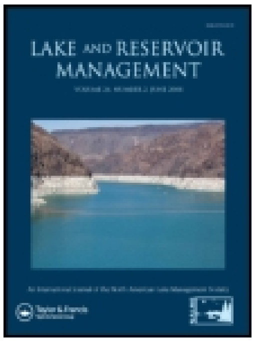

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Eutrophication and cyanobacteria blooms in run-of-river impoundments in North Carolina, U.S.A.

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Abstract

Touchette, B.W., J.M. Burkholder, E.H. Allen, J.L. Alexander, C.A. Kinder, C. Brownie, J. James and C.H. Britton. 2007. Eutrophication and cyanobacteria blooms in run-of-river impoundments in North Carolina, U.S.A. Lake and Reserv. Manage. 23:179-192.

We compared monthly data taken during the dry summer growing season of 2002 in 11 potable water supply reservoirs (19-85 years old based on year filled) within the North Carolina Piedmont, including measures of watershed land use, watershed area, reservoir morphometry (depth, surface area, volume), suspended solids (SS), nutrient concentrations (total nitrogen, TN; total Kjeldahl nitrogen, TKN; nitrate + nitrite, $NO_3^- + NO_2^-$; total phosphorus, TP; total organic carbon), phytoplankton chlorophyll *a* (chl*a*) concentrations, cyanobacteria assemblages, and microcystin concentrations from monthly data taken during the dry summer 2002 growing season. The reservoirs were considered collectively or as two subgroups by age as “mod.” (moderate age, 19-40 years post-fill, n = 5) and “old” (74-85 yr post-fill, n = 6). The run-of-river impoundments were meso-/eutrophic and turbid (means 25-125 µg TP/L, 410-1,800 µg TN/L, 3-70 µg chl*a*/L and 5.7-41.9 mg SS/L). Under drought conditions in these turbid systems, there was a positive relationship between chl*a* and both TN and TP, supported by correlation analyses and hierarchical ANOVA models. The models also indicated significant positive relationships between TN and TP, and between SS and both TP and TN. Agricultural land use was positively correlated with TKN for the reservoirs considered collectively, and with TN, TKN, TP, and chl*a* in mod. reservoirs. In models considering the reservoirs by age group, TN:TP ratios were significantly lower and $NO_3^- + NO_2^-$ was significantly higher in old reservoirs, and these relationships were stronger when reservoir age was used as a linear predictor. Cyanobacteria assemblages in the two reservoir age groups generally were comparable in abundance and species composition, and comprised 60-95% (up to 1.9×10^6 cells/mL) of the total phytoplankton cell number. Potentially toxic taxa were dominated by *Cylindrospermopsis raciborskii* and *C. philippinensis*. Although known microcystin producers were low in abundance, microcystin (< 0.8 µg/L) was detected in most samples. TP and chl*a* were significant predictors of total cyanobacterial abundance. The data suggest that at present these turbid, meso-/eutrophic reservoirs have moderate cyanobacteria abundance and low cyanotoxin (microcystin) levels over the summer growing season, even in low-precipitation seasons that favor cyanobacteria. Accelerated eutrophication from further watershed development is expected to promote increased cyanobacterial abundance and adversely affect the value of these reservoirs as potable water supplies.

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Key words: chlorophyll *a*, cyanobacteria, eutrophic, microcystin, nitrogen, nutrients, phosphorus, reservoirs, turbid

Eutrophication, defined here as the increase in biological productivity in surface waters promoted by elevated phosphorus (P) and nitrogen (N) levels, has been well described during the aging process for run-of-river impoundments, and consists of three phases (Kimmel and Groeger 1986, Holz *et al.* 1997). The trophic upsurge period immediately after filling is characterized by relatively high biological productivity, with spikes in nutrient loading from decomposition of flooded areas. The second phase, trophic depression with a decline in productivity, is followed by a trophic equilibrium phase characterized by productivity levels typically less than those attained during trophic upsurge (Kimmel and Groeger 1986, Holz *et al.* 1997). Most previous research has focused in the initial two phases, whereas little is known about reservoir aging within the trophic equilibrium phase. Generalizations about the three phases assume that external nutrient loadings remain relatively constant over time (Kimmel and Groeger 1986). Productivity in the trophic equilibrium phase would be expected to increase or decrease if external nutrient loads change (Kimmel and Groeger 1986, Holz *et al.* 1997).

In reservoirs as in natural lakes, trophic status generally is determined by external nutrient loadings as modified by morphology, hydrology and suspended sediments (Edmondson 1961; Vollenweider 1975; Cuker *et al.* 1987, 1990; Wetzel 2001; Jones *et al.* 2004). Reservoirs can be highly variable in response to nutrient enrichment, however, and temporal variation is important to consider in data interpretation (Turner *et al.* 1983, Yoo *et al.* 1995, Knowlton and Jones 2006). Although reservoirs age by the same processes as natural lakes, their rate of aging tends to be accelerated (Ryder 1978, Thornton *et al.* 1990, Popp and Hoagland 1995). Run-of-river impoundments generally receive higher loadings of sediments and nutrients (Kimmel and Groeger 1986, Thornton *et al.* 1990, Holz *et al.* 1997), and they are often sited in densely populated or rapidly developing areas, and/or in regions with intensive agricultural practices (Cooke *et al.* 1993, Thornton *et al.* 1990). Many reservoirs in North America are located at intermediate latitudes (23-40°N), where soils are highly erodable and watersheds maintain relatively high nutrient export (Canfield and Bachmann 1981, Kennedy and Walker 1990).

Changes in reservoir trophic status can be tightly linked to anthropogenic alterations within the watershed, rather than the natural, gradual accumulation of nutrients and sediments (Kimmel and Groeger 1986). As a consequence, many reservoirs constructed within the past 50 yr have filled in rapidly because of high sediment loading, and are expected to have a lifespan for designated uses of only ~100 yr (Popp and Hoagland 1995). Previous studies have shown that during the trophic upsurge period in the succession of run-of-river impoundments, increased phytoplankton productivity in response to elevated nutrients can consist mostly of cyanobacteria. For example, Hergenrader (1980) noted that cyanobacteria usually represented more than 95% of the total phytoplankton biovolume (dominated by *Microcystis*, *Aphanizomenon*, and *Anabaena* spp.) during the summer months in recently created reservoirs (four reservoirs evaluated within 6 yr after filling). Some reservoirs may undergo a shift from cyanobacteria toward increased abundance of flagellated algae during the trophic equilibrium phase, often under elevated sediment loadings (Cuker 1987, Burkholder 1992, Holz *et al.* 1997). Nevertheless, reservoirs with low to substantial sedimentation can also maintain cyanobacteria populations indefinitely, especially under high nutrient loading (Burkholder 1992). Some species can convert N_2 gas to ammonia internally, and typically have higher P requirements in comparison to other phytoplankton (DeNobel *et al.* 1997). Therefore, when inorganic nitrogen is limiting in freshwaters, low N:P ratios can often promote blooms of diazotrophic cyanobacteria (Smith 1983, Chorus and Bartram 1999, but see Geider and La Roche 2002). High-biomass cyanobacteria blooms commonly cause fish kills via oxygen deficits during dark periods (Bartram *et al.* 1999), and various bloom-forming cyanobacteria also commonly produce toxins that can adversely impact aquatic life (Chorus and Bartram 1999).

Relatively few studies have been conducted in reservoirs during the trophic equilibrium phase of eutrophication, mostly focused on accumulation of sediments and associated contaminants (Dendy *et al.* 1973, Rodgers *et al.* 1995, Popp *et al.* 1996, Bennett *et al.* 2002). Less is known about cyanobacteria assemblages in reservoirs affected by moderate sediment loading, which is characteristic of many reservoirs used for potable water supplies and recreation in the southeastern U.S. The objective of this study was to evaluate the water quality, cyanobacteria assemblages, and cyanotoxin microcystin concentrations in reservoirs of different age within the trophic equilibrium phase of eutrophication in the North Carolina Piedmont. We assessed the influence of reservoir morphometry (depth, surface area, volume), watershed area and land use, and reservoir age on nutrient concentrations, suspended solids concentrations, and phytoplankton chlorophyll *a* during a summer growing season under drought conditions.

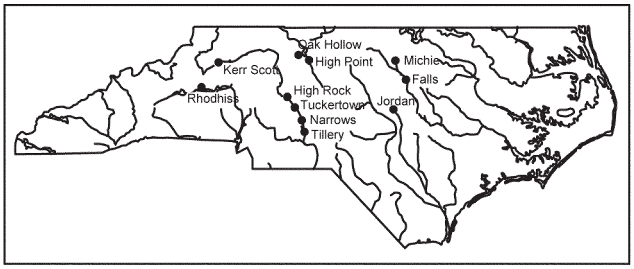

Figure 1.-Locations of reservoirs sampled.

Materials and methods

Study areas

Eleven North Carolina reservoirs (Fig. 1) were evaluated monthly from June - August 2002 for cyanobacteria, cyanotoxins, and background environmental conditions. The study was conducted near the end of a three-year drought that was evaluated as the worst sustained by North Carolina in more than 100 yr (North Carolina State Office of Climatology 2004, Southeast Regional Climate Center 2004). Thus, light reduction, nutrient sequestering, and coflocculation influences of suspended sediments on phytoplankton assemblages (Hoyer and Jones 1983, Burkholder 1992) were minimal. The selected reservoirs were considered collectively, and also were subdivided into two groups (Table 1), as reservoirs of moderate age, filled ~19-40 yr ago (1962-1983, designated as "mod."); and older reservoirs, filled ~74-85 yr ago (1917-1928, designated as "old"). Mod. and old reservoirs were within the same river basins with exception of Lake Rhodhiss, which was the only impoundment within the Catawba River basin. They ranged from 290-5,790 ha and 220-6,375 ha in area, respectively. Flushing rate data were not available for most reservoirs. With exception of High Point Lake, reservoirs in the old subgroup had comparable ratios of watershed

Table 1.-Reservoirs evaluated in this study grouped by age (moderate and old), year constructed, upstream watershed area, mean depth, surface area, volume, basin drainage area, and primary land use within drainage area (percent - forested, agriculture [crop/pasture], and urban).[a] Data compiled from NC DENR (2000, 2002, 2003, 2004a).[b]

Reservoir	Year Filled	River Basin	Mean Depth (m)	Surface Area (ha)	Volume (m^3)	Drainage (km^2)	Land Use (%)[c]
Moderate							
Kerr Scott	1962	Yadkin	11.9	590	189×10^6	1,638	Forest (78)/ Agric. (15)/ Urban (5)
Tuckertown[d]	1962	Yadkin	30.2	1,030	172×10^6	10,506	Forest (55)/ Agric. (27)/ Urban (13)
Oak Hollow	1972	Cape Fear	7.0	290	11×10^6	82	Forest (27)/ Agric. (20)/ Urban (47)
Jordan[d]	1981	Cape Fear	4.9	5,790	265×10^6	4,367	Forest (50)/ Agric. (24)/ Urban (19)
Falls[d]	1983	Neuse	5.0	5,055	55×10^6	1,998	Forest (59)/ Agric. (18)/ Urban (13)
Old							
Narrows[d]	1917	Yadkin	14.0	2,165	344×10^6	10,555	Forest (55)/ Agric. (27)/ Urban (13)
Rhodhiss[d]	1925	Catawba	6.1	1,425	84×10^6	2,824	Forest (76)/ Agric. (10)/ Urban (10)
Michie	1926	Neuse	8.0	220	16×10^6	426	Forest (64)/ Agric. (23)/ Urban (6)
High Rock	1927	Yadkin	4.9	6,375	314×10^6	9,985	Forest (54)/ Agric. (27)/ Urban (13)
Tillery	1928	Yadkin	7.2	2,130	207×10^6	10,747	Forest (55)/ Agric. (27)/ Urban (12)
High Point	1928	Cape Fear	4.9	120	5×10^6	877	Forest (21)/ Agric. (10)/ Urban (66)

[a] Remaining land use was mostly as grassland and wetland.

[b] Sampling locations were as follows: Kerr Scott – reservoir boat ramp and dock (N36°08.115', W81°13.454'), and footbridge below dam (N36°07.811', W81°13.754'); Tuckertown – off boat ramp on Highway 8 (N35°30.141', W80°11.465') and off Tuckertown Road (N35°29.521', W80°10.488'); Oak Hollow – end of Centennial Street off Highway 68 (N36°00.745', W80°00.097'), and Washburn Dam, Festival Park off Highway 68 (N36°00.588', W79°59.425'); Jordan – Seaforth Recreation Area (N35°43.633', W79°02.006'), and entrance to Seaforth Recreation Area off Highway 64 (N35°44.204', W79°02.466'); Falls – Highway 50 boat ramp (N35°58.432', W78°39.299'), and boat ramp area off Six Forks at Upper Barton Creek (N36°01.258', W78°41.505'); Narrows – Badin Park (N35°24.918', W80°06.893'), and Gar Creek boat ramp (N35°25.521', W80°08.545'); Rhodhiss – near Lenoir water treatment plant (N35°47.082', W81°29.145'), and near Granite Falls water treatment plant (N35°47.178', W81°26.413'); Michie – off Bahama Road park area boat dock (N36°10.336', W78°51.462'), and Wilkins/Bahama Road picnic area (N36°10.457', W78°51.776'); High Rock – Southmont-Abbott's Creek boat access (N35°38.859', W80°15.619'), and Flat Swamp public access (N35°38.602', W80°11.142'); Tillery – end of Bowers Road off Highway 52 (N35°15.388', W80°07.350'), and Norwood Road boat ramp (N35°14.776', W80°06.887'); High Point – City Lake Park dam (N35°59.678', W79°57.004'), and East Fork Road bridge (N36°00.539', W79°56.578').

[c] Agric. = agriculture.

[d] Influenced by point source(s) discharges listed by type and classification(major, $\geq 3.785 \times 10^6$ liters [1 million gallons] per day) as follows: Tuckertown Reservoir – municipal potable water treatment plant (WTP, minor) and industrial process and commercial (IPC, minor); Jordan Lake – municipal WTP (minor); Falls Lake – municipal wastewater treatment plant (major); Narrows Reservoir – IPC (minor); and Lake Rhodhiss – WTP (minor).

area to reservoir volume, roughly indicating flushing rate, but this ratio varied by ~9-fold within the mod. subgroup.

Individual watersheds for each of the selected reservoirs were created in ArcGIS 9.1 using the 8-digit Hydrologic Unit Code (HUC) sub-basin boundary layer from the U.S. Geological Survey (USGS). Sub-basin boundaries in this layer were used as templates to create larger sub-basins that extended from the reservoir to the uppermost part of its watershed within the Neuse, Cape Fear, Yadkin, or Catawba River Basins. USGS National Land Cover data for 2001 were downloaded and combined with the sub-basin boundaries in a GIS interface. The land use classification system for each dataset was then modified to include seven general categories: urban, agricultural, forested, grassland, water, wetland, and barren/disturbed. Once the land cover categories were reclassified, the Spatial Analyst "tabulate area" function was used to calculate the area of each land class within each of reservoir sub-basins.

Environmental conditions

Two sites in each reservoir were evaluated monthly during the summer season (June - August) for physical/chemical conditions (temperature, pH, dissolved oxygen, oxidation-reduction potential [ORP]) using a YSI multiprobe water quality system (model 6600EDS; YSI Environmental Inc., Yellow Springs, OH). The YSI multiprobe was calibrated on each date of use. Samples for nutrient analyses, chlorophyll *a*, microcystin concentrations, and cyanobacteria assemblages were collected using an integrated water-column sampler modified from Cuker *et al.* (1990). Integrated water-column samples were taken to account for vertical distribution of cyanobacteria through the euphotic zone. The water-column sampler was thoroughly rinsed with site water prior to sampling. Samples were maintained in darkness on ice for transport to the laboratory, and were refrigerated or frozen as appropriate until analysis (U.S. Environmental Protection Agency [EPA] 1993, American Public Health Association [APHA] *et al.* 1998).

The state-certified laboratory at the NC State University Center for Applied Aquatic Ecology analyzed suspended solids (SS) and nutrient samples by approved methods. Total SS were held at 4°C, filtered within 7 days, and measured gravimetrically following APHA *et al.* (1998) method APHA 2540D (practical quantitation limit, 2 mg/L). Nitrogen samples were analyzed with a TrAAcs 800 Continuous Flow Analyzer (Bran+Leubbe; Buffalo Grove, IL - now Seal Analytical, Mequon, WI). Samples for nitrate+nitrite (NO_3^-+NO_2^-, hereafter NO_x^-) analysis were frozen and analyzed within 1-2 months (modification of EPA method 353.2 / APHA 4500 NO3 F; practical quantitation limit, 6 µg NO_x^-/L - U.S. EPA 1993, APHA 1998). For samples held longer than 1 month, a reservoir-specific correction factor was applied to adjust the concentration, based on comparison of data from 1-month vs. 2-month holding times (3-7% difference). Samples for total Kjeldahl nitrogen analysis (TKN = free NH_3 + organic N) were assayed as in Burkholder *et al.* (2006), using a modification of EPA method 351.2 (samples held at -20°C and not preserved with sulfuric acid; practical quantitation limit, 140 µg N/L - U.S. EPA 1993). Values for total nitrogen (TN) were calculated as TKN +NO_x^-.

Phosphorus samples were analyzed using a QuikChem 8000 Flow Injection Analyzer (Lachat Instruments; Milwaukee, WI). Samples for total phosphorus (TP) analysis were frozen at -20°C until digestion and analysis, using a modification of EPA method 365.1 / APHA method 4500 PE (practical quantitation limit, 10 µg P/L - U.S. EPA 1993, APHA *et al.* 1998). TN and TP values were used to calculate TN:TP ratios (molar basis – Wetzel 2001). Total organic carbon (TOC) samples were analyzed with an Apollo 9000 combustion analyzer (Tekmar-Dohrmann, Cincinnati, OH). Preserved samples were refrigerated and analyzed following EPA method 415.1 / APHA 5310B (practical quantitation limit, 2 mg C/L; U.S. EPA 1993, APHA 1998).

Phytoplankton abundance, cyanobacteria assemblages, and cyanotoxins

Samples for analysis of chlorophyll *a* (chl*a*), an indicator of total phytoplankton biomass (Wetzel and Likens 2001), were transported to the laboratory in darkness on ice. Samples were filtered under low vacuum (Whatman GF/C filters, 8-10 psi) and low light (20 µmol photons/m^2/s) within 24 hr of collection, and were stored frozen with desiccant until analysis. Chl*a* was extracted in 90% basic acetone (Wetzel and Likens 2001), and fluorescence was determined using a Turner 10-AU fluorometer (Turner Designs, Sunnyvale, CA) (EPA method 445.0; practical quantitation limit, 1 µg/L - U.S. EPA 1997).

Microcystin concentrations were assessed using the enzyme-linked immunosorbent assay (ELISA; Chu *et al.* 1990; microcystin plate kit EP 022 – EnviroLogix, Inc., Portland, ME; sensitive to microcystin-LR, -YR, -RR variants and nodularin; practical quantitation limit 0.147 µg/L), followed by secondary confirmation for some samples using protein phosphatase-1 inhibition assays (quantitation limit 50 ng/L; Yoo *et al.* 1995). For the protein phosphatase inhibition assay, we used the catalytic subunit of protein phosphatase I (No. 1636758; Roche Diagnostics Corporation, Indianapolis, IN) (Sim and Mudge 1993, An and Carmichael 1994). Although there were some variations in toxin levels detected between ELISAs and protein phosphatase-1 inhibition assays, the relative trends were consistent; that is, samples that had elevated microcystin levels based on ELISAs also had more protein phosphatase-1 inhibition. Because ELISAs are more widely used to monitor microcystin (Spoof 2005), we report the ELISA data for this analysis.

Samples collected for analysis of total phytoplankton and the cyanobacteria assemblage were preserved in the field with 1% acidic Lugol's solution (Vollenweider 1974) and were transported to the laboratory in darkness at 4°C. Samples from dates/locations with chl*a* concentrations ≥ 15 µg/L (n = 17, from 6 reservoirs) were quantified for cyanobacteria cell numbers and taxa composition using light microscopy (Olympus IX70 research microscope, 600×) and Palmer-Maloney chambers (Wetzel and Likens 2001). Cyanobacteria abundance was compared to data for total phytoplankton cell number provided by the North Carolina Department of Environment and Natural Resources [NC DENR] (2004b).

Statistics

Correlation analyses (PROC CORR – SAS Institute, Inc. 1999) and linear regressions were used to examine relationships between biological parameters and physical/chemical factors. Differences among the two reservoir age groupings in selected physical/chemical factors, phytoplankton chl*a* and total cyanobacterial abundance were detected with a hierarchical ANOVA (PROC MIXED, SAS Institute, Inc. 1999). Physical parameters considered were SS concentrations, watershed area, watershed land use, reservoir morphometry (mean depth, surface area, volume) and watershed area per unit reservoir volume; chemical factors included TN, TKN, $NO_3^- + NO_2^-$, TP, TN:TP ratio, TOC and chl*a*. Model fitting included testing effects for reservoir, site within reservoir and sampling month to account for correlations between sites on the same lake and between repeated measures at the same site. Variables were log-transformed except that cyanobacterial abundance, age, reservoir depth, and percentage land use (urban, agricultural, forested) were not transformed; and reservoir surface areas were square-root transformed. Alternatively, rather than using age group as a class variable, the age of each reservoir was calculated and used in some models as a linear predictor. Statistical analyses were performed at a level of significance of $\alpha \leq 0.05$.

Results

This study was conducted during the third year of a 100-year record drought (North Carolina State Office of Climatology 2004), with warm conditions and reduced reservoir flushing that would promote cyanobacteria blooms (Burkholder 2002). Mean surface water conditions during June - August 2002 ranged as follows: temperature, 26.3-31.3°C; pH, 7.4-9.1; ORP, 405-545 mV (Table 2). Although reservoirs in the North Carolina Piedmont generally are turbid from excessive suspended sediment loading (Cuker *et al.* 1990, Burkholder *et al.* 1998), the dry conditions promoted some clearing of the water column. The reservoirs were meso-/eutrophic and turbid (means 25-125 µg TP/L, 410-1,800 µg TN/L, 2-7 mg TOC/L, 3-70 µg chl*a*/L, 4.8-9.6 mg DO/L, and 5.7-41.9 mg SS/L) (Table 2, Fig. 2; Wetzel 2001). Moderately aged (mod.) reservoirs averaged ~400-900 µg TN/L and 25-55 µg TP/L; old reservoirs averaged ~350-1,800 µg TN/L and 25-130 µg TP/L. NO_x^- levels were ~six-fold higher in old reservoirs than in mod. reservoirs (Table 3). TN:TP ratios ranged from 11.7 ± 0.8 (High Rock Reservoir), indicative of a transition between N and P limitation, to 25.8 ± 2.3 (Falls Lake), suggestive of P limitation (Wetzel 2001). Turbidity varied by ~ten-fold (June data only), averaging from 6.3 ± 3.3 NTU (mean ± 1 SE, Lake Tillery) to 66.4 ± 33.7 NTU (Lake Michie); median turbidity among all reservoirs was ~14 NTU.

Table 2.-Environmental conditions (temperature, pH, dissolved oxygen, oxidation-reduction potential, [ORP, rounded to the nearest five mV], suspended solids [SS], total organic carbon [TOC], and total nitrogen:total phosphorus [TN:TP] ratios) in the surface waters of the eleven reservoirs. Data are given as means ± 1 standard error (SE) over the three-month study (n = 4-6).

Reservoir	Temp. (°C)	pH	DO (mg/L)	ORP (mV)	SS (mg/L)	TOC (mg/L)	TN:TP
Mod. (Group 1)							
Kerr Scott	26.3 ± 4.8	7.5 ± 0.7	7.3 ± 0.1	460 ± 20	5.7 ± 2.5	2 ± 0	19.8 ± 3.6
Tuckertown	29.6 ± 0.7	9.1 ± 0.2	9.0 ± 2.6	425 ± 15	11.8 ± 1.5	4 ± 0	16.3 ± 2.3
Oak Hollow	31.3 ± 0.5	8.1 ± 0.3	5.9 ± 0.9	535 ± 20	15.2 ± 5.1	4 ± 0	22.7 ± 3.0
Jordan	27.6 ± 0.6	8.0 ± 0.1	4.8 ± 0.6	515 ± 10	29.2 ± 12.7	7 ± 0	21.0 ± 0.7
Falls	28.8 ± 0.9	7.7 ± 0.2	6.3 ± 0.5	545 ± 25	9.0 ± 3.1	6 ± 0	25.8 ± 2.3
Old (Group 2)							
Narrows	30.7 ± 1.8	8.6 ± 0.2	8.1 ± 0.4	435 ± 10	23.2 ± 11.0	4 ± 1	17.2 ± 2.7
Rhodhiss	30.1 ± 0.2	8.7 ± 0.1	7.6 ± 0.5	490 ± 70	7.6 ± 0.1	3 ± 0	13.4 ± 0.6
Michie	29.1 ± 1.7	7.4 ± 0.4	5.8 ± 1.4	500 ± 15	34.1 ± 8.2	7 ± 1	20.0 ± 1.2
High Rock	29.8 ± 1.3	9.0 ± 0.4	8.3 ± 1.0	425 ± 10	41.9 ± 0.0	4 ± 0	11.7 ± 0.8
Tillery	29.9 ± 1.2	9.1 ± 0.3	9.6 ± 0.9	405 ± 15	4.8 ± 0.2	3 ± 0	18.2 ± 1.9
High Point	30.6 ± 0.6	8.4 ± 0.2	6.4 ± 0.7	470 ± 0	9.3 ± 0.1	4 ± 0	20.1 ± 0.7

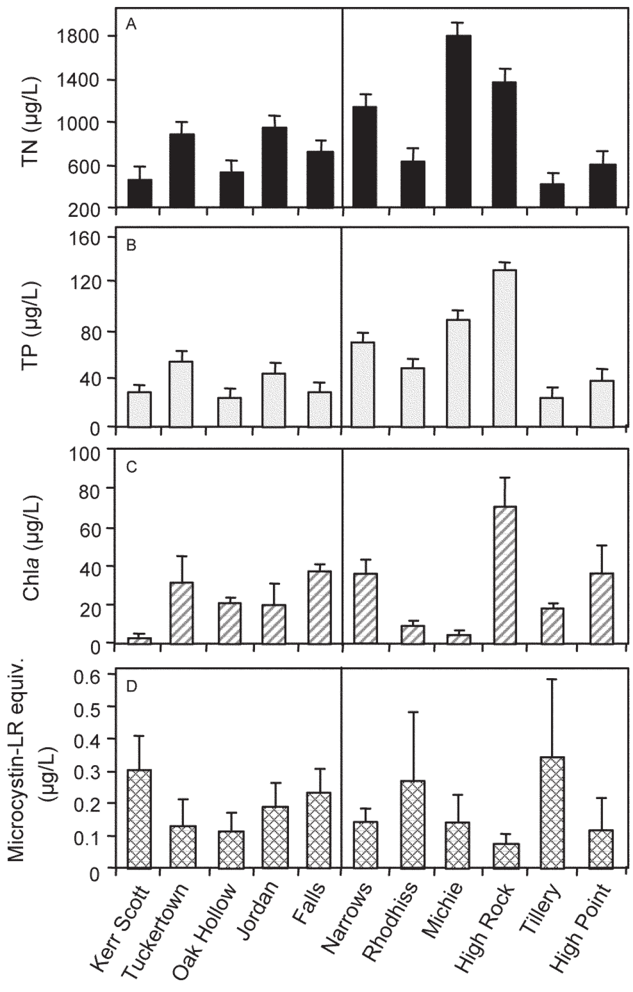

Figure 2.-For individual reservoirs within each reservoir grouping (moderate age, mod. – left panel, old – right panel), concentrations of (A) total nitrogen (TN), (B) total phosphorus (TP), (C) phytoplankton (suspended microalgal) chlorophyll *a*, and microcystin. Data are given as means ± 1 SE.

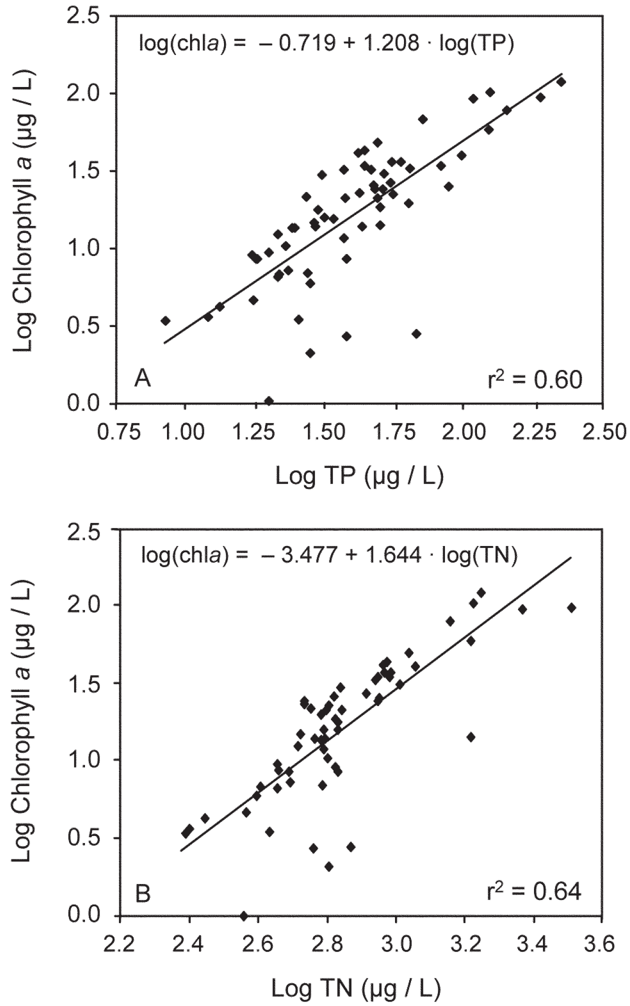

Figure 3.-Linear regression analysis showing the relationship between log(chlorophyll *a*) and (A) log(total phosphorus) and (B) log(total nitrogen) for the reservoirs considered collectively.

Under drought conditions in these turbid systems, there was a positive relationship between chl*a* and both TN and TP (Fig.3), supported by correlation analyses and hierarchical ANOVA models (Table 3). The hierarchical ANOVA models also indicated significant positive relationships between TN and TP, and between SS and both TP and TN (Table 3). Statistical analyses did not indicate significant influences of watershed drainage area (DA) and reservoir morphometric parameters on water quality from this small data set, except for a significant positive correlation between watershed DA and TOC in old reservoirs, and between reservoir surface area and TOC in mod. reservoirs (Table 3). Agricultural land use was positively correlated with TKN for the reservoirs considered collectively, and with TN, TKN, TP, and chl*a* in mod., but not old, reservoirs. In hierarchical ANOVA models considering the reservoirs by age group, TN:TP ratios were significantly lower and NO_x^- was significantly higher in old reservoirs, and these relationships were stronger when reservoir age was used as a linear predictor (Table 3).

Cyanobacteria were the dominant photosynthetic plankters in all reservoirs, and contributed 60 to 95% of the total phytoplankton cells. High abundances of *C. raciborskii* and several other cyanobacterial species generally coincided with high chl*a* concentrations (correlation analysis: $r = 0.62$, $p = 0.01$ for phytoplankton chl*a* vs. total cyanobacterial abundance) and with TP ($r = 0.61$, $p = 0.009$; $n = 17$, from 6 reservoirs) (Table 3). In hierarchical ANOVA models, TP and chl*a* were significant predictors of total cyanobacterial abundance ($p = 0.003$ and 0.016, respectively) (Table 3). About 24% of the samples had chl*a* values equal to or in excess of the state standard for acceptable water quality (>

Table 3.-Statistically significant analyses, including (A) correlations (SAS Institute, Inc. 1999) based upon monthly averages (2 sites per reservoir) as an initial approach to examine potential relationships[a], considering two reservoir subgroups (moderately aged: 5 reservoirs, n = 10; old: 6 reservoirs, n = 12) and all reservoirs (n = 22); (B) hierarchical ANOVA models with month as a repeated measure factor; and (C) hierarchical ANOVA models using reservoir age group as a class variable versus reservoir age as a linear predictor.

A. Correlations

Moderate Reservoirs	**Pearson Correlation Coefficient**	***p* value**
SS vs. TP	+ 0.65	0.044
SS vs. TN, TKN	+ 0.67	0.03 to 0.04
TP vs. chl*a*	+ 0.63	0.053
TN vs. TP	+ 0.93	0.0001
TN or TKN vs. chl*a*	+ 0.77 to + 0.83	0.003 to 0.010
TKN vs. TOC	+ 0.67	0.033
NO_x^- vs. TOC	– 0.88	0.0008
TOC vs. chl*a*	+ 0.81	0.004
Age vs. TOC	– 0.86	0.002
Reservoir SA vs. TOC	+ 0.81	0.004
Agric. land use vs. TN, TKN	+ 0.68 to 0.73	0.017 to 0.030
Agric. land use vs. TP	+ 0.69	0.029
Agric. land use vs. chl*a*	+ 0.85	0.002
Old Reservoirs	**Pearson Correlation Coefficient**	***p* value**
SS vs. TP	+ 0.90	< 0.0001
SS vs. TN, TKN	+ 0.95	< 0.0001
SS vs. chl*a*	+ 0.83	0.0008
SS vs. TOC	+ 0.74	0.006
TP vs. chl*a*	+ 0.89	0.0001
TN vs. TP	+ 0.96	< 0.0001
TN or TKN vs. chl*a*	+ 0.88 to + 0.89	< 0.0001
TN or TKN vs. TOC	+ 0.60	0.038 to 0.039
Reservoir DA vs. TOC	– 0.61	0.034
All Reservoirs	**Pearson Correlation Coefficient**	***p* value**
SS vs. TP	+ 0.80	< 0.0001
SS vs. TN, TKN	+ 0.85	< 0.0001
SS vs. chl*a*	+ 0.72	< 0.0001
SS vs. TOC	+ 0.55	0.008
TP vs. chl*a*	+ 0.77	< 0.0001
TN vs. TP	+ 0.94	< 0.0001
TN or TKN vs. chl*a*	+ 0.84 to + 0.86	< 0.0001
TN or TKN vs. TOC	+ 0.55 to + 0.59	0.004 to < 0.008
TOC vs. chl*a*	+ 0.61	0.003
Chl*a* vs. cyanobacterial abundance[b]	+ 0.62	0.008
TP vs. cyanobacterial abundance[b]	+ 0.61	0.009
Age vs. NO_x^-	+ 0.61	0.003
Age vs. TN:TP	– 0.56	0.007
Reservoir DA vs. TN:TP	– 0.43	0.048
Agric. land use vs. TKN	+ 0.43	0.045

Table 3.-Continued

B. Hierarchical ANOVA models (*p* values, all reservoirs collectively)				
Parameter	**SS**	**TP**	**TN**	**Chl*a***
SS	----	< 0.0001	< 0.0001	< 0.0001
TP		----	< 0.0001	< 0.0001
TN			----	< 0.0001
Cyanobacterial abundance[b]		0.003		0.016

C. Hierarchical ANOVA models (*p* values)[c]		
Parameter	**Age Group (Class Variable)**	**Age (Linear Predictor)**
NO_x^-	0.0630	0.0183
TN:TP	0.0218	0.0120

[a] DA = watershed drainage area; SA = reservoir surface area; agric = agricultural.
[b] Cyanobacterial abundance was determined when chl*a* concentrations were ≥ ~15 µg/L (maximum ~120 µg/L; n = 17, from 6 reservoirs).
[c] The model using age as a linear predictor was also run for TN:TP ratios versus age without Lakes High Rock and Rhodhiss, which had the lowest ratios, and age remained a significant effect ($p = 0.0492$).

Table 4.-Common cyanobacteria taxa (found in ≥ 5% of samples) and their abundance as cell number in moderately aged (mod.) reservoirs in June-August 2002 (arranged by frequency of occurrence among samples; also showing means ± 1 SE, ranges, and taxonomic authority). Asterisks (*) indicate diazotrophs.

Species	**Freq. (%)**	**Abundance (cells x 10⁴/mL)**	**Range (cells x 10⁴/mL)**	**Taxonomic Authority**
**Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju	100	11.00 ± 2.60	0.40 – 20.00	Saker *et al*. (1999)
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová et Cronberg	80	11.00 ± 2.50	0.75 – 22.00	Komárek and Anagnostidis (2005)
**Pseudanabaena limnetica* (Lemmermann) Komárek	80	34.00 ± 11.00	0.65 – 90.00	Komárek and Anagnostidis (2005)
Aphanocapsa delicatissima W. et G.S. West	70	14.00 ± 3.00	4.50 – 28.00	Komárek and Anagnostidis (1999)
Chroococcus dispersus (Keissler) Lemmermann	70	4.60 ± 0.75	1.90 – 7.70	Komárek and Anagnostidis (1999)
**Anabaena planktonica* Brunnthaler	45	1.30 ± 0.27	0.40 – 2.05	Geitler (1932), Li *et al*. (2000)
Chroococcus limneticus Lemmermann	45	2.40 ± 1.10	0.16 – 7.30	Komárek and Anagnostidis (1999)
Merismopedia tenuissima Lemmermann	45	2.50 ± 0.20	2.00 – 3.30	Komárek and Anagnostidis (1999)
Planktolyngbya regularis Komárková-Legnerová et Tavera	45	2.45 ± 0.65	0.33 – 4.90	Komárek and Anagnostidis (2005)
Glaucospira sp.	45	0.90 ± 0.25	0.17 – 1.65	Komárek *et al*. (2003)
**Anabaena circinalis* Rabenhorst	35	0.62 ± 0.10	0.41 – 0.83	Geitler (1932)
**Aphanizomenon gracile* (Lemmermann) Lemmermann	25	1.90 ± 0.90	0.02 – 3.80	Hindak (2000)
Aphanothece clathrata W. et G.S. West	20	4.70 ± 1.90	0.66 – 8.70	Komárek and Anagnostidis (1999)
**Cylindrospermopsis philippinensis* (Taylor) Komárek	20	2.20 ± 0.06	2.05 – 2.30	Komarková-Legnerova and Tavera (1996)
**Anabaena* spp.	5	0.83	-----	Komárek and Anagnostidis (1989)
**Cylindrospermopsis catemaco* Komárková-Legnerová et Tavera	5	9.10	-----	Komárková-Legnerová and Tavera (1996)
Microcystis aeruginosa (Kützing) Kützing	5	0.03	-----	Komárek and Anagnostidis (1999)
Planktolyngbya spp.	5	8.30	-----	Komárek and Anagnostidis (2005)

Table 5.-Common cyanobacteria taxa[a] (found in ≥ 5% of samples) and their abundance as cell number in the old reservoirs during June-August 2002 (frequency of occurrence among samples, means ± 1 SE, and ranges). Asterisks (*) indicate diazotrophs.

Species	Freq. (%)	Abundance (cells x 10^4/mL)	Range (cells x 10^4/mL)
Planktolyngbya limnetica	70	7.23 ± 2.94	0.40 – 22.80
Aphanocapsa delicatissima	65	19.00 ± 5.70	1.57 – 51.70
Chroococcus dispersus	65	80.40 ± 20.00	0.70 – 17.00
**Cylindrospermopsis raciborskii*	65	10.20 ± 3.20	1.80 – 27.10
Merismopedia tenuissima	50	8.00 ± 4.60	0.41 – 38.70
Pseudanabaena limnetica	50	39.60 ± 15.60	1.70 – 108.00
**Anabaena* spp.	35	4.00 ± 1.37	0.50 – 8.70
Aphanothece clathrata	30	2.70 ± 0.64	1.32 – 5.45
Merismopedia glauca (Ehrenberg) Kützing[a]	30	4.05 ± 0.99	2.00 – 8.27
Chroococcus limneticus	20	1.87 ± 0.50	0.41 – 3.47
Planktolyngbya spp.	20	5.66 ± 0.92	3.70 – 7.60
Glaucospira sp.	20	0.54 ± 0.14	0.25 – 0.83
**Anabaena planktonica*	15	0.37 ± 0.10	0.16 – 0.57
**Aphanizomenon gracile*	15	0.58 ± 0.26	0.10 – 1.49
Gloeothece spp.	15	0.83	-----
Planktolyngbya circumcreta (G.S. West) Anagnostidis et Komárek[a]	15	2.70 ± 1.01	0.83 – 6.20
Planktolyngbya regularis	15	0.54 ± 0.14	0.25 – 0.83
Pseudanabaena spp.	15	10.90 ± 2.93	4.65 – 17.10
**Anabaena circinalis*	5	0.02	-----
**Anabaenopsis tanganyikae* (G.S. West) V. Miller[a]	5	0.66	-----
Aphanocapsa pulchra (Kützing) Rabenhorst[a]	5	11.30	-----
Arthrospira sp.	5	0.58	-----
**Komvophoron minutum* (Skuja) Anagnostidis et Komárek[a]	5	1.00	-----
Microcystis aeruginosa	5	4.14	-----

[a] Taxonomic references consulted: *Merismopedia glauca*, *Aphanocapsa pulchra* - Komárek and Anagnostidis (1999); *Planktolyngbya circumcreta*, *Komvophoron minutum* - Komárek and Anagnostidis (2005); *Anabaenopsis tanganyikae* – Geitler (1932).

40 µg/L; NC DEHNR 1996), and 6 of the 11 reservoirs had mean chl*a* concentrations of 30 µg/L or more. Microcystin concentrations were comparable in mod. and old reservoirs (0.14 ± 0.02 µg/L and 0.24 ± 0.03 µg/L, respectively; and concentrations consistently were less than < 0.8 µg/L (Fig. 2). Similar cyanobacteria assemblages occurred in mod. and old reservoirs considering both taxa composition and abundance (Tables 4, 5), although mean abundance was about two-fold higher in old reservoirs (~1.118 × 10^6 cells/mL and 2.169 × 10^6 cells/mL, respectively, in mod. and old reservoirs).

Dominant taxa (found in at least 50% of samples) in both reservoir sub-groups included *Aphanocapsa delicatissima*, *Chroococcus dispersus*, *Cylindrospermopsis raciborskii*, *Planktolyngbya limnetica*, and *Pseudanabaena limnetica*. Some species, such as *Anabaenopsis tanganyikae*, *Cylindrospermopsis philippinensis*, *Cylindrospermopsis catemaco*, *Gloeothece* spp. and *Merismopedia glauca*, were detected in reservoirs of one group but not the other. Bloom-forming species (considered as ≥ 1 × 10^5 cells/mL – Chorus and Bartram 1999, Oliver and Ganf 2000) consisted of *Pseudanabaena limnetica*, *Aphanocapsa delicatissima*, *Cylindrospermopsis raciborskii*, *Planktolyngbya limnetica*, and *Cylindrospermopsis catemaco* in mod. reservoirs, and *Chroococcus dispersus*, *Pseudanabaena limnetica*, *A. delicatissima*, *C. raciborskii*, and *Pseudanabaena* spp. in old reservoirs. The two reservoir subgroups differed in relative abundance of coccoid, filamentous nonheterocytous, and heterocytous taxa. In mod. reservoirs, heterocytous taxa averaged more than half (53%) of the total cyanobacterial cells, followed by coccoid (25%) and nonheterocytous filamentous forms (22%). In contrast, coccoid taxa dominated the cyanobacterial assemblages of old reservoirs (averaging 61% of the total cyanobacterial cells), followed by heterocytous taxa (31%) with nonheterocytous taxa averaging 8% of the total.

Discussion

This study focused on a set of turbid reservoirs within the trophic equilibrium phase of eutrophication (Kimmel and Groeger 1986). Throughout this phase in reservoirs, nutrient inputs commonly increase over time in urbanizing watersheds (Kimmel and Groeger 1986, Holz *et al.* 1997) that characterize the North Carolina Piedmont (*e.g.*, urban land cover increased by ~230,000 acres from 1982 to 1997 within the Neuse River basin; NC DENR 2002). Nevertheless, analysis of the small dataset from this study indicated that agricultural land use still influenced the water quality of these reservoirs, not surprising since agriculture remains a major source of nutrient pollution in most regions of the U.S. despite rapidly expanding urbanization (U.S. EPA 1998, 2000; Lunetta *et al.* 2005). For example, in the Neuse River watershed, agricultural lands contribute 55% of total annual nonpoint source N loadings, followed by forested lands (23%), and urban areas which are rapidly expanding in the upper watershed (21%; Line *et al.* 2002, Lunetta *et al.* 2005).

Use of reservoir age as a linear predictor provided additional support for a significant positive relationship between reservoir age and NO_x^-, and a significant negative relationship between reservoir age and TN:TP ratios indicating that, as expected, reservoirs become more eutrophic over time (Wetzel 2001). The estimated chl*a* yield per unit TP in these reservoirs (0.19 µg chl*a*/µg TP) was comparable to values reported for natural lakes and other reservoirs: *e.g.*, 0.07 µg chl*a*/µg TP in lakes of Japan and North America (Dillon and Rigler 1974); 0.21 µg chl*a*/µg TP in lakes of northern and western Europe and North America (Prairie *et al.* 1989); 0.43 µg chl*a*/µg TP in reservoirs in the midwestern U.S. (Hoyer and Jones 1983); 0.21 µg chl*a*/µg TP in tributary reservoirs in the Tennessee River Valley, U.S. (Cox 1984). The estimated chl*a* yield per unit TN in NC reservoirs (0.0003 µg chl*a*/µg TN) was about 3- to 10-fold lower than reported for natural lakes (*e.g.*, Canfield 1983, Pridmore *et al.* 1985, Prairie *et al.* 1989). Phosphorus is more important than nitrogen in influencing chl*a* concentrations in many lentic systems (Wetzel 2001, Kalff 2002, Malve and Qian 2006). The significant relationship between chl*a* and both TP and TN in these reservoirs ($r^2 = 0.60$ and 0.64, respectively) suggests that both nutrients are important factors influencing phytoplankton production in periods of reduced flushing and turbidity. Other factors such as light availability, flushing and herbivory would also be expected to be important controlling factors (Cuker *et al.* 1987, Cuker and Hudson 1992, Wetzel 2001). The data additionally suggest that N and P should be co-managed (National Research Council 2000) to reduce algal blooms and cyanobacteria in these turbid systems, which frequently exceed the state's chl*a* standard for acceptable water quality (~one-fourth of the samples in this study; Burkholder 2006).

Cyanobacteria dominated the phytoplankton cell numbers in the summer growing season of this low-precipitation year, averaging 65-95% (usually > 75%) of the total cells. Chorus and Bartram (1999) provided guidance about cyanobacteria cell densities in reference to World Health Organization (WHO) recommendations as follows: to prevent irritative/allergenic effects, $< 2 \times 10^4$ cells/mL; and for a moderate health alert (especially for swimming adults), $\geq 1 \times 10^5$ cells/mL. Oliver and Ganf (2000) stated that in potable and recreational waters, a cyanobacteria bloom is "frequently defined in terms of cell concentrations that cause a nuisance to humans, and a lower limit may be set at ca. 10 mg/m^3 of chlorophyll *a* (ca. 20,000 cells/mL)." We commonly encountered mean densities of potentially toxic cyanobacteria greater than 10^5 cells/mL in both mod. and old reservoirs, mainly as *Cylindrospermopsis raciborskii* along with other potentially toxic species.

Diazotrophic taxa were well represented at the moderate TN:TP ratios found in these reservoirs. As these systems become more eutrophic, diazotrophs may be favored because they are not as adversely affected by inorganic N limitation toward the end of the summer growing season; they have relatively low DO requirements in comparison to other algae; they are favored by waters with lower TN:TP ratios; and they can regulate their vertical station in the water column (Reynolds 1984, Chorus and Bartram 1999). Coccoid taxa may be favored, however, under conditions of frequent, high episodic suspended sediment loading (Burkholder 1992). In general, the moderate sedimentation rates characteristic of North Carolina reservoirs (*e.g.*, 0.3-2.4 cm/yr; Royall 2003), together with the nutrient-enriched conditions, may have contributed to cyanobacterial dominance. Of the reservoirs included in this study, Lake Michie was among the highest in sedimentation (loss of 20% of the volume between 1922 and 1996; United States Geological Survey [USGS] 1995). Sediment yields have been reported at ~420 tonnes/km^2/yr in the Cape Fear River basin, and at ~660-700 tonnes/km^2/yr in the Yadkin and Catawba River basins (USGS 1995). By comparison, erosion rates can vary from ~50 tonnes of sediment/km^2/yr in wooded areas, to ~1,175 tonnes/km^2/yr in rural areas, to 23,500 tonnes/km^2/yr in urban areas (Kautzman and Cavaroc 1973). The distinction between moderate and high sedimentation rates is important when considering phytoplankton assemblage composition (Cuker *et al.* 1990, Burkholder 1992, Lind *et al.* 1992). For example, in a study of phytoplankton assemblages over time during the trophic equilibrium phase in reservoirs, Holz *et al.* (1997) noted a shift in dominance from cyanobacteria (in the 1970s) to flagellated chlorophytes (in the 1990s). This shift was attributed to light attenuation from elevated suspended sediments. In the present study, the drought conditions would have depressed episodic sediment loading and favored cyanobacteria (Cuker *et al.* 1990).

Microcystin cyanotoxins can be produced by various cyanobacteria found in North Carolina waters (Burkholder 1992, NC DENR 2004b), such as *Anabaena flos-aquae*, *A. circinalis*, *Microcystis aeruginosa*, *Nostoc rivulare*, and certain *Oscillatoria* spp. (reviewed in Burkholder 2002). Among ~80 known microcystins (Zurawell *et al.* 2005), microcystin-LR is commonly considered in potable water safety issues, and WHO (1998) has recommended a limit of 1 μg microcystin-LR/L in drinking water to protect public health. Microcystin concentrations were below that limit throughout the study, but microcystin was detected in nearly all samples. Cyanobacteria abundance and composition, and microcystin generally were comparable in mod. and old reservoirs. Chorus *et al.* (2001) and Zurawell *et al.* (2005) noted that microcystin content in field populations of cyanobacteria can differ among strains, and in the same strain depending upon nutrient availability and physiological status. Literature reports vary regarding influences of N on cyanotoxins levels. Sivonen (1990) found that microcystin-LR production increased in cultured *Oscillatoria agardhii* with increasing NO_x^-. However, anatoxin-a production in cultured *Aphanizomenon* sp. and *Anabaena* sp. were highest under nitrogen-free conditions (Rapala *et al.* 1993). In eutrophic and hypereutrophic lakes in Alberta, Canada, Kotak *et al.* (1995, 2000) reported strong inverse relationships between microcystin concentrations and NO_x^- or NH_4^+ levels, but microcystin concentrations were much higher than in this study.

Although microcystin was targeted for measurement in this study, microcystin-producing taxa were much lower in abundance than the potentially toxic species, *Cylindrospermopsis raciborskii*. Moreover, recent more frequent (weekly) measurements in Falls Lake have detected saxitoxin as well as low levels of microcystin (M. Shehee, North Carolina Department of Health and Human Services, personal communication, March 2007). The present study, limited to 11 reservoirs over a dry summer growing season, provides initial insights about the influence of watershed land use, reservoir age and reservoir morphometry on water quality and cyanobacteria assemblages in the North Carolina Piedmont. We plan to continue to examine these relationships by assessing additional potable water supply and recreational reservoirs over multiple years, including consideration of other toxins such as cylindrospermopsin, anatoxin-a and saxitoxin as well as microcystins.

Overall, this study indicates that at present, these turbid, meso-/eutrophic reservoirs have only moderate cyanobacteria abundance and low cyanotoxin (microcystin) levels, even in the low-precipitation summer growing season analyzed. Nevertheless, the significant relationships between phytoplankton chl*a* and cyanobacterial abundance, and between both parameters and TP, together with the fact that nearly one-fourth of the samples had chl*a* values equal to or in excess of the state standard for acceptable water quality, indicate that when under drought conditions with reduced turbidity and flushing, these reservoirs may respond similarly as natural lakes to nutrient over-enrichment (Dillon and Rigler 1975, Vollenweider 1975, Jones and Bachmann 1976, Hoyer and Jones 1983, Wetzel 2001). Considering that cyanotoxin production can be stimulated by nutrient enrichment (Zurawell 2005, Gobler *et al.* 2007), these potentially toxic taxa may adversely affect the utility of these impoundments for potable water supplies and recreational activities as eutrophication progresses.

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