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Chapter 2

Environmental Factors that Influence Cyanobacteria and Geosmin Occurrence in Reservoirs

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http://dx.doi.org/10.5772/54807

1. Introduction

Phytoplankton are small to microscopic, free-floating algae that inhabit the open water of freshwater, estuarine, and saltwater systems. In freshwater lake and reservoirs systems, which are the focus of this chapter, phytoplankton communities commonly consist of assemblages of the major taxonomic groups, including green algae, diatoms, dinoflagellates, and cyanobacteria. Cyanobacteria are a diverse group of single-celled organisms that can exist in a wide range of environments, not just open water, because of their adaptability [1-3]. It is the adaptability of cyanobacteria that enables this group to dominate the phytoplankton community and even form nuisance or harmful blooms under certain environmental conditions [3-6]. In fact, cyanobacteria are predicted to adapt favorably to future climate change in freshwater systems compared to other phytoplankton groups because of their tolerance to rising temperatures, enhanced vertical thermal stratification of aquatic ecosystems, and alterations in seasonal and interannual weather patterns [7, 8]. Understanding those environmental conditions that favor cyanobacterial dominance and bloom formation has been the focus of research throughout the world because of the concomitant production and release of nuisance and toxic cyanobacterial-derived compounds [4-6, 7-10]. However, the complex interaction among the physical, chemical, and biological processes within lakes, reservoirs, and large rivers often makes it difficult to identify primary environmental factors that cause the production and release of these cyanobacterial by-products [9].

1.1. Hydrologic controls

Hydrologic processes control the delivery and retention of nutrients and suspended sediments to lakes and reservoirs, which influence the composition of phytoplankton and zooplankton
communities within those lakes and reservoirs [11-16]. One of the major hydrologic processes that constrain nutrient retention and availability to the phytoplankton community is the flushing rate, related to the lake or reservoir volume and inflow from streams and rivers [17]. Residence times range from several months to many years in natural lakes and from days to weeks in reservoirs [18]. The shorter residence times in reservoirs tend to lower phytoplankton abundances when compared to natural lakes [12,16]. Conversely, climatically or anthropogenically induced lengthening of residence times in reservoirs can promote episodic cyanobacterial dominance [14,16]. Further clouding the relation between hydrologic processes and cyanobacterial abundances, stream or river inflows control the transport of suspended sediment into lakes and reservoirs and affect the phytoplankton community structure and physiology whereby excessive turbidity attributed to the suspended sediment loadings can shift the community towards cyanobacteria [19].

1.2. Environmental risk associated with cyanobacteria

While most lakes and reservoirs have multiple uses, about two-thirds of the United States population drinks water treated from surface-water sources and, of those, the majority of the largest public utilities obtain their drinking water from lakes and reservoirs [20]. Taste-and-odor episodes are common in lakes and reservoirs used for drinking water throughout the United States [1,5-6, 21-23]. Taste-and-odor episodes are often sporadic, and intensities vary spatially [6,23]. Cyanobacterial production of trans-1, 10-dimethyl-trans-decalol (geosmin), and 2-methylisoborneol (MIB), which produce musty, earthy tastes and odors in drinking water, represents one of the primary causes of taste-and-odor complaints to water suppliers [24]. Compounds that produce taste and odor in drinking water are not harmful; therefore, taste-and-odor problems are a palatability, rather than health, issue for drinking water systems.

Geosmin and MIB can be produced by cyanobacteria and certain other bacteria. Three genera of actinomycetes, a type of bacteria found ubiquitously in soils and also present in the aquatic environment, are important sources of geosmin and MIB: Microbispora, Nocardia, and Streptomyces [6,22]. Genera of cyanobacteria that contain known geosmin- and MIB-producing species include Anabaena, Planktothrix, Oscillatoria, Aphanizomenon, Lyngbya, Symploca [6,25-26], and Synechococcus [21]. Geosmin and MIB are problematic in drinking water because the human taste-and-odor detection threshold for these compounds is extremely low (10 nanograms per liter (ng/L)) [27-29], and conventional water-treatment procedures (particle separation, oxidation, and adsorption) typically do not reduce concentrations below the threshold level [24].

If cyanobacteria are identified as the source of geosmin and MIB, human health concerns arise because these cyanobacterial-derived compounds frequently co-occur with cyanobacterial-derived toxins (cyanotoxins). Although many species of cyanobacteria capable of producing geosmin or MIB are also capable of producing toxins, most species are not capable of producing taste-and-odor compounds and cyanotoxins simultaneously [4,30-32]. Cyanotoxins generally are associated with a bloom formation of a toxin-producing cyanobacterial species. Less frequently, cyanobacterial releases of geosmin and MIB that are not associated with cyano-
toxins, have been linked to seasonal periods of high transparency (clear-water phase) attributed to heavy zooplankton grazing [6, 33-36].

The biological function of these cyanobacterial-derived compounds is not well known, but production of geosmin and MIB are reported to occur during active growth and extracellular release during stationary periods, cellular senescence, or cell lysis [26]. The release of cyanotoxins and taste-and-odor compounds by cyanobacteria simply may be a mechanism for the removal of excess metabolites during periods of environmental stress [1,3]. The possibility that cyanotoxins, geosmin, and MIB may contribute to the distribution, abundance, and survival of cyanobacteria in the environment has been investigated [1,35,37]. Secondary metabolites may deter herbivore grazing and shift grazing pressure toward chemically undefended cyanobacterial and algal species, but that allelopathic role is more often attributed to cyanotoxins [37]. If, however, the availability of chemically undefended algae and cyanobacteria is limited (for example, during seasonally heavy zooplankton grazing events that can produce a clear-water phase), it is possible that a shift could occur in the herbivore community toward species that consume chemically defended cyanobacteria [34-39].

1.3. Purpose

Cyanobacterial blooms can be stimulated by human activities that introduce excessive nutrients or modifies the flushing rate in a lake or reservoir [5,9,12-16,40]. Therefore, focus of most research has been on nutrient-enriched, eutrophic to hypereutrophic lake systems that experience cyanobacteria blooms at least seasonally, including agriculturally dominated watersheds of the Midwestern United States [14, 31-32,41-44] and Florida [40,45-47]. Production and release of geosmin often have been reported to occur during periods when cyanobacteria dominated the phytoplankton community and often produced species-specific blooms [5,9,25,40-49]. Environmental factors that have been reported to enhance the ability of cyanobacteria to dominate the phytoplankton community include decreased availability of nitrogen, increased phosphorus concentrations, low total nitrogen to phosphorus ratios, reduced light availability (turbidity), warmer water temperatures, greater water column stability, and longer residence times [1,4-6,9,31-32,44-50].

Two cascading reservoirs that serve as drinking water supplies experienced periodic taste-and-odor problems although the reservoirs were not excessively enriched in nutrients, did not experience observable blooms [51-54], and, therefore, did not appear to fit the existing chemical models for cyanobacterial-dominated systems [9,14,31-32,41-44]. The two reservoirs are located in a rural watershed in the Piedmont region of Spartanburg County, South Carolina. Three synoptic surveys and a 2-year seasonally intensive study of limnological conditions in Lake William C. Bowen (Lake Bowen) and Municipal Reservoir #1 (Reservoir #1) were conducted from 2005 to 2009 to assess the chemical, physical, and biological processes that influenced the occurrence of cyanobacteria and cyanobacterial-derived compounds geosmin, MIB, and microcystin, a common cyanotoxin [52,54].
2. Methods

Water quality and phytoplankton community structure in Lake Bowen and Reservoir #1 were monitored synoptically in 2005 and 2006 and intensively from May 2007 to June 2009 to assess the conditions associated with cyanobacterial, geosmin, MIB, and microcystin occurrence. Water samples were collected near the surface (1-meter (m) depth) and near the bottom (6-m depth, where sufficiently deep) at selected transects (fig. 1). Euphotic-zone composite samples were collected during winter, spring, and summer 2009 only to compare to the corresponding surface samples. Samples were collected and processed using U. S. Geological Survey (USGS) protocols and guidelines described in [29]. Discrete depth samples were collected at three locations across each transect (25, 50, and 75 percent of transect width) using Van Dorn samplers and were compositd to create a depth-specific sample. Transparency (Secchi disk depth) and light attenuation (to determine euphotic zone depth) were measured at the time of
sampling. Lake profile measurements of fluorescence (an estimate of chlorophyll), specific conductance, pH, dissolved oxygen concentrations, and water temperature were collected at 1-m depth intervals along each transect. Reservoir sampling frequency varied seasonally with 67 percent of the samples collected during the peak algal growth period (spring to late summer) [54]. Samples were analyzed for nutrients, major ions, organic carbon, phytoplankton biomass, chlorophyll $a$, pheophytin $a$, dissolved geosmin and MIB, total microcystin, and actinomycetes concentrations. Analytical methods for chemistry and algal taxonomy are described in detail in [54]. The degree of stratification was quantified for each sampling event by computing the relative thermal resistance to mixing (RTRM) at 1-m depth intervals from the lake profile of water temperature at the time of sampling at each site. The RTRM for each 1-m depth interval was computed as the ratio of the density difference between water at the top and the bottom of the 1-m depth interval divided by 0.000008 (the density difference between water at 5 and 4 °C; [18,55-56]. The maximum RTRM for that lake profile was used as a measure of the degree of stratification at that site for that sampling event.

Bivariate and multivariate nonparametric techniques were utilized for data exploration. Data preparation included assigning censored values the same rank and ranked below estimated and quantitative (detections above the laboratory reporting limit or LRL) values [57-58]. Estimated values that are semiquantitative detections below the LRL were assigned the same rank, that is, above censored values but below detected values [57-58]. For biotic data, analyses were done using cell biovolumes (in cubic micrometers per milliliter); preliminary analyses of cell densities (cells per milliliter) yielded similar results but are not provided in this chapter. For chemical, physical, and a subset of phytoplankton data, the Kruskal-Wallis (KW) test was applied to the data to determine if a statistical difference existed (alpha level = 0.05) among groups of data, and the Tukey’s Studentized Range test was used to identify which group or groups were different [57-58]. Data from selected sites were evaluated by the Kendall tau correlation procedure to measure the strength of the monotonic bivariate relation between the environmental factors and geosmin concentrations, microcystin concentrations, and cyanobacteria biovolumes [57-58].

Phytoplankton assemblage data were evaluated using multivariate techniques described in [59]. Prior to evaluation, assemblage data were transformed using a fourth root transformation. Hypotheses of temporal (seasonal, annual) and spatial (depth, reservoir location) similarities in the taxonomic composition and biovolumes of phytoplankton communities were examined with a series of non-metric multi-dimensional scaling (NMDS) and one-way analysis of similarity (ANOSIM) tests [59-60]. Prior to plotting the sample patterns of the complex relations among phytoplankton groups and species in 2- and 3-dimensional (2-D, 3-D, respectively) space using NMDS, between-sample similarity (or dissimilarity) coefficients were computed and a triangular matrix constructed [60]. The goodness-of-fit of the NMDS was measured as a stress value, whereby stress < 0.1 corresponds to an effective ordination in 2-D space and < 0.2 is useful but should be superimposed with grouping from a hierarchical cluster analysis to verify [60]. Therefore, the hierarchical Cluster analysis with the SIMPROF option of the cyanobacterial assemblages was superimposed on the NMDS for this study. The statistical test that was used to determine differences among phytoplankton groups and
species was ANOSIM, based on a Global R statistic [60]. Global R statistic falls between -1 and 1 whereby R equal to zero indicates completely random grouping of phytoplankton assemblages while R equal to 1 indicates that all assemblages of a group are more similar to each other than to assemblages from another group [60]. Significant R values (p-value < 0.05) indicate the R value is significantly different from zero [60]. The last step of the multivariate analysis was to link the cyanobacterial assemblage data to the environmental data using RELATE and the global BEST test procedures [59,60]. RELATE provided a Spearman rho correlation analysis between the similarity matrices of the cyanobacterial assemblage and environmental data. Prior to analysis, a logarithmic (base 10) transformation was applied to environmental data. Subsets of explanatory environmental variables and a fixed resemblance matrix computed from the cyanobacterial data were used as input into the BEST model to determine the best combination of variables that explains the observed cyanobacterial assemblages [59,60]. The stepwise procedure Bio-ENV was employed [60].

On the basis of the results of the exploratory statistical analysis, one site in Lake Bowen and one site in Reservoir #1 were selected to develop a regression model to estimate geosmin concentrations by using environmental factors as explanatory variables. The Lake Bowen site was located nearest the dam and represented the quality of water released to Reservoir #1 (fig. 1). The Reservoir #1 site was located near its dam and represents the quality of water near the raw water intake for the R.B. Simms Water Treatment Plant (fig. 1). Because of the high percentage of censored geosmin concentrations at both sites, ordinary least squares regression was not used to develop a multiple linear regression model. Instead, the multiple logistic regression approach was used to identify environmental factors that best explained the likelihood of geosmin concentrations exceeding the human detection threshold of 10 ng/L [57]. Variables selected for input into the multiple logistic regression analysis included those identified in the Kendall tau correlation analysis and those that could be easily measured by Spartanburg Water as part of their watershed monitoring program. The best equation for each reservoir was selected on the basis of the Pearson Chi-square Statistic (goodness of fit greater at lower statistics and higher p-values), the Hosmer-Lemeshow Statistic (goodness of fit greater at lower statistics and higher p-values), and the minimum Likelihood Ratio Test Statistic, which tests how well an equation fits the data by summing the squares of the Pearson residuals (goodness of fit greater at lower p-values). Model output provided a Logit P result, whereby Logit P results greater than 0.5 resulted in a positive response (geosmin concentrations exceeded the human detection threshold of 10 ng/L) and less than 0.5 resulted in a reference response (geosmin concentrations were below the human detection threshold of 10 ng/L).

3. Reservoir hydrology

Lake Bowen and Reservoir #1 are relatively small, shallow (4.8 and 2.3 m depths, respectively) cascading impoundments of the South Pacolet River in Spartanburg County, South Carolina (fig. 1) [52,61]. At the full-pool elevation, Lake Bowen has a surface area of 621 hectares (ha) and has 53.2 kilometers (km) of shoreline. Lake Bowen releases spillage (overflow) at the dam directly into Reservoir #1 and by controlled releases at depth from gated conduits (flow-
through). Reservoir #1 is substantially smaller and older than Lake Bowen. At the full-pool elevation, Reservoir #1 has a surface area of 110 ha and 21.1 km of shoreline. Water from these reservoirs is treated by Spartanburg Water at the R. B. Simms Water Treatment Plant. The water-treatment facility and raw water intake are located on Reservoir #1. Recreational activities are allowed on Lake Bowen but are prohibited on Reservoir #1.

3.1. Hydrologic conditions in the reservoirs

Inflow to Lake Bowen from the South Pacolet River at the USGS streamgaging station 02154790 generally represented 70 to 85 percent of the total inflow into Lake Bowen on the basis of the synoptic streamflow measurements of minor tributaries (fig. 1) [53]. Unit-area streamflows (computed as the measured synoptic streamflow divided by the drainage areas at the site) were similar among the South Pacolet River and its minor tributaries. Therefore, daily mean unit-area streamflow at the South Pacolet River streamgaging station was multiplied by the total drainage area above Lake Bowen (207 km\(^2\)) to extrapolate the total inflow to Lake Bowen from 2005 to 2009. Daily residence times in Lake Bowen were computed by dividing the daily mean inflow to Lake Bowen by the daily mean water volume in Lake Bowen (determined using the stage-volume curve developed by the USGS [54] and the daily mean water level at the USGS streamgaging station 02154950). Outflow from Lake Bowen was considered the major inflow to Reservoir #1 and was computed by the summation of daily volume of water associated with spillage (overflow) and controlled releases. However, the estimated Lake Bowen inflow did not represent all the inflow to Reservoir #1 because it did not include contributions from a tributary to the south of Reservoir #1. Therefore, Reservoir #1 outflow also was considered. Determining outflow from Reservoir #1 was further complicated by the confluence of the South and North Pacolet Rivers to form the Pacolet River just below the Reservoir #1 dam. So, outflow from Reservoir #1 was estimated by computing a 30-day moving window average of differences in streamflow between the Pacolet River USGS streamgaging station (02155500) and North Pacolet River USGS streamgaging station (02154500).

3.2. Climate controls on hydrology

The climate of the Pacolet River Basin is classified as temperate [62-63], with 30-year mean annual precipitation of 127.6 centimeters (cm) and mean annual temperature of 15.6 degrees Celsius (°C) [64]. The study area was under severe to extreme drought conditions from September 2007 through December 2008, with annual precipitation amounts that were less than 38 percent of the long-term mean (48.7 and 31.0 cm for 2007 and 2008, respectively) [64]. Annual mean precipitation in 2009 was near the 30-year mean annual precipitation.

As is common for reservoirs, computed residence times were relatively short and averaged 0.60 year in Lake Bowen (estimated from inflow) and 0.26 year in Reservoir #1 (estimated from outflow) for the 5-year study period (2005 – 2009) (fig. 2A). Maximum residence times of 1.04 years in Lake Bowen and 0.80 year in Reservoir #1 occurred in 2008 when drought conditions were prevalent in Spartanburg County (fig. 2A). The maximum residence times were concomitant with major changes in chemical and biological conditions in both reservoirs.
Longer residence times in 2008 were attributed to lower water contributions from the South Pacolet River to Lake Bowen and less water being released from Reservoir #1. This lower water contribution affected the nutrient transport from the watershed to the reservoirs, with much lower annual loads of nitrogen and phosphorus being delivered in 2008 (fig. 2B,C) [53]. Annual total nitrogen loads decreased an order of magnitude from 117,600 kilograms per year (kg/yr) in 2005 (wet period) to 14,000 kg/yr in 2008 (drought period). In 2008, the annual total phosphorus load of 795 kg/yr was only 5 percent of the 2005 total phosphorus load. While annual nitrate plus nitrite loads decreased four-fold from 35,380 kg/yr in 2005 to 8,740 kg/yr in 2008, the 2008 nitrate plus nitrite load represented a greater percentage of the total nitrogen load (62 percent) than in 2005 (30 percent).

**Figure 2.** (A) Annual and mean annual residence times in Lake William C. Bowen (Lake Bowen) and Municipal Reservoir #1 (Reservoir #1) and annual (B) nitrogen and (C) phosphorus loadings from the South Pacolet River into Lake Bowen, from 2005 to 2009, Spartanburg County, South Carolina.
4. General limnological conditions

Overall, Lake Bowen and Reservoir #1 can be classified as warm monomictic reservoirs that stratify from early June to early-to-late October in locations where depths exceed 5 m (for detailed bathymetry, see [61]). From 2005 to 2009, thermal stratification occurred seasonally in the deeper, downgradient regions of the reservoirs [52,54]. Maximum RTRMs that were representative of strongly stratified conditions (greater than 80) were prevalent during the summer season, but were near zero from November through the winter season for these sites [18]. During periods of stratification, the hypolimnion became anoxic (dissolved-oxygen concentrations below 1 milligram per liter (mg/L)) in both reservoirs.

Water column transparencies were greatest at deep, downgradient sites in Lake Bowen and Reservoir #1, but lower at the shallower, upgradient sites in Reservoir #1 (KW p < 0.001). Statistically significant seasonal patterns in transparencies were identified in Lake Bowen and Reservoir #1, with the greatest transparencies in the spring and the least in the fall (KW p < 0.001). Median transparencies were about 1.6 m in the downgradient regions and 1.0 m in the upgradient regions of the reservoirs; however, maximum transparencies of 3.7 m in Lake Bowen and 2.0 m in Reservoir #1 were measured in May 2008.

Overall, Lake Bowen and Reservoir #1 had statistically similar chlorophyll $a$ and nutrient concentrations that were indicative of mesotrophic conditions (KW p > 0.10) [65-66]. Maximum nutrient concentrations remained below 0.034 mg/L for total phosphorus and 0.70 mg/L for total nitrogen in the reservoirs for the study period [54]. Maximum chlorophyll $a$ concentration was 26 micrograms per liter ($\mu$g/L) and occurred in Lake Bowen, but median concentrations were below 9 $\mu$g/L at all sites in both reservoirs. Maximum chlorophyll $a$ concentrations in both reservoirs were indicative of eutrophic conditions [18,65]. Chlorophyll-to-phosphorus ratios (Chl:TP) that are substantially less than 1 indicate phytoplankton are not phosphorus limited [41,67]. Median chlorophyll-to-total-phosphorus ratios ranged from 0.5 to 0.7 for all sites in Lake Bowen and Reservoir #1 and indicated phosphorus concentrations were approaching limiting conditions. Median TN:TP ratios ranged from 20 to 24 in Lake Bowen and from 13 to 18 in Reservoir #1. Based on these median TN:TP ratios, potential phosphorus limitation (TN:TP >17) was common in Lake Bowen and co-limitation (17 < TN:TP > 10) by phosphorus and nitrogen was common in Reservoir #1 [65]. Low total-nitrogen-to-total-phosphorus (TN:TP) ratios (generally below 29:1) were consistent with environmental conditions reported to favor nitrogen-fixing cyanobacteria (fig. 3C, 3D) [40,45,47].

Seasonal variation in dissolved inorganic nitrogen (DIN; sum of dissolved nitrate plus nitrite and ammonia) concentrations indicated periods of limitation and enrichment in readily bioavailable forms of nitrogen for most phytoplankton groups (fig. 3A, 3B). DIN concentrations were consistently low in the reservoirs near the surface (1 m) during the spring and summer of 2008, when compared to other spring periods. Elevated DIN concentrations were observed near the bottom relative to the surface (not shown in fig. 3) and were attributed to increased dissolved ammonia in the anoxic hypolimnion [54]. Statistically, dissolved ammonia concentrations were highest in the fall and lowest in the spring and summer (KW p < 0.001), and dissolved nitrate plus nitrite concentrations
were highest in the winter and spring and lowest in the summer and fall (KW p < 0.001). However, the winter 2009 sample period also had dissolved ammonia concentrations comparable to the fall sample periods (fig. 3A). Increases in total phosphorus concentrations in the hypolimnion relative to the epilimnion during summer were evident in both reservoirs (KW p < 0.001), and especially was pronounced during the summer of 2008. Hypolimnentic increase in total phosphorus concentrations was concurrent with the development of strong stratification (KW p < 0.001; maximum RTRMs greater than 100) and anoxic conditions in the summer and early fall, indicating a source of phosphorus from the bed sediment during that seasonal period [66]. Hypolimnetic increases in total phosphorus concentrations were as high as 0.017 mg/L in Lake Bowen and 0.012 mg/L in Reservoir #1. Dissolved nitrate plus nitrite concentrations were highest in the winter (KW p < 0.001), while total nitrogen and TN:TP ratios were statistically lower during the summer (KW p < 0.001) (fig. 3). For all depths and sites, total organic nitrogen concentrations were statistically greater during the spring and winter than during the summer and fall (KW p = 0.003) (fig. 3).

Overall, chlorophyll a, total nitrogen, and phosphorus concentrations observed in Lake Bowen and Reservoir #1 were not indicative of reservoir systems that would experience cyanobacterial dominance of the phytoplankton community, based on empirical models developed in [9] (fig. 3). Instead, the empirical models predicted that cyanobacteria would represent less than 20 percent of the phytoplankton community based on the observed chlorophyll and nutrient levels. One exception based on the empirical model was for observed TN:TP ratios that indicated greater likelihood of cyanobacterial dominance [9] (fig. 3C, 3D), whereby TN:TP ratios were generally below 29:1 and were consistent with environmental conditions reported to favor nitrogen-fixing cyanobacteria [40,45,47].

5. Phytoplankton community structure

Overall, no statistical differences were identified in total phytoplankton and cyanobacterial biovolumes among sites and depths in Lake Bowen and Reservoir #1 (KW p > 0.20). Median total phytoplankton biovolumes ranged from 1,853,000 to 2,480,000 cubic micrometers per milliliter (µm³/mL) in Reservoir #1 and from 1,869,000 to 2,292,000 µm³/mL in Lake Bowen. Cyanobacterial biovolumes were an order of magnitude lower, with median biovolumes ranging from 127,700 to 180,000 µm³/mL in Reservoir #1 and from 111,800 to 123,700 µm³/mL in Lake Bowen. Maximum cyanobacterial biovolumes occurred during the summer (July to August), but did not always result in cyanobacteria dominating the phytoplankton community (KW p < 0.001). In fact, median percentages of the cyanobacterial fraction of the total phytoplankton community were less than 10 percent in all samples from all sites, indicating cyanobacteria rarely dominated the phytoplankton community and verifying the empirical model results described in the last section [9] (fig. 4). However, during drought conditions in July 2008 in Lake Bowen, cyanobacteria represented as much as 59 percent of the total phytoplankton community in Lake Bowen (fig. 4). During the same time period, cyanobacteria dominance was less pronounced in Reservoir #1 (31 to 44 percent of the total phytoplankton community...
community) than Lake Bowen. Additionally, surface algal blooms were not observed during the study period.

Within the cyanobacteria group, genera that contained known geosmin-producing species and known toxin-producing species were present at all sites. Biovolumes of these genera, however, varied seasonally and annually. Known geosmin-producing genera identified in the two reservoirs included *Planktolyngbya, Aphanizomenon, Synechococcus, Pseudoanabaena, Oscillatoria*, and *Anabaena*, many of which also can produce toxins. Other known toxin-producing genera identified in the two reservoirs included *Cylindrospermopsis, Synechocystis, Microcystis*, and *Aphanacapsa*. Median percentages of known geosmin-producing genera in the cyanobacterial group ranged from 48 to 59 percent among all sites. Fractions of all known geosmin-producing genera in the cyanobacterial group were similar among sites and depths (KW p > 0.90). However, seasonal differences were identified that indicated greater fractions of known geosmin-producing genera occurred during the spring and winter and the least fractions occurred during the fall (KW p < 0.001).
Figure 4. Seasonal variation in (A) dissolved geosmin concentrations, (B) total microcystin concentrations, (C) fraction of geosmin-producing genera within the Cyanophyta (cyanobacteria) group, and (D) fraction of cyanobacteria in the total phytoplankton for sites near the surface in Lake William C. Bowen and Municipal Reservoir #1, Spartanburg County, South Carolina, 2005 to 2009. Red dashed line represents the laboratory reporting level for dissolved geosmin (< 0.005 µg/L) total microcystin (< 0.010 µg/L).

Results of the ANOSIM tests for the differences among samples grouped by reservoir, season, year, and depth position indicated significant and relatively strong temporal variation (particularly seasonal variation) in phytoplankton assemblages in terms of biovolumes of algal divisions, all Cyanophyta genera, and the 6 known geosmin-producing Cyanophyta genera. Overall, phytoplankton assemblages of all divisions and genera within the Cyanophyta division had low Global R values (< 0.22) when grouped by reservoir and depth position, indicating relatively random grouping (table 1). Conversely, the same assemblages had the highest Global R values (> 0.40) when grouped by season (table 1). The greatest seasonal differences were for genera within the Cyanophyta genera (including genera with known geosmin-producing species). Once the taxonomic assemblage of known-geosmin producing Cyanophyta genera became restricted by season (spring only) and depth position (spring only, surface depth only), no differences among samples were identified (table 1). In summary, multivariate statistical analysis indicated that taxonomic assemblages of the phytoplankton community (represented by the major algal divisions) and the cyanobacterial community varied season to season and, to a lesser degree, year to year. The above-mentioned taxonomic assemblages between the two reservoirs and between the two depth positions were similar. This seasonal pattern of variability is consistent with the pattern identified in the Kruskal-Wallis analysis of the chemical and physical data.
Table 1. Nonparametric Analysis of Similarity (one-way) results for 5 phytoplankton taxonomic assemblages in relation to 4 factors of reservoir group, season, year, and depth position for Lake William C. Bowen and Municipal Reservoir #1, Spartanburg County, South Carolina, May 2007 to June 2009. Bold italicized text highlights most significant relation between groups and phytoplankton assemblages. Global R statistic falls between -1 and 1 whereby R equal to zero indicates completely random grouping of phytoplankton assemblages whereas R equal to 1 indicates that all assemblages of a factor are more similar to each other than to assemblages from another factor [60]. Significant R values (p-value < 0.05) indicate the R value is significantly different from zero.

Four NMDS 2-D plots of the cyanobacterial assemblages as Cyanophyta genera were constructed and samples were grouped by reservoir (fig. 5A), season (fig. 5B), depth (fig. 5C), and season by year (fig. 5D) to illustrate the ANOSIM results. The hierarchical Cluster analysis with the SIMPROF option of the cyanobacterial assemblages was superimposed on the NMDS, producing 5 distinct assemblages at 60 percent similarity [59, 60]. Comparison of the cyanobacterial assemblages in Lake Bowen and Reservoir #1 and at the 3 depths identified samples that were fairly evenly distributed throughout the 5 cluster groups (fig. 5A,5C). Euphotic-zone composite samples were collected during winter, spring, and summer 2009 only. Therefore, as determined by ANOSIM and illustrated by NMDS, reservoir and depth were not considered to be major factors that explain the differences in cyanobacterial assemblages (fig. 5A, 5C). Seasonal changes explained most of the differences in cyanobacterial assemblages among the 5 cluster groups, however, spring and fall samples were split into two distinct groups (fig. 5B). The best explanatory factor was the combined season and year that identified the spring 2008 cyanobacterial assemblage as a separate group from the spring 2009 and 2007 samples. Additionally, the two distinct fall 2007 groups were determined to be cyanobacterial assemblages associated with pre- and post-overturn periods, with the post-overturn samples plotting to the upper left of the pre-overturn samples shown in Figure 5D.
Figure 5. Non-metric multi-dimensional scaling (NMDS) ordinations of cyanobacterial assemblages in surface, euphotic zone, and bottom depths at sites in Lake William C. Bowen and 3 sites in Municipal Reservoir #1, Spartanburg County, South Carolina, May 2007 to June 2009, based on (A) reservoir, (B) season, (C) depth, and (D) season and year. Fourth-root transformation was applied to the cyanobacterial assemblage data prior to construction of the Bray Curtis resemblance matrix for the NMDS ordination. Goodness-of-fit of the NMDS is measured by the 2-dimensional stress of 0.11, which is indicative of relatively good ordination but to verify, cluster groups, determined by Hierarchical Cluster analysis program with the SIMPROF option in PRIMER, were superimposed [60].

6. Geosmin, MIB, and microcystin occurrence

Geosmin was the most commonly detected taste-and-odor compound in Lake Bowen and Reservoir #1 during the study period from May 2006 to June 2009 [51]. However, about 35 percent of the samples in Lake Bowen and more than 44 percent of the samples in Reservoir #1 had non-detectable geosmin concentrations (< 0.005 µg/L). Median geosmin concentrations ranged from < 0.005 to 0.006 µg/L at the study sites. MIB rarely was detected (median concentrations were less than the laboratory reporting level of 0.005 µg/L at all sites). When present, MIB was at very low concentrations with maximum MIB concentrations ranging from 0.005 to 0.014 µg/L.

As is often observed in surface-water systems, geosmin concentrations in Lake Bowen and Reservoir #1 exhibited strong annual and seasonal variability during the study, with peak geosmin concentrations occurring in the spring (KW p < 0.001; fig. 4A). Maximum geosmin concentrations occurred in spring 2008 (April and May 2008) at all sites in both reservoirs. During the spring of 2008, maximum geosmin concentrations ranged from 0.060 to 0.100 µg/L near the surface in Lake Bowen, which were 6 to 10 times greater than the human
detection threshold of 10 ng/L (0.010 µg/L). Maximum geosmin concentrations near the surface in Reservoir #1 were about half (0.035 to 0.050 µg/L) the maximum concentration observed in Lake Bowen during the same spring 2008 period. Annual maximum geosmin concentrations tended to re-occur in the April–May period, but in 2009, the annual maximum geosmin concentrations occurred in March (fig. 4). Nonetheless, the spring (March-to-May) period of annual maximum geosmin concentrations was not concurrent with the period of annual maximum cyanobacterial biovolume (fig. 4A, D). Cyanobacteria were present in both reservoirs during the peak geosmin period in the spring of 2008, but represented less than 20 percent of total phytoplankton biovolume (fig. 4D). The peak geosmin period was concurrent with a peak in the fraction of known geosmin-producing genera in the cyanobacteria group (fig. 4A, C). Microcystin rarely was detected. Maximum microcystin concentrations of 0.30 and 0.40 µg/L were observed in Lake Bowen and Reservoir #1, respectively, during the summer of 2008 immediately following the peak geosmin period (fig. 4B). Microcystin concentrations were below concentrations of concern of 1 µg/L for drinking water or 20 µg/L for recreational activities [4]. No statistical differences existed in geosmin, MIB, and microcystin concentrations and in cyanobacterial biovolume between Lake Bowen and Reservoir #1, among sites within each reservoir, and between depths at a site (KW p > 0.70).

7. Environmental factors influencing geosmin concentrations and cyanobacterial biovolumes

In Lake Bowen, elevated geosmin concentrations near the surface were correlated significantly (p < 0.05) with deep (greater than 6 m) mixing zone (\(Z_m\)) depths (Kendall tau (\(\tau\)) = 0.60), lower euphotic zone-to-mixing zone depth (\(Z_{eu}:Z_m\)) ratios (\(\tau\) = –0.47), and elevated dissolved-oxygen concentrations (\(\tau\) = 0.42), which are environmental conditions indicative of unstratified conditions in Lake Bowen. In relation to phytoplankton community structure, elevated geosmin concentrations in Lake Bowen were correlated to greater *Oscillatoria* biovolumes (\(\tau\) = 0.60), reduced algal biomass (dry weight) (\(\tau\) = –0.61), and lower total phytoplankton biovolumes (\(\tau\) = –0.4). This disparate pattern of increased cyanobacterial biovolumes and overall reduced phytoplankton biovolumes during periods of elevated geosmin was not in line with past geosmin occurrence research [25,41,43,44]. Conversely, geosmin concentrations were not correlated to residence times, nutrient concentrations, chlorophyll \(a\) concentrations, actinomycetes concentrations, other known geosmin-producing genera of cyanobacteria, fraction of known geosmin-producing genera in the cyanobacteria group, or cyanobacterial biovolumes in Lake Bowen.

In Reservoir #1, elevated geosmin concentrations near the surface were correlated to elevated dissolved-oxygen and chloride concentrations (\(\tau\) = 0.55 and 0.58, respectively). Elevated geosmin concentrations were correlated to lower pheophytin \(a\) concentrations (\(\tau\) = –0.61) and to reduced algal biomass (dry weight) (\(\tau\) = –0.53). Elevated geosmin concentrations were correlated to greater fraction of geosmin-producing genera in the cyanobacteria group (\(\tau\) = 0.56); however, total cyanobacterial biovolumes were not correlated to geosmin concentra-
tions. As was determined for Lake Bowen, geosmin concentrations were not correlated to residence times, nutrient, chlorophyll \(a\), or actinomycetes concentrations in surface samples.

Sites in Lake Bowen had geosmin concentrations correlated to the known geosmin-producing genus *Oscillatoria* within the cyanobacteria group but not to actinomycetes concentrations, suggesting that cyanobacteria were the probable source of geosmin. Sites in Reservoir #1 also had geosmin concentrations that correlated to known geosmin-producing genera as a fraction of the overall cyanobacteria group. Again, no significant correlation existed between geosmin and actinomycetes concentrations in either reservoir.

Cyanobacterial biovolumes tended to be greatest during the summer when the reservoirs were stratified (KW \(p < 0.001\)). In contrast to elevated geosmin concentrations, environmental factors indicative of stratified or stable water column conditions, including warmer water temperatures (\(\tau = 0.86\)), lower \(Z_m\) (\(\tau = -0.84\)), greater \(Z_{eu}/Z_m\) ratios (\(\tau = 0.88\)), lower dissolved-oxygen concentrations (\(\tau = -0.52\)), and maximum RTRM (\(\tau = 0.91\)) correlated strongly with elevated cyanobacterial biovolumes in Lake Bowen. Additionally, in contrast to elevated geosmin concentrations, cyanobacterial biovolumes in Lake Bowen correlated with changes in nutrient levels in the reservoirs. Elevated cyanobacterial volumes were correlated to lower nitrogen concentrations (as ammonia, nitrate plus nitrite, total organic nitrogen, and TN) and lower TN:TP ratios. Elevated cyanobacterium biovolume in both reservoirs correlated strongly to elevated hypolimnetic to epilimnetic TP ratios (\(\tau = 0.55\)) that was considered indicative of release of phosphorus from the sediment by biotic or abiotic processes during anoxic conditions. Elevated cyanobacterial biovolumes in surface samples from Reservoir #1 had similar correlative relations to environmental factors as cyanobacterial biovolumes in Lake Bowen, with the exception of no correlation to TN:TP ratios and a negative correlation to TP (\(\tau = -0.51\)).

On the basis of 30-day moving window averages, elevated cyanobacterial biovolumes in Lake Bowen and Reservoir #1 were correlated to longer residence times (computed from inflow; \(\tau = 0.69\) and 0.50, respectively) and lower overflow volumes (\(\tau = -0.61\) and -0.44, respectively), indicative of low-flow or drought conditions. Biovolumes of known geosmin- and toxin-producing cyanobacteria genera, including *Cylindrospermopsis*, *Planktolyngbya*, *Synechococcus*, *Synechocystis*, and *Aphanizomenon* (Lake Bowen only), correlated with greater cyanobacteria biovolumes and were the dominant taxa in the cyanobacteria group.

The RELATE and BEST multivariate analyses also were used to link cyanobacterial assemblages to environmental factors by testing the hypothesis of no relation between multivariate pattern of two datasets (table 2) [59,60]. The RELATE procedure was used to evaluate potential correlations between three assemblage measures (all phytoplankton divisions, all genera and genera with known-geosmin producing species only in the Cyanophyta division,) and 14 potential explanatory factors. Specifically, resemblance matrices for phytoplankton division, Cyanophyta genera, and known geosmin-producing Cyanophyta genera assemblages for all seasons and for spring only were correlated to the resemblance matrices for 14 selected environmental variables using Spearman rho correlation analysis (table 2) [59]. Strongest correlations were identified for the potential explanatory variables and Cyanophyta genera (\(\rho = 0.526\)) and known geosmin-producing Cyanophyta genera (\(\rho = 0.436\)) during the 3 spring seasons (2007, 2008, 2009), which indicated that differences in cyanobacterial assemblages can
be explained by differences in environmental factors during the spring period (table 2). Statistically significant \((p < 0.001)\) but weak correlations were identified between environmental factor and phytoplankton divisions and Cyanophyta genera when all seasons were considered (table 2). The BEST analysis, which incorporated the stepwise search algorithm (BVSTEP), was used to select the environmental variables that best explain the cyanobacterial assemblages during the spring season. BEST analysis was performed by comparing fixed resemblance matrices of springtime Cyanophyta assemblages (all genera and genera with known geosmin-producing species only) with resemblance matrices of a combination of up to 14 variables, with the final result being the best set of explanatory variables that explain structure in the assemblage data. A six-variable model was selected that best explained the assemblages of all Cyanophyta genera in the two reservoirs during the spring \((\rho = 0.601)\) (table 2). The explanatory variables included specific conductance, the two forms of DIN (ammonia and nitrate plus nitrite), chlorophyll \(a\), pheophytin \(a\) (degradate of chlorophyll \(a\)), and iron (table 2). A four-variable model was selected to best explain the assemblages of only known geosmin-producing genera of Cyanophyta \((\rho = 0.663)\) and included magnesium, nitrate plus nitrite, chlorophyll \(a\):pheophytin \(a\) ratio (undegraded to degraded pigment), and geosmin (table 2).

<table>
<thead>
<tr>
<th>Phytoplankton Taxonomic Assemblages</th>
<th>RELATE Analysis on Matched Resemblance Matrices</th>
<th>BEST Analysis on Phytoplankton Fixed Resemblance Matrix and Selected Explanatory Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton Divisions, all season</td>
<td>Spearman Rho 0.275 P-value 0.001</td>
<td>Spearman Rho 0.601 P-value 0.001 Explanatory Variables Specific conductance, ammonia, nitrate plus nitrite, chlorophyll (a), pheophytin (a), iron</td>
</tr>
<tr>
<td>Cyanophyta genera, all seasons</td>
<td>0.197 0.001</td>
<td>-- -- --</td>
</tr>
<tr>
<td>Cyanophyta genera, spring only</td>
<td>0.526 0.001</td>
<td>0.601 0.001</td>
</tr>
<tr>
<td>Cyanophyta genera with known geosmin-producing genera, spring only</td>
<td>0.436 0.001</td>
<td>0.663 0.001</td>
</tr>
</tbody>
</table>

Table 2. RELATE and BEST tests for linkages between springtime cyanobacterial assemblages and environmental factors for Lake William C. Bowen and Municipal Reservoir #1, Spartanburg County, South Carolina, May 2007 to June 2009.

Factor-specific relations were depicted using NMDS ordination of cyanobacterial assemblages superimposed with bubble plots of selected environmental factors (fig. 6). Maximum geosmin concentrations occurred during the spring of 2008 when the distinct cyanobacterial assemblage was present in Lake Bowen and Reservoir #1 (fig. 6A, 6C). During that same period, nitrate plus nitrite concentrations were reduced when compared to other spring periods but not as
reduced as fall and summer periods (fig. 6E). Minimum iron concentrations were associated with the spring 2008 cyanobacterial assemblage when compared to spring 2007 and 2009 assemblages (fig. 6B). Less evident differences in chlorophyll a and pheophytin a concentrations were identified in the bubble plots for all cyanobacterial assemblages (fig. 6 D, F).

Production and release of geosmin often have been reported to be related to periods when cyanobacteria dominated the phytoplankton community and often produced species-specific blooms [5,9,25,40-49]. In turn, cyanobacterial dominance has been attributed to several environmental factors that allow the cyanobacteria to thrive more successfully than other phytoplankton groups, including decreased availability of nitrogen, increased phosphorus
concentrations, reduced light availability, warmer water temperatures, greater water column stability, and longer residence times (1,4-6,9,31-32,44-50). In Lake Bowen and Reservoir #1, cyanobacterial biovolumes were related to many of these reported environmental factors as well as increased total phosphorus in the hypolimnion attributed to sediment release during anoxic conditions (internal phosphorus cycling). Elevated dissolved geosmin concentrations, however, were not related to increased cyanobacterial biovolumes, and cyanobacteria rarely dominated the total phytoplankton community. Therefore, another mechanism was needed to explain the increased production and release of geosmin in April and May of 2008 for Lake Bowen and Reservoir #1.

One plausible explanation of elevated geosmin concentrations in 2008 is related to a pattern of greater transparencies observed in both reservoirs (more pronounced in Lake Bowen) concurrent with annual maximum dissolved geosmin concentrations in the spring. In fact, during the spring of 2008, maximum transparencies (3.7 m in Lake Bowen and 2.0 m in Reservoir #1) coincided with the period of peak dissolved geosmin production when maximum geosmin concentrations were 100 ng/L in Lake Bowen and 50 ng/L in Reservoir #1. That springtime pattern of greater transparency is consistent with a clear-water phase usually attributed to heavy zooplankton grazing of the phytoplankton community and has been reported commonly in mesotrophic reservoirs [33, 35]. Because zooplankton data were not available, the occurrence of this process could not be confirmed directly. Nonetheless, the relation between elevated dissolved geosmin concentrations and environmental factors other than deeper transparencies also suggested zooplankton grazing could be a mechanism for the direct or indirect release of geosmin from cyanobacteria into the dissolved phase. In both reservoirs, elevated dissolved geosmin concentrations were correlated to environmental factors not only indicative of greater light penetration (greater euphotic zone depths) but also reduced algal biomass and total phytoplankton biovolumes. Geosmin release from cyanobacterial cells has been associated with cellular senescence or cell lysis [26] and with periods of environmental stress [1,3], all of which are consistent with heavy zooplankton grazing. While cyanobacteria generally are not considered favorable food for zooplankton, a shift in the zooplankton community toward less selective predation is probable because of the increased competition for prey that would accompany a heavy zooplankton grazing event [35-39].

Another indication of heavy zooplankton grazing was the coincidence of the lowest total phytoplankton and cyanobacterial biovolumes near the surface (1-m depth) during the period of maximum dissolved geosmin concentrations in the spring of 2008.

Predator-driven natural selection of cyanobacterial genera with chemical-defense capabilities could further contribute to elevated dissolved geosmin concentrations. During the period of maximum geosmin concentrations, three genera with known geosmin-producing strains in the cyanobacteria group (Oscillatoria, Aphanizomenon, and Synechococcus) were the dominant cyanobacteria taxa in both reservoirs. Increasing grazing pressure with decreasing prey alternatives would be expected to trigger chemical defenses in this surviving population. While grazing of Oscillatoria and Aphanizomenon by zooplankton is not well documented, the genus Synechococcus consists of picoplankton that are important contributors to pelagic freshwater ecosystems and have been shown to be grazed by zooplankton [34,68].

In combination with or separate from heavy springtime zooplankton grazing, nutrient (N, P) limitation also could explain seasonal changes to the phytoplankton communities [12,69,70]. Limited supply of the biologically available nitrogen has been proposed as a major factor for the presence of nitrogen-fixing cyanobacteria [9,14,47]. During the 2008 drought period, Lake Bowen received reduced DIN loading (predominately in the form of nitrate plus nitrite) from the watershed (fig. 2) and experienced lower-than-normal springtime DIN concentrations in both reservoirs. Concurrently, total phosphorus concentrations remained relatively consistent in both reservoirs (fig. 3).

8. Multiple logistic regression model of geosmin concentrations

Two multiple logistic regression models (MLogModel) were developed that estimated the occurrence of dissolved geosmin concentrations above the human detection threshold of 10 ng/L (0.010 µg/L) on the basis of the multivariate and Kendall tau correlation analyses. Even though significant correlation existed between geosmin and several phytoplankton taxonomic variables (for example, total phytoplankton and Oscillatoria biovolumes), explanatory variables used as input for the MLogModels were limited to more easily or quickly measured chemical constituents and hydrodynamic properties. These selected, easily measured variables would be included in the watershed management and monitoring strategy for the reservoirs. Two models were labeled MLogRModel 1—developed from data at the surface (1 m) depth at Lake Bowen and MLogRModel 2—developed from data at the surface depth (1 m) in Reservoir #1. Although many of the same environmental factors were used as explanatory variables in initial model runs, the final explanatory variables that provided the best fit model varied among the two sites.

The best fit model for MLogModel 1 for Lake Bowen was the following:

\[
\text{Logit} \ P = -4.691 + 2.184 \times Z_m - 24.419 \times \sqrt{\text{TN}} + 0.0351 \times \text{Overflow - Flowthrough},
\]

where \(Z_m\) is mixing zone depth in meters, \(\sqrt{\text{TN}}\) is the square root of the total nitrogen concentration in milligrams per liter, and Overflow – Flowthrough is the 30-day prior moving window average of overflow minus the 30-day prior moving-window average of flowthrough at Lake Bowen dam, in million gallons per day. Overall, MLogModel 1 determined that the likelihood of dissolved geosmin concentrations exceeding the human detection threshold in Lake Bowen occurred during greater mixing zone depths (unstratified conditions), greater spillage or overflow at Lake Bowen dam rather than controlled releases or flowthrough (indicative of higher water levels), and by lower total nitrogen concentrations. Of the three explanatory variables, only mixing zone depth was correlated significantly to geosmin concentrations in the exploratory data analysis. The MLogModel 1 correctly estimated the likelihood of geosmin concentrations exceeding the human detection threshold 83 percent of the time and not exceeding the human detection threshold 100 percent of the time, resulting in an overall sensitivity of 94 percent (table 3).
The best fit model for MLogModel2 for Reservoir #1 was the following:

$$\text{Logit } P = -20.098 - 5.970 \times \log_{10} \text{MR1 Outflow} + 4.444 \times Z,$$

(2)

where $\log_{10}[\text{MR1 Outflow}]$ is the logarithm of the 30-day prior moving window average of outflow from Reservoir #1, in million gallons per day, and $Z_{eu}$ is the euphotic zone depth, in meters. Euphotic zone depth extended from the surface of the water downward to a depth where light intensity fell to 1 percent of that at the surface. In Reservoir #1, greater euphotic zone depth and reduced outflow from Reservoir #1 provided the best estimation of dissolved geosmin concentrations above the human detection threshold in MLogModel2. Of the two explanatory variables, only euphotic zone depth was correlated significantly to geosmin concentrations. The MLogModel 2 was less sensitive than MLogModel 1. When applied to the dataset, MLogModel 2 correctly estimated the likelihood of geosmin concentrations exceeding the human detection threshold 67 percent of the time and not exceeding the human detection threshold 90 percent of the time, resulting in an overall sensitivity of 85 percent (table 3). The reduced sensitivity of the MLogModel2 for Reservoir #1 relative to the other model was attributed, in part, to the fact that the site had a much lower number of observed positive responses (geosmin concentrations above the human detection threshold) than the other site, which decreased the ability of the regression model to accurately estimate that response.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Predicted Reference</th>
<th>Predicted Positive</th>
<th>Totals</th>
<th>Diagnostic</th>
<th>Percent</th>
<th>Hosmer-Lemshow (p-value)</th>
<th>Pearson Chi-Squared (p-value)</th>
<th>Likelihood Ratio Test (p-value)</th>
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</thead>
<tbody>
<tr>
<td><strong>MLogModel 1 for Lake Bowen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual Reference Responses</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>Specificity</td>
<td>100</td>
<td>9.07 (0.336)</td>
<td>15.49 (0.216)</td>
<td>12.12 (0.007)</td>
</tr>
<tr>
<td>Actual Positive Responses</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>Sensitivity</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>12</td>
<td>5</td>
<td>17</td>
<td>Overall</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **MLogModel 2 for Reservoir #1** | | | | | | | | |
| Actual Reference Responses | 9 | 1 | 10 | Specificity | 90 | | | |
| Actual Positive Responses | 1 | 2 | 3 | Sensitivity | 67 | | | |
| Totals | 10 | 3 | 13 | Overall | 85 | | | |

**Table 3.** Classification tables for the multiple logistic regression models developed to estimate the likelihood of geosmin concentrations exceeding the human detection threshold of 10 ng/L in Lake William C. Bowen (MLogModel 1) and in Municipal Reservoir #1 (MLogModel 2), Spartanburg County, South Carolina, 2005 to 2009.
Overall, MLogModel 1 indicated greater likelihood for both dissolved geosmin to exceed the human detection threshold during periods of lower nitrogen concentrations. MLogModel 1 indicated a geosmin exceedence during periods of higher water levels in Lake Bowen (greater spillage or overflow compared to controlled releases or flowthrough at Lake Bowen dam). Conversely, MLogModel 2 indicated a greater likelihood of threshold exceedences by dissolved geosmin concentrations during periods of reduced outflow from Reservoir #1 and greater light penetration. It also should be noted that the calibration dataset for each logistic model had a small sample size (less than 20 samples) and was collected during a hydrologic period of extremely low- to average-flow conditions. The small sample size and extreme hydrologic conditions potentially may limit the applicability of these models for above average and, especially, high-flow conditions.

9. Conclusions

The occurrence of dissolved geosmin was studied in two reservoirs in Spartanburg County, South Carolina, from August 2005 to June 2009. Lake Bowen and Reservoir #1 are relatively shallow, meso-eutrophic, warm monomictic, cascading impoundments on the South Pacolet River. Overall, water-quality conditions and phytoplankton community assemblages were similar between the two reservoirs but differed seasonally and annually. Median dissolved geosmin concentrations in the reservoirs ranged from 0.004 to 0.006 µg/L, below the human detection threshold of 0.010 µg/L. Annual maximum dissolved geosmin concentrations tended to occur between March and May. In this study, peak dissolved geosmin production occurred in April and May 2008, ranging from 0.050 to 0.100 µg/L at the deeper reservoir sites near the dams. The peak geosmin period coincided with drought conditions that extended the water residence time in both reservoirs and reduced the nutrient inputs. In situ production of geosmin by cyanobacteria was the most probable source of elevated geosmin concentrations in Reservoir #1 and Lake Bowen. Elevated cyanobacterial biovolumes in the reservoirs that were present during the summer of 2008 were related to many environmental factors that have been previously reported to enhance cyanobacterial dominance of the phytoplankton community, including decreased availability of nitrogen, increased phosphorus concentrations, reduced light availability, warmer water temperatures, greater water column stability, and longer residence times. However, unlike previous research, elevated dissolved geosmin concentrations did not coincide with increased cyanobacterial biovolumes and cyanobacteria were not dominating the total phytoplankton community. Therefore, another mechanism was needed that could explain the increased production and release of geosmin in April and May of 2008. Heavy springtime zooplankton grazing and nutrient (N, P, iron) limitation were two plausible mechanisms that could explain seasonal changes to the phytoplankton communities and associated geosmin production in the spring period [12,69,70].

In Lake Bowen, elevated geosmin concentrations near the surface were correlated to environmental conditions indicative of unstratified conditions (higher dissolved-oxygen concentrations and greater $Z_m$). In relation to phytoplankton community structure, elevated geosmin concentrations were correlated to greater Oscillatoria biovolumes, a genus of cyanobacteria
with known geosmin-producing species. Elevated geosmin concentrations were correlated to reduced algal biomass and lower total phytoplankton biovolumes. In Reservoir #1, elevated geosmin concentrations near the surface were correlated to greater dissolved-oxygen concentrations and to reduced algal biomass. Rather than a specific genus of cyanobacteria, elevated geosmin concentrations in Reservoir #1 were correlated to a greater fraction of geosmin-producing genera in the cyanobacteria group. However, total cyanobacterial biovolumes were not correlated to geosmin concentrations.

In contrast to elevated geosmin concentrations in surface samples from Lake Bowen and Reservoir #1, environmental factors indicative of stratified or stable water column conditions, including warmer water temperatures, lower $Z_m$, greater $Z_m/Z_e$ ratios, lower dissolved-oxygen concentrations, and maximum RTRM, correlated strongly with elevated cyanobacterial biovolumes. Additionally, in contrast to elevated geosmin concentrations in surface sample, elevated cyanobacterial biovolumes correlated with changes in nutrient levels in the reservoirs, including lower nitrogen concentrations (as ammonia, nitrate plus nitrite, total organic nitrogen, and TN) and elevated hypolimnetic TP concentrations relative to the epilimnetic TP concentrations (considered indicative of release of phosphorus from the sediment during anoxic conditions). In both reservoirs, elevated cyanobacterial biovolumes were correlated to longer residence times, indicative of low-flow or drought conditions, and lower overflow volumes, indicative of lower water levels. A greater fraction of cyanobacteria in the total phytoplankton community and biovolumes of known geosmin- and toxin-producing cyanobacteria genera, including *Cylindrospermopsis*, *Planktolyngbya*, *Synechococcus*, *Synechocystis*, and *Aphanizomenon* correlated with the greater cyanobacteria biovolumes and were the dominant taxa in the cyanobacteria group. During the summer 2008 when these genera dominated the phytoplankton community, low-level (< 0.5 µg/L) microcystin concentrations also were observed in the two reservoirs.

The BEST analysis selected a six-variable model that best explained the assemblages of all Cyanophyta genera in the two reservoirs during the spring ($\rho = 0.601$). The explanatory variables included specific conductance, the two forms of DIN (ammonia and nitrate plus nitrite), chlorophyll a and its degradate, pheophytin a, and iron (table 2). NMDS bubble plots indicated less nitrate plus nitrite and iron concentrations during the spring 2008 when compared to spring 2007 and 2009 that may be attributed to the change in the assemblage of Cyanophyta genera and greater geosmin concentrations. A four-variable model was selected to best explain the assemblages of only known geosmin-producing genera of Cyanophyta ($\rho = 0.663$) and included magnesium, nitrate plus nitrite, chlorophyll a:pheophytin a ratio (undegraded to degraded pigment), and geosmin.

Logistic regression models indicated geosmin concentrations had the greatest probability (83 percent model sensitivity) of exceeding 10 ng/L during periods of greater overflow (higher water levels in Lake Bowen) relative to flowthrough releases at the dam, lower total nitrogen, and unstratified conditions (greater mixing zone depths) in Lake Bowen. Conversely, in the source water in Reservoir #1, geosmin concentrations above 10 ng/L were more probable (only a 67 percent model sensitivity) during periods of lower outflow but greater light penetration (euphotic zone depth, that correlated to transparency). Fewer periods of geosmin concentra-
tions exceeding 10 ng/L (only 3 compared to 6 at the other site) could have produced the reduced sensitivity, poorer fit, and apparent inverse relations of elevated geosmin concentrations to hydrodynamic conditions relative to Lake Bowen and the raw water. It also should be noted that the calibration dataset for the logistic model had a small sample size (less than 20 samples) and was collected during a hydrologic period of extremely low-flow to average conditions. The small sample size and extreme hydrologic conditions potentially may limit the applicability of these models for above average and, especially, high-flow conditions.

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