

Five-Year Analysis (2019-2023) of Anatoxin-a (ATX-a) concentrations in Lake Anna, Virginia, USA: Implications for Future Freshwater Cyanotoxin Monitoring

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Abstract

Harmful algal blooms (HABs) are an increasing concern for the health of freshwater systems worldwide. Cyanobacterial HABs (cyanoHABs) have recently been reported in Lake Anna, Virginia, and low levels of the neurotoxin anatoxin-a (ATX-a) have been observed. Here, we provide a comprehensive report of ATX-a concentrations at multiple locations in the upper tributaries and northern sections of Lake Anna over five consecutive years (2019–2023). ATX-a concentrations ranged from 0.053 to 1.64 ppb, and 14% (13/91) of samples exceeded Virginia's drinking water advisory threshold of 0.4 ppb. Elevated ATX-a concentrations were most frequently observed in shallow northern tributaries during summer, coinciding with periods of increased cyanobacterial abundance. ATX-a was quantified using two different methods, each with a distinct limit of detection (LOD). Statistical analyses demonstrated a strong positive association between water temperature and ATX-a concentration. For each 1 °C increase in temperature, there was a 37% (1.37-fold) increase in ATX-a concentration ($p < 0.001$) when accounting for potentially toxigenic cyanobacteria (PTOX) counts, zone, and year. To our knowledge, this study represents the first published multi-year record of ATX-a occurrence in a lake in the USA. Our findings highlight the need for continued and expanded ATX-a monitoring in Lake Anna, VA to provide essential data to water quality experts, health departments, and community members. Future research, coupled with increased funding and public engagement, are needed to mitigate the future impact of cyanotoxins on the Lake Anna community.

Introduction

Freshwater harmful algal blooms (HABs) are often caused by an overgrowth of toxin-producing cyanobacteria (Moreira, Vasconcelos and Antunes, 2022; Reid et al., 2024). Climate change factors, including increased temperatures and the influx of nutrients from rainfall, are important contributors to HABs (Moreira, Vasconcelos and Antunes, 2022). Cyanotoxins produced by cyanobacterial HABs (cyanoHABs) include, but are not limited to, anatoxin-a (ATX-a), beta-methylamino-L-alanine (BMAA), cylindrospermopsin, lyngbyatoxin, microcystins, and saxitoxin. The most commonly detected neurotoxins produced by cyanoHABs are ATX-a and BMAA (Al-Sammak et al., 2014). These neurotoxins, both of which block and/or disrupt neurotransmitter function pose a significant threat to the health of wildlife, domestic animals, and humans (Christensen and Khan, 2020; Nugumanova et al., 2023). Currently, few guidelines exist for recreational exposure limits to ATX-a. The World Health Organization (WHO) has issued a short-term provisional advisory of 30 ppb for drinking water and 60 ppb for recreational exposure (US EPA, 2019). The WHO drinking water advisory value is substantially higher than those adopted by several U.S. states. For example, states with 10-day drinking water health advisory limits that are more conservative than the WHO drinking water threshold of 30 ppb, include Minnesota (0.1 ppb), Ohio (0.3 ppb), and Virginia (0.4 ppb) (US EPA, 2018b). Recreational water guidelines in states that have established threshold values for ATX-a vary widely and range from 1 ppb to 80 ppb. Although Lake Anna is not used as a source for drinking water, the Virginia drinking water advisory threshold provides a conservative and health-protective benchmark that is relevant to communities surrounding Lake Anna and is therefore used as a reference throughout this study.

The first cyanobacteria advisory issued by the Virginia Department of Health (VDH) for Lake Anna was in 2018 (McCotter, 2019; Luck, 2021). The lake is a man-made freshwater reservoir that was completed in 1972, when the North Anna River dam was finished (Salisbury, 2020). It was constructed primarily to cool a nuclear power plant in the southern part of Lake Anna. The Virginia (VA) Department of Environmental Quality (DEQ) has monitored water quality at the lake since its creation in 1972. Water samples for VDH's cyanobacterial monitoring have been collected by DEQ during routine water quality assessments at Lake Anna since 2013. Water quality parameters such as dissolved oxygen, temperature, and phosphorus have also been monitored by the Lake Anna Civic Association (LACA) since 2002 with two decades of recorded data (since 2005). DEQ and LACA have collaborated since 2002 to monitor the lake at more than 30 established locations. Their partnership is formalized through a Memorandum of Agreement (MOA) enacted in 2002. The MOA is updated every five years. Since the first report of cyanoHABs at Lake Anna, VDH, with the help of DEQ, and the Phytoplankton Analysis Laboratory (PhytoLab) at Old Dominion University (ODU) has been involved in a respond and report program at Lake Anna. Public reporting of a potential HAB, or routine monitoring by DEQ have prompted sample collections. VDH has then supported ODU to analyze samples and issue advisories based on guidance. In 2022, Lake Anna was added to the EPA's (Environmental Protection Agency) list of impaired waterways, with five impaired areas listed, including the Contrary Creek and Freshwater Creek watersheds (EPA, 2021; Chesapeake Bay Foundation, 2022). As of 2025, advisories for Lake Anna were based solely on toxin concentrations (VDH, 2025). In previous years, the guidance on issuing an advisory included the presence of potentially toxigenic (PTOX) species of cyanobacteria at cell densities of above 100,000 cells mL⁻¹ (VDH, 2025).

Here, we provide a comprehensive report of ATX-a concentrations at multiple locations in the upper tributaries and northern sections of Lake Anna where cyanoHABs have been reported over five consecutive years (2019–2023). We hypothesized that ATX-a concentrations in Lake Anna vary with sampling location, month, and interannually. To test this hypothesis, we analyzed collected water samples and gathered available data. ATX-a and water quality data was compiled from multiple sources including DEQ, VDH, ODU, LACA, and a prior published work using drone-based water sampling methods (Hanlon et al., 2022). The objectives of this study were to: (1) collect and analyze water samples from Lake Anna for ATX-a from different locations, months, and years, (2) review two methods of ATX-a detection and quantification (liquid chromatograph triple quadrupole mass spectrometry (LC-MS/MS) and enzyme-linked immunosorbent assay (ELISA)), and (3) examine potential factors that could be used to predict the presence of ATX-a in Lake Anna to assist with communicating potential risks to water quality experts and community members. To our knowledge, this is the first published report documenting a five-year record of ATX-a in a lake in the United States. The results emphasize the need for continued and expanded ATX-a monitoring in Lake Anna, VA to properly inform water quality experts, health departments, community members, and other stakeholder groups. Future research, coupled with increased funding and public engagement, will be crucial to mitigate the impact of HABs in the region.

Materials and Methods

Lake Anna water sampling

The water samples analyzed in this study were collected from Lake Anna, Virginia, USA during summer, between 2019 and 2023. Lake Anna northern tributaries were classified into four distinct lake zones (Fig. 1). We analyzed 91 samples with detectable ATX-a concentrations gathered from 14 stations over the five- year period (**Supplementary Table 1**). The Virginia Department of Health (VDH) Beaches Harmful Algal Bloom (HAB) map (Fig. 1, **inset**) shows the HAB advisory status for Lake Anna on Sept. 12, 2024 ('Algal Bloom Surveillance Map', 2024). Red areas of the lake denote areas with a recreation advisory. Circles on the VDH inset map show testing site locations routinely used by VA DEQ to gather water samples. Red circles indicate specific cell densities (100,000 cells/mL) of VDH targeted potentially toxic cyanobacteria (PTOX) taxa or toxin concentrations above the advisory threshold (ATX-a > 8 ppb, saxitoxin (STX) > 4 ppb, microcystin (MC) > 8 ppb, cylindrospermopsin (CYL) > 15 ppb). Green circles indicate testing sites with reported values below the threshold values.

Collection methods

Lake Anna water samples were collected primarily in summer by field personnel and by drone (**Supplementary Table 1**). Drone-based collections were made in 2019 and 2020 from the surface of the water and followed the methods of Hanlon et al. 2022 (Fig. 2A). DEQ and LACA collections carried out by field personnel from 2020–2023 were subsurface collected, at 0.3 m below the surface (Fig. 2B). DEQ has continuously sampled 11 of the 14 sites shown in Fig. 1 as part of routine water quality monitoring at Lake Anna since 2002. The three remaining sites have been sampled by LACA since 2002. While the map site abbreviations show traditional DEQ collection sites with a "D" and LACA sites with an "L" (Fig. 3), this nomenclature is not indicative of the actual group who performed the collection for this study. The State Park Beach (SPB) site and the collection site at "the splits" (DY) has been used by both DEQ and LACA. Because LACA is a DEQ Level 3 Citizen Water Quality Monitoring Partner, LACA's data can be used by DEQ and other regulatory agencies for environmental assessments.

All lake water collections were stored in the dark on ice immediately after collection. Subsurface samples collected by DEQ and LACA were gathered by DEQ, and in collaboration with VDH were delivered to the Phytoplankton Analysis Laboratory (PhytoLab) at Old Dominion University (ODU) for further analysis. Two bottles with 500 mL of lake water preserved with a ratio of 1:100 Lugol's iodine solution were held on ice during transport to the lab at ODU for phytoplankton analysis. A 5 mL aliquot of each of the 21 drone samples were frozen and shipped to the Lumigen Center (Wayne State University, Detroit, MI) for LC-MS/MS analysis. Aliquots of 28 subsurface samples collected by LACA by hand were also frozen and shipped to the Lumigen Instrument Center. For the remaining 42 subsurface collected samples, aliquots of lake water with PTOX cells were frozen in 2 mL glass amber tubes prior to ELISA analysis at ODU.

ATX-a analyses

Two different methods were used to detect ATX-a in this collection of samples. LC-MS/MS (LOD 0.050 ppb) detection of ATX-a was done at the Lumigen Instrument Center, MI. ELISA detection (LOD 0.15 ppb) was performed with the Abraxis kit at ODU. ATX-a concentrations from both methods are shown for each location in Fig. 3 and combined by zone in Fig. 4. For the LC-MS/MS methods, three freeze-thaw cycles were performed prior to ATX-a analysis. The samples were run on a Thermo Scientific TSQ Altis™ triple quadrupole mass spectrometer (Thermo Scientific, Waltham, MA, United States) with an EQuan MAX Plus™ system. The LC-MS/MS method that was run was a modified from US EPA Method 545 (US EPA, 2019). The internal standard, L-Phenylalanine-D5, was run with the samples and the LOD for ATX-a was 0.05 ppb. Details of the cyanotoxin analysis protocol can be found in Hanlon et al. (2022).

Taxonomic enumerations at ODU

Taxonomic enumerations of cyanobacteria taxa and counts were conducted by the PhytoLab at ODU. The density counts were conducted on an Olympus CX23 microscope under phase contrast at 400x to the lowest taxonomic level possible following morphological features described by Komárek (Komárek, J and Anagnostidis, K, 1998, 2005; Komárek, J, 2013). Only filamentous and colonial taxa were counted and reported as per the VDH contract with the PhytoLab at ODU. Unicellular and picocyanobacteria were not counted in these samples. Results are then reported to VDH to be included in their HAB Map (LeGresley and McDermott, 2010). This compiled data was used to visualize the relative abundance of PTOX cyanobacteria taxa at the classification of genus (Fig. 5) and order (**Supplemental Fig. 1**).

16S DNA diversity analysis

A bulk 1 L surface water sample was collected in Terry's run (Zone 1) on Sept. 10, 2019, and was stored frozen. After thawing the bottle, a 14 mL aliquot of the bulk water sample was used for a DNA extraction with the Qiagen DNeasy PowerWater kit (Qiagen #14900). The purified DNA was sent to MR DNA (Molecular Research, Shallowater, TX) for 16S diversity sequencing with new-515f (5'GTGYCAGCMGCCGCGGTAA3') and new-805r (5'GGACTACNVGGGTWCTAAT3') prokaryotic primers. Sequence data was processed in the working pipeline by MR DNA. Briefly, short reads (< 150 base pairs), ambiguous reads, and primer sequences were removed. Sequences were dereplicated and denoised, and chimeras were removed. The zero-radius Operational Taxonomic Units (zOTUs) were identified using BLASTn with a curated NCBI (www.ncbi.nlm.nih.gov) database. The resulting sequences were compiled into "counts" and "percentages" files. In this study, the OTU counts file from MR DNA (zotus.fa.prokaryote.OTU) was modified in Open Refine, <https://citeas.org/cite/OpenRefine> (Ham, 2013). Modifications included separating taxonomic names into separate columns and creating a facet of sequence reads with 98% to 100% homology. Background corrections for no template control (ntc) were removed in excel with a Power Query imported data sheet. *Raphidiopsis* is synonymous to *Cylindrospermopsis* (Zheng et al., 2023) therefore, read counts recorded under both names were combined and shown as *Raphidiopsis*. The relative abundance of the resulting prokaryotic phyla at the level of genus is shown in **Supplementary Fig. 2**. The eight taxa with a relative abundance above 3% are listed in ascending order: *Azohydromonas*, *Streptomyces*, *Polynucleobacter*, *Microcystis*, *Aphanothece*, *Raphidiopsis*, *Sediminibacterium*, and *Synechococcus*.

Statistical Analyses

Statistical analyses were performed in R using RStudio (v2025.09.2 + 418). An ordinary least squares (OLS) linear regression model was used to evaluate associations between ATX-a concentration and environmental predictors. Natural log-transformed ATX-a concentration (ppb) was specified as the response variable. Predictor variables included water temperature (°C), log1p-transformed PTOX cell density counts, zone, and year. Due to limited sample sizes at individual sampling locations, sites were aggregated and analyzed by zone rather than treated as distinct locations. Only complete cases containing ATX-a concentration, temperature, and PTOX density data were included in the analysis (n = 39). Model assumptions were evaluated prior to inference. Normality of residuals was assessed using the Shapiro-Wilk test, and homoskedasticity was evaluated using the Breusch-Pagan test. Regression coefficients were exponentiated and reported (**Supplementary Table 3**) as fold-changes ($\exp[\beta]$) with 95% confidence intervals (CIs). To account for the small sample size and potential model misspecification, heteroskedasticity-consistent (HC) standard errors were calculated using the HC3 estimator, and statistical inference was based on the resulting robust p-values.

Results

Lake Anna water sample collections

Twenty-one drone samples (surface) and 70 subsurface (0.3 m below) samples were collected over 5 years (2019–2023) from the northern tributaries of Lake Anna, were analyzed for ATX-a (Figs. 3 and 4). The yearly sample counts were as follows: 12 samples from 2019, 36 from 2020, 34 from 2021, four from 2022, and five from 2023 (**Supplementary Table 1**, Fig. 3). The northern tributaries of Lake Anna were divided into lake Zones 1–4 (Fig. 3). Two location sites were located in Zone 1, the Terry's Run branch. Four samples were collected from L37, and 16 samples were collected from D10 for a total of 20 samples from Zone 1 (Fig. 3). Zone 2 was in the Upper Pamunkey branch where 34 samples were collected from the three collection sites: 8 samples from L38, 5 samples from D24, and 21 samples from D11. Eleven samples were collected in Zone 3; 7 samples were collected from the Mid and Lower section of the Pamunkey branch (5 at D719 and 2 at D612), 2 samples from the State Park Beach (SPB) site and 2 samples from the area referred to as the "splits" (DY). Zone 4 included five sampling sites along the North Anna Branch in which a total of 26 total samples was collected: 1 sample at L43, 12 at D13, 8 at D12, 3 at D14 and 1 at L47.

ATX-a concentrations from Lake Anna water samples

Samples with measurable ATX-a were collected on 2 dates in 2019, one in August and one in September; 9 dates from June to August in 2020; 11 dates from June to October 2021; 3 dates in 2022, one in May, June, and August; and 1 date in June in 2023. The cumulative concentration of ATX-a (ppb) measured in each of the four lake zones (LC-MS/MS and ELISA) is shown in Fig. 4 for the years 2019 to 2023. ATX-a concentrations ranged from 0.053 to 1.64 ppb (mean = 0.27 ppb), with the highest concentration located in Zone 4 (Fig. 3D), and the lowest concentration sample located in Zone 2 (Fig. 3C). The highest measured ATX-a concentration (1.64 ppb) was from a subsurface water sample collected at site D13 (Fig. 3) in the North Anna branch (Zone 4) on June 18, 2021. The lowest ATX-a sample (0.053 ppb) was from a surface sample collected from L38 on September 10, 2019. ATX-a concentrations above 0.250 ppb made up 31% (28/91) of the sample set and were found in Zones 1, 2, and 4, but not in Zone 3.

Cyanobacteria cell counts

PTOX cyanobacteria cell count information from 2019 to 2023 was used as defined by VDH and analyzed at the level of genus (Fig. 5) and order (**Supplementary Fig. 1**). The cyanobacteria cell counts were summed for each year, for all collections at Lake Anna. The identified taxa were used to calculate mean relative abundance of cyanobacteria. The histogram for each year, shows the relative abundance of each classification. There are 14 genera (Fig. 5), that represent four different levels of order (**Supplementary Fig. 1**).

16S Diversity analysis of lake water DNA

The OTUs present above 3% from a single water sample on September 10, 2019, are shown in **Supplementary Fig. 2**. *Synechococcus* was the most abundant genus with a mean relative abundance of 29.9%. The other taxa present at levels higher than 3% were *Sediminibacterium* (9.88%), *Raphidiopsis* (previously named *Cylindrospermopsis*, 9.24%), *Aphanizomenon* (9.0%), *Microcystis* (7.45%), *Polynucleobacter* (6.81%), *Streptomyces* (6.51%) and *Azohydromonas* (3.83%).

Statistical Analyses

Model diagnostics indicated that assumptions of linear regression were adequately met. Residuals were normally distributed, as supported by the Shapiro-Wilk test ($p = 0.092$), and no evidence of heteroskedasticity was detected based on the Breusch-Pagan test ($p = 0.17$). Visual inspection of diagnostic plots supported model adequacy. Scatterplots of ATX-a concentration versus water temperature across the four zones demonstrated a consistent positive relationship, with no evidence of strong nonlinearity. Residuals plotted against fitted values showed no systematic patterns, indicating stable variance across the range of fitted values. Normality of residuals was supported by both the quantile-quantile (Q-Q) plot and the residual histogram, which showed approximate symmetry and alignment with theoretical expectations. Collectively, these diagnostics indicate that the OLS model provided an appropriate fit to the data (**Supplementary Fig. 3a-e**).

Water temperature was a strong positive predictor of ATX-a concentration ($p < 0.0001$). A one-degree increase in temperature (°C) was associated with a 37% increase in ATX-a concentration (95% CI: 22–54%) (**Supplementary Table 3**). PTOX density exhibited a positive association with ATX-a concentration, with a one-unit increase in log1p (pTOX) corresponding to a 21% increase in ATX-a concentration (95% CI: 3–41%; $p = 0.051$) (**Supplementary-Table 3**). Spatial differences in ATX-a concentration were observed among zones. Zone 3, the area surrounding the State Park, exhibited significantly lower ATX-a

concentrations relative to Zone 1, Terry's Run ($p < 0.05$). In contrast, Zone 2, Upper Pamunkey, was estimated to have slightly higher ATX-a concentrations, while Zone 4, the North Anna branch, showed no clear difference relative to Zone 1, Terry's Run.

Discussion

Harmful cyanoHABs are an increasing concern for the health of freshwater systems worldwide (Chapra et al., 2017; Sha et al., 2021; Wiley and McPherson, 2023). In this study, we present a comprehensive analysis of ATX-a concentrations at multiple locations in the upper tributaries and northern sections of Lake Anna, VA, over five consecutive years (2019–2023). To our knowledge, this is the first published report documenting a five-year record of ATX-a in a US Lake. These findings contribute to the growing body of research on waterbodies with reported ATX-a levels, including sites in Brazil (Becker et al., 2010), Australia (John et al., 2019), and elsewhere in the United States (Nebraska and Texas) (Al-Sammak et al., 2014; Perri et al., 2024). Reservoirs, which provide drinking water to millions of people worldwide, have been increasingly scrutinized for the presence of cyanobacterial toxins in the interest of protecting human populations (Becker et al., 2010; Al-Sammak et al., 2014). Becker et al. (2010) reported the first detection of ATX-a in *Anabaena crassa* during a bloom in the Faxinal Reservoir, the primary water source for the city of Caxias do Sul in Brazil. In Nebraska reservoirs, 24.3% (94/387) of the samples tested were found to have measurable levels of neurotoxins (0.8–3.2 ppb) (Al-Sammak et al., 2014). This study also documented the co-occurrence of ATX-a, BMAA, and DABA with microcystin in at least one of two years in six of the eight reservoirs tested. In Australia, ATX-a was first reported in surface water samples collected when cyanobacterial cells were > 1000 cells/mL across 67 locations in Victoria between 2010 and 2017 (John et al., 2019). Concentrations, of ATX-a measured using the Abraxis ELISA kits, ranged from 0.15 to 1.6 ppb, with two samples exceeding 4.0 ppb (John et al., 2019). Additionally, the *anaC* (anatoxin-a synthase C) gene was detected in 31 of the 67 sampled locations. In the United States, Perri et al., (2024) reported the first detection of dihydroanatoxin-a, an ATX-a congener, in filamentous cyanobacterial mat material collected from the Lady Bird Lake reservoir in Texas. Using LC-MS, they analyzed anatoxins, microcystin, cylindrospermopsin, and saxitoxins in both water and mat material. Their study also demonstrated that dhATX exhibited greater toxicity than ATX-a in an ingestion study (Puddick et al., 2021).

Fourteen percent (13 of 91) of Lake Anna water samples surpassed 0.4 ppb, the drinking water advisory limit in Virginia, while 77% (70 of 91) exceeded the most stringent state limit of 0.1 ppb (in Minnesota). Current federal guidelines in the US do not specify a safe concentration limit for ATX-a in recreational or drinking water (US EPA, 2018a) while a few states have set advisory limits for ATX-a in drinking water (US EPA, 2018b). California has a notification level (NL) advisory of 4 ppb for drinking water, while Vermont has set the limit for drinking water at 0.5 ppb (US EPA, 2018b; Vermont Dept. of Health, 2018). Virginia's advisory limit for ATX-a in drinking water is 0.4 ppb (VDH, 2025). The lowest advisory limits are found in Ohio and Minnesota, with ATX-a limits of 0.3 ppb and 0.1 ppb, respectively (US EPA, 2018b). All samples from Lake Anna, VA with detectable levels of ATX-a were below the recommended recreational use value advisory limit of 8 ppb for Virginia. Recreational use threshold guidelines for ATX-a vary across states, ranging from 1 ppb to 80 ppb. Washington has the lowest recreational threshold at 1 ppb, while other states set limits between 7 ppb and 80 ppb. States with recreational use limits for ATX-a at or below 80 ppb include Minnesota (7 ppb), Indiana (8 ppb), Ohio (8 ppb), Pennsylvania (8 ppb), Virginia (8 ppb), Vermont (10 ppb), Colorado (15 ppb), New Jersey (15 ppb), Oregon (15 ppb), Utah (15 ppb), California (20 ppb), Montana (20 ppb), Nevada (20 ppb), Texas (60 ppb), Wyoming (60 ppb), and West Virginia (80 ppb) (US EPA, 2018b).

In this study, the highest ATX-a concentrations were consistently observed in the northern tributaries of Lake Anna, where there have been reports of PTOX-producing cyanoHABs. Expanding water testing to encompass all areas of the lake year-round could provide a more comprehensive understanding of the spatial and temporal occurrence of ATX-a in relation to PTOX bloom events (Ho and Michalak, 2020; OneHealth, 2020). From 2019 to 2024, the most abundant PTOX taxa recorded for Lake Anna were primarily in the order *Nostocales*, followed by *Synechococcales*, *Oscillatoriales*, and *Chroococcales* (Supplementary-Fig. 1). Comparable work by (Savadova-Ratkus et al., 2022) on two shallow eutrophic lakes (maximum depths 4.4–4.5 m) identified fifteen unique putative PTOX species from 248 cyanobacterial isolates, spanning the orders *Chroococcales*, *Oscillatoriales*, and *Nostocales*. Genera characteristic of nutrient-rich environments: *Aphanizomenon*, *Planktothrix*, *Limnothrix*, and *Planktolyngbya*, were dominant in both lakes studied. In the monodominant Lake Širvys, *Planktothrix* prevailed and was associated with ATX-a concentrations up to 9.7 times higher than in the polidominant Lake Jieznas, where toxin production was more variable and STX predominated. Seasonal succession patterns in the polidominant lake included *Limnothrix* dominance in early summer, *Planktolyngbya* in September, and *Aphanizomenon gracile* peaking in August alongside *P. agardhii* and *Microcystis aeruginosa*. Laboratory monocultures of these taxa produced higher toxin levels than observed in situ, underscoring the role of community interactions and environmental conditions in modulating toxin production. If Lake Anna were to shift from a polidominant to a monodominant system dominated by a high-ATX-a-producing genus such as *Planktothrix* or *Aphanizomenon*, toxin concentrations could substantially increase, potentially elevating risks to human, wildlife, and ecosystem health while reducing seasonal biodiversity within the cyanobacterial community. *Aphanizomenon* had the highest density of cell counts at Lake Anna for all five years.

While warmer seasonal temperatures can enhance cyanobacterial growth, the duration of elevated summer heat can also influence toxin production within individual lakes (Ho and Michalak, 2020). As climate trends drive temperature shifts, Lake Anna's mid-latitude location may influence its sensitivity to stratification dynamics (Bukaveckas, Buikema and Cameron, 2025). Analyzing data from over 100 Virginia reservoirs dating back to the 1970s, Bukaveckas et al. (2025) found that prolonged stratification can restrict nutrient mixing and limit oxygen exchange between surface and bottom waters. Accelerated surface warming in deeper parts of the lake, where bottom cooling does not occur as in glacial lakes at higher latitudes, may intensify thermal gradients and stratification, potentially leading to oxygen depletion in bottom layers. Additional water quality measurements collected across different areas of the lake indicate a higher potential for HABs in the northern tributaries compared to the main body of the lake (LACA, 2024). Water temperature at each sampling location was measured and recorded by LACA beginning in 2020.

The strong positive relationship observed between water temperature and ATX-a concentration provides a mechanistic basis for the spatial and seasonal patterns observed across the northern lake tributaries. Surface temperatures in Zones 1–4 can reach 32°C by mid to late August, coinciding with elevated ATX-a concentrations. The regression analysis indicated that each 1° C increase in temperature was associated with a 37% increase in ATX-a concentration,

underscoring temperature as a primary driver of toxin variability during the warmer summer months. The positive association between PTOX density and ATX-a concentration further supports the linkage between cyanobacteria and the presence of ATX-a, especially when lake temperatures are elevated.

Seasonal warming in late summer appears to have played a role in shaping both the magnitude and spatial extent of ATX-a occurrence, with the shallower tributaries experiencing earlier and more pronounced thermal increases than the deeper and more centrally located regions. The northern tributary zones exhibited distinct differences in ATX-a concentration. Terry's Run, Upper Pamunkey, and the North Anna branch (Zones 1, 2, and 4) are relatively shallow with depths as low as 1.5 meters and are positioned further west than the main body of the lake. These attributes allow the northern tributaries to warm earlier in the season than the State Park beach and the splits area (Zone 3), located above the main body of the lake. These tributary conditions likely contribute to enhanced cyanobacterial growth and toxin production. In contrast, Zone 3 had significantly lower ATX-a concentrations relative to Zone 1, a pattern that aligns with cooler temperatures and greater hydraulic connectivity near deeper portions of the main lake. Insights from other systems highlight how extreme hydrological events can abruptly alter cyanobacterial bloom dynamics. For example, flooding in a shallow floodplain lake of the Danube disrupted the intensity and abundance of a *Raphidiopsis* bloom, leading to reduced cyanobacterial growth (Stević et al., 2025). Such events can act as large-scale physical disturbances, potentially flushing out biomass or altering nutrient availability. In Lake Anna, continued monitoring of the tributaries before and after comparable environmental disturbances, such as heavy rainfall or hydrologic pulses, would help clarify how such events influence ATX-a concentrations and bloom persistence.

Between 2019 and 2023, ATX-a concentrations in Zones 1, 2, and 4 exceeded the VDH drinking water advisory threshold of 0.4 ppb. The VDH HAB map for September 2024 indicates that this trend continued, with testing parameters in the same regions surpassing advisory thresholds. Expanding ATX-a testing across all areas of the lake would confirm the detectable presence or absence of ATX-a more comprehensively, both spatially and temporally. Reid et al., (2024) observed that algal biomass in water samples is highly mobile and can shift rapidly between sampling moments due to water movement. A 2023 bloom in Lake Lough Neagh, the largest lake in the UK, was predominately caused by *Microcystis aeruginosa* (Reid et al., 2024). In contrast, previous blooms in this Northern Ireland lake were dominated by *Planktothrix*, suggesting that one or more of the eighteen identified HAB drivers triggered an unexpected shift in the lake's bacterioplankton communities (Reid et al., 2024). Our findings indicate that spatial and temporal variations in ATX-a levels at Lake Anna are influenced by factors such as water temperature, and cyanobacteria composition.

ATX-a concentrations were determined using two different testing methods, each with a distinct LODs. Limitations in ATX-a detection methods include the availability of testing options, associated costs, and the timely turnaround of toxin results (Mass.gov, 2015). Although LC-MS/MS testing can provide results within two days, samples must be shipped to specialized facilities capable of processing them immediately upon arrival. The cost of LC-MS/MS testing can be prohibitive, with prices reaching over \$500 USA per sample (Mass.gov, 2015; EPA, 2016). The ELISA test, by contrast, can also be completed within 1–2 days, though samples must still be transported to a designated testing facility. While ELISA is generally less expensive than LC-MS/MS, its current LOD (using the Abraxis test kit) is 0.15 ppb, whereas LC-MS/MS can detect concentrations as low as 0.05 ppb. To compare the performance of both methods, we analyzed a sample collected from location D11 in Zone 2 on June 23, 2021, using both techniques. The LC-MS/MS result was 0.240 ppb ATX-a, while the ELISA result was 0.293 ppb. Additionally, two other sites, D10 in Zone 1 and D11 in Zone 2, were tested with LC-MS/MS on August 3, 2021, and with ELISA on August 5, 2021. At site D10 (Zone 1), LC-MS/MS detected 0.140 ppb ATX-a, whereas ELISA measured 0.233 ppb. At site D11 (Zone 2), LC-MS/MS detected 0.220 ppb, while ELISA showed 0.155 ppb. These results suggest that both methods consistently detect ATX-a at or above 0.15 ppb in Lake Anna. One limitation of our ATX-a samples above the LOD (> 0.15 or > 0.05 ppb) was the small sample size. Given this constraint, we retained 39 samples that met the criteria for complete cases, ie: samples that included ATX-a concentration, temperature, and cell density counts. Expanding the number of sampling sites and increasing collection frequency would enhance the comprehensiveness of the study (Reid et al., 2024). Supplementing the dataset with repeated sampling across different spatial and temporal scales, both within and outside of the HAB zones, would improve the reliability of the results (Li et al., 2020; Reid et al., 2024).

For over two decades, the citizen scientists from LACA have been collecting and testing water at Lake Anna, monitoring trends, and keeping homeowners informed. After the first observed HAB in 2018, the Lake Anna Advisory Committee (LAAC) hired a testing company to assess metrics related to lake health and HABs (Solitude Lake Management, 2022; LACA, 2024). As a partner in the effort, LACA contributed funding to the study. Between fiscal years 2020 to 2024, LACA raised over \$200,000 to fund various technological demonstrations aimed at mitigating the growth of cyanobacteria populations. In 2021, LACA also funded an extensive study on Lake Anna that included cyanobacteria identification. During a HAB event, the dominant species identified were *Pseudoanabaena*, *Raphidiopsis*, and *Planktolyngbya* (Solitude Lake Management, 2022).

A major knowledge gap in HAB research, both at Lake Anna and more broadly, is the direct correlation between specific toxins and their associated taxa during bloom events. Confirming specific toxin production in a controlled laboratory setting could lead to more reliable methods for identifying the cyanobacteria responsible for ATX-a production (Mahmood and Carmichael, 1987; Furey et al., 2003). This challenge is consistent with broader calls from Burford et al. (2020), who emphasize the urgent need to refine our understanding of cyanoHABs in the context of global climate change. Their perspective highlights the high physiological flexibility of cyanobacteria in response to environmental shifts and the importance of studying them across scales, from molecular mechanisms to ecosystem dynamics. They note that current predictive models often fail to account for the complex interactions between rising temperatures and water column stratification, both of which can significantly influence cyanobacterial behavior. Process-based research, combined with high-resolution monitoring data and data assimilation techniques, could improve forecasting and strengthen early-warning systems. These tools would be directly applicable to managing blooms at Lake Anna.

In parallel, Carey et al. (2025) present a comprehensive framework identifying five key challenges in developing real-time phytoplankton bloom forecasting systems, including predictive modeling, cyberinfrastructure, uncertainty quantification, co-production of decision-support tools, and forecasting training. Addressing these bottlenecks is critical for translating scientific advances into operational tools for water managers. For lakes like Lake Anna, integrating real-time in situ and satellite-derived data into cloud-based systems could enable ensemble model execution and uncertainty analysis, with results informing

timely management interventions. Long-term sustainability of such systems would require not only cross-disciplinary collaboration but also the kind of community engagement and funding continuity that LACA has already demonstrated.

Christensen et al. (2024) outline a USGS strategic science vision for HAB research that aligns closely with both the scientific and community-based priorities at Lake Anna. Their framework identifies short- and long-term opportunities for understanding environmental drivers, toxin production mechanisms, and exposure pathways, while advancing predictive modeling and mitigation strategies. The emphasis on interdisciplinary collaboration, stakeholder engagement, and capacity building mirrors the existing partnerships among LACA, LAAC, and state agencies, and provides a roadmap for scaling these efforts regionally and nationally.

In 2022, the Virginia General Assembly allocated \$3.5 million to the Virginia DEQ for a four-year study investigating the sources of HABs in both the Shenandoah River and Lake Anna. Additionally, LACA played a key role in securing \$1.5 million in funding from the Virginia state budget for Lake Anna for fiscal years 2024 and 2025. This funding, overseen by the LAAC, will support efforts to remediate excess phosphorus levels in areas of the lake that have experienced recurring cyanobacteria blooms since 2019. In parallel, the Virginia General Assembly appropriated \$150,000 in 2024 to support a collaborative study at Smith Mountain Lake (SML) led by the DEQ, Virginia Tech, and the Smith Mountain Lake Association. The report submitted for the first year of funding developed a comprehensive framework for HAB assessment at SML (Walker et al., 2025). The project integrated over 62,000 DEQ water-quality records (1994–2024), analyzed long-term nutrient and land-use data, and employed SWAT watershed modeling to evaluate phosphorus and sediment inputs. The study's recommendations, ranged from nutrient control upgrades and erosion mitigation to educational outreach and deployment of real-time monitoring buoys. This report provides a valuable model for lake management and interagency collaboration that could be adapted to other reservoirs. Another successful example of citizen science in freshwater monitoring is the Lake Erie Volunteer Science Network (LEVSN), which manages 184 monitoring locations around the lake (Cleveland Water Alliance, 2014). LEVSN collaborates with the Cleveland Water Alliance to share best practices, test new technologies, and standardize efforts across jurisdictions. Local organizations engage community volunteers in monitoring water quality and addressing key water resource concerns. These organizations include watershed experts, park systems, conservation districts, and government agencies (Cleveland Water Alliance, 2014). One such group, the Huron River Council, has played a critical role in developing standardized procedures and advocating for shared water quality data for informed environmental decision-making (Baxter, 2024).

Our findings highlight the need for continued and expanded ATX-a monitoring in Lake Anna, VA to better inform water quality experts and community members (Wiley and McPherson, 2023; Reid et al., 2024). Future research, combined with increased funding and public engagement (OneHealth, 2020; Sha et al., 2021; Stević et al., 2025), will be crucial in mitigating the impact of HABs on the Lake Anna community. Reliable funding for ongoing lake sampling and toxin testing would allow for more comprehensive analyses, helping to establish long-term trends in lake health. Such trends, whether for Lake Anna or other freshwater lakes, could provide valuable insights for citizens and stakeholders on the effects of funding and management practices over time. The community-based model in use at Lake Anna could be applied in other regions facing a range of environmental threats. Strong communication between groups, effective monitoring strategies, and proactive funding efforts can help communities build resilience against challenges such as watershed pollution, flooding, and wildfires.

Conclusion

Our study highlights the significant presence of ATX-a in Lake Anna, VA, particularly in the northern tributaries, where higher concentrations of this neurotoxin have been consistently observed during the warmer summer months. The five-year dataset provides crucial insight into the temporal and spatial distribution of ATX-a, underscoring the need for continued and expanded monitoring efforts across the lake. Despite some limitations in sampling frequency and method sensitivity, the results indicate that not only are PTOX cyanobacteria present in the lake, the neurotoxin, ATX-a is present and this warrants ongoing vigilance. Future research, coupled with increased funding for water quality testing and public engagement, is essential to develop a more comprehensive understanding of the drivers of HABs in Lake Anna and to establish effective management strategies. The support from local stakeholders, including the LAAC and the Virginia General Assembly, will play a pivotal role in mitigating the impact of HABs on the lake's ecosystem and the health of its surrounding communities.

Declarations

Ethics Approval.

All authors have read, understood, and complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors.

Competing interests.

HL is a member of the Lake Anna Civic Association and the Lake Anna Advisory Committee. Research was conducted without financial or commercial relationships. The remaining authors declare no conflict of interest.

Clinical trial number.

Not applicable.

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Author Contribution

RH and DS planned the field experiments for Lake Anna. RH, HL, JR, HG, and DS participated in field campaigns. HL, MS, JT, MM, and LS contributed to compilation of data for Lake Anna. JB and JW performed LC-MS/MS cyanotoxin analysis. RH coordinated sample compilation and managed analysis. RH and DS lead the writing of the manuscript. All authors provided manuscript feedback.

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Data Availability

The authors confirm that the data supporting the findings of this study are available in an online data repository. Seven CSV files were deposited in the repository at VtechData on December 8, 2025 (<https://data.lib.vt.edu/>), Virginia Tech's institutional repository for research data. This repository offers DOI assignment and complies with journal standards. These data can be found at the private link here: [<https://figshare.com/s/43cc93fadc56ca849631>] (<https://figshare.com/s/43cc93fadc56ca849631>)

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Figures

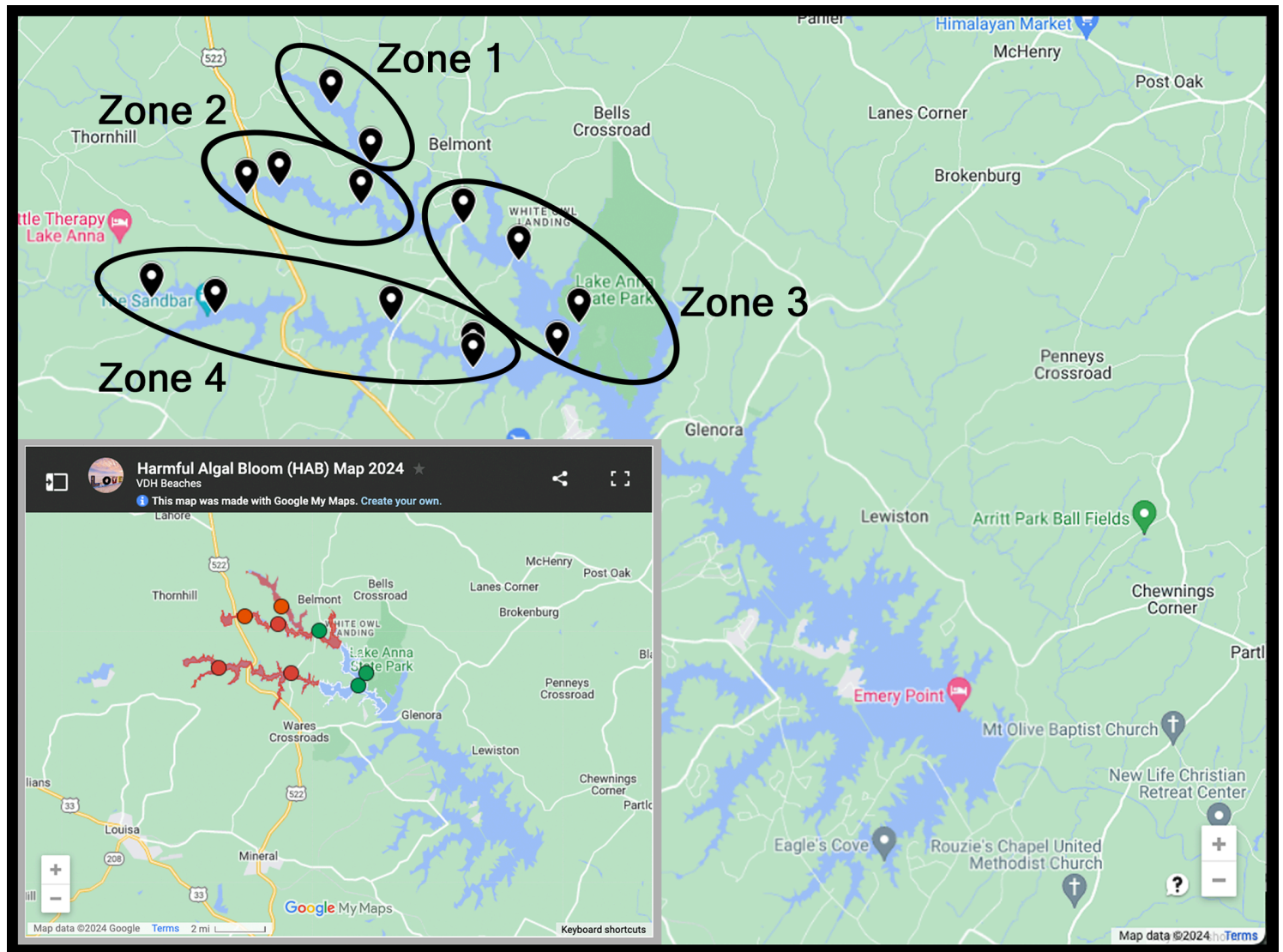


Figure 1

Map of Lake Anna, Virginia showing 14 water collection sites as black and white pins (Google maps, 2024). Zone 1 was located in the Terry's Run branch. Zone 2 was in the Upper Pamunkey branch. Zone 3 included Mid and Lower-Pamunkey, and Upper Lake Anna sites, including the State Park Beach (SPB) and "the splits" (DY). Zone 4 included sites along the North Anna branch. The inset shows the VDH Beaches HAB map for Lake Anna on Sept. 12, 2024. The red circles denote testing sites that had high PTOX counts or toxins above safe levels. The red areas of Lake Anna show the areas with an Advisory in Effect for Sept. 2024. The green circles represent testing areas that were below advisory thresholds.

A. Schmale Lab
Surface water collections



B. LACA
Subsurface collections at 0.3 m



Figure 2

Images showing a drone surface water collection (A) and a water sample collection by hand from 0.3 m below the surface of the lake (B).

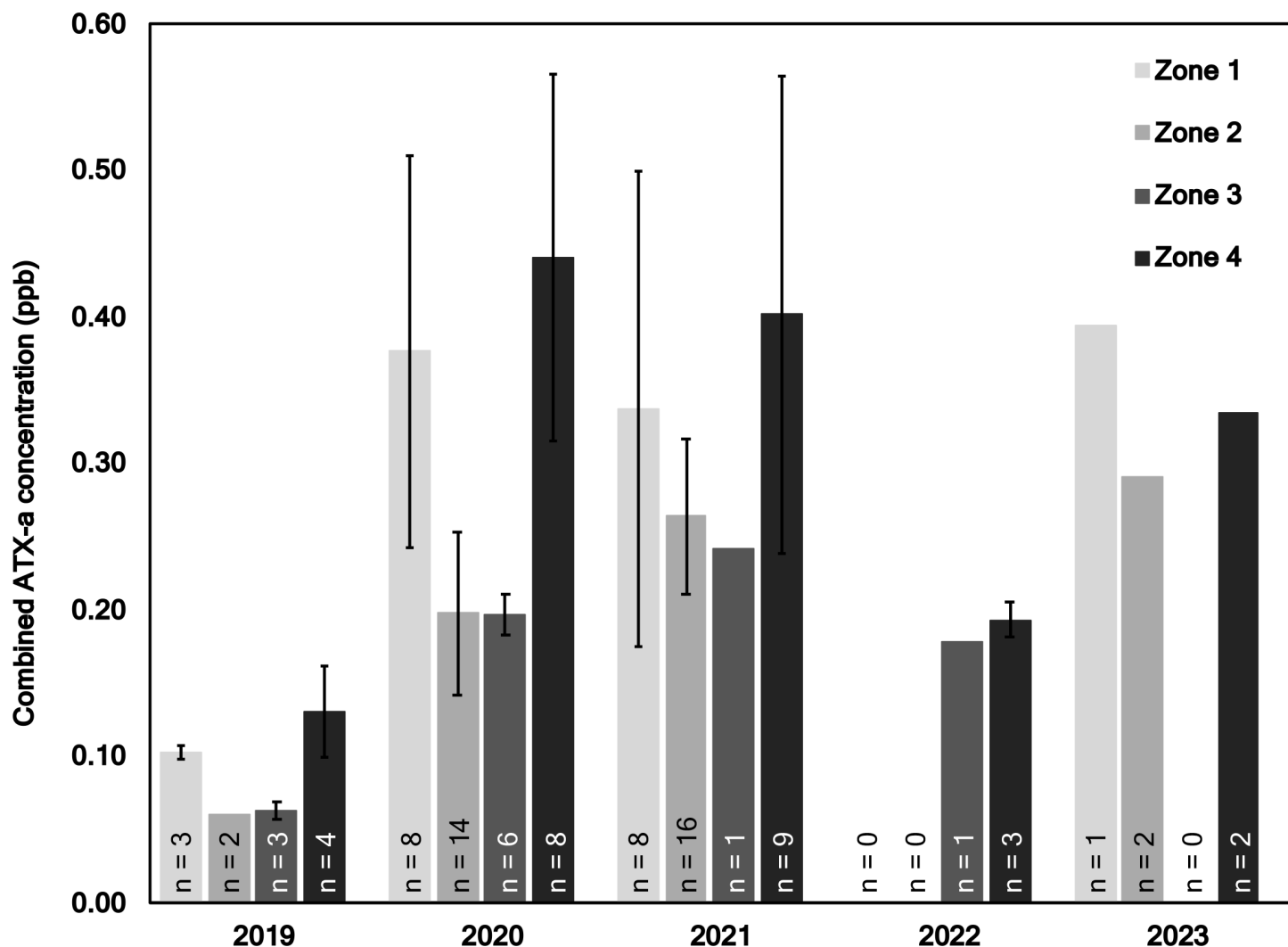


Figure 4

Histogram chart with average ATX-a concentration on the y-axis and lake zones, grouped by year, on the x-axis. For each year, ATX-a concentrations determined by both LC-MS/MS (LOD 0.050 ppb) and the Abrixis ELISA (LOD 0.15 ppb) were combined and shown in grayscale with lake zones 1 to 4 represented by progressively deeper shades of gray, respectively. Lake Zone 1 refers to two sampling sites in the upper branch of Terry's Run. Lake Zone 2 includes 3 testing locations in the Upper Pamunkey Brach. Lake Zone 3 includes a location in Mid-Pamunkey, Lower-Pamunkey, the State Park Beach, and the splits testing site. Lake Zone 4 is comprised of 5 sampling sites along the North Anna Branch. Standard error of the mean is shown for sample set values of 3 or higher. The sample set total is shown as the n value in each zone across the five years.

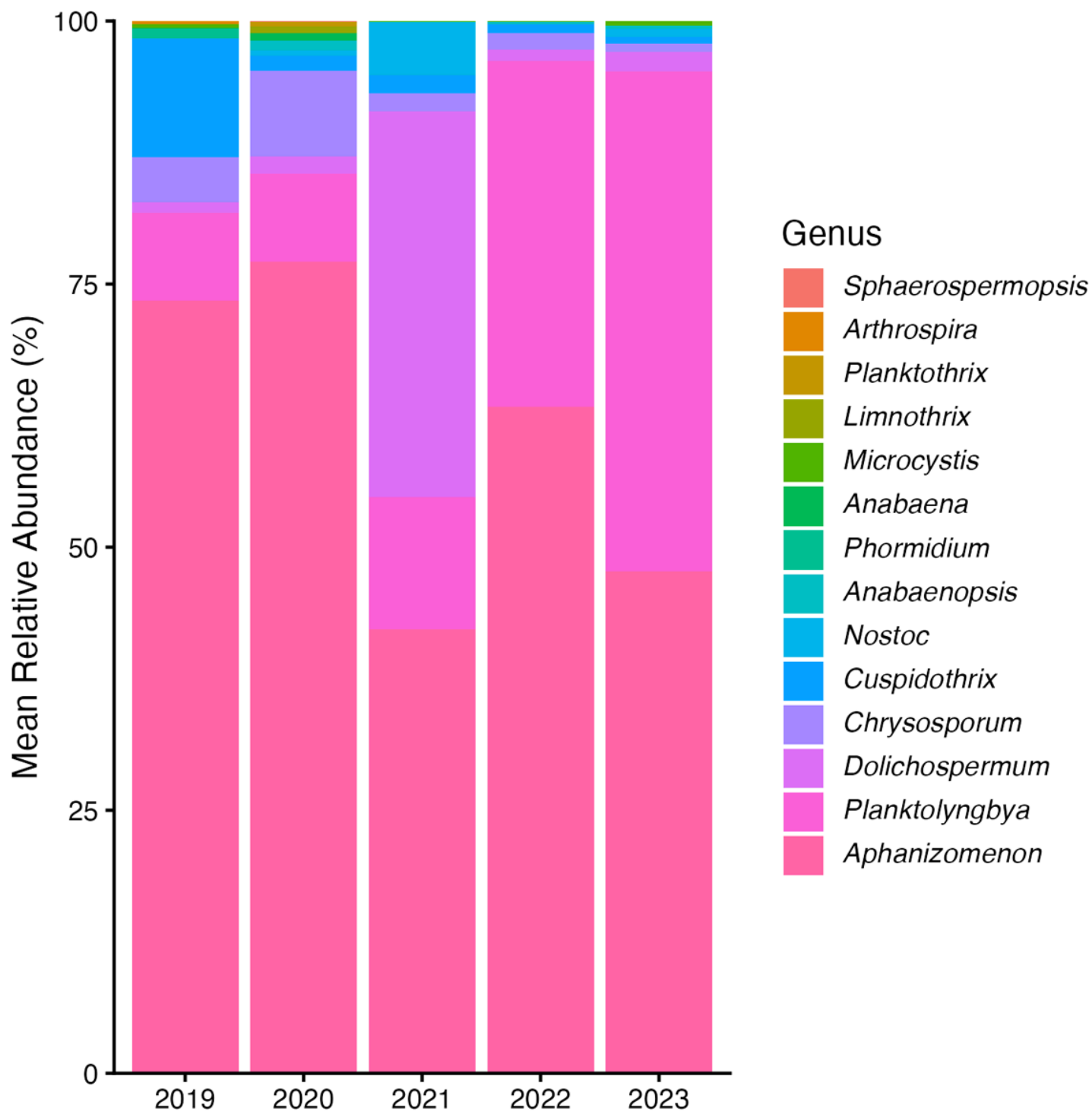


Figure 5

Stacked bar chart for targeted potentially toxigenic (PTOX) cyanobacteria cell counts as reported by VDH. Collection of water samples was carried out by DEQ and cyanobacteria counts were performed at ODU. Cell density counts for each year were combined at the level of genus and shown as relative abundance of the total for each year. *Aphanizomenon* and *Planktolyngbya* were the most abundant with 60.8% and 21.9% relative abundance. The other genera in descending order were: *Dolichospermum*, *Chrysosporum*, *Cuspidothrix*, *Nostoc*, *Anabaenopsis*, *Phormidium*, *Anabaena*, *Microcystis*, *Limnothrix*, *Planktothrix*, *Arthrospira*, *Sphaerospermopsis*.

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