TAHOE HAB PRIVATE CITIZENS' GROUP

January 4, 2023

To: Office of Environmental Health Hazard Assessment (California) – Rebecca Stanton, Lahontan Waterboards Region 6 – Mary Fiore Wagner, Russell Norman, Robert Tucker, TRPA – Dennis Zabaglo, Paul Nielsen, Kimberly Chevallier, Shay Navarro, Julie Regan, El Dorado County Public Health Officer – Dr. Nancy Williams, Placer County Interim Health Officer – Dr. Rob Oldham, Washoe County Director of Health Department – Nancy Diao

Regarding: Transmittal of a technical report by the Ecotoxicology Laboratory, of the University of New Hampshire, A Preliminary Investigation of Cyanobacteria Toxins in Lake Tahoe Water and Aerosols, (Lake Tahoe Cyanotoxin Report: Summer 2022)

The increasing incidence of toxic cyanobacteria in lakes has become a concern for pets, wildlife, and humans worldwide. The detection of cyanobacteria cells and cyanobacteria toxins in the aerosols emitted from freshwater lakes has raised questions about the role of aerosols in the transport of cyanotoxins to the environment as well as their potential health risk. Despite this, the levels of cyanobacteria toxins in the Lake Tahoe water and the surrounding air are not monitored regularly if at all. The attached study was initiated at the request of a private citizens' group, concerned about possible health risks associated with cyanobacteria at Lake Tahoe and the Serene lakes.

The specific project goals were:

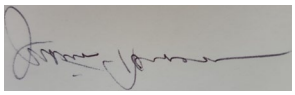
1. Determine whether detectable levels of three cyanotoxins, i.e., microcystins (MCs), anatoxin-a (ATX), and β-N-methylamino-L-alanine (BMAA) are present in samples of lake water and aerosols.
2. Estimate the cyanotoxin levels in lake water using two size fractions, unfiltered whole lake water and filtered through a 50 μm (micron) mesh.
3. Measure cyanotoxins in lake aerosols employing two methods.

In addition to the well documented threat to pets, wildlife and humans from cyanobacteria, studies now suggest links to neurological disorders, such as ALS. Cyanobacteria blooms are well documented in Lake Tahoe confirming that a threat exists. The findings of this study are limited in both scope and time. They represent a snapshot of cyanotoxins in the lake water and in the air during late summer at two selected sites in Lake Tahoe. Follow-up research is needed to better identify and understand the producers of the observed toxins and the ongoing and real threat to the environment at Lake Tahoe.

For additional information or discussion, please contact Trish from the Public Facebook Library page: Tahoe Area ALS/MND Research Library where you can find over 105 research articles on the connection between toxic cyanobacteria blooms and neurodegeneration. You can also reach out via e-mail: TahoeHAB@gmail.com or by phone: 775.301.7567.

Sincerely,

Trish Elise Jett Ron Grassi



Tobi Tyler

Julie K.

Tahoe HAB Private Citizens' Group
Lake Tahoe, California
Attachment: Lake Tahoe Cyanotoxin Report: Summer 2022, Photographs of Weeds around Lake Tahoe

A Preliminary Investigation of Cyanobacteria Toxins in Lake Tahoe Water and Aerosols

Lake Tahoe Cyanotoxin Report: Summer 2022

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Introduction

The increasing incidence of toxic cyanobacteria in lakes has become a concern for pets, wildlife, and humans worldwide (Paerl et al., 2011). The detection of cyanobacteria cells and cyanobacteria toxins in the aerosols emitted from freshwater lakes (Murby & Haney, 2015) and the Baltic Sea (Lewandowska et al., 2017) has raised questions about the role of aerosols in the transport of cyanotoxins to the environment as well as their potential health risk (Stommel et al., 2013). Although toxic cyanobacteria are often associated with high nutrient levels and surface blooms, cyanobacteria and their toxins are surprisingly present in lakes with a wide range of trophic conditions including clear, oligotrophic lakes (Langley, 2019; McQuaid, 2019). Clusters of persons diagnosed with ALS have been correlated with levels of cyanobacteria (Caller et al. 2008; Torbick et al. 2018), suggesting a possible link between cyanotoxins and neurological disorders.

Lake Tahoe is well known for its extremely clear water, although water transparency has been declining due to gradual eutrophication. A recent increase in atmospheric deposition of nutrients rich in nitrogen has also caused increased growth of picoplankton in the lake (Mackey et al. 2013). However, the levels of cyanobacteria toxins in the Lake Tahoe water and the surrounding air are not monitored regularly. The present study was initiated at the request of a

private citizen's group, concerned about possible health risks associated with cyanobacteria at Lake Tahoe and the Serene lakes.

1. **Specific Project Goals**:

    1) Determine whether detectable levels of three cyanotoxins, i.e., microcystins (MCs), anatoxin-a (ATX), and β-N-methylamino-L-alanine (BMAA) are present in samples of water and lake aerosols collected from two sampling locations in Lake Tahoe (see Figure 1 for location of the sampling sites). Additional water samples were also collected from sites in the Tahoe Keys, as well as from the nearby Serene Lakes.

    2) Estimate the cyanotoxin levels in lake water using two size fractions, i.e., unfiltered whole lake water (WLW) and WLW filtered through a 50 μm (micron) mesh (<50 μm). The unfiltered WLW includes the large cyanobacteria that frequently form surface blooms, whereas the <50 μm fraction includes the smallest cyanobacteria (picocyanobacteria or PCY) that are most easily aerosolized and transmitted through the air because of their small size. Thus, a comparison of toxin concentrations in the two water size fractions can help identify the type of cyanobacteria that are most important in toxin production in a particular lake.

    3) Measure cyanotoxins in lake aerosols employing two methods. First, in July, we collected aerosol samples directly from the two Lake Tahoe collection sites using

the in situ Compact Lake Aerosol Monitor, i.e., CLAM (Carter, 2022). A second aerosol testing was carried out under controlled conditions in the laboratory with water samples collected from Lake Tahoe in August that were refrigerated and shipped overnight to the Ecotoxicology Laboratory at the University of New Hampshire in Durham, NH. The in-laboratory assay has two major benefits. First it allows for the testing of aerosols from a simple water collection requiring minimum sampling equipment or training. Secondly, testing the lake water under laboratory conditions, eliminates many of the uncontrolled variables of field aerosol collections, such as wind, temperature, and humidity. It is a standardized method of aerosol testing that is influenced primarily by water quality conditions and does not reflect the on-lake conditions at the time of collection, such as meteorological conditions. The lab aerosol assay is likely a conservative estimate of aerosolized toxins, as it does not include possible wind-generated aerosols that may also occur during windy conditions.

METHODS

Study Sites

Lake Tahoe is a 49,469-hectare (122,240-acre) freshwater lake with a maximum depth of 501 meters or 1,645 ft (USDA Forest Service Lake Tahoe Basin Mgt Unit, 2022). The surface elevation of this oligotrophic, alpine lake is 1,897 meters (6,225 ft).

This research was carried out in 2022 at two main sites on Lake Tahoe, with Tahoe Keys Site 1 located at Monterey Drive and Site 2 located at Valhalla Pier (Figure 1). On-site aerosol and water collections took place at Site 1 and Site 2 on July 19th and 20th, respectively. Grab samples taken just below the water surface were also collected in July from two additional sites in the Tahoe Keys (July 20th). Grab samples were also collected on July 21 from four sites at the Serene Lakes (Figure 1 & 2), two interconnected lakes located approximately 28 km northwest of Lake Tahoe at an elevation of 2,106 m (6,910 ft), On August 2nd, water was collected from both the Tahoe Keys Site 1 and the Valhalla Pier (Site 2) and was shipped

refrigerated overnight to Durham, NH where aerosol collection and water sampling was conducted in the lab on August 3rd.

Figure 1 Map of South Lake Tahoe with sampling locations labeled with letters corresponding to the site numbers listed in Table 1 (Google Maps, 2022b)

Figure 2 Map of Serene Lakes with sample locations labeled with letters corresponding to the site numbers listed in Table 1. Map shows Lake Serena on the left and Lake Dulzura on the right.(Google Maps, 2022a)

Table 1 List of all sampling sites at Lake Tahoe and at Serene Lakes

Full Name and Site #	Address	Coordinates	Nickname	Samples collected
South Lake Tahoe Keys (Site #1)	Monterey Drive	38°55'54.4"N 120°00'25.3"W	Tahoe Keys	Air & Water
Valhalla Pier at Camp Richardson (Site #2)	Jameson Beach Road	38°56'25.4"N 120°02'21.5"W	V. Pier	Air & Water
Tahoe Keys (Site #3)	White Sands	38°56'08.2"N 120°00'42.3"W	Keys Site 2	Water
Tahoe Keys (Site #4)	Aloha Drive	38°56'05.7"N 120°01'01.3"W	Keys Site 3	Water
Serene Main (Site #5)	Island Way & Serene Road	39°17'53.5"N 120°23'10.9"W	Lake Serena	Water
Serena Creek (Site #6)	Serene Road	39°17’39.6”N 120°22’59.8”W	Lake Dulzura 1	Water
Serene Public Dock (Site #7)	Sierra Road	39°17'58.6"N 120°22'53.2"W	Lake Dulzura 2	Water
Lake D. Storm Drain (Site #8)	Island Way & Serene Road	39°29'76.3"N 120°38'50.9"W	Lake Dulzura 3	Water

Field Aerosol Collection

Aerosols were collected using a modified version of the University of New Hampshire Center for Freshwater Biology’s Compact Lake Aerosol Monitor (CLAM) (Figure 3). The basic design of the unit is based on a filter-collection aerosol device described by Murby & Haney (2016) and later modified by Langley (2019). Additionally, the CLAM used in the present study contained three in-tandem water traps to capture toxins that were not retained on the filter. Operationally, a portable air pump (Gillian BDX-II Air Sampler, Sensidyne, LP, Clearwater, FL) in the CLAM draws air from the surface of the water through a funnel and wind screen to minimize wind effects. Air passes into a system of 2 mm diam (ID) Tygon tubing and through a

Whatman GFF 25 mm diameter glass fiber filter to collect airborne particulates. Before use, the GFF filters were rinsed with 15 mL of Milli-Q water then combusted at 500 ˚C for 1 hour, resulting in sterilization and a reduction of the effective pore size from 0.6 to 0.3 μm (Nayar & Chou, 2003). Toxins retained on the GFF filter were operationally defined as "particulate" toxins.

After passing through the GFF filter, the air was bubbled via stainless-steel air diffusers (pore size 2 μm) through a series of three in-tandem traps, made from 60 mL Luer lock syringes, each containing 17 mL of Milli-Q water. Toxins retained in the traps were operationally defined as "dissolved" toxin under the assumption that the prefiltered air contained primarily extracellular toxins small enough to pass through the 0.3 μm filter. Milli-Q water was used as the trap solvent because microcystins (MC), β-Methylamino-L-alanine (BMAA), and anatoxin-a (ATX) are water-soluble molecules. Each CLAM collected triplicate samples, with three GFF filters, three sets of liquid traps, and three independent pumps. Air pumps collected at a flow rate of 1 L min^{-1} for 4 h, sampling approximately 0.24 m^3 air per collection. Immediately following the collection period, GFF filters and water from the liquid traps were removed, put on ice during transportation, and frozen at -20 °C within 8 hours of collection. Samples remained frozen until analyzed.

Figure 3. Compact Lake Aerosol Monitor (CLAM) set up on a temporary platform during a collection. Funnels and wind screens are positioned directly on the surface of the water. The three sets of filters, liquid traps, and pumps are inside the CLAM box.

In-Lab Aerosol Collection

Aerosol collections were carried out in the lab for the second round of sampling. Lake water was collected from the surface of the water at the same two sites at Tahoe Keys Site 1

(Monterey Drive) and Valhalla Pier (Site 2). The water was put on ice and shipped overnight to Durham, NH. Once in the lab, the water was poured into 3 individual 1 L flasks. Each flask was connected to the same filter, trap system, and pump as described in the field aerosol collection description. Each flask had an inlet filter (0.22 μm PTFE membrane filter) to filter the air entering the flask from the lab. Aerosol pumps were set at 1 LPM and collections ran for 4 h in a temperature-controlled fume hood at 20 °C.

Water Sampling for Toxins and Pigments

At the two main Tahoe sites (Tahoe Keys Site 1 and Valhalla Pier), water fractions collected included unfiltered whole lake water (WLW) and < 50 μm (Figure 4). At each sampling site, a 60 mL sample of surface WLW was collected and mixed thoroughly using a PETG Nalgene bottle. Bottles were frozen and shipped overnight to Durham, NH. Once at the lab in Durham, NH, samples were thawed and mixed thoroughly. From that bottle, 30 mL were collected as a WLW sample, unfiltered lake water that includes all planktonic organisms present in the water. An additional 30 mL was passed through a 53 μm mesh Nitex net to remove large debris and bloom-forming cyanobacteria. The filtrate (30 mL) was collected as a < 50 μm sample. This entire process was repeated for triplicates. At all additional sites described above, a single surface grab of WLW was collected in PETG Nalgene bottles. All samples were frozen at -20 °C and remained frozen until processed.

The accessory cyanobacteria pigments, phycocyanin and phycoerythrin were measured with a handheld fluorometer (Aquafluor, Turner Instruments Inc., San Jose, CA) on the two water fractions (WLW and < 50 μm). Phycocyanin, an accessory pigment to chlorophyll, is a blue-green protein pigment that is characteristic of cyanobacteria. Phycoerythrin is a red assessory pigment present in many cyanobacteria taxa and often associated with the

picocyanobacteria. Assessory pigments can indicate the presence of cyanobacteria and be used as surrogates for cyanobacteria biomass (Leland & Haney, 2018) as well as correlated with the concentration of cyanobacteria toxins (Leland et al., 2019). The Aquafluor fluorometer was calibrated in the lab using purified phycocyanin and phycoerythrin. The handheld fluorometer excitation wavelengths were 595 nm for phycocyanin and 545 nm for phycoerythrin.

All samples were subjected to a single freeze-thaw cycle (Leland & Haney, 2018) and brought to room temperature (20-25 °C) before fluorometric measurements were taken. Phycocyanin and phycoerythrin in water and aerosol samples were measured prior to sample concentration (see Toxin Analysis).

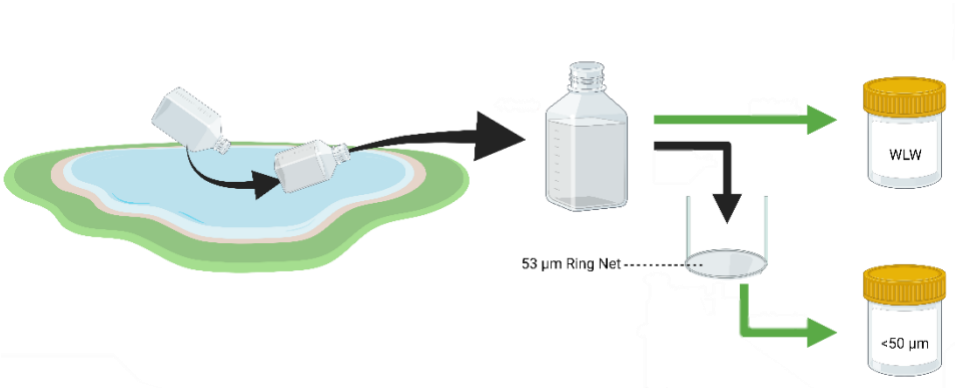

Figure 4. Flowchart showing the procedure for collecting water samples (WLW and < 50 µm) as described above. Created with BioRender.com, modified from thesis (Carter, 2022).

Environmental Parameters

Water temperature and air temperature were determined at all sites on each sampling date. Temperatures throughout the 4 h collection periods were measured every 10 min with HOBO data loggers (ONSET, Bourne, MA) Additionally, wind direction was recorded during field aerosol collections.

Toxin Analysis

All aerosol samples and water samples were tested for microcystins (MC), anatoxin-a (ATX) and β-Methylamino-L-alanine (BMAA), using the ELISA (enzyme-linked immunosorbent assay) method. The ELISA is a sensitive clonal antibody method that is specific to MC (QuantiPlate Kit for detection of Microcystin - High Sensitivity, EnviroLogix Inc, Portland, ME), BMAA (BMAA, ELISA, 96-test, Eurofins Abraxis Inc., Warminster, PA) and ATX (Anatoxin-a (VFDF), ELISA, 96-test, Eurofins Abraxis Inc., Warminster, PA). Prior to performing the ELISA test, samples were processed and concentrated with SpeedVac concentrators to bring low-toxin samples to the limit of detection of the ELISA tests. Throughout processing and concentration, samples were repeatedly weighed to calculate final concentration factors. Sample concentrations ranged from 5-145x, depending on the type of sample.

Aerosol filters were processed by cutting each filter individually into 12 equal slices using scissors and tweezers that were cleaned between each sample using 70% ethanol and Milli-Q water. Each filter was placed in a 2.0 mL microcentrifuge tube and 1.8 mL of Milli-Q water was added. Toxins were then extracted from the filters using three freeze- thaw-vortex-sonicate (FTVS) cycles to rupture the cell walls and release intracellular toxins. For each FTVS cycle, microcentrifuge tubes were frozen at -20 °C, then placed in a 40 °C water bath to thaw. Fully thawed samples were vortexed for 10 s (Vari-Whirl Mixer, level 6, VWR Scientific, Radnor, PA) and sonicated for 3 min (Ultrasonic Bath CPX/CPXH series. Thermo Fisher Scientific, Waltham, MA). The liquid portion of the samples was then transferred into a new 1.5 mL microcentrifuge tube without the filters. To remove any remaining filter material from the liquid samples, the 1.5 mL microcentrifuge tubes were centrifuged for 3 min at 10,000 RPM (Gusto Mini Centrifuge, Vernon Hills, IL) and the supernatant was carefully removed, avoiding filter debris, and placed

into a new microcentrifuge tube. Repeating this step once more ensured that the maximum volume of liquid sample was collected free of any remaining filter debris. Extracted toxin samples were then concentrated to 0.35 mL using speed vacuums (Thermo Fisher Scientific™ Savant™ SpeedVac™, and Savant Speedvac Concentrator Sc100 Centrifugal Evaporator, Thermo fisher Scientific, Inc., Waltham, MA) resulting in a concentration factor of ~5x. Samples were stored at -20 °C until toxin analysis.

Immediately following the 4-h aerosol collection, the water from the three liquid aerosol trap samples (approx. 17 mL) in each CLAM unit were transferred to three 20-mL PET clear plastic vials. The CLAM trap samples were processed in the lab with three repetitions of the same FTVS cycle as described above. The ~17-mL water trap samples were concentrated to 1 mL using the speed vacuum systems described above. Each set of three individual trap samples (1 mL each, originating from the same trap system) were then combined to form a single replicate, 3-mL sample. Each 3-mL sample was then further concentrated to a final volume of 0.35 mL, resulting in a final concentration of ~145x. Exact concentration factors for each sample were calculated gravimetrically. Concentrated trap samples were stored in 1.5 mL microcentrifuge tubes at -20 °C until toxin analysis.

Water samples previously thawed for fluorometry were refrozen in 20 mL vials. These samples were processed with the three FTVS cycles described above. Water samples were then concentrated using the above speed vacuums from 18 mL to 1 mL. Samples were transferred into 1.5 mL microcentrifuge tubes and concentration was continued to a final volume of 0.35 mL or ~51x and stored at -20 °C until toxin analysis.

In final preparation for the ELISA testing, all processed samples were thawed, vortexed and centrifuged (3 min, 10,000 RPM) to remove remaining particulate matter and minimize

solids in the ELISA test. ELISA tests for MC, ATX and BMAA were run on the same concentrated sample. ELISA testing followed the test procedures supplied by each of the test manufacturers. Optical densities of all samples and standards were measured using an 800TS Microplate Reader and Gen 5 Microplate Reader and Imager Software (Agilent Technologies, Winooski VT) with a wavelength of 450 nm. Standards provided in the ELISA kits were included in the toxin analysis. Standard curves with optical densities versus toxin concentrations were fitted with a four-parameter logistic equation. Toxin concentrations were based on the estimates from the standard curves. All standard curves had adjusted $R^2 > 0.99$. Final toxin concentrations were adjusted for the SpeedVac concentrations and for the volume of air sampled.

Statistical Analysis

Data were recorded and managed in Excel. Graphs were created with SigmaPlot 12.5 (SYSTAT Software Inc., Chicago, IL). Statistical analyses were conducted in SigmaPlot 12.5 and JMP (SAS Institute Inc., Cary, NC). Analyses in SigmaPlot 12.5 included one-way ANOVA and All Pairwise Multiple Comparison Procedures using Tukey's Post Hoc tests that were used to identify significant differences in toxicity between different dates and locations within sample types. Data that failed normality or equal variance were reanalyzed using the non-parametric Kruskal-Wallis One-Way ANOVA on Ranks.

RESULTS

Table 2 Aerosol collection details and environmental information. Site numbers correspond to those in Table 1. Time of collection is in PST for field collections on July 19 and July 20 and EST for in lab collections on August 2.

Date of Aerosol Collection	Location and Site #	Time of Collection	Type of Collection	Air Temp Avg (°C)	Water Temp Avg (°C)	Wind Direction
19-Jul	Tahoe Keys (Site 1)	10:19 a- 2:20 p	Field	37.7	27.6	S
20-Jul	Valhalla Pier (Site 2)	10:30 a- 2:26 p	Field	39.1	21.6	S/SW
2-Aug	Tahoe Keys (Site 1)	10:30 a- 2:30 p	In Lab	20.9	20.1	N/A (in lab)
2-Aug	Valhalla Pier (Site 2)	10:25 a - 2:25 p	In Lab	20.9	20.1	N/A (in lab)

Accessory Pigments

Phycocyanin (PC) was detected at the Tahoe Keys and average PC increased between July and August (Figure 5). PC was not detected at the Valhalla Pier on either collection date. Phycoerythrin (PE), a pigment often associated with picocyanobacteria (cyanobacteria generally defined by their small size, <2 μm), was detected at both the Tahoe Keys and the Valhalla Pier in July and in August (Figure 6). The highest average levels of PE were measured at the Tahoe Keys in July and decreased between July and August. The lowest average levels of PE were measured at Valhalla Pier in July.

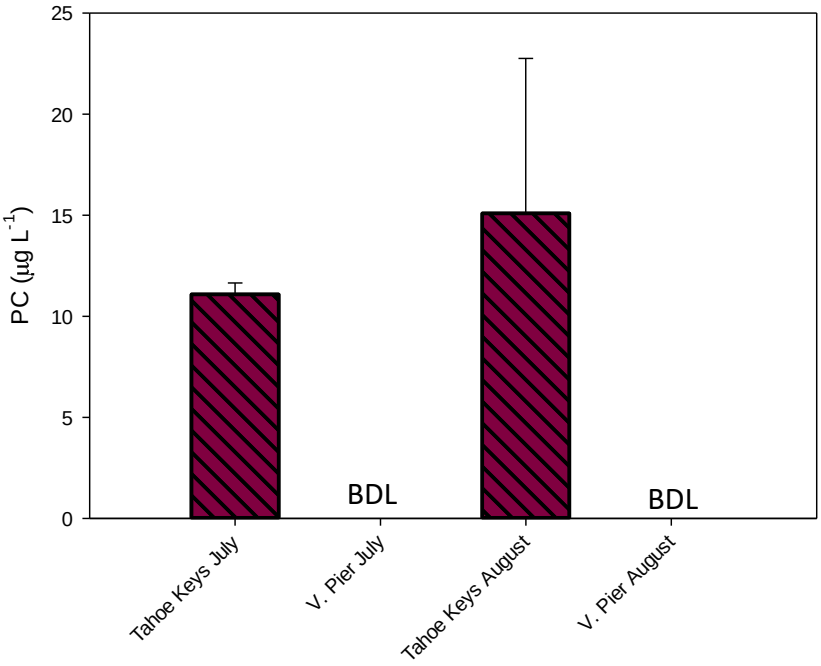

Figure 5 Average PC (µg L^{-1}) detected in whole lake water (WLW) samples at the Tahoe Keys Site 1 and Valhalla Pier in July and August 2022. Samples at Valhalla Pier were below the detectable limit. Error bars represent ± 1 standard error of the mean.

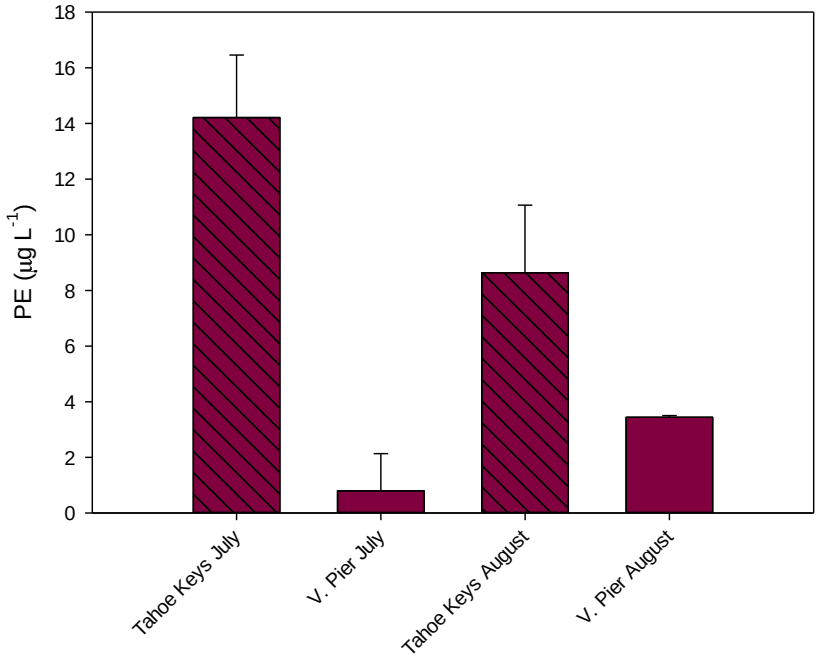

Figure 6 Average PE (µg L^{-1}) detected in whole lake water (WLW) samples at the Tahoe Keys (Site 1) and Valhalla Pier (Site 2) in July and August 2022. Error bars represent ± 1 standard error of the mean.

Cyanotoxins

Microcystins (MC) in Lake Water

MC concentrations in the water at the two main sites (Tahoe Keys Site 1 and Valhalla Pier) ranged from 1.25 - 3.81 ng MC L^{-1} (Table 3), concentrations that are below the currently recommended notification level of 30 ng MC L^{-1} in California (Notice May 3, 2021, California OEHHA). There were no detectable MCs in either water fractions at Valhalla Pier in the July or August samples. However, visible bloom-like material collected in the water near the shoreline at Valhalla Pier in July had an MC concentration of 16.89 ng MC L^{-1}. MC concentrations at the Tahoe Keys Site 1 in July was associated with the small <50 µm fraction, indicating the dominant MC-producing cyanobacteria at that time were not large colonial or filamentous forms. MC increased in both water fractions between July and August, however only the small-sized fraction (<50 µm) increased significantly ($p = 0.038$, Figure 7) suggesting the increase in MC concentration in August was likely due to small non-bloom-forming cyanobacteria.

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Table 3 Mean MC concentrations in the whole lake water (WLW) and the <50 µm water fractions ± SE. BDL represents samples with toxin concentrations that could not be estimated as they were below the detectable limit.

MC Concentrations (ng MC L^{-1}) ± SE			
Date (2022)	Location	WLW	<50 µm
19-Jul	Tahoe Keys Site 1	1.36 ± 0.62	1.25 ± 0.07
20-Jul	Valhalla Pier	BDL	BDL
2-Aug	Tahoe Keys Site 1	3.81 ± 0.87	1.82 ± 0.12
2-Aug	Valhalla Pier	BDL	BDL

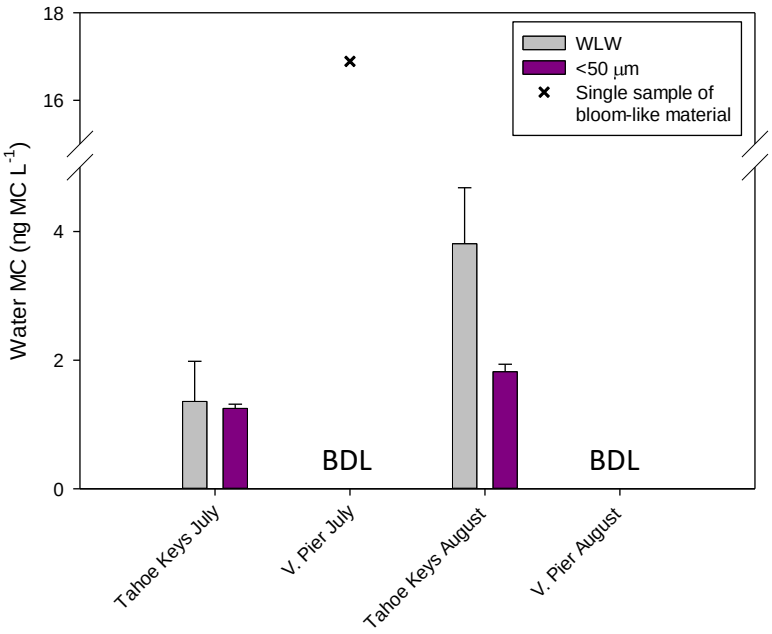

Figure 7 Average MC (ng MC L^{-1}) detected in water samples in 2022. This includes both unfiltered whole lake water (WLW), and water passed through a <50 µm mesh filter. There were no significant differences in MC concentrations in the WLW between any dates or locations (p = 0.206). Between the July and August sampling dates, MC concentration in the <50 µm fraction increased significantly at the Tahoe Keys Site 1 (p = 0.038). Error bars represent +SE. Missing bars are a result of all samples of the date/location being below the detectable limit

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MCs in Lake Aerosols

Despite the low MC levels in Keys Site 1 and below-detectable levels at Valhalla Pier, MCs were detected in the aerosols from both sampling locations (Figure 8). This is consistent with the findings of Langley (2019) that microcystins aerosolize most efficiently in oligotrophic lakes with the lowest lake water MC concentrations. Average total aerosolized MC ranged from 560.03-914.65 pg MC m^{-3} across both sampling dates and locations in Lake Tahoe (Table 4). These toxin concentrations are generally comparable to average summer MC levels found in aerosols (357.4 & 498.8 pg MC m^{-3}) at two meso-eutrophic lakes in Cape Cod, MA (Carter 2022). Particulate MC concentrations on the aerosol filters were only detectable during the July collections. Soluble aerosolized MC in the water traps remained relatively consistent across all collections with the maximum aerosolized MC occurring at Valhalla Pier in August (Figure 8).

Aerosolized MC was consistently dominated by soluble MC collected in the water traps. However, due to high sample variance, MC concentrations did not differ significantly between water traps ($p = 0.056$) or air filters. Aerosolized particulate MC was not detected at the Valhalla Pier site (Table 4).

Table 4 Aerosolized MC (pg m^{-3}) concentrations in water traps, aerosol filters, and combined (total) ± SE. BDL represents samples with toxin concentrations that could not be estimated as they were below the detectable limit. Values without SE represent a single sample that was above the detectable limit. Readings below detectable limits were included in averages as zeros when one or more of its replicates were readable. Aerosol toxins at Tahoe Keys are from Site 1.

MC Concentrations (pg MC m^{-3}) ± SE				
Date (2022)	Location	Water Traps	Aerosol Filters	Total Aerosols
19-Jul	Tahoe Keys	601.09 ± 192.11	39.21 ± NA	640.30 ± 173.76
20-Jul	Valhalla Pier	702.56 ± 135.37	168.68 ± 22.15	871.25 ± 81.51
2-Aug	Tahoe Keys	560.03 ± 164.78	BDL	560.03 ± 164.78
2-Aug	Valhalla Pier	914.65 ± 96.76	BDL	914.65 ± 96.76

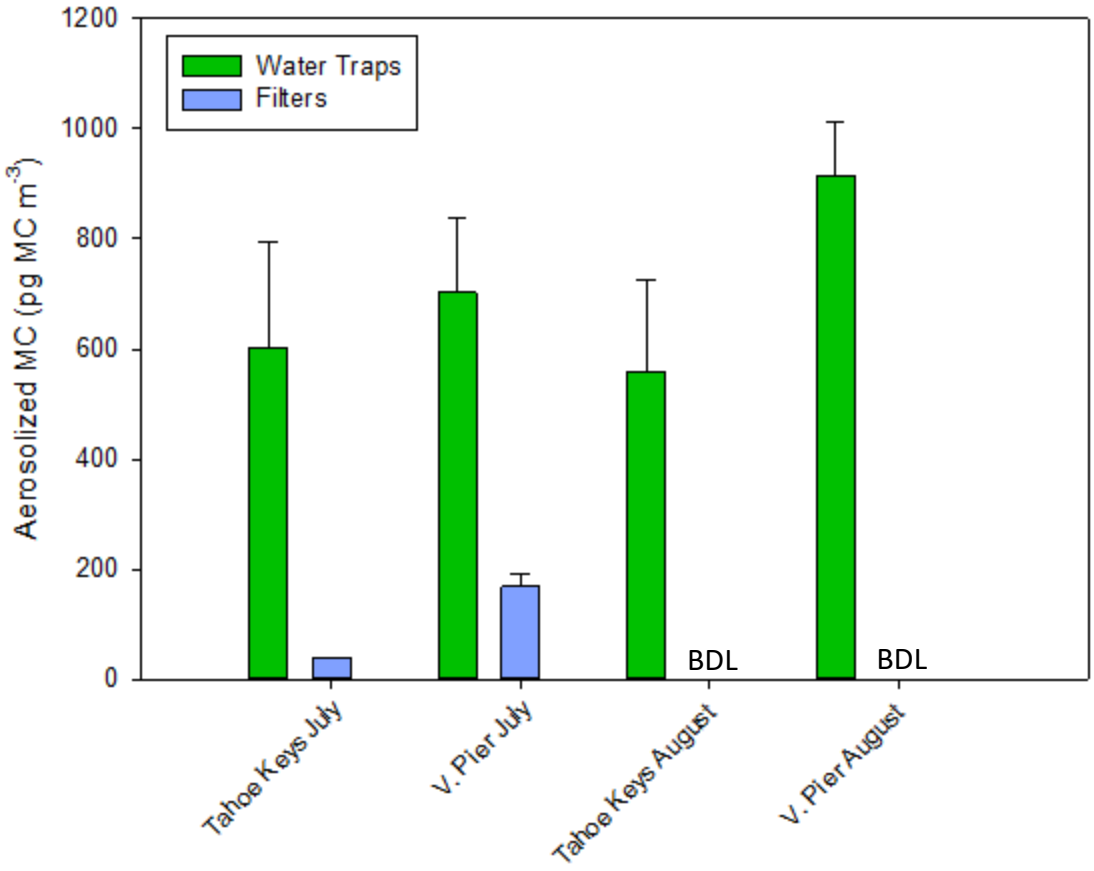

Figure 8 Average aerosolized MC (pg MC m^{-3}) in 2022 across all sampling dates and locations. No significant difference in toxin concentrations in water traps (p = 0.056). Not enough detectable values to test for significant differences between filters. Error bars represent +1 standard error of the mean.

Anatoxin-a (ATX) in Lake Water

ATX concentrations measured in the water at the two main sites (Tahoe Keys Site 1 and Valhalla Pier) ranged from 0.02-2.00 µg ATX L^{-1} (Table 5). There was no detectable ATX in either water fractions (WLW or <50 µm), or in the bloom-like material at Valhalla Pier in July. ATX concentration in the <50 µm fraction at the Tahoe Keys site was significantly higher in August (p = 0.035, Figure 9). The <50 µm fraction in the Tahoe Keys in August was also significantly higher than that of the Valhalla Pier in August (p = 0.048). ATX concentrations in the WLW were significantly higher at the Tahoe Keys (both July and August) than at Valhalla

Pier in August ($p < 0.001$, Figure 9). The increase from July to August in ATX in the small <50 µm fraction from 35% to 89% of the whole lake water ATX suggests either a higher ATX production in August by small cyanobacteria or possibly a release of ATX into the water from the cyanobacteria cells.

Table 5 ATX concentrations (µg L^{-1}) in the whole lake water (WLW) and the <50 µm water fractions ± SE in 2022. BDL represents samples with toxin concentrations that could not be estimated as they were below the detectable limit. Values without SE represent a single readable sample and 2 replicates whose toxin concentrations were below the detectable limit.

ATX Concentrations (µg ATX L^{-1}) ± 1 SE			
Date (2022)	Location	WLW	<50 µm
19-Jul	Tahoe Keys	2.00 ± 0.13	0.699 ± 0.04
20-Jul	Valhalla Pier	BDL	BDL
2-Aug	Tahoe Keys	1.81 ± 0.06	1.62 ± 0.17
2-Aug	Valhalla Pier	0.02 ± 0.01	0.57 ± NA

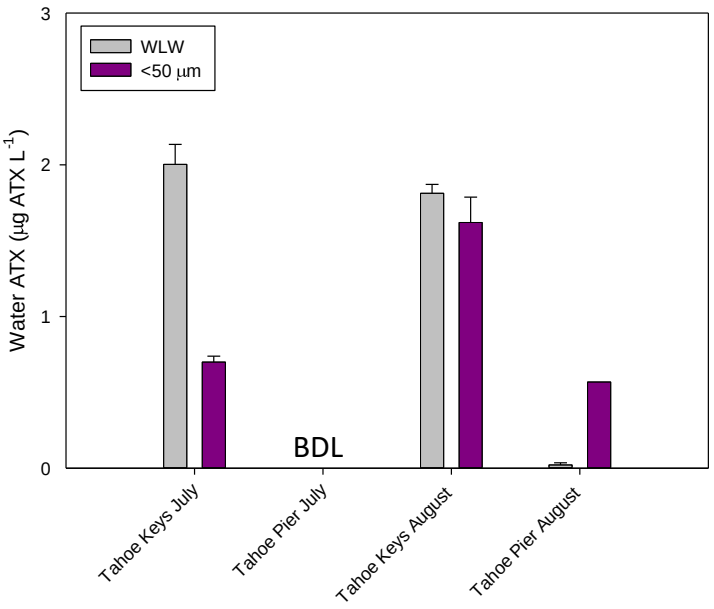

Figure 9 Average ATX (µg ATX L^{-1}) detected in water samples in 2022. This includes both unfiltered, whole lake water (WLW), and water which passed through a <50 µm mesh filter. Concentrations of ATX in the WLW at the Tahoe Keys found in July and August were significantly higher than concentrations of ATX in the WLW at Valhalla Pier ("Tahoe Pier" in graph) in August ($p < 0.001$). In August, ATX measured in the <50 um fraction at the Tahoe Keys was significantly higher than both the ATX measured at the Tahoe Keys in July ($p = 0.035$) and the ATX measured at the Valhalla Pier in August ($p = 0.048$). Error bars represent +SE.

ATX in Lake Aerosols

Average total aerosolized ATX ranged from 6.91-91.35 ng ATX m^{-3} across both sampling dates and locations (Table 6). ATX concentrations on the aerosol filters were only detectable for the Tahoe Keys 1 collections and were relatively low (<2% of total ATX). In July, soluble aerosolized ATX in the water trap was slightly higher at the Tahoe Keys than Valhalla Pier. However, in August, aerosol ATX concentrations at the Tahoe Keys more than doubled, and at Valhalla Pier increased more than 13-fold to 91.35 ng ATX m^{-3}, surpassing the concentration of aerosolized ATX estimated at the Tahoe Keys (Figure 10).

Table 6 Aerosolized ATX (ng ATX m^{-3}) concentrations in all forms (water traps, aerosol filters, and combined (total)) ± SE in 2022. BDL represents samples with toxin concentrations that could not be estimated as they were below the detectable limit. Values without SE represent a single sample that was above the detectable limit. Readings below detectable limits were included in averages as zeros when one or more of its replicates were readable.

ATX Concentrations (ng ATX m^{-3}) ± SE				
Date (2022)	Location	Water Traps	Aerosol Filters	Total Aerosols
19-Jul	Tahoe Keys	8.16 ± 1.62	0.14 ± NA	8.30 ± 1.54
20-Jul	Valhalla Pier	6.91 ± 0.61	BDL	6.91 ± 0.61
2-Aug	Tahoe Keys	18.86 ± 5.82	0.09 ± NA	18.94 ± 5.82
2-Aug	Valhalla Pier	91.35 ± 37.65	BDL	91.35 ± 37.65

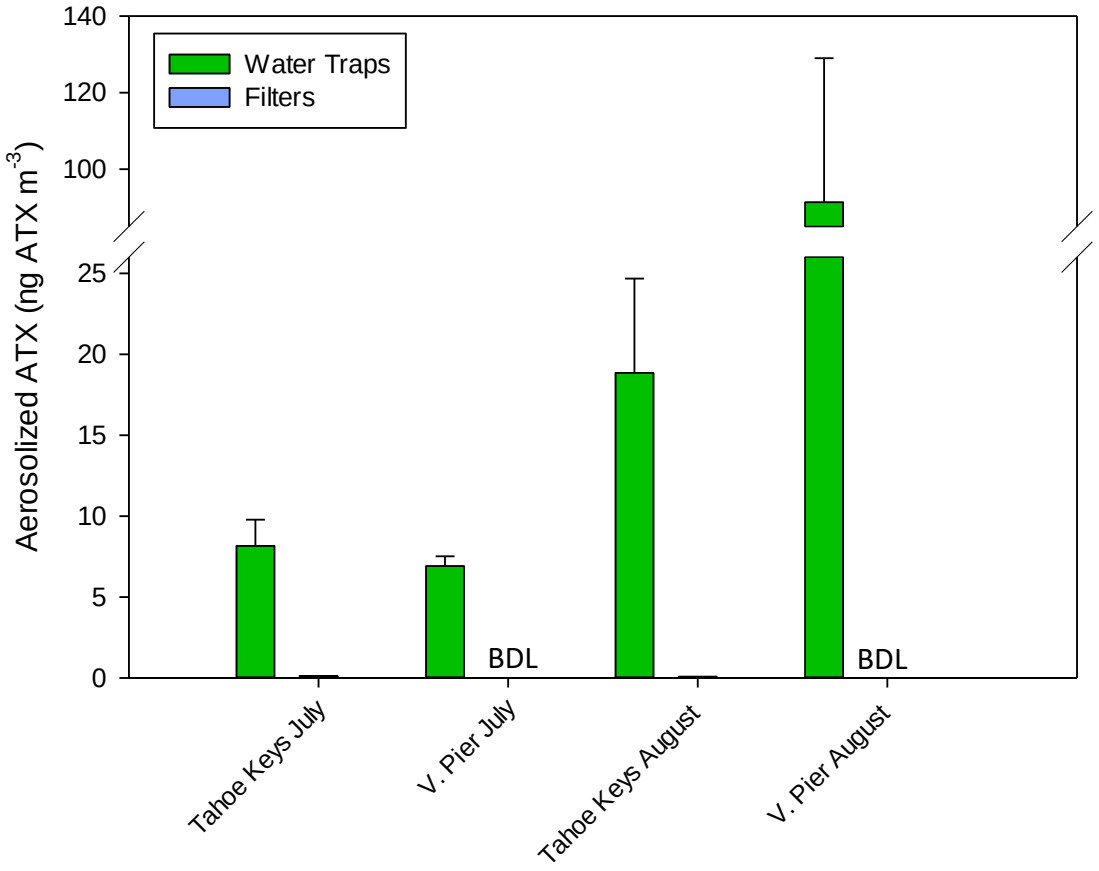

Figure 10 Average aerosolized ATX (ng ATX m^{-3}) across all sampling dates and locations in 2022. Toxin concentrations in water traps did not differ significantly ($p = 0.183$). There were insufficient data to test for significant differences between filters. Error bars represent +SE. Missing data bars are a result of all samples of the date/location being below the detectable limit and data bars without a SE bar indicate a single readable sample and 2 replicates below the detectable limit. Tahoe Keys samples are from Site 1. Note the break in the Y axis (Aerosolized ATX) to accommodate the high ATX concentrations at Valhalla Pier in August.

β-Methylamino-L-alanine (BMAA) in Lake Water

BMAA concentrations were detected in the lake water at the two primary Lake Tahoe sites (Tahoe Keys Site 1 and Valhalla Pier). Concentrations ranged from 0.07-0.59 µg BMAA L^{-1} (Table 7). Most of the BMAA was in the small <50 µm size fraction (differences between WLW and the small fraction did not differ significantly ($p>0.05$), indicating BMAA was probably produced primarily by the small cyanobacteria such as the picocyanobacteria rather

than the larger, bloom-forming cyanobacteria, In July, BMAA in the water was higher at the Tahoe Keys than at Valhalla Pier (Figure 11). However, when this water was tested again in August, BMAA concentration at the Tahoe Keys had decreased slightly whereas Valhalla Pier increased and surpassed that of the Tahoe Keys following the same pattern seen with the aerosolized BMAA (Table 7, Figure 11). In August, Valhalla Pier had significantly higher concentrations of BMAA in the <50 µm fraction than the Valhalla Pier in July ($p = 0.008$) and the Tahoe Keys in August ($p = 0.011$). On the July sampling date at Valhalla Pier, bloom-like material was observed and sampled in the near-shore water. This "scum" material had a BMAA concentration of 2.62 µg BMAA L^{-1} (Figure 11), more than four times the highest water BMAA concentration found at in the water at Valhalla Pier (Table 7).

Table 7 Average BMAA concentrations (µg BMAA L^{-1}) in the whole lake water (WLW) and the <50 µm water fractions ± SE at the two primary sampling locations in Lake Tahoe.

BMAA Concentrations (µg BMAA L^{-1}) ± SE			
Date (2022)	Location	WLW	<50 µm
19-Jul	Tahoe Keys	0.22 ± 0.05	0.41 ± 0.06
20-Jul	Valhalla Pier	0.09 ± 0.04	0.07 ± 0.01
2-Aug	Tahoe Keys	0.17 ± 0.02	0.14 ± 0.03
2-Aug	Valhalla Pier	0.36 ± 0.05	0.59 ± 0.09

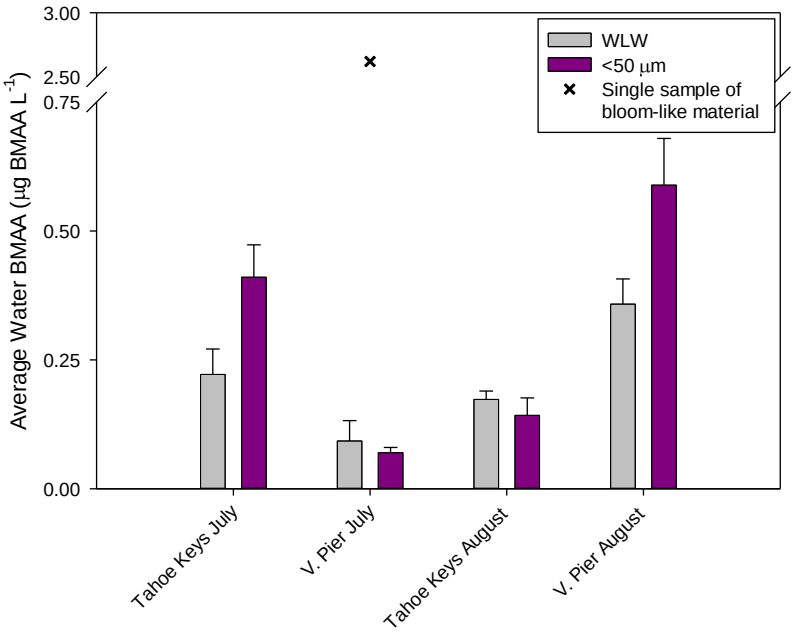

Figure 11 Average BMAA (µg BMAA L^{-1}) detected in water samples in 2022. This includes both unfiltered, whole lake water (WLW), and water which passed through a <50 µm mesh filter. BMAA concentrations in the WLW fractions were not significantly different across sampling dates or location (p = 0.079). In August, Valhalla Pier had significantly higher concentrations of BMAA in the <50 µm fraction than the Tahoe Keys in August (p = 0.011) and the Valhalla Pier in July (p = 0.008). Error bars represent ± 1 SE.

β-Methylamino-L-alanine (BMAA) in Lake Aerosols

Average total aerosolized BMAA ranged from 47.77-189.87 ng BMAA m^{-3} across both sampling dates and locations (Table 8, Figure 12). Aerosolized particulate BMAA concentrations detected on the aerosol filters remained relatively consistent with no significant differences across all sampling dates or locations (p = 0.726, Figure 12). In July, total aerosolized BMAA was higher at the Tahoe Keys Site 1 than at Valhalla Pier. However, in August, when this water was tested in the laboratory for aerosolized BMAA concentrations at the Tahoe Keys Site 1 decreased slightly whereas Valhalla Pier increased and surpassed that of the Tahoe Keys Site 1 (Figure 12), a trend also seen in the BMAA in the water samples (Table 7, Figure 11). In August, aerosolized soluble BMAA concentrations (water traps) at Valhalla Pier were significantly higher than at the Tahoe Keys Site 1 (p = 0.042).

In contrast to the other two aerosolized cyanotoxins examined, aerosolized BMAA had relatively higher amounts of particulate BMAA (filter BMAA), ranging from 22.3-21.6% at the Keys Site 1 to 52.9-21.0% at the Valhalla Pier, in July and August respectively. The relatively high particulate BMAA in the aerosols, supports the tentative hypothesis that the BMAA was aerosolized in PCY cells, whereas MC and ATX may become airborne primarily in the dissolved form.

Drivers of Aerosol Production at Lake Tahoe

It can be assumed that multiple variables contribute to the aerosolization of cyanobacteria cells and toxins. Based on field data, Langley (2018) found the difference between air and water temperature as well as the concentration of microcystins in the water were significant predictors of aerosolized microcystins collected from nine New England lakes. Of the factors examined in this study, the concentration of BMAA in the whole lake water (WLW) was the best predictor of the concentration of soluble BMAA in the lake aerosols, accounting for 97% of the variation in aerosol BMAA (simple linear regression, p=0.009, adj r^2=0.972. n=4, Shapiro Wilkinson Normality test passed). The relationship between whole lake water and soluble BMAA from CLAM water traps was:

$$Soluble\ aerosol\ BMAA\ (ng\ BMAA\ m^{-3}) = -19.783 + \left(478.731 \times WLW\ BMAA(\mu g\ L^{-1})\right)$$

Note, however, that this relationship is based on a limited data set (n=4) and could be strengthened with measurements of water and aerosol BMAA covering more locations over a broader temporal period. Relationships between aerosolized and water concentrations of MC and ATX were not established because air and water samples below detectable concentrations of these toxins could not be included in the model.

Table 8 Average aerosolized BMAA (ng m^{-3}) concentrations in all forms (water traps, aerosol filters, and combined (total)) ± SE measured in 2022. Readings below detectable limits were included in averages as zeros.

Aerosol BMAA Concentrations (ng BMAA m^{-3}) ± SE				
Date (2022)	Location	Water Traps	Aerosol Filters	Total Aerosols
19-Jul	Tahoe Keys 1	77.46 ± 20.51	17.30 ± 16.18	94.76 ± 33.38
20-Jul	Valhalla Pier	31.25 ± 3.73	16.52 ± 8.69	47.77 ± 11.73
2-Aug	Tahoe Keys 1	60.07 ± 7.64	12.98 ± 6.59	73.05 ± 12.80
2-Aug	Valhalla Pier	156.90 ± 33.91	32.98 ± 13.55	189.87 ± 24.71

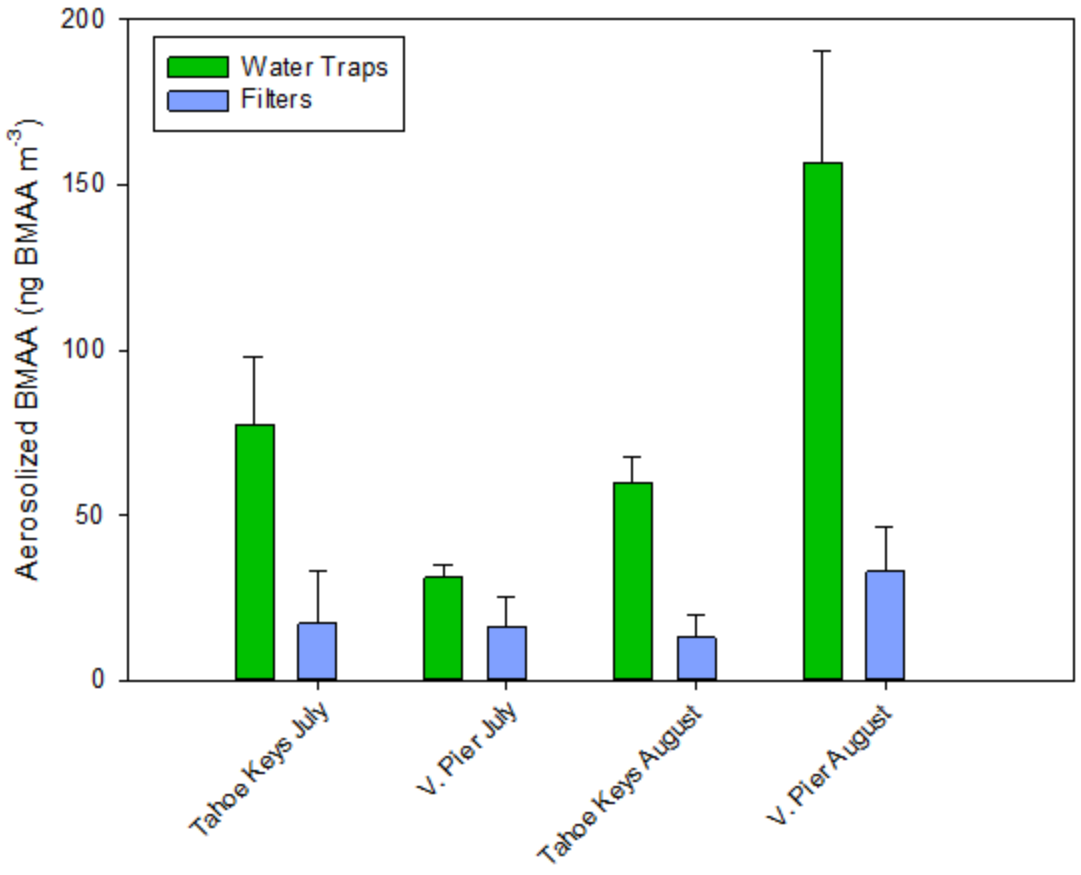

Figure 12 Average aerosolized BMAA (ng BMAA m^{-3}) across all sampling dates and locations in 2022. Valhalla Pier had a significantly higher concentration of aerosolized BMAA in August than in July ($p = 0.042$). Tahoe Keys samples are from Site 1. Error bars represent ± 1 standard error of the mean.

Additional Water Sampling Sites

Table 9 Cyanobacterial toxin concentrations in WLW samples collected from additional sites. These values are estimates from a single sample rather than an average of replicate samples. The first two rows show the average toxin ± SE in WLW samples collected from the main two sites (Tahoe Keys Site 1 and Valhalla Pier Site 2) on July 19th and 20th for comparison. BDL represents samples with toxin concentrations that could not be estimated as they were below the detectable limit of the ELISA test used. Samples were collected at the Lake Tahoe sites on July 19-20, 2022, and at the four Serine Lakes sites on July 21, 2022.

Site Name	Site Location	MC (ng MC L^{-1})	ATX (µg ATX L^{-1})	BMAA (µg BMAA L^{-1})
Tahoe Keys (Site #1)	*Monterey Drive*	*1.36 ± 0.62*	*2.00 ± 0.13*	*0.22 ± 0.05*
Valhalla Pier (Site #2)	*Valhalla Pier*	*BDL*	*BDL*	*0.09 ± 0.04*
Tahoe Keys (Site #3)	White Sands	0.55	0.42	0.43
Tahoe Keys (Site #4)	Aloha Drive	6.26	BDL	0.73
Serene Main #5	Island Way	1.59	0.02	0.38
Serena Creek #6	Serene Road	BDL	0.01	0.43
Serene Public Dock #7	Sierra Road	BDL	BDL	0.48
Lake D. Storm Drain #8	Sierra Road	BDL	0.25	21.01
CALOEHHA Guideline Values Drinking Water*		30 ng/L (0.03 µg/L) NL**	4	NA***

*California EPA's Office of Environmental Health Hazard Assessment (OEHHA 2021)
** Interim Notification Limit
***Guideline values not available

Additional Site Water Cyanotoxins (Table 9)

Exploratory grab water samples were collected from six sampling locations to determine whether detectable concentrations of the three cyanotoxins tested in this study were present. Since each site has only a single sample, no statistical comparisons were made between samples and locations.

Overall, MCs were generally low or non-detectable in the single water samples from the additional Key sites. Within the Keys, Keys Site 4 had the highest MC concentration (6.26 ng MC L^{-1}). Tahoe Keys Site 3 had the highest ATX concentration (0.42 μg ATX L^{-1}). BMAA in all grab samples from the additional sites was higher than at the two primary Lake Tahoe sites. The highest BMAA value was at the Tahoe Keys Site 4 (0.73 μg BMAA L^{-1}). The highest BMAA concentration (21.01 μg BMAA L^{-1}) in the "scum" sample taken at a storm drain at the Lake Dulzura Site 8 was roughly 10 times the BMAA concentration in the water at Keys Site 1 (Table 9).

Health Implications

Cyanotoxins in the Water

Table 10 provides an overview of the range of the three cyanobacteria toxins measured in the water and lake aerosols at Lake Tahoe. Also provided in Table 10 are estimates of guideline values for maximum allowable concentrations of MC and ATX in the water as well as daily intake limits recommended by the World Health Organization and the California EPA OEHHA. Microcystins were detected but in low concentrations at the Keys Site 1 (ca. 1-2 ng L^{-1}). Water MC was highest at Keys Site 4 (Table 9), ca 6 ng MC L^{-1}, but all MC values were well below the interim drinking water notification value of 30 ng (0.030 μg L^{-1}). Microcystins were below detectable limits in the lake water at Valhalla Pier. Anatoxin-a was highest at Keys Site 1 (2 μg L^{-1}) and detected at lower concentrations at several other sampling sites (Table 9). All water ATX values were below the OEHHA guideline value of 4 μg L^{-1}). BMAA was detected in the water from at all sampling sites and highest in a storm drain in the Serene Lakes area. The "scum" sample from that site had the highest BMAA concentration in this study (ca. 21 μg L^{-1}). Microscopic examination of the scum sample revealed several cyanobacteria taxa, including

filamentous benthic cyanobacteria as well as colonial picoplankton. It is also a reminder that cyanobacteria toxins are also often produced by bottom-dwelling and attached forms of cyanobacteria that are not commonly found floating in plankton. Although isolated toxic scums such as found at Storm Drain Site 8 (Table 9) should be avoided or handled with care, they are generally not representative of overall lake conditions, at least in the water column. It is difficult to assess quantitatively the potential health implications of the neurotoxic BMAA found in this study, since guideline values have not been established for water concentrations of BMAA.

Cyanotoxins in the air

Do aerosolized cyanotoxins represent a human health risk? Aerosols have been proposed as an important transport medium for both marine and freshwater toxins. For example, the highly potent palytoxins and the associated cells of the marine dinoflagellate *Ostreopsis* have been found in marine aerosols coming from the Mediterranean Sea (Ciminiello et al. 2014).

Both cyanobacteria cells and their toxins have been measured in the aerosols generated from a wide range of lakes, during the day and at night (Backer et al. 2008; Murby and Haney 2016; Langley 2019; Carter 2022).

Aerosolized toxins represent a potentially important route of exposure. For example, aerosols may travel some distance from the body of water, possibly exposing persons not engaging in on-lake shoreline activities. Also, the transfer of toxins such as microcystins via lung tissue may be up to 10 times more efficient than for toxins and cells that are passed through the digestive system (Wood and Dietrich 2011). The dominance of dissolved form of aerosolized toxins in this study would also contribute to the transfer efficiently once the toxins have entered the body. Due to the paucity of studies directed at measuring exposure to the aerosolized cyanotoxins, as well as the absence of established guidelines, it is not possible to directly evaluate the risk of

aerosolized cyanotoxins to human health. Simplified and very rough estimates of the potential contribution of aerosolized toxins to the total toxin exposure can be calculated assuming a worst-case scenario where a person would be breathing the same concentrations of toxins that are emitted from lake over a 24-hour period. Average adult human daily breathing rates (air inhalation) vary by individual size, age and activity rate, but generally range from about 10 to 20 m^3 air breathed per day for adults (https://www.epa.gov/expobox/exposure-assessment-tools-routes-inhalation). Assumptions and limitations of calculating daily potential cyanotoxin inhalation estimates are listed in the heading for Table 10.

Estimates of the potential daily "worst case "inhalation rates for the three aerosolized cyanotoxins at Lake Tahoe were lowest for microcystins (8.4-9.6 ng MC inhaled per day at Tahoe Keys 1 and 1.3-1.4 ng MC per day at Valhalla Pier, approximately $1/8^{th}$ to $1/20^{th}$ of the maximum daily MC intake (Table 10). Potential inhalation of anatoxiins at Tahoe Keys 1 were less than $1/100^{th}$ of the daily allowable ingestion of ATX for children and adult females (Table 10). Concentrations of aerosolized BMAA were highest of the three toxins measured with the maxima at Tahoe Keys 1 at 94.4 ng m^{-3}) and at Valhalla Pier at 189.9 ng m^{-3}. Estimated potential inhalation ranged from 1.1 to 1.4 μg d^{-1} for Tahoe Keys and 1.1 to 2.4 μg d^{-1} at Valhalla Pier. As mentioned earlier, there are no published guideline values for ingestion or inhalation of BMAA.

Table 10 Cyanobacterial toxin concentrations in whole lake water(WLW) samples and in lake aerosols at the two primary collection sites in Lake Tahoe in July and August 2022. Water and aerosol values represent the range of concentrations for the two sampling dates. Values in parentheses are Guidance Values (GV) from organizations/agencies listed at the bottom of the table. "Potential Inhalation" represents the aerosol toxin concentration times the assumed inhalation rate for adults with intermediate activity (15 m^3 air per day), Values in parentheses under Potential Inhalation GVs (in parentheses) are based on the daily ingestion guidance values for each toxin per unit (kg) body weight (μg toxin kg^{-1} d^{-1}). Potential Inhalation values are allowable daily intake values calculated for the body weight of a 35 lb. (16 kg) child, an adult 120 lb. (54 kg) female and a 155 lb. (70 kg). male. It is also assumed that the aerosol concentrations based on 4-h aerosol collections are representative of a 24-h period. Note that guideline daily intake value estimates for aerosols 1) are based on oral ingestion of toxins and do not adjust for the efficiency of toxin transfer through the lungs, 2) are estimates based on results from relatively short term experiments testing for non-cancerous effects, 3) although "uncertainty factors" are generally included in the calculation of guidance values, they do not adjust for multiple sources of a single toxin, such as exposure to cyanotoxins in foods and potential multiplicative or synergistic interactions of simultaneous exposure to more than one toxin, such as BMAA, ATX and mercury. Guidance values were not available for BMAA.

Site Name	MC	ATX	BMAA
Tahoe Keys 1 Water Range. ($\mu g\ L^{-1}$)	*0.0014-0.0038 (0.03[a])*	*1.8-2.00 (4[a])*	*0.17-0.22*
Tahoe Keys 1 Aerosol Range. ($ng\ m^{-3}$)	*0.560-0.640*	*8.3-18.9*	*73.0-94.4*
Tahoe Keys 1 Potential Inhalation ($\mu g\ d^{-1}$)	*0.0084-0.0096 (0.64, 2.16, 2.8)[b]*	*0.12-0.28 (13, 45, 58)[a]*	*1.1-1.4 (GVs NA)*
Valhalla Pier Water Range (($\mu g\ L^{-1}$)	*BDL*	*BDL*	*0.09 ± 0.04*
Valhalla Pier Aerosol Range ($ng\ m^{-3}$)	*0.871-0.914*	*6.9-91.4*	*47.8-189.9*
Valhalla Pier Potential Inhalation ($\mu g\ d^{-1}$)	*0.013-0.014 (0.64, 2.16, 2.8)[b]*	*0..10-1.4 (13, 45, 58)[a]*	*1.12-2.38 (GVs NA)*

a: CalEPA OEHHA b: World Health Organization

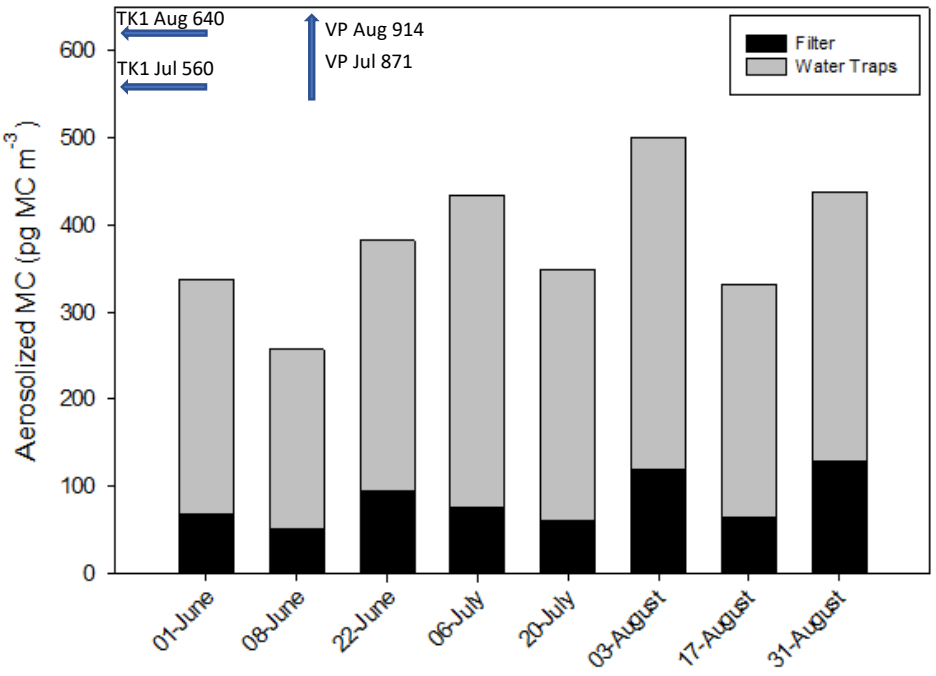

Figure 13. A comparison of Lake Tahoe MC aerosol concentrations with average concentrations of MC (ng MC L^{-1}) in Walkers Pond (mesotrophic) and Lower Mill Pond (meso-eutrophic) during the 2021 sampling season.. From Carter 2022. Aerosol concentrations of MC were higher at both Tahoe Keys 1 (TK1) and Valhalla Pier (VP), despite lower concentrations of MC in the water.

Summary

1. A preliminary investigation of cyanobacterial toxins in Lake Tahoe was carried out on July 19-20 and August 2, 2022. The study was a collaborative effort by the University of New Hampshire Ecotoxicology Laboratory and a private citizens' group from Lake Tahoe.
2. The cyanobacterial toxins, microcystin (MC), anatoxin-a (ATX), and β-Methylamino-L-alanine (BMAA) were detected in the lake water at Tahoe Keys 1, but MC was below detection limits at Valhalla Pier. All three toxins, MC, ATX and BMAA were present in the lake aerosols sampled at the two primary sampling sites (Tahoe Keys 1 and Valhalla Pier). Additional exploratory water samples from six additional sites in Lake Tahoe Keys

and the two nearby Serene Lakes also contained detectable levels of cyanotoxins, some at higher concentrations than at the two primary Lake Tahoe sites.

3. The presence of cyanobacteria was confirmed by the levels of the accessory phycobilin pigments phycocyanin (PC) and phycoerythrin (PE). PC concentrations varied from 11.1 ± 0.56 to 15.10 ± 7.66 μg PC L^{-1} in the Keys Site 1 in July and August, respectively, but was not detectable at Valhalla Pier. At Keys Site 1, PE concentrations were highest in July and lower in August (14.2 ± 2.24 vs 8.64 ± 2.43 μg PE L^{-1}).
4. Whole lake water concentrations of MCs were generally low at the Tahoe Keys Site 1 (1.36 ± 0.62–3.81 ± 0.87 ng MC L^{-1}) and below the detection limit at the Valhalla Pier sampling site on the two sampling dates in July and August. However, MCs were present in the lake aerosols ranging from 560.03 ± 164.78 to 640.30 ± 173.76 pg MC m^{-3} at the Tahoe Keys Site 1 and 871.25 ± 81.51 to 914.65 ± 96.76 pg MC m^{-3} at Valhalla Pier. The relatively high aerosol MC at Valhalla Pier compared to the low or non-detectable MC concentrations in the water raises important questions about the mechanism underlying aerosolization. It is likely that the form of MC in the water at the Valhalla Pier site was efficiently aerosolized, such as an abundance of the dissolved form of the MC toxin. It is also possible that other conditions promoted the aerosolization of MC, such as the type of picocyanobacteria present. Also, climatic and geographic conditions associated with Lake Tahoe, such as low humidity and the high-altitude of the lake may contribute to an enhanced aerosolization.
5. Whole lake water concentrations of anatoxin-a (ATX) at the Tahoe Keys Site 1 ranged from 1.81 ± 0.06 to 2.00 ± 0.13 μg ATX L^{-1} and below the limit of detection in July, to 0.02 ± 0.01 μg ATX L^{-1} at the Valhalla Pier in August. Concentrations of ATX in the lake

aerosols ranged from 8.30 ± 1.54 to 18.94 ± 5.82 ng ATX m^{-3} at the Tahoe Keys Site 1 and 6.91 ± 0.61 to 91.35 ± 37.65 ng ATX m^{-3} at Valhalla Pier.

6. Whole lake water concentrations of BMAA at the Tahoe Keys Site 1 ranged from 0.17 ± 0.02 to 0.22 ± 0.05 µg BMAA L^{-1} and from 0.09 ± 0.04 to 0.36 ± 0.05 µg BMAA L^{-1} at the Valhalla Pier in August. BMAA in the lake aerosols ranged from 73.05 ± 12.80 to 94.76 ± 33.38 ng BMAA m^{-3} at the Tahoe Keys Site 1 and 47.77 ± 11.73l in July to 189.87 ± 24.71 ng BMAA m^{-3} at Valhalla Pier in August. Compared to MC and ATX, a larger proportion of aerosolized BMAA was in the particulate BMAA form retained on the CLAM filters at Valhalla Pier, suggesting that toxigenic picoplankton cells may have accounted for much of the higher aerosolized BMAA concentrations at Valhalla Pier.
7. The concentration of BMAA in the whole lake water was a strong predictor of the soluble BMAA in the lake aerosols, accounting for approximately 97% of the variation in aerosol BMAA. This relationship needs to be examined further with a larger data set as it could be a useful tool for forecasting the concentration of toxins in the lake aerosols.
8. Our findings confirm that the three cyanotoxins, MC, ATX and BMAA were present in both the water and aerosols sampled from Lake Tahoe. Despite relatively small concentrations of microcystins in the Lake Tahoe water, concentrations in the air were comparable to lakes with higher productivity and lakes with higher concentrations of microcystins in the water (Figure 13). The toxins found in the aerosols at the two Tahoe Lake sites appear to reflect the type of cyanobacteria, i.e., dominance of larger bloom-forming cyanobacteria at the Tahoe Keys 1 site versus small, picocyanobacteria at the more open-lake at Valhalla Pier. Based on examination of the water size fractions (whole lake water and <50 µm) and the form of the toxin (particulate and dissolved) in the air, it appears

that much of aerosolized MC and ATX may be derived from dissolved toxins in the water, whereas aerosolized BMAA was associated with a higher percentage of the particulate form of the toxin, presumably from picocyanobacteria cells present in the lake water.

9. Daily inhalation rates of the cyanotoxins were estimated, based on toxin concentrations in the air and assumed inhalation rates. Estimates of the potential daily "worst case" inhalation rates for the three aerosolized cyanotoxins at Lake Tahoe were lowest for microcystins (8.4-9.6 ng MC inhaled per day at Tahoe Keys 1 and 1.3-1.4 ng MC per day at Valhalla Pier, approximately $1/8^{th}$ to $1/20^{th}$ of the maximum daily MC intake (Table 10). aPotential inhalation of anatoxins at Tahoe Keys 1 were less than $1/100^{th}$ of the daily allowable ingestion of ATX for children and adult females (Table 10). Aerosolized BMAA concentrations were highest of the three toxins measured with the maximum concentration at Tahoe Keys 1at 94.4 ng m^{-3} and at Valhalla Pier at 189.9 ng m^{-3}. Estimated potential toxin inhalation ranged from 1.1-1.4 μg d^{-1} for Tahoe Keys and 1.1to 2.4 μg d^{-1} at Valhalla Pier. Note, however, that the intake estimated as the potential toxin inhalation does not include simultaneous exposure from other sources of the toxins, such as water and food. There are no published guideline values for ingestion or inhalation of BMAA that can be applied to the findings in this report.

   It should be stressed that the findings of this study are limited in both scope and time. They represent a snapshot of cyanotoxins in the lake water and in the air during late summer at two selected sites in Lake Tahoe. Follow-up research is needed to better identify and understand the producers of the observed toxins. The research described in this report focused on conditions at the southern region of Lake Tahoe and is not necessarily representative of other locations in the lake. Future work should include

more intensive sampling in time and space to determine possible toxin "hot spots" within the lake and times of the year when toxins in the water and air are highest. Ideally, additional research would include a search for the relevant physical, chemical, and biological factors that "drive" aerosolization of toxins in the lake. A useful goal would be to develop a model that would use environmental variables, such as water temperature, water toxins levels and cyanobacteria pigments to predict when toxins in the air reach levels of concern. It would also be valuable to examine the presence of other toxic substances in the air that may act synergistically with cyanobacteria toxins (e.g., mercury), thereby amplifying possible health risks. Finally, to address more directly the question of potential health risks from breathing lake aerosols, a study could be undertaken to monitor the levels of toxic aerosols present in the homes and nearby communities.

Acknowledgements

Many thanks are due to the following people who assisted with financial and logistical of this research project during the summer, 2022. Special thanks to Trish from Tahoe Area ALS/MND Research Library who generated interest and was a major driving force for the project.
The following acknowledgement list was provided by Trish from Tahoe:

Alex Beane, Deborah Beane and Michael Beane
Elise Fett
Eric Geisler
Friends of the West Shore (FOWS)-Lake Tahoe: Ron Grassi, Rocky Gunderson, Dana Schneider, Larry Schuler and Judi Tornese
George Binney
Julie Soules
Nate Neerhof, Claudia Polsky and Team from Berkeley Law
Our Donors on GoFundMe: Help Us Research the Cause of ALS in Lake Tahoe!!
Richard McHenry and California Sport Fishing Alliance
Tahoe Area Hazardous Algae Blooms on Facebook: Dana Schneider
Tobi Tyler
Trish from Tahoe Area ALS/MND Research Library on Facebook
Everyone else who contributed but wished to remain anonymous
Also, thanks to the following for their interest and suggestions concerning the study
Bo Landin
Dr. Elijah Stommel
Dr. Paul Alan Cox, Dr. Sandra Banack, Dr. James Metcalf and the Team at Brain Chemistry Labs

Finally, the authors wish to thank Haley Fong in the UNH Ecotoxicology Lab for her help with preparation of toxin samples and for analyzing and graphing the assessory pigments.

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Footnote:

Units of measure used in this study include:
1 microgram (μg) = 1000 nanograms (ng) = 1,000,000 picograms (pg)