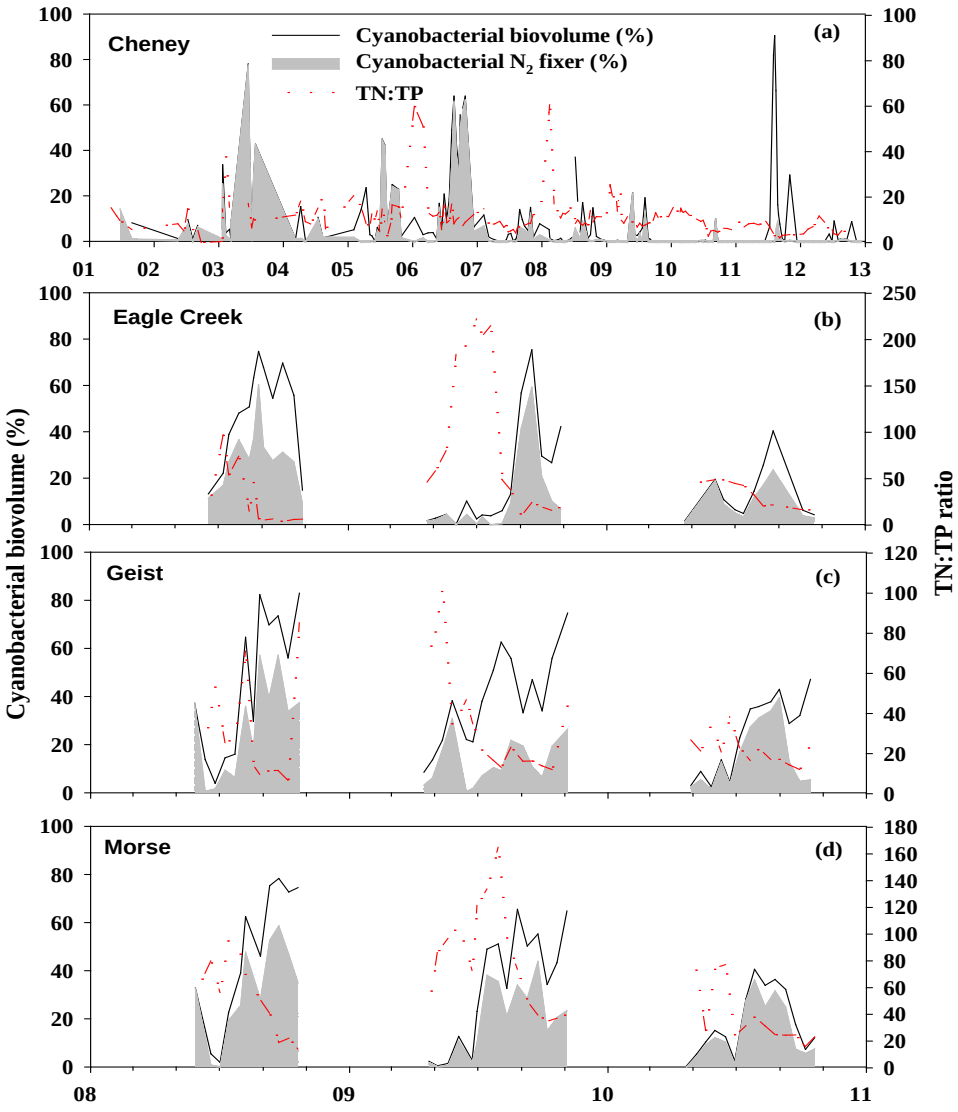
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6/18/2008 | 2.5 | 6.5 | 7.7 | 10.5 | 4.9 | 3.8 | 2.8 | 5.6 | 5.6 | 2.8 | 5.5 | 7.7 | 2.5 |
| 7/7/2008 | 4.65 | 11.1 | 9.1 | 9.3 | 4.9 | 3.3 | 2.7 | 5.6 | 5.6 | 2.9 | 4.3 | 6.2 | 4.7 |
| 7/21/2008 | 2.5 | 11.2 | 11.1 | 11.5 | 4.9 | 4.7 | 3.8 | 5.6 | 5.6 | 3.5 | 6.0 | 8.3 | 2.5 |
| 7/28/2008 | 1 | 10.9 | 10.5 | 10.1 | 4.9 | 4.7 | 11.0 | 5.6 | 5.6 | 6.4 | 3.1 | 5.3 | 1.0 |
| 8/18/2008 | 1 | 0.5 | 6.9 | 4.4 | 4.9 | 1.8 | 3.2 | 5.6 | 5.6 | 2.5 | 3.6 | 4.0 | 1.6 |
| 9/2/2008 | 5 | 9.8 | 10.3 | 6.2 | 4.9 | 3.3 | 4.3 | 5.6 | 5.6 | 3.5 | 4.3 | 4.7 | 5.0 |
| 9/17/2008 | 1 | 7.9 | 7.7 | 4.9 | 4.9 | 2.3 | 1.1 | 5.6 | 5.6 | 2.6 | 2.5 | 4.8 | 1.0 |
| 10/1/2008 | 2.8 | 8.8 | 8.3 | 5.6 | 4.9 | 3.3 | 5.0 | 5.6 | 5.6 | 2.6 | 6.8 | 5.4 | 2.8 |
| 10/15/2008 | 1 | 2.7 | 5.5 | 5.8 | 4.9 | 2.3 | 2.8 | 5.6 | 5.6 | 2.7 | 2.4 | 5.0 | 1.0 |
| 11/4/2008 | 1 | 4.5 | 4.2 | 4.4 | 4.9 | 1.8 | 3.4 | 5.6 | 5.6 | 3.2 | 2.9 | 4.7 | 0.3 |
| 12/2/2008 | 1 | -0.7 | 3.1 | 2.2 | 4.9 | 2.3 | 1.6 | 5.6 | 5.6 | 2.5 | 1.5 | 3.7 | 1.0 |
| 1/6/2009 | 2.7 | -19.1 | 0.5 | -4.8 | 4.9 | 4.0 | 2.6 | 5.6 | 5.6 | 4.3 | 4.2 | 7.4 | 2.7 |
| 1/20/2009 | 1 | 16.1 | 8.4 | 7.2 | 4.9 | 2.3 | 3.9 | 5.6 | 5.6 | 4.6 | 4.1 | 7.5 | 1.0 |
| 2/2/2009 | 1 | 1.5 | 9.8 | 2.4 | 4.9 | 2.3 | 3.8 | 5.6 | 5.6 | 6.9 | 4.9 | 7.4 | 1.0 |
| 2/18/2009 | 9.7 | 1.0 | 7.5 | 2.5 | 4.9 | 7.7 | 10.8 | 5.6 | 5.6 | 7.3 | 9.1 | 7.8 | 9.7 |
| 2/25/2009 | 8.2 | 3.0 | 8.1 | 3.4 | 4.9 | 7.3 | 10.3 | 5.6 | 5.6 | 6.0 | 8.7 | 7.4 | 8.2 |
| 3/3/2009 | 17 | 1.3 | 8.4 | 3.0 | 4.9 | 7.2 | 13.1 | 5.6 | 5.6 | 6.6 | 12.9 | 8.4 | 17.0 |
| 3/9/2009 | 11.6 | 1.1 | 7.9 | 3.5 | 4.9 | 7.0 | 10.1 | 5.6 | 5.6 | 5.8 | 8.6 | 7.5 | 16.3 |
| 3/16/2009 | 8.6 | 8.1 | 10.9 | 8.3 | 4.9 | 7.1 | 9.3 | 5.6 | 5.6 | 7.8 | 9.2 | 8.8 | 2.7 |
| 3/25/2009 | 8.5 | 2.0 | 8.3 | 5.0 | 4.9 | 6.6 | 3.1 | 5.6 | 5.6 | 5.6 | 8.2 | 7.2 | 11.1 |
| 4/8/2009 | 4.3 | 10.2 | 8.3 | 6.9 | 4.9 | 5.7 | 7.5 | 5.6 | 5.6 | 5.0 | 5.6 | 5.8 | 11.1 |
| 4/29/2009 | 1 | 4.2 | 7.3 | 5.7 | 4.9 | 2.3 | 2.0 | 5.6 | 5.6 | 6.0 | 3.3 | 5.3 | 1.0 |
| 5/27/2009 | 1 | 12.8 | 6.8 | 8.8 | 4.9 | 2.3 | 2.2 | 5.6 | 5.6 | 5.6 | 4.2 | 4.7 | 1.0 |
| 6/9/2009 | 6.2 | 2.7 | 4.8 | 4.9 | 4.9 | 2.8 | 2.8 | 5.6 | 5.6 | 2.8 | 5.5 | 5.2 | 6.2 |
| 6/23/2009 | 4.7 | 7.3 | 7.3 | 11.8 | 4.9 | 5.5 | 5.8 | 5.6 | 5.6 | 4.6 | 7.0 | 8.6 | 4.7 |
| 7/7/2009 | 14.3 | 5.1 | 11.3 | 8.2 | 4.9 | 4.4 | 4.9 | 5.6 | 5.6 | 10.5 | 11.7 | 10.5 | 14.3 |
| 7/21/2009 | 4.6 | 10.8 | 9.0 | 8.3 | 4.9 | 4.3 | 3.1 | 5.6 | 5.6 | 5.0 | 5.9 | 7.1 | 4.6 |
| 8/5/2009 | 1 | 4.8 | 7.3 | 4.3 | 4.9 | 2.2 | 2.0 | 5.6 | 5.6 | 2.7 | 2.9 | 4.3 | 1.0 |
| 8/24/2009 | 1 | 7.1 | 7.8 | 5.8 | 4.9 | 2.3 | 2.8 | 5.6 | 5.6 | 2.5 | 2.1 | 4.3 | 1.0 |
| 9/2/2009 | 1 | 10.8 | 9.2 | 7.2 | 4.9 | 4.1 | 4.4 | 5.6 | 5.6 | 2.9 | 3.9 | 5.6 | 1.0 |
| 9/16/2009 | 1 | 2.3 | 6.1 | 1.8 | 4.9 | 2.2 | 1.6 | 5.6 | 5.6 | 2.5 | 2.1 | 5.3 | 1.0 |
| 10/5/2009 | 1 | 12483.1 | 675.0 | 4688.8 | 4.9 | 5.3 | 97.0 | 5.6 | 5.6 | 2.7 | 7.1 | 4.5 | 0.4 |
| 10/19/2009 | 1 | 2.2 | 3.9 | 4.8 | 4.9 | 2.3 | 2.7 | 5.6 | 5.6 | 2.7 | 2.7 | 4.2 | 1.0 |
| 11/23/2009 | 1 | -0.9 | 3.6 | 4.0 | 4.9 | 2.2 | 1.0 | 5.6 | 5.6 | 2.5 | 1.2 | 4.0 | 1.0 |
| 12/16/2009 | 1 | 1.1 | 2.7 | 3.5 | 4.9 | 2.3 | 1.3 | 5.6 | 5.6 | 2.5 | 1.4 | 3.8 | 1.0 |
| 1/12/2010 | 1 | 5.2 | 3.9 | 7.4 | 4.9 | 1.0 | 2.7 | 20.6 | 5.6 | 8.7 | 4.1 | 5.4 | 1.0 |
| 2/10/2010 | 3.1 | 7.2 | 9.2 | 7.5 | 4.9 | 2.4 | 3.4 | 5.6 | 5.6 | 3.0 | 3.8 | 7.5 | 3.1 |
| 2/17/2010 | 3.8 | 10.7 | 16.0 | 14.5 | 18.9 | 3.6 | 4.3 | 5.6 | 18.6 | 15.8 | 4.8 | 7.4 | 3.8 |
| 2/24/2010 | 5 | 29.2 | 17.4 | 22.5 | 33.7 | 4.1 | 29.7 | 5.6 | 18.6 | 16.2 | 17.4 | 8.2 | 5.4 |
| 3/3/2010 | 4.8 | 23.2 | 15.6 | 14.1 | 23.1 | 4.4 | 5.0 | 5.6 | 18.6 | 16.3 | 7.9 | 9.0 | 4.8 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3/10/2010 | 5.8 | 12.8 | 11.8 | 5.6 | 9.7 | 6.3 | 7.4 | 5.6 | 18.6 | 6.9 | 11.1 | 9.0 | 4.6 |
| 4/12/2010 | 5.9 | 9.2 | 8.4 | 6.7 | 4.9 | 4.9 | 5.3 | 5.6 | 5.6 | 4.4 | 6.5 | 6.8 | 5.4 |
| 5/18/2010 | 1 | 5.0 | 6.3 | 4.7 | 4.9 | 2.3 | -0.9 | 5.6 | 5.6 | 2.9 | 2.1 | 3.7 | 1.0 |
| 6/17/2010 | 3.5 | -8.5 | 4.4 | 0.5 | 4.9 | 3.1 | 2.7 | 5.6 | 5.6 | 5.9 | 6.7 | 6.0 | 3.5 |
| 6/30/2010 | 3.5 | -0.6 | 5.6 | 6.9 | 4.9 | 3.2 | 3.6 | 5.6 | 5.6 | 2.8 | 5.4 | 8.0 | 3.5 |
| 7/14/2010 | 1 | 6.9 | 6.0 | 3.7 | 4.9 | 1.8 | 4.8 | 5.6 | 5.6 | 5.5 | 7.2 | 6.7 | 2.5 |
| 7/29/2010 | 1 | -11.5 | 4.5 | 3.1 | 4.9 | 2.3 | 2.5 | 5.6 | 5.6 | 7.0 | 4.7 | 6.6 | 1.0 |
| 8/12/2010 | 1 | 4.6 | 2.8 | 3.6 | 4.9 | 2.3 | 3.0 | 5.6 | 5.6 | 3.6 | 2.6 | 4.1 | 1.0 |
| 8/26/2010 | 1 | 2.1 | 2.0 | 1.8 | 4.9 | 2.5 | 3.3 | 5.6 | 5.6 | 2.7 | 2.4 | 4.4 | 1.0 |
| 9/8/2010 | 2 | 14.0 | 1.8 | 4.4 | 4.9 | 3.7 | 3.0 | 5.6 | 5.6 | 2.4 | 3.0 | 4.4 | 1.0 |
| 9/22/2010 | 1 | 9.7 | -1.0 | 2.9 | 4.9 | 2.3 | 2.4 | 5.6 | 5.6 | 2.4 | 1.5 | 4.6 | 1.0 |
| 10/4/2010 | 1 | 11.2 | -1.1 | 5.3 | 4.9 | 2.3 | 2.5 | 5.6 | 5.6 | 2.7 | 3.4 | 4.8 | 1.0 |
| 10/18/2010 | 1 | 5.0 | -3.9 | 3.1 | 4.9 | 2.0 | 1.7 | 5.6 | 5.6 | 2.4 | 1.5 | 5.1 | 1.0 |
| 11/15/2010 | 4.6 | 8.5 | -3.1 | 2.5 | 4.9 | 3.3 | 3.8 | 5.6 | 5.6 | 2.5 | 2.9 | 4.8 | 4.6 |
| 12/6/2010 | 1 | 6.2 | -5.1 | 2.7 | 4.9 | 2.3 | 2.0 | 5.6 | 5.6 | 2.5 | 1.5 | 4.5 | 1.0 |
| 1/18/2011 | 1 | 64.8 | -6.0 | 24.2 | 4.9 | 5.3 | 44.4 | 5.6 | 5.6 | 2.5 | 6.7 | 5.0 | 1.2 |
| 2/14/2011 | 1 | -0.3 | -2.8 | -0.4 | 4.9 | 2.3 | -0.6 | 5.6 | 5.6 | 2.5 | 2.6 | 4.8 | 1.0 |
| 3/14/2011 | 4.7 | -9.4 | -0.8 | 0.3 | 4.9 | 3.4 | 0.5 | 5.6 | 5.6 | 2.6 | 4.9 | 4.5 | 4.7 |
| 4/11/2011 | 3.9 | 8.4 | -0.3 | 7.2 | 4.9 | 2.6 | 4.4 | 5.6 | 5.6 | 2.5 | 5.6 | 5.0 | 3.9 |
| 5/11/2011 | 1 | -4.4 | -0.9 | 1.2 | 4.9 | 2.1 | 2.6 | 5.6 | 5.6 | 2.5 | 2.4 | 3.9 | 1.0 |
| 6/13/2011 | 1 | -3.2 | -1.7 | 1.0 | 4.9 | 1.9 | -4.2 | 5.6 | 5.6 | 2.8 | 3.9 | 5.2 | 1.2 |
| 7/11/2011 | 1 | -3.1 | -1.4 | 2.9 | 4.9 | 2.3 | 3.6 | 5.6 | 5.6 | 3.7 | 3.9 | 6.6 | 1.0 |
| 8/1/2011 | 2 | 2.3 | 4.8 | 5.8 | 4.9 | 3.7 | 2.9 | 5.6 | 5.6 | 4.2 | 4.4 | 5.1 | 2.3 |
| 8/8/2011 | 3.2 | 1.2 | 7.0 | 7.4 | 4.9 | 4.5 | 2.3 | 5.6 | 5.6 | 10.2 | 7.5 | 8.1 | 3.2 |
| 8/15/2011 | 5.6 | 5.6 | 4.3 | 4.8 | 4.9 | 4.3 | 4.6 | 5.6 | 5.6 | 6.5 | 5.9 | 5.9 | 5.6 |
| 8/30/2011 | 5.1 | -10.9 | -3.0 | 0.0 | 4.9 | 5.0 | 3.4 | 5.6 | 5.6 | 2.5 | 5.2 | 4.8 | 5.1 |
| 9/6/2011 | 9 | 5.7 | 0.4 | 5.7 | 4.9 | 7.7 | 5.9 | 5.6 | 5.6 | 5.9 | 8.0 | 6.3 | 9.0 |
| 9/20/2011 | 3.7 | 0.9 | 1.8 | 9.2 | 4.9 | 5.0 | 4.5 | 5.6 | 5.6 | 5.3 | 6.8 | 7.4 | 3.7 |
| 10/4/2011 | 3.2 | 13.2 | 3.8 | 8.6 | 4.9 | 4.5 | 10.2 | 5.6 | 5.6 | 8.6 | 6.5 | 6.5 | 3.2 |
| 11/1/2011 | 2.2 | 15.2 | 2.6 | 7.4 | 4.9 | 4.4 | 8.1 | 5.6 | 5.6 | 2.7 | 5.5 | 5.4 | 3.1 |
| 12/12/2011 | 1 | 15.6 | -0.3 | 4.9 | 4.9 | 3.0 | 7.6 | 5.6 | 5.6 | 2.5 | 4.7 | 5.0 | 1.8 |
| 1/18/2012 | 2 | 3.0 | -2.2 | 1.6 | 4.9 | 1.9 | 2.0 | 5.6 | 5.6 | 2.5 | 3.3 | 4.9 | 2.0 |
| 2/14/2012 | 2 | 0.1 | -0.4 | 1.0 | 4.9 | 1.6 | 2.9 | 5.6 | 5.6 | 2.5 | 2.5 | 4.1 | 2.0 |
| 3/12/2012 | 1 | -0.2 | 6.3 | 4.6 | 4.9 | 2.3 | 3.1 | 5.6 | 5.6 | 4.6 | 4.0 | 6.8 | 1.0 |
| 4/16/2012 | 1 | 7.0 | -0.4 | 1.4 | 4.9 | 2.3 | 3.4 | 5.6 | 5.6 | 2.5 | 3.1 | 3.8 | 1.0 |
| 5/16/2012 | 2 | 2.1 | 3.7 | 5.1 | 4.9 | 3.3 | -0.9 | 5.6 | 5.6 | 2.5 | 3.2 | 4.0 | 2.0 |
| 6/11/2012 | 3.5 | -0.1 | 2.0 | 2.3 | 4.9 | 2.2 | 2.9 | 5.6 | 5.6 | 2.9 | 3.3 | 5.5 | 3.5 |
| 6/27/2012 | 1 | 4.4 | 4.3 | 4.2 | 4.9 | 2.8 | 3.2 | 5.6 | 5.6 | 3.0 | 2.5 | 6.5 | 1.0 |
| 7/9/2012 | 2.9 | 6.0 | 6.5 | 7.6 | 4.9 | 3.8 | 3.5 | 5.6 | 5.6 | 8.0 | 4.2 | 8.9 | 2.9 |
| 7/31/2012 | 1 | -2.6 | 4.3 | 4.4 | 4.9 | 2.3 | 4.1 | 5.6 | 5.6 | 4.1 | 2.6 | 4.9 | 1.0 |

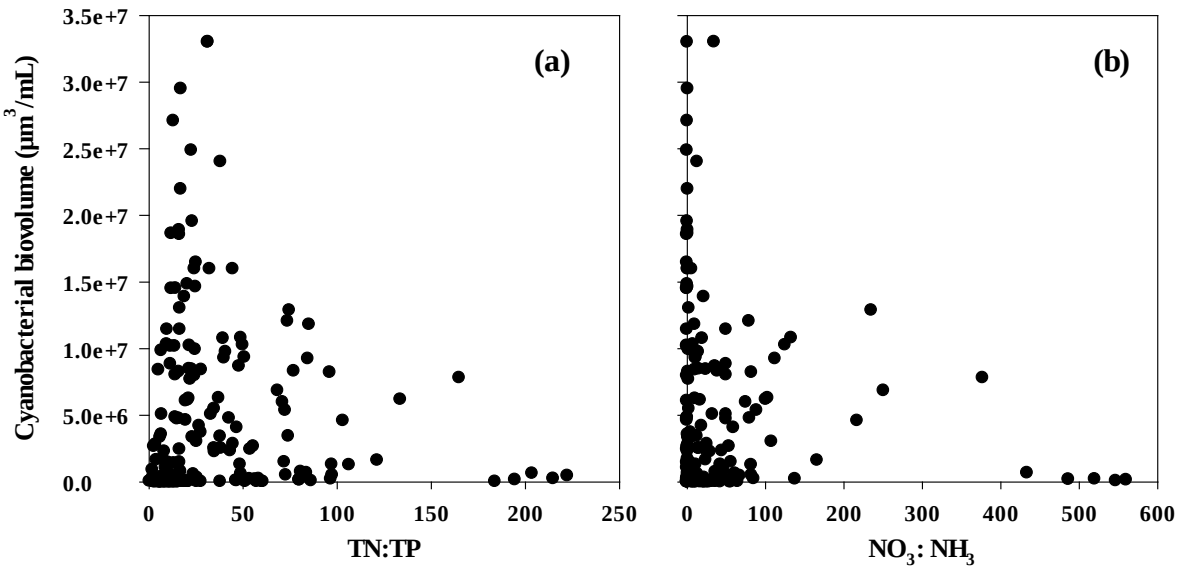
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8/21/2012 | 2.1 | 9.9 | 4.6 | 9.1 | 4.9 | 3.4 | 3.8 | 5.6 | 5.6 | 3.6 | 3.4 | 4.8 | 2.1 |
| 9/19/2012 | 3.4 | 8.9 | 4.4 | 9.3 | 4.9 | 3.7 | 4.5 | 5.6 | 5.6 | 10.7 | 7.6 | 7.6 | 2.4 |
| 10/16/2012 | 4.2 | 17.1 | 4.4 | 13.2 | 4.9 | 4.5 | 21.0 | 5.6 | 5.6 | 7.3 | 10.2 | 6.7 | 1.2 |
| 11/13/2012 | 1.3 | 4.7 | 2.4 | 7.2 | 4.9 | 2.6 | 3.3 | 5.6 | 5.6 | 7.6 | 4.4 | 6.8 | 1.3 |
| 12/11/2012 | 0.5 | 3.2 | 1.9 | 5.9 | 4.9 | 0.7 | 4.8 | 5.6 | 5.6 | 4.6 | 2.6 | 5.7 | 0.5 |
| 1/15/2013 | 2.8 | 10.3 | 3.8 | 5.9 | 4.9 | 2.0 | 4.2 | 5.6 | 5.6 | 6.7 | 7.3 | 7.7 | 2.8 |
| 1/23/2013 | 0.5 | 5.9 | 4.5 | 5.7 | 4.9 | 1.8 | 2.4 | 5.6 | 5.6 | 6.7 | 5.2 | 7.7 | 0.5 |
| 2/12/2013 | 2.3 | 2.1 | 3.7 | 4.5 | 4.9 | 1.5 | 4.4 | 5.6 | 5.6 | 5.6 | 2.9 | 7.1 | 2.3 |
| 3/19/2013 | 2.1 | -2.0 | 4.1 | 4.6 | 4.9 | 1.2 | 4.4 | 5.6 | 5.6 | 4.7 | 3.6 | 5.9 | 2.1 |
| 4/9/2013 | 0.5 | 3.0 | 4.9 | 7.3 | 4.9 | 1.8 | 2.8 | 5.6 | 5.6 | 5.1 | 2.8 | 5.4 | 0.5 |
| 5/7/2013 | 1.7 | 1.9 | 5.0 | 4.4 | 4.9 | 3.0 | 2.9 | 5.6 | 5.6 | 5.4 | 5.2 | 4.8 | 1.7 |
| 6/6/2013 | 0.5 | 5.4 | 4.4 | 7.1 | 4.9 | 4.3 | 2.7 | 5.6 | 5.6 | 7.0 | 6.0 | 6.6 | 11.2 |
| 7/8/2013 | 12.5 | 13.2 | 7.8 | 13.3 | 4.9 | 7.1 | 12.7 | 5.6 | 5.6 | 13.1 | 12.0 | 10.1 | 12.5 |
| 8/6/2013 | 17.5 | 14.6 | 8.2 | 10.4 | 4.9 | 6.7 | 17.8 | 5.6 | 5.6 | 8.2 | 5.7 | 7.3 | 2.9 |
| 8/19/2013 | 3.9 | 13.6 | 7.5 | 5.0 | 4.9 | 5.2 | 4.5 | 5.6 | 5.6 | 6.2 | 4.8 | 5.4 | 3.9 |
| 9/9/2013 | 12.6 | 15.4 | 9.9 | 7.8 | 4.9 | 8.5 | 10.9 | 5.6 | 5.6 | 7.3 | 12.9 | 8.5 | 12.6 |
| 9/25/2013 | 50 | 12.3 | 8.1 | 6.8 | 4.9 | 6.9 | 39.4 | 5.6 | 5.6 | 15.0 | 28.2 | 9.4 | 50.0 |
| 10/21/2013 | 54.1 | 15.0 | 6.7 | 8.7 | 4.9 | 7.6 | 46.7 | 5.6 | 5.6 | 14.8 | 32.7 | 10.1 | 54.1 |
| 11/6/2013 | 27.9 | 13.0 | 5.6 | 5.3 | 4.9 | 6.9 | 25.6 | 5.6 | 5.6 | 13.4 | 18.2 | 8.1 | 27.9 |
| 11/18/2013 | 16.5 | 10.7 | 5.0 | 3.6 | 4.9 | 6.7 | 29.2 | 5.6 | 5.6 | 3.5 | 5.7 | 5.3 | 16.1 |
| 12/12/2013 | 5.8 | 12.2 | 3.4 | 2.0 | 4.9 | 6.3 | 5.4 | 5.6 | 5.6 | 3.4 | 6.0 | 6.0 | 5.8 |
| 2/19/2014 | 2.5 | 5.9 | 2.0 | 0.6 | 4.9 | 3.8 | 2.9 | 5.6 | 5.6 | 2.6 | 3.3 | 4.6 | 2.5 |
| 3/19/2014 | 1.9 | 7.9 | 12.0 | 11.7 | 14.7 | 3.2 | 1.8 | 5.6 | 18.6 | 9.3 | 7.2 | 7.7 | 1.9 |
| 4/16/2014 | 2.1 | 11.9 | 9.7 | 9.5 | 4.9 | 7.6 | 8.7 | 5.6 | 5.6 | 5.8 | 6.1 | 7.1 | 7.4 |
| 5/20/2014 | 0.5 | 10.9 | 8.1 | 6.5 | 4.9 | 6.6 | 4.7 | 5.6 | 5.6 | 4.7 | 5.0 | 5.4 | 4.5 |
| 7/10/2014 | 4.3 | 1.8 | 7.4 | 6.5 | 4.9 | 5.6 | 3.2 | 5.6 | 5.6 | 5.9 | 5.2 | 7.9 | 4.3 |
| 7/22/2014 | 16.6 | 9.2 | 8.4 | 8.9 | 4.9 | 6.7 | 12.2 | 5.6 | 5.6 | 8.2 | 12.0 | 7.6 | 16.6 |
| 8/5/2014 | 4.3 | 8.4 | 9.5 | 9.2 | 4.9 | 5.6 | 5.0 | 5.6 | 5.6 | 3.8 | 4.7 | 5.9 | 4.3 |
| 9/16/2014 | 11.8 | 9.1 | 8.7 | 8.7 | 4.9 | 8.2 | 7.7 | 5.6 | 5.6 | 6.5 | 9.5 | 7.2 | 11.8 |
| 10/28/2014 | 5 | 1.0 | 6.1 | 4.1 | 4.9 | 6.8 | 3.5 | 5.6 | 5.6 | 5.3 | 9.3 | 6.4 | 12.0 |
| 11/20/2014 | 13.1 | 17.1 | 8.6 | 7.9 | 4.9 | 7.0 | 25.9 | 5.6 | 5.6 | 6.2 | 7.5 | 7.5 | 4.2 |
| 12/16/2014 | 11.1 | 3.1 | 5.0 | 6.8 | 4.9 | 9.8 | 8.3 | 5.6 | 5.6 | 6.9 | 9.6 | 7.2 | 11.1 |
| 1/13/2015 | 7.6 | 3.3 | 1.5 | 1.3 | 4.9 | 6.3 | 3.2 | 20.6 | 5.6 | 14.2 | 9.3 | 8.3 | 7.6 |
| 2/10/2015 | 5 | 8.5 | 8.4 | 5.8 | 4.9 | 6.3 | 5.6 | 20.6 | 5.6 | 14.1 | 9.7 | 8.7 | 5.0 |
| 3/10/2015 | 2.7 | 11.0 | 7.6 | 5.8 | 4.9 | 6.7 | 8.3 | 20.6 | 5.6 | 14.1 | 12.2 | 8.7 | 5.0 |
| 4/15/2015 | 0.5 | 3.8 | 5.8 | 6.2 | 4.9 | 5.2 | 1.3 | 5.6 | 5.6 | 4.3 | 4.9 | 4.8 | 7.1 |
| 6/9/2015 | 8.4 | 14.3 | 10.6 | 7.2 | 4.9 | 7.1 | 8.7 | 5.6 | 5.6 | 10.6 | 9.0 | 8.8 | 8.4 |

## Appendix B: Chapter 2 Supplemental Figures and Tables

**Supplemental Figure 1.** Temporal dynamics of relative cyanobacterial biovolume, relative cyanobacterial $N_2$-fixer biovolume, and the TN:TP ratio (by weight) in the four study reservoirs used in the study. Numbers on the x-axis represent the last two digits of the year post 2000 for Cheney (a) and Eagle Creek, Geist, and Morse (b-d), respectively.



**Supplemental Figure 2.** The relationship between cyanobacterial biovolume and the TN:TP (a) and $NO_3$:$NH_3$ ratio (b).



**Supplemental Table 1.** Limit of detection (LOD), number (No.) of samples below the LOD, and total number of samples in each data set for each reservoir.

| Reservoir | Analyte | Analytical method | LOD | Half of LOD | Date range | No. below LOD | No. of samples |
|---|---|---|---|---|---|---|---|
| Indy reservoirs* | $NO_3$ | EPA 300.0 | 0.02 mg/L | 0.01 mg/L | all | 25 | 117 |
| Indy reservoirs* | $NH_3$ | EPA 365.4 | 0.02 mg/L | 0.01 mg/L | all | 30 | 117 |
| Cheney | $NO_3$ | EPA 300.0 | 0.01 mg/L | 0.005 mg/L | all | 25 | 175 |
| Cheney | $NH_3$ | APHA 4500-$NH_3$ D | 0.03 mg/L | 0.015 mg/L | all | 53 | 176 |
| All reservoirs | Microcystin | ELISA$ | 0.1 µg/L | 0.05 µg/L | all | 126 | 283 |
| Eagle Creek | Geosmin | APHA 6040D - GC/MS^ | 2 ng/L | 1 ng/L | all | 2 | 37 |
| Geist & Morse | Geosmin | APHA 6040D - GC/MS^ | 3 ng/L | 1.25 ng/L | all | 5 | 80 |
| Eagle Creek | MIB | APHA 6040D - GC/MS^ | 2 ng/L | 1 ng/L | all | 2 | 37 |
| Geist & Morse | MIB | APHA 6040D - GC/MS^ | 3 ng/L | 1.25 ng/L | all | 12 | 80 |
| Cheney | Geosmin | APHA 6040D - GC/MS^ | 3 ng/L | 1.5 ng/L | May 2001-May 2002 | 3 | 6 |
| Cheney | Geosmin | Zimmerman et al. 2002 - GC/MS^# | 5 ng/L | 2.5 ng/L | June 2002-July 2008 | 60 | 90 |
| Cheney | Geosmin | Zimmerman et al. 2002 - GC/MS^# | 2 ng/L | 1 ng/L | August 2008-August 2012 | 45 | 81 |
| Cheney | MIB | Zimmerman et al. 2002 - GC/MS^# | 2 ng/L | 1 ng/L | August 2008-August 2012 | 81 | 81 |
| Cheney | Geosmin/MIB | Zimmerman et al. 2002 - GC/MS^# | 1 ng/L | 0.5 ng/L | September 2012–December 2012 | 4 | 4 |
| Cheney | Geosmin/MIB | Zimmerman et al. 2002 - GC/MS^# | 1 ng/L | 0.5 ng/L | September 2012–December 2012 | 0 | 4 |
| Cheney | MIB | Zimmerman et al. 2002 - GC/MS^# | 5 ng/L | 2.5 ng/L | May 2001-July 2008 | 92 | 96 |

# Zimmerman et al. 2002 uses similar GC/MS procedures as APHA 6040D

^ GC/MS = gas chromatography/mass spectrometry

* Indy reservoirs include Eagle Creek, Geist, and Morse reservoirs surrounding Indianapolis, IN

$ ELISA = enzyme-linked immunosorbent assay

**Supplemental Table 2.** Cyanobacteria, and their capability to fix $N_2$, present in the study. Asterisks indicate species where the capability to fix $N_2$ is likely strain specific.

| *Genus species* | $N_2$-fixer? | *Genus species* | $N_2$-fixer? |
|---|---|---|---|
| **Order: Chroococcales** | **0/39** | **Order: Oscillatoriales** | **8/12** |
| *Aphanocapsa delicatissima* | No | *Lyngbya contorta* | Yes |
| *Aphanocapsa holsatica* | No | *Lyngbya limnetica* | Yes |
| *Aphanocapsa incerta* | No | *Lyngbya sp.* | Yes |
| *Aphanocapsa planctonica* | No | *Oscillatoria anguina* | Yes* |
| *Aphanocapsa pulchra* | No | *Oscillatoria limnetica* | Yes* |
| *Aphanocapsa sp.* | No | *Oscillatoria nitida* | Yes* |
| *Aphanothece clathrata* | No | *Oscillatoria sp.* | Yes* |
| *Aphanothece minutissima* | No | *Oscillatoria tenuis* | Yes* |
| *Aphanothece sp.* | No | *Phormidium sp.* | No |
| *Aphanothece stagnina* | No | *Planktothrix cf. agardhii* | No |
| *Chroococcus cf. aphanocapsoides* | No | *Planktothrix rubescens* | No |
| *Chroococcus dispersus* | No | *Planktothrix sp.* | No |
| *Chroococcus limneticus* | No | | |
| *Chroococcus minimus* | No | **Order: Pseudanabaenales** | **8/9** |
| *Chroococcus minutus* | No | *Leptolyngbya sp.* | Yes |
| *Chroococcus sp.* | No | *Limnothrix redekei* | No |
| *Chroococcus turgidus* | No | *Planktolyngbya circumcreta* | Yes |
| *Coelosphaerium dubium* | No | *Planktolyngbya contorta* | Yes |
| *Coelosphaerium kuetzingianum* | No | *Planktolyngbya limnetica* | Yes |
| *Coelosphaerium sp.* | No | *Planktolyngbya sp.* | Yes |
| *Dactylococcopsis fascicularis* | No | *Pseudanabaena galeata* | Yes* |

| | | | |
|---|---|---|---|
| *Gomphosphaeria aponina* | No | *Pseudanabaena limnetica* | Yes* |
| *Gomphosphaeria lacustris* | No | *Pseudanabaena sp.* | Yes* |
| *Gomphosphaeria sp.* | No | | |
| *Gomphosphaeria virieuxii* | No | **Order: Nostocales** | **19/20** |
| *Merismopedia elegans* | No | *Anabaena circinalis* | Yes |
| *Merismopedia glauca* | No | *Anabaena flos-aquae* | Yes |
| *Merismopedia hyalina* | No | *Anabaena helicoidea* | Yes |
| *Merismopedia sp.* | No | *Anabaena limnetica* | Yes |
| *Merismopedia tenuis* | No | *Anabaena planctonica* | Yes |
| *Merismopedia tenuissima* | No | *Anabaena sp.* | Yes |
| *Microcrocis sp.* | No | *Anabaena spiroides* | Yes |
| *Microcystis aeruginosa* | No | *Anabaena viguieri* | Yes |
| *Microcystis flos-aquae* | No | *Anabaenopsis circularis* | Yes |
| *Microcystis incerta* | No | *Anabaenopsis elenkinii* | Yes |
| *Microcystis sp.* | No | *Anabaenopsis sp.* | Yes |
| *Snowella lacustris* | No | *Aphanizomenon elenkinii* | Yes |
| *Woronichinia karelica* | No | *Aphanizomenon flos-aquae* | Yes |
| | | *Aphanizomenon issatchenkoi* | Yes |
| **Order: Synechococcales** | **1/6** | *Aphanizomenon sp.* | Yes |
| *Cyanogranis basifixa* | No | *Cylindrospermopsis raciborskii* | Yes |
| *Rhabdoderma irregulare* | No | *Cylindrospermopsis sp.* | Yes |
| *Rhabdoderma lineare* | No | *Cylindrospermum cf. musicola* | Yes |
| *Rhabdoderma sigmoidea* | No | *Cylindrospermum sp.* | Yes |
| *Synechococcus sp.* | Yes* | *Raphidiopsis curvata* | No |
| *Synechocystis aquatilis* | No | | |

## Appendix C: Chapter 3 Supplemental Tables

**Supplemental Table 1:** Keywords used to search for relevant studies. All 3 phytoplankton keywords were combined using the "OR" search function in each search. Each search paired the phytoplankton keywords with either the herbicide, pesticide, PPCP, or PAH & PCB keywords to find relevant studies. PPCPs = pharmaceutical and personal care products (including antibiotics); PAHs & PCBs = polycyclic aromatic hydrocarbons and polychlorinated biphenyls.

| Phytoplankton keywords | Herbicide keywords | Pesticide keywords | PPCP keywords | PAH & PCB keywords |
|---|---|---|---|---|
| cyanobacteria | herbicide | pesticide | antibiotic | polycyclic aromatic hydrocarbon |
| blue green algae | atrazine | fungicide | pharmaceutical | polychlorinated biphenyl |
| blue-green algae | glyphostate | insecticide | personal care product | PAH |
| \| | Roundup | organophosphorus | PPCP | PCB |
| \| | metribuzin | pyrethoid | PCP | Aroclor |
| \| | terbuthylazine | azocyclotin | amoxicillin | pyrene |
| \| | TBA | cypermethin | ciprofloxacin | phenanthrene |
| \| | 2,4-Dichlorophenoxyacetic acid | dimethoate | erythromycin | naphthalene |
| \| | 2,4 D | endosulfan | tetracyclin | \| |
| \| | mecoprop | fenitrothion | tylosin | \| |
| \| | MCPP | fentin acetate | spiramycin | \| |
| \| | MCPA | propargite | triclosan | \| |
| \| | dicamba | propiconazole | ibuprofen | \| |
| \| | picloram | pyridaphenthion | quinolones | \| |
| \| | clopyralid | thionazin | paracetamol | \| |
| \| | triclopyr | triazophos | diclofenac | \| |
| \| | acrolein | benalaxyl | \| | \| |
| \| | fluroxypyr | hexaconazole | \| | \| |
| \| | sulfosate | carbaryl | \| | \| |
| \| | imazquin | carbofuran | \| | \| |
| \| | imazethapyr | carbosulfan | \| | \| |
| \| | imazapyr | propoxur | \| | \| |
| \| | chlorimuron | metlocarb | \| | \| |
| \| | chlorsulfuron | chlorpyrifos | \| | \| |
| \| | nicosulfuron | terbufos | \| | \| |
| \| | primisulfuron | methamidophos | \| | \| |

| | | | | |
|---|---|---|---|---|
| — | thifensulfuron | isoprocarb | — | — |
| — | tribenuron | flumetralin | — | — |
| — | sulfometuron | methidathion | — | — |
| — | metsulfuron | diazinon | — | — |
| — | halosulfuron | phoxim | — | — |
| — | flumetsulam | metalaxyl | — | — |
| — | clomazone | chlorothalonil | — | — |
| — | amitrole | cymoxanil | — | — |
| — | norflurazon | dimethoate | — | — |
| — | fluridone | trichlorphon | — | — |
| — | fenoxaprop | fosetyl-aluminum | — | — |
| — | fluazifop | — | — | — |
| — | quizalofop | — | — | — |
| — | clethodim | — | — | — |
| — | sethoxydim | — | — | — |
| — | paraquat | — | — | — |
| — | diquat | — | — | — |
| — | acifluorfen | — | — | — |
| — | fomesafen | — | — | — |
| — | lactofen | — | — | — |
| — | oxyfluorfen | — | — | — |
| — | bentazon | — | — | — |
| — | flufosinate | — | — | — |
| — | simazine | — | — | — |
| — | cyanazine | — | — | — |
| — | prometon | — | — | — |
| — | hexazinone | — | — | — |
| — | terbacil | — | — | — |
| — | bromacil | — | — | — |
| — | linuron | — | — | — |
| — | diuron | — | — | — |
| — | tebuthiuron | — | — | — |
| — | bentazon | — | — | — |
| — | bromoxynil | — | — | — |
| — | pyridate | — | — | — |
| — | butylate | — | — | — |

| | | | | |
|---|---|---|---|---|
| — | pebulate | — | — | — |
| — | cycloate | — | — | — |
| — | acetochlor | — | — | — |
| — | alachlor | — | — | — |
| — | metolachlor | — | — | — |
| — | propachlor | — | — | — |
| — | dimethenamid | — | — | — |
| — | bensulide | — | — | — |
| — | napropamide | — | — | — |
| — | pronamide | — | — | — |
| — | dichlobenil | — | — | — |
| — | dithiopyr | — | — | — |
| — | molinate | — | — | — |
| — | methabenzthiazuron | — | — | — |
| — | irgarol | — | — | — |

**Supplemental Table 2.** Persistent Organic Pollutant (POP) class, authors of studies, publication year of studies, response of cyanobacteria to compound, POP compound studied, and study scale, respectively, of studies used to create frequency histograms.

| POP class | Authors | Year | Results | Compound | Study scale |
|---|---|---|---|---|---|
| Fungicide | Ma et al. | 2004 | (-) Cyano | Fentin acetate | Lab EC 50 |
| Fungicide | Guanzonjr and Nakahara | 2002 | (+) Cyano | Isoprothiolane | Lab EC 50 |
| Fungicide | Ma et al. | 2011 | (+) Cyano | Chlorothalonil | Lab EC 50 |
| Fungicide | Ma et al. | 2011 | (+) Cyano | Cymoxanil | Lab EC 50 |
| Fungicide | Ma et al. | 2011 | (+) Cyano | Fosetyl-aluminum | Lab EC 50 |
| Fungicide | Ma et al. | 2011 | (+) Cyano | Metalaxyl | Lab EC 50 |
| Fungicide | Ma et al. | 2008 | (+) Cyano | Propiconazole | Lab EC 50 |
| Fungicide | Abdel-Hamid et al. | 1996 | (0) Cyano | Propiconazole | Field mesocosms |
| Fungicide | Ma | 2005 | (0) Cyano | Fentin-hydroxide | Lab EC 50 |
| Fungicide | Ma et al. | 2011 | (0) Cyano | Benalaxyl | Lab EC 50 |
| Fungicide | Ma et al. | 2011 | (0) Cyano | Hexaconazole | Lab EC 50 |
| Fungicide | Peterson et al. | 1994 | (0) Cyano | Propiconazole analytical | Lab EC 50 |
| Fungicide | Peterson et al. | 1994 | (0) Cyano | Propiconazole formulation | Lab EC 50 |
| Herbicide | Deblois et al. | 2013 | (-) Cyano | Atrazine | Lab EC 50 |
| Herbicide | Herman et al. | 1986 | (-) Cyano | Atrazine | Field mesocosms |
| Herbicide | Leboulanger et al. | 2011 | (-) Cyano | Diuron | Lab micro/nanocosms |
| Herbicide | Leboulanger et al. | 2011 | (-) Cyano | Paraquat | Lab micro/nanocosms |
| Herbicide | Melendez et al. | 1993 | (-) Cyano | Diquat | Lab micro/nanocosms |
| Herbicide | Nystrom et al. | 1999 | (-) Cyano | Chlorsulfuron | Lab EC 50 |
| Herbicide | Nystrom et al. | 1999 | (-) Cyano | Metsulfuron-methyl | Lab EC 50 |
| Herbicide | Paule et al. | 2013 | (-) Cyano | Alachlor | Lab micro/nanocosms |
| Herbicide | Peterson et al. | 1994 | (-) Cyano | Diquat | Lab EC 50 |
| Herbicide | Peterson et al. | 1997 | (-) Cyano | Diquat | Lab EC 50 |
| Herbicide | Philips et al. | 1992 | (-) Cyano | Diquat | Lab EC 50 |
| Herbicide | Pratt and Barreiro | 1998 | (-) Cyano | Diquat | Lab micro/nanocosms |
| Herbicide | Shehata et al. | 1993 | (-) Cyano | Gardoprim and Gesapax | Lab bioassay |
| Herbicide | Tadonleke et al. | 2009 | (-) Cyano | Diuron | Lab bioassay |

| | | | | | |
|---|---|---|---|---|---|
| Herbicide | Weiner et al. | 2004 | (−) Cyano | Atrazine | Lab bioassay |
| Herbicide | Zhang et al. | 2013 | (−) Cyano | Paraquat | Lab EC 50 |
| Herbicide | AbdEl-Aty and El-Dib | 2009 | (+) Cyano | Cyanazine | Lab EC 50 |
| Herbicide | Abdel-Hamid et al. | 1996 | (+) Cyano | Chlorsulfuron | Field mesocosms |
| Herbicide | Abou-waly et al. | 1991 | (+) Cyano | Atrazine | Lab EC 50 |
| Herbicide | Abrantes et al. | 2008 | (+) Cyano | Alachlor | Lab bioassay |
| Herbicide | Berard et al. | 1999 | (+) Cyano | Atrazine | Lab bioassay |
| Herbicide | Berard et al. | 2003 | (+) Cyano | Atrazine | Lab micro/nanocosms |
| Herbicide | Berard et al. | 2003 | (+) Cyano | Irgarol 1051 | Lab micro/nanocosms |
| Herbicide | Caquet et al. | 2005 | (+) Cyano | Fomesafen | Field mesocosms |
| Herbicide | Caquet et al. | 2005 | (+) Cyano | Fomesafen and Agral 90 | Field mesocosms |
| Herbicide | DeLorenzo et al. | 1999 | (+) Cyano | Atrazine | Field mesocosms |
| Herbicide | DeLorenzo et al. | 1999 | (+) Cyano | Deethylatrazine | Field mesocosms |
| Herbicide | Fairchild et al. | 1998 | (+) Cyano | Alachlor | Lab EC 50 |
| Herbicide | Fairchild et al. | 1998 | (+) Cyano | Metolachlor | Lab EC 50 |
| Herbicide | Fairchild et al. | 1998 | (+) Cyano | Metribuzin | Lab EC 50 |
| Herbicide | Forlani et al. | 2008 | (+) Cyano | Glyphosate | Lab bioassay |
| Herbicide | Guanzon jr and Nakahara | 2002 | (+) Cyano | Chlomitrofen | Lab EC 50 |
| Herbicide | Gustavson et al. | 2003 | (+) Cyano | Metribuzin | Lab EC 50 |
| Herbicide | Lurling and Roessink | 2006 | (+) Cyano | Metribuzin | Lab bioassay |
| Herbicide | Ma et al. | 2004 | (+) Cyano | Ethephon | Lab EC 50 |
| Herbicide | Ma et al. | 2008 | (+) Cyano | Flumetralin | Lab EC 50 |
| Herbicide | Magnusson et al | 2012 | (+) Cyano | Diuron | Lab micro/nanocosms |
| Herbicide | Maule and Wright | 1984 | (+) Cyano | Chlorpropham | Lab bioassay |
| Herbicide | McClellan et al. | 2008 | (+) Cyano | Diuron | Lab micro/nanocosms |
| Herbicide | Murdock et al. | 2012 | (+) Cyano | Atrazine | Lab bioassay |
| Herbicide | Nagai et al. | 2013 | (+) Cyano | 2,4 Dichlorophenol | Lab bioassay |
| Herbicide | Ni et al. | 2014 | (+) Cyano | Mesotrione | Lab EC 50 |
| Herbicide | Pannard et al. | 2009 | (+) Cyano | Atrazine | Lab bioassay |
| Herbicide | Perez et al. | 2007 | (+) Cyano | Glyphosate | Field mesocosms |
| Herbicide | Pesce et al. | 2010 | (+) Cyano | Diuron | Empirical field study |

| | | | | | |
|---|---|---|---|---|---|
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Atrazine | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Chlorsulfuron | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Cyanazine | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Glyphosate | Lab EC 50 |
| Herbicide | Peterson et al. | 1997 | (+) Cyano | Hexazinone | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Hexazinone | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Metribuzin | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Metsulfuron-methyl | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Simazine | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (+) Cyano | Tebuthiuron | Lab EC 50 |
| Herbicide | Powell et al. | 1991 | (+) Cyano | Glyphosate | Lab bioassay |
| Herbicide | Slijkerman et al. | 2005 | (+) Cyano | Linuron | Field mesocosms |
| Herbicide | Sun et al. | 2013 | (+) Cyano | Glyphosate-isopropylammonium | Lab EC 50 |
| Herbicide | Vera et al. | 2014 | (+) Cyano | Glyphosate | Lab bioassay |
| Herbicide | Vera et al. | 2010 | (+) Cyano | Glyphosate | Field mesocosms |
| Herbicide | Vera et al. | 2012 | (+) Cyano | Glyphosate | Field mesocosms |
| Herbicide | Zhang et al. | 2008 | (+) Cyano | Irgarol 1051 | Lab EC 50 |
| Herbicide | Abdel-Hamid et al. | 1996 | (0) Cyano | Glyphosate | Field mesocosms |
| Herbicide | Andrus et al. | 2013 | (0) Cyano | Atrazine | Empirical field study |
| Herbicide | Blanck et al. | 2009 | (0) Cyano | Irgarol 1051 | Empirical field study |
| Herbicide | Brain et al. | 2012 | (0) Cyano | Atrazine | Lab bioassay |
| Herbicide | Carder and Hoagland | 1998 | (0) Cyano | Alachlor | Artificial outdoor streams |
| Herbicide | Carder and Hoagland | 1998 | (0) Cyano | Atrazine | Artificial lab streams |
| Herbicide | Chalifour and Juneau | 2011 | (0) Cyano | Atrazine | Lab bioassay |
| Herbicide | Daam et al. | 2009 | (0) Cyano | Linuron | Field mesocosms |
| Herbicide | Eriksson et al. | 2009 | (0) Cyano | Irgarol 1051 | Artificial lab streams |
| Herbicide | Fairchild et al. | 1998 | (0) Cyano | Atrazine | Lab EC 50 |
| Herbicide | Guasch and Sabater | 1998 | (0) Cyano | Atrazine | Lab EC 50 |
| Herbicide | Guasch et al. | 1998 | (0) Cyano | Atrazine | Empirical field study |
| Herbicide | Hartgers et al. | 1998 | (0) Cyano | Mixture of Atrazine, Diuron, and Metalochlor | Lab micro/nanocosms |

| | | | | | |
|---|---|---|---|---|---|
| Herbicide | Lipok et al. | 2010 | (0) Cyano | Glyphosate | Lab EC 50 |
| Herbicide | Lockert et al. | 2006 | (0) Cyano | Atrazine | Lab bioassay |
| Herbicide | Maule and Wright | 1984 | (0) Cyano | 2-methyl-4-chlorophenoxyacetic acid | Lab bioassay |
| Herbicide | Maule and Wright | 1984 | (0) Cyano | Atrazine | Lab bioassay |
| Herbicide | Maule and Wright | 1984 | (0) Cyano | Diuron | Lab bioassay |
| Herbicide | Maule and Wright | 1984 | (0) Cyano | Glyphosate | Lab bioassay |
| Herbicide | Maule and Wright | 1984 | (0) Cyano | Propanil | Lab bioassay |
| Herbicide | Mohr et al. | 2008 | (0) Cyano | Irgarol 1051 | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Ethametsulfuron-methyl | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Imazethapyr | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Metolachlor | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Picloram | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Triasulfuron | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Triclopyr | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | 2,4 Dichlorophenoxyacetic aid | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | 2-methyl-4-chlorophenoxyacetic acid | Lab EC 50 |
| Herbicide | Peterson et al. | 1994 | (0) Cyano | Acrolein | Lab EC 50 |
| Herbicide | Sabater and Carrasco | 1997 | (0) Cyano | Chlorsulfuron | Lab EC 50 |
| Herbicide | Sabater and Carrasco | 1998 | (0) Cyano | Molinate | Lab EC 50 |
| Herbicide | Saxton et al. | 2011 | (0) Cyano | Glyphosate | Lab bioassay |
| Herbicide | Seguin et al. | 2002 | (0) Cyano | Atrazine | Field mesocosms |
| Herbicide | Stachowski-Haberkorn et al. | 2008 | (0) Cyano | Glyphosate | Field mesocosms |
| Herbicide | Villeneuve et al. | 2011 | (0) Cyano | Diuron | Artificial outdoor streams |
| Herbicide | Zananski et al. | 2010 | (0) Cyano | Atrazine | Lab bioassay |
| PAH | De Morais et al. | 2014 | (+) Cyano | Pentachlorophenol | Lab EC 50 |
| PAH | De Morais et al. | 2014 | (+) Cyano | Pentachlorophenol | Lab bioassay |
| PAH | Echeveste et al. | 2010 | (−) Cyano | Pyrene | Lab EC 50 |
| PAH | Echeveste et al. | 2010 | (−) Cyano | Phenanthrene | Lab EC 50 |

| | | | | | |
|---|---|---|---|---|---|
| PAH | Echeveste et al. | 2011 | (−) Cyano | Mixture of acenaphtene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo(k)fluoranthene, benzo[ghi]perylene, benzo(a)pyrene, dibenzo[a,h]anthracene, chrysene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, pyrene, phenanthrene | Lab EC 50 |
| PAH | Zhu et al. | 2012 | (+) Cyano | Mixture of Naphthalene, Phenanthrene, and Pyrene | Lab bioassay |
| PCB | Kostel et al. | 1999 | (+) Cyano | Aroclor 1242 | Artificial lab streams |
| Pesticide | DeLorenzo et al. | 1999 | (−) Cyano | Endosulfan | Field mesocosms |
| Pesticide | Leboulanger et al. | 2011 | (−) Cyano | Fenitrothion | Lab micro/nanocosms |
| Pesticide | Ma | 2005 | (−) Cyano | Azocyclotin | Lab EC 50 |
| Pesticide | Ma et al. | 2005 | (−) Cyano | Thionazin | Lab EC 50 |
| Pesticide | Ma et al. | 2004 | (−) Cyano | Triazophos | Lab EC 50 |
| Pesticide | Guanzon jr and Nakahara | 2002 | (+) Cyano | Fenitrothion | Lab EC 50 |
| Pesticide | Guanzon jr and Nakahara | 2002 | (+) Cyano | Tri-n-butyltin chloride | Lab EC 50 |
| Pesticide | Ma et al. | 2006 | (+) Cyano | Carbosulfan | Lab EC 50 |
| Pesticide | Ma et al. | 2008 | (+) Cyano | Isoprocarb | Lab EC 50 |
| Pesticide | Ma et al. | 2006 | (+) Cyano | Propoxur | Lab EC 50 |
| Pesticide | Sun et al. | 2013 | (+) Cyano | Dimethoate | Lab EC 50 |
| Pesticide | Sun et al. | 2013 | (+) Cyano | Phoxim | Lab EC 50 |
| Pesticide | Sun et al. | 2013 | (+) Cyano | Trichlorfon | Lab EC 50 |
| Pesticide | Wendt-Rasch et al. | 2003 | (+) Cyano | Cypermethrin | Field mesocosms |
| Pesticide | Abdel-Hamid et al. | 1996 | (0) Cyano | Dimethoate | Field mesocosms |
| Pesticide | Ma | 2005 | (0) Cyano | Beta-cyfluthrin | Lab EC 50 |
| Pesticide | Ma | 2005 | (0) Cyano | Cyhexatin | Lab EC 50 |
| Pesticide | Ma | 2005 | (0) Cyano | Fenbutatin-oxide | Lab EC 50 |
| Pesticide | Ma et al. | 2006 | (0) Cyano | Carbaryl | Lab EC 50 |
| Pesticide | Ma et al. | 2006 | (0) Cyano | Carbofuran | Lab EC 50 |
| Pesticide | Ma et al. | 2005 | (0) Cyano | Diazinon | Lab EC 50 |
| Pesticide | Ma et al. | 2005 | (0) Cyano | Methidathion | Lab EC 50 |
| Pesticide | Ma et al. | 2006 | (0) Cyano | Metolcarb | Lab EC 50 |

| | | | | | |
|---|---|---|---|---|---|
| Pesticide | Ma et al. | 2005 | (0) Cyano | Phoxim | Lab EC 50 |
| Pesticide | Ma et al. | 2008 | (0) Cyano | Propargite | Lab EC 50 |
| Pesticide | Peterson et al. | 1994 | (0) Cyano | Carbaryl | Lab EC 50 |
| Pesticide | Peterson et al. | 1994 | (0) Cyano | Carbofuran | Lab EC 50 |
| Pesticide | Sabater and Carrasco | 2001 | (0) Cyano | Fenitrothion | Lab EC 50 |
| Pesticide | Sabater and Carrasco | 2001 | (0) Cyano | Pyridaphenthion | Lab EC 50 |
| Pesticide | Tien and Chen | 2012 | (0) Cyano | Chlorpyrifos | Lab EC 50 |
| Pesticide | Tien and Chen | 2012 | (0) Cyano | Methamidophos | Lab EC 50 |
| Pesticide | Tien and Chen | 2012 | (0) Cyano | Terbufos | Lab EC 50 |
| PPCP | Brezovsek et al. | 2014 | (−) Cyano | Cisplatin | Lab EC 50 |
| PPCP | Lawrence et al. | 2005 | (−) Cyano | Caffeine | Lab bioassay |
| PPCP | Lawrence et al. | 2005 | (−) Cyano | Carbamazepine | Lab bioassay |
| PPCP | Lawrence et al. | 2007 | (−) Cyano | Dicolfenac | Lab bioassay |
| PPCP | Lawrence et al. | 2005 | (−) Cyano | Furosemide | Lab bioassay |
| PPCP | Lawrence et al. | 2005 | (−) Cyano | Ibuprofen | Lab bioassay |
| PPCP | Lawrence et al. | 2012 | (−) Cyano | Acetaminophen and Diclofenac | Lab bioassay |
| PPCP | Brezovsek et al. | 2014 | (+) Cyano | Etoposide | Lab EC 50 |
| PPCP | Brezovsek et al. | 2014 | (+) Cyano | Imatnib mesylate | Lab EC 50 |
| PPCP | Brezovsek et al. | 2014 | (+) Cyano | 5-fluorouracil | Lab EC 50 |
| PPCP | Drury et al. | 2013 | (+) Cyano | Triclosan | Artificial lab streams |
| PPCP | Lawrence et al. | 2007 | (+) Cyano | Dicolfenac | Lab bioassay |
| PPCP | Nietch et al. | 2013 | (+) Cyano | Triclosan | Artificial outdoor streams |
| PPCP | Pomati et al. | 2004 | (+) Cyano | Ibuprofen | Lab bioassay |
| PPCP | Proia et al. | 2013 | (+) Cyano | Dicolfenac | Field mesocosms |
| PPCP | Proia et al. | 2013 | (+) Cyano | Ibuprofen | Field mesocosms |
| PPCP | Proia et al. | 2013 | (+) Cyano | Paracetamol | Field mesocosms |
| PPCP | Proia et al. | 2013 | (+) Cyano | Triclosan | Field mesocosms |
| PPCP | Yergeau et al. | 2012 | (+) Cyano | Gemfibrozil | Lab bioassay |
| PPCP | Wilson et al. | 2003 | (0) Cyano | Tergitol NP 10 | Lab bioassay |
| PPCP | Wilson et al. | 2003 | (0) Cyano | Triclosan | Lab bioassay |
| PPCP (antibiotic) | Baumann et al. | 2015 | (−) Cyano | Clarithromycin | Lab EC 50 |

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| PPCP (antibiotic) | Ebert et al. | 2011 | (−) Cyano | Ciprofloxacin | Lab EC 50 |
| PPCP (antibiotic) | Gonzalez pleiter et al. | 2013 | (−) Cyano | Amoxicillin | Lab bioassay |
| PPCP (antibiotic) | Gonzalez pleiter et al. | 2013 | (−) Cyano | Norfloxacin | Lab bioassay |
| PPCP (antibiotic) | Gonzalez pleiter et al. | 2013 | (−) Cyano | Quinolones levofloxacin | Lab bioassay |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Benzylpenicillin (penicillin G) | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Chlortetracycline | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Olaquindox | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Spiramycin | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Streptomycin | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Tetracycline | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Tiamulin | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen | 2000 | (−) Cyano | Tylosin | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen et al. | 2000 | (−) Cyano | Streptomycin | Lab EC 50 |
| PPCP (antibiotic) | Halling-Sorensen et al. | 2000 | (−) Cyano | Tylosin | Lab EC 50 |
| PPCP (antibiotic) | Lui et al. | 2012 | (−) Cyano | Spiramycin | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Amoxicillin | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Flumequine | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Oxolinic acid | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Oxytetracycline | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Sarafloxacin hydrochloride | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Sulfadiazine | Lab EC 50 |
| PPCP (antibiotic) | Lutzhoft et al. | 1999 | (−) Cyano | Trimethoprim | Lab EC 50 |
| PPCP (antibiotic) | Qian et al. | 2012 | (−) Cyano | Streptomycin | Lab EC 50 |
| PPCP (antibiotic) | Robinson et al. | 2005 | (−) Cyano | Fluoroquinolone | Lab EC 50 |
| PPCP (antibiotic) | van der Grinten et al. | 2010 | (−) Cyano | Flumequine | Lab bioassay |
| PPCP (antibiotic) | van der Grinten et al. | 2010 | (−) Cyano | Streptomycin | Lab bioassay |
| PPCP (antibiotic) | van der Grinten et al. | 2010 | (−) Cyano | Sulphamethoxazole | Lab bioassay |
| PPCP (antibiotic) | van der Grinten et al. | 2010 | (−) Cyano | Trimethoprim | Lab bioassay |
| PPCP (antibiotic) | Ebert et al. | 2011 | (+) Cyano | Enrofloxacin | Lab EC 50 |
| PPCP (antibiotic) | Gonzalez pleiter et al. | 2013 | (+) Cyano | Tetracycline | Lab bioassay |
| PPCP (antibiotic) | Halling-Sorensen et al. | 2000 | (+) Cyano | Trimethoprim | Lab EC 50 |

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| PPCP (antibiotic) | Kolar et al. | 2014 | (+) Cyano | Oxytetracycline | Lab EC 50 |
| PPCP (antibiotic) | Kolar et al. | 2014 | (+) Cyano | Trimethoprim | Lab EC 50 |
| PPCP (antibiotic) | Lui et al. | 2014 | (+) Cyano | Amoxicillin | Lab EC 50 |
| PPCP (antibiotic) | Lui et al. | 2012 | (+) Cyano | Amoxicillin | Lab EC 50 |
| PPCP (antibiotic) | Pinckney et al. | 2013 | (+) Cyano | Tylosin | Lab bioassay |
| PPCP (antibiotic) | Stoichev et al. | 2011 | (+) Cyano | Minocycline | Lab EC 50 |
| PPCP (antibiotic) | van der Grinten et al. | 2010 | (+) Cyano | Oxytetracycline | Lab bioassay |
| PPCP (antibiotic) | van der Grinten et al. | 2010 | (+) Cyano | Tylosin | Lab bioassay |
| PPCP (antibiotic) | Wan et al. | 2015 | (+) Cyano | Erythromycin | Lab bioassay |
| PPCP (antibiotic) | Wan et al. | 2014 | (+) Cyano | Levofloxacin | Lab bioassay |
| PPCP (antibiotic) | Yang et al. | 2013 | (+) Cyano | Tetracycline | Lab bioassay |
| PPCP (antibiotic) | Yergeau et al. | 2012 | (+) Cyano | Erythromycin | Lab bioassay |
| PPCP (antibiotic) | Wilson et al. | 2003 | (0) Cyano | Ciprofloxacin | Lab bioassay |