INVESTIGATION OF MULTIPLE CYANOTOXINS IN TOXIC LAKE AEROSOLS

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ III
TABLE OF CONTENTS ............................................................................................................. V
LIST OF TABLES ...................................................................................................................... vi
LIST OF FIGURES ................................................................................................................... vii
ABSTRACT .............................................................................................................................. IX
INTRODUCTION ....................................................................................................................... 1
METHODS ................................................................................................................................ 6
Study Sites ................................................................................................................................. 6
Field Aerosol Collection .......................................................................................................... 11
Water Sampling ......................................................................................................................... 14
Environmental Parameters ..................................................................................................... 17
Toxin Analysis .......................................................................................................................... 19
Statistical Analysis .................................................................................................................. 22
RESULTS .................................................................................................................................. 24
Seasonal Occurrence of Cyanotoxins in Water and Air ........................................................... 24
Water Microcystins .................................................................................................................. 24
Aerosolized Microcystins ......................................................................................................... 28
Water BMAA ............................................................................................................................ 32
Aerosolized BMAA .................................................................................................................. 37
Water Anatoxin-a ...................................................................................................................... 41
Aerosolized Anatoxin-a .......................................................................................................... 46
Potential Drivers of Toxic Aerosol Production ...................................................................... 50
Biological Factors and Insignificant Climatic Factors ............................................................ 50
Microcystins at Walkers Pond ................................................................................................. 51
Microcystins at Lower Mill Pond ............................................................................................ 53
BMAA at Walkers Pond ........................................................................................................... 54
BMAA at Lower Mill Pond ....................................................................................................... 55
ATX at Walkers Pond .............................................................................................................. 56
ATX at Lower Mill Pond .......................................................................................................... 58
Summary of Potential Drivers of Toxin Aerosol Production: .................................................. 60
DISCUSSION ............................................................................................................................ 62
Aerosolized Toxin .................................................................................................................... 62
Microcystin Concentrations .................................................................................................... 62
BMAA Concentrations ........................................................................................................... 63
Anatoxin-a Concentrations ..................................................................................................... 63
Potential Environmental Drivers of Aerosolized Cyanotoxins .............................................. 64
Potential Environmental Drivers of Aerosolized Microcystins ............................................. 65
Potential Environmental Drivers of Aerosolized BMAA ....................................................... 68
Potential Environmental Drivers of Aerosolized ATX .......................................................... 70
Summary and Comparison of Potential Environmental Drivers Between Aerosolized Toxins ............................................................................................................................................ 73
Mechanisms of Aerosolization ............................................................................................... 74
Evaluation of the Compact Lake Aerosol Monitor ................................................................. 75
Atmospheric Loading and Health Implications ..................................................................... 77
Major Findings: ....................................................................................................................... 82
REFERENCES ........................................................................................................................... 83
LIST OF TABLES

TABLE 1. MC CONCENTRATIONS IN ALL WATER FRACTIONS THROUGHOUT THE 2021 SAMPLING SEASON AT WALKERS POND AND LOWER MILL POND (BREWSTER, MA) .................................................. 25
TABLE 2. AEROSOLIZED MC CONCENTRATIONS THROUGHOUT THE 2021 SAMPLING SEASON AT WALKERS POND AND LOWER MILL POND (BREWSTER, MA) ................................................................. 29
TABLE 3. BMAA CONCENTRATIONS IN ALL WATER FRACTIONS THROUGHOUT THE 2021 SAMPLING SEASON AT WALKERS POND AND LOWER MILL POND (BREWSTER, MA) ...................34
TABLE 4. AEROSOLIZED BMAA CONCENTRATIONS THROUGHOUT THE 2021 SAMPLING SEASON AT WALKERS POND AND LOWER MILL POND (BREWSTER, MA) ...................................................... 39
TABLE 5. ATX CONCENTRATIONS IN ALL WATER FRACTIONS THROUGHOUT THE 2021 SAMPLING SEASON AT WALKERS POND AND LOWER MILL POND (BREWSTER, MA) .......................................................... 43
TABLE 6. AEROSOLIZED ATX CONCENTRATIONS THROUGHOUT THE 2021 SAMPLING SEASON AT WALKERS POND AND LOWER MILL POND (BREWSTER, MA) .......................................................... 48
TABLE 7. ESTIMATIONS OF TOTAL DAILY INHALATION OF MC, BMAA, AND ATX AT WALKERS POND AND LOWER MILL POND ................................................................. 79
TABLE 8. ESTIMATIONS OF TOTAL DAILY ATMOSPHERIC LOADING OF MC, BMAA, AND ATX AT WALKERS POND AND LOWER MILL POND ................................................................. 81
LIST OF FIGURES

FIGURE 1. MAP OF CAPE COD, MASSACHUSETTS SHOWING LOCATION OF THE MILL POND COMPLEX IN BREWSTER, MA.................................................................6
FIGURE 2. MILL PONDS COMPLEX IN BREWSTER, MA SHOWING WALKERS POND (A), UPPER MILL POND (B), AND LOWER MILL POND (C)..................................................................7
FIGURE 3. WALKERS POND IN BREWSTER, MA..................................................................8
FIGURE 4. WALKERS POND LANDING IN BREWSTER, MA.........................................................9
FIGURE 5. LOWER MILL POND IN BREWSTER, MA.................................................................10
FIGURE 6. PRIVATE DOCK AND SAMPLING EQUIPMENT USED FOR SAMPLE COLLECTION AT LOWER MILL POND IN BREWSTER, MA.................................................................11
FIGURE 7. COMPACT LAKE AEROSOL MONITOR (CLAM) SET UP ON A TEMPORARY PLATFORM DURING A COLLECTION.................................................................13
FIGURE 8. DIAGRAM OF THE INSIDE OF THE CLAM...........................................................14
FIGURE 9. FLOWCHART SHOWING THE PROCEDURE FOR COLLECTING WATER SAMPLES........16
FIGURE 10. ONSET HOBO DATA MONITORS ATTACHED TO A STAKE TO MEASURE TEMPERATURE AND LIGHT INTENSITY.................................................................18
FIGURE 11. SET OF THREE EVAPORATION PANS SET OUT NEXT TO THE CLAM DURING AEROSOL COLLECTION.................................................................19
FIGURE 12. MICROCYSTINS IN WATER FRACTIONS AT WALKERS POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................26
FIGURE 13. MICROCYSTINS IN WATER FRACTIONS AT LOWER MILL POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................27
FIGURE 14. AVERAGE CONCENTRATION OF MC IN ALL WATER FRACTIONS AT WALKERS POND AND LOWER MILL POND DURING THE 2021 SAMPLING SEASON..........................28
FIGURE 15. AEROSOLIZED MICROCYSTINS AT WALKERS POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................30
FIGURE 16. AEROSOLIZED MICROCYSTINS AT LOWER MILL POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................31
FIGURE 17. AEROSOLIZED MC IN ALL FORMS AT WALKERS POND AND LOWER MILL POND DURING THE 2021 SAMPLING SEASON.................................................................32
FIGURE 18. BMAA IN WATER FRACTIONS AT WALKERS POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................35
FIGURE 19. BMAA IN WATER FRACTIONS AT LOWER MILL POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................36
FIGURE 20. AVERAGE CONCENTRATION OF BMAA IN ALL WATER FRACTIONS AT WALKERS POND AND LOWER MILL POND DURING THE 2021 SAMPLING SEASON..........................37
FIGURE 21. AEROSOLIZED BMAA AT WALKERS POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................39
FIGURE 22. AEROSOLIZED BMAA AT LOWER MILL POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................40
FIGURE 23. AEROSOLIZED BMAA IN ALL FORMS AT WALKERS POND AND LOWER MILL POND DURING THE 2021 SAMPLING SEASON.................................................................41
FIGURE 24. ATX IN WATER FRACTIONS AT WALKERS POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................44
FIGURE 25. ATX IN WATER FRACTIONS AT LOWER MILL POND (BREWSTER, MA) DURING THE 2021 SAMPLING SEASON.................................................................45
ABSTRACT

INVESTIGATION OF MULTIPLE CYANOTOXINS IN TOXIC LAKE AEROSOLS

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Aerosols from freshwater lakes with toxigenic cyanobacteria pose a potentially serious threat to humans and wildlife. The most studied route of exposure to cyanotoxins is direct consumption (i.e., drinking contaminated water or eating contaminated food). However, a less studied but a potentially important route of exposure is inhalation of aerosolized cyanotoxins. Three toxins, produced by cyanobacteria, examined in this study were hepatoxic microcystins (MC), and the neurotoxins \( \beta \)-Methylamino-L-alanine (BMAA), and anatoxin-a (ATX).

Using a compact lake aerosol monitor (CLAM), this study measured levels of aerosolized cyanotoxins (MC, BMAA, and ATX) at two shallow and inter-connected lakes, Walkers Pond and Lower Mill Pond (Brewster, MA, USA), biweekly throughout a sampling season (June 1-August 31). Simultaneously, toxin concentrations in lake water fractions as well as climatic parameters (temperature differential, light intensity, evaporation rate, wind velocity, and humidity) were recorded during each sample collection. The objectives of this study were to 1) determine the presence of multiple cyanotoxins in lake-generated aerosols, and 2) gain a better understanding of lake aerosols and the mechanisms behind aerosolization.
Microcystins, BMAA, and anatoxin-a were detected in lake aerosols of Walkers Pond and Lower Mill Pond on all eight sampling dates from June 1 to August 31, 2021. Mean concentrations of aerosolized cyanotoxins (ng m\(^{-3}\)) were generally higher at Walkers Pond for all three toxins: MC 0.50 ± 0.06 vs. 0.36 ± 0.02, BMAA 45.1 ± 8.9 vs 24.7 ± 4.3, and ATX 6.2 ± 0.8 vs. 3.9 ± 0.6, for Walkers Pond and Lower Mill Pond respectively. A consistent characteristic of the aerosolized toxins in both lakes was the dominance of the operationally defined “dissolved toxin” form (<0.3 µm filtered air captured in the water traps versus “particulate” form retained on a 0.3 µm filter), making up 79.65-98.45% on average of the aerosolized toxins for all three cyanotoxins.

Although perhaps the most logical factor, toxin concentration in the water alone was not the best determinant of aerosolized toxins. For example, MCs at Walkers Pond were best predicted using a two-parameter model including both air-water temperature differential and MC concentration in the Picoplankton (0.3-2.0 µm) water fraction (p = 0.004, Adj R\(^2\): 0.84), suggesting that the Pico fraction was the main source of aerosolized MCs in this case.

Concentrations of BMAA in aerosols from Walkers Pond were best predicted by a three-parameter model including BMAA concentration in the <50 µm water fraction, evaporation rate, and air-water temperature differential (p = 0.023, Adj R\(^2\): 0.88). At Lower Mill Pond, aerosolized BMAA was correlated with BMAA in the Pico (0.3-2.0 µm) water fraction (p = 0.014, Adj R\(^2\): 0.605), again, suggesting that the Pico fraction may be a major source of aerosolized cyanotoxins.

Aerosolized ATX at Walkers Pond was not correlated with any individual or combinations of environmental parameters measured in this study. However, at Lower Mill
Pond, aerosolized ATX was correlated with both evaporation rate (p = 0.006, Adj R^2: 0.76), and wind velocity separately (p = 0.021, Adj R^2: 0.55).

The difference in significant environmental parameters between different toxins and lake systems highlights the uniqueness of toxin occurrence and potential aerosolization mechanisms of these cyanotoxins. The difference in significant environmental parameters of a single toxin between the lakes highlights the individuality of the two lakes despite being in close proximity and connected.

This is the first reported study to detect MC, BMAA and ATX simultaneously in lake aerosols. Insights gained regarding potential drivers and possible mechanisms will expand our limited knowledge of cyanotoxin aerosolization. Multiple, simultaneous mechanisms of aerosolization, both of a single toxin and between different toxins, is a novel finding and needs to be explored further. The presence of multiple cyanotoxins in aerosols from a single lake will affect how cyanotoxin exposure estimates are approached in the future.
INTRODUCTION

Freshwater ecosystems are not exempt from the damage caused by climate change and pollution on the environment. As a result of these anthropogenic effects, nutrients in excess concentrations are being loaded into natural freshwater lakes (Smith et al., 1999). These nutrients allow potentially harmful organisms, like cyanobacteria, to thrive in the ecosystem and often has adverse effects (Paerl et al., 2011).

Cyanobacteria are phylum of photosynthetic prokaryotes, formerly known as blue-green algae. Present all over the globe, cyanobacteria inhabit freshwater, marine, and terrestrial ecosystems. In a natural lake ecosystem, excess nutrient availability such as nitrogen and phosphorus can lead to a considerable increase in cyanobacteria population (Paerl et al., 2011; Smith et al., 1999). Often, high concentrations of cyanobacteria are visible in aquatic systems as cyanobacterial blooms. Bloom-forming cyanobacteria have larger cells which can be easily visualized without magnification. However, cyanobacteria also exist in a smaller size fraction, known as picocyanobacteria, typically measuring 0.2 – 2.0 µm in size (Jakubowska & Szeląg-Wasielewska, 2015; Lewandowska et al., 2017).

Cyanobacteria are unique in their ability to produce cyanobacterial toxins as secondary metabolites (Harada, 2004). These cyanotoxins have negative impacts on organisms in the ecosystem therefore reducing competition and increasing access to vital nutrients (Wiegand & Pflugmacher, 2005). A single cyanobacterium can produce multiple cyanotoxins simultaneously and can change the production ratio of various toxins (Merel et al., 2013; Namikoshi et al., 2003). Both bloom-forming cyanobacteria and picocyanobacteria are capable of producing
cyanotoxins. Cyanotoxins are more commonly associated with bloom-forming cyanobacteria and are well-studied due to their size and visibility. Picocyanobacteria are often overlooked due to their small size but have also been found to produce potent cyanotoxins in significant quantities (Jakubowska & Szelaż-Wasielewska, 2015). Research at the University of New Hampshire’s Center for Freshwater Biology is currently focused on the cyanobacterial toxins microcystins (MC), β-Methylamino-L-alanine (BMAA), and anatoxin-a (ATX) because of their toxicity and prevalence.

Microcystin (MC) is a hepatotoxin produced by cyanobacteria that causes cellular damage to various mammalian organs. The majority of ingested MC accumulates in the liver and can result in tumor-promoting toxic mechanisms and development of non-alcoholic liver disease (He et al., 2022; McLellan & Manderville, 2017). The mode of action of MC involves inhibiting protein phosphatases by binding to the protein phosphatases (de Figueiredo et al., 2004; Vesterkvist et al., 2012). This inhibition can lead to cellular damage and tumor promotion. The World Health Organization (WHO) set provisional tolerable daily intake of microcystin-LR at 0.04 µg kg⁻¹ d⁻¹ and provisional lifetime drinking water, short-term drinking water and recreational guideline for adults at 1 µg L⁻¹, 12 µg L⁻¹, and 24 µg L⁻¹ respectively (Chorus & Welker, 2021). No regulatory guidelines have been set for inhalation of MC.

BMAA is a neurotoxin produced by cyanobacteria that has been linked to neurodegenerative symptoms consistent with those of amyotrophic lateral sclerosis (ALS) and Alzheimer’s disease (Caller et al., 2009; Torbick et al., 2017). BMAA was first linked to progressive neurological diseases after discovering its presence in cycad seeds in Guam (Cox et al., 2003). When the central nervous system is exposed to BMAA, it can be mistaken by the cellular machinery as L-serine. L-serine is usually incorporated into proteins but if BMAA is
incorporated instead, production of neurofibrillary tangles and amyloid deposits are promoted (Cox et al., 2005, 2018; Dunlop et al., 2013). Both neurofibrillary tangles and amyloid deposits are common physical manifestations of neurodegenerative diseases and can be induced by ingestion of BMAA (Cox et al., 2016). No regulatory guidelines have been set by the World Health Organization for the ingestion or inhalation of BMAA.

Anatoxin-a is a neurotoxin produced by cyanobacteria known as the “Very Fast Death Factor” due to its acute, and potentially deadly, toxicity (Chorus & Welker, 2021; Christensen & Khan, 2020). Exposure to high concentrations of ATX often leads to interference of nerve cells resulting in damage to the nervous system (Christensen & Khan, 2020). Anatoxin-a acts as a neurotransmitter (acetylcholine) and binds with its receptor (Kotak & Zurawell, 2009). This causes overstimulation of respiratory muscles. In high concentrations, this overstimulation can result in deadly respiratory failure and suffocation (D’anglada et al., 2016; Kotak & Zurawell, 2009).

Regulatory ATX guidelines for recreational and drinking water for adults range from 1–80 µg L⁻¹ (Cyanobacterial Toxins: Anatoxin-a and Analogues. Background Document for Development of WHO Guidelines for Drinking-Water Quality and Guidelines from Safe Recreational Water Environments, 2020; Dewine & Stevenson, 2020; Farrer, 2015; Ministry of Health, 2008). Most commonly, values closer to 1 µg L⁻¹ have been adopted as the recommended limit of ATX in drinking water for adults. The World Health Organization (WHO) has set the adult provisional short-term drinking water reference value for anatoxin-a at 30 µg L⁻¹ and adult provisional recreational water reference value at 60 µg L⁻¹ (WHO et al., 2021). No regulatory guidelines have been set for inhalation of anatoxin-a.
Organisms can be exposed to cyanotoxins through a variety of different pathways. Direct consumption such as drinking contaminated water or consuming aquatic life residing in affected systems is the most studied route of exposure. However, a less studied, but possibly more prevalent, route of exposure is inhalation of toxic aerosols. 

Aerosols are small liquid droplets or particles suspended in the air containing viruses, single cellular organisms (bacteria and protozoa), and multicellular organisms (fungi and algae) (Sahu et al., 2015). Aerosols can also contain toxic cyanobacteria (Murby & Haney, 2015). Toxic aerosols may be readily inhaled by humans and animals that reside near contaminated water, but little is known on this potential exposure. Aerosols from lake surfaces may contain dissolved toxins (extracellular toxins) and particulate toxins (intracellular) (McLellan & Manderville, 2017). Studies have documented the presence of singular toxins such as microcystins, BMAA and anatoxin-a in the air (Cheng et al., 2007; Murby & Haney, 2015; Scott et al., 2018; Sutherland et al., 2021). However, aerosol production is variable, and little is known about the factors influencing the aerosolization process.

Hypothesized mechanisms of cyanobacteria aerosolization include wind driven aerosols, microbubble bursting and passive evaporation (Blanchard & Syzdek, 1972; Cheng et al., 2007; Dueker et al., 2011; Stommel et al., 2013, Medina-Pérez et al., 2021; Murby & Haney, 2015; Sahu et al., 2015). However, aerosolization of cyanobacteria is a complicated process and it is unlikely the process can be explained by one mechanism exclusively. It is more probable that cyanobacteria aerosolize through a dynamic combination of multiple mechanisms. This process is also influenced by varying climatic factors (Medina-Pérez et al., 2021; Wiśniewska et al., 2021). Hypothesized climatic factors that may regulate aerosol production include wind speed and direction, light intensity, air and water temperature, and humidity. Complex biological
factors such as cyanobacteria community structure, cell size and chemical composition may also influence cyanobacterial aerosolization (Lewandowska et al., 2017; Wiśniewska et al., 2021).

Past studies have hypothesized inhalation of aerosolized cyanotoxins may have a 10-fold increase in toxicity when compared to ingested cyanotoxins due to a more direct route of absorption in the body (Stommel et al., 2013; Wood & Dietrich, 2011). This suggests that aerosolization of cyanotoxins is a potentially significant route of exposure that is not currently considered in exposure estimates. In addition, inhalation is a more constant route of exposure than oral ingestion suggesting that current cyanotoxin exposures are notably underestimated.

The University of New Hampshire Center for Freshwater Biology developed field and laboratory methods to measure the rate of release of cyanobacterial toxins from aquatic systems and into the air (Murby & Haney, 2015). The major objectives of this study were to 1) quantify the concentrations of microcystins, β-Methylamino-L-alanine, and anatoxin-a in freshwater lake aerosols, and 2) gain a better understanding of lake aerosols and the mechanisms behind aerosolization by identifying climactic variables that influence concentration of aerosols containing microcystins, β-Methylamino-L-alanine, and anatoxin-a. Gaining a better understanding of the factors that influence aerosolization of cyanotoxins will help inform management of this public health risk.
METHODS

Study Sites

This research was carried out at two freshwater lake sites, Walkers Pond, and Lower Mill Pond. Both study sites are in Brewster, MA and are a part of the Mill Ponds Complex (Figure 1). The Mill Ponds Complex is a system of connected ice block depression (kettle) lakes with sandy substrate bottoms flowing from Walkers Pond to Stony Brook via Upper and Lower Mill Ponds (Figure 2). Walkers Pond and Lower Mill Pond were sampled biweekly between June 08, 2021, and August 31, 2021, with an additional sampling date on June 01, 2021, for a total of eight sampling dates.

Figure 1. Map of Cape Cod, Massachusetts showing location of the Mill Pond Complex in Brewster, MA (41.7292° N, -70.1666° W) (Google Maps, 2022a).
Walkers Pond is a 41.28-hectare (102-acre) with a maximum depth of 2.4 meters (Brewster Ponds Coalition, 2022). This mesotrophic lake was dominated by *Dolichospermum* for most of the 2021 sampling season, although small populations of *Woronichinia*, *Microcystis*, and *Aphanizomenon* were occasionally present (Association to Preserve Cape Cod & Brewster Ponds Coalition, 2021). Walkers Pond is open to the public for kayaking, sailing, boating, and fishing. The public Walkers Pond Landing (41.7202° N, -70.1320° W) was used to access and collect
samples at Walkers Pond (Figure 3 and Figure 4). The aerosol collection was done on a temporary platform installed 3 m from shore at a depth of 0.5 m. The platform was installed so the aerosol collector sat with the wind screen touching the surface of the water and was completely exposed to the sun during all collection periods. For each collection, the platform was installed, using landmarks to ensure the location was consistent. All other samples were collected from the site of the aerosol collector. Sampling took place 9:30 am-1:30 pm (EST).

Figure 3. Walkers Pond in Brewster, MA. Pinned location shows Walkers Pond Landing where sampling took place (41.7202° N, -70.1320° W) (Google Maps, 2022d).
Figure 4. Walkers Pond Landing in Brewster, MA (41.7202° N, -70.1320° W) where sampling took place with an aerosol collector installed in the location used on each sampling date.

Lower Mill Pond is a 20.40-hectare (50.4-acre) with a maximum depth of 3.9 m (Brewster Ponds Coalition, 2022). This mesotrophic lake was dominated by Dolichospermum throughout the entire sampling season aside from a brief shift in dominance to Microcystis in early July. Small populations of Woronichinia and Oscillatoria were occasionally present (Association to Preserve Cape Cod & Brewster Ponds Coalition, 2021). The northern shore of Lower Mill Pond is dammed and leads to Stony Brook (Figure 5). Lower Mill Pond is open to the public for kayaking, boating, and fishing. Sampling at Lower Mill Pond took place at the end of a 3 m-long private dock on the east shore (Figure 5 and Figure 6) (41.7396° N, 70.1074° W). Water depth at the end of the dock was approximately 0.5 m. The dock at Lower Mill Pond was
surrounded by shrubs and trees overhead providing partial wind protection and shade throughout the sampling periods. Sampling took place 9 am-1 pm (EST).

Figure 5. Lower Mill Pond in Brewster, MA. “A” shows the location of the Lower Mill Pond dam leading to Stony Brook (41.7414° N, -70.1149° W). “B” shows the location of the private dock where sampling took place (41.7396° N, -70.1074° W) (Google Maps, 2022).
Figure 6. Private dock and sampling equipment used for sample collection at Lower Mill Pond in Brewster, MA (41.7396° N, -70.1074° W).

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 7 and Figure 8). The basic design of the unit is based on a filter collection aerosol device described by Murby & Haney (2015) and later modified by Langley (2019). 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. A wind screen minimizes wind effects. Air passes into a system of 2 mm diam (ID) Tygon tubing (Figure 8) and through a Whatman GFF 25 mm diameter glass fiber filter to collect 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 to 0.3 μm (Nayar & Chou, 2003). Toxins captured by 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. Toxin retained in the traps was 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 m³ 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 7. 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.
Figure 8. Diagram of the inside of the CLAM showing one of the three sets that is stored inside a CLAM. Scale and position of the arm is not accurate in this diagram and should only be used for context to understand the orientation of the traps. Red arrows represent air flow through the system. Small circles represent aerosolized particles which are not visible. Created with BioRender.com.

Water Sampling

Water fractions collected include whole lake water (WLW), < 50 μm, 0.3-2.0 μm, and < 0.3 μm to represent all organisms present in the water, the Pico fraction, and the dissolved fraction, respectively (Figure 9). Aerosol samples included toxin captured on the filters (particulate), toxin captured in the water traps (dissolved), and both combined (total).

Directly next to the CLAM, a 90 mL sample of surface whole lake water (WLW) was collected and mixed thoroughly using a PETG Nalgene bottle. From that bottle, 30 mL were
collected as a WLW sample. This WLW sample was unfiltered lake water and thus represents all organisms present in the water. An additional 60 mL were passed through a 53 μm mesh Nitex net. Of the filtrate, 30 mL were collected as a < 50 μm sample. The remaining 30 mL were passed through a 2 μm TTTP Isopore membrane filter (MilliporeSigma, Burlington, MA) using a 60 mL syringe and attached 25 mm Swinney polypropylene filter holder. To collect the “Pico fraction” (0.3-2.0 μm), 30 mL of the < 2 μm filtrate was filtered through a 0.3 μm pre-ignited GFF filter using a portable vacuum pump (Gillian BDX-II Air Sampler, Sensidyne, LP, Clearwater, FL). The filtrate was collected as the < 0.3 μm fraction. The 0.3-2 μm fraction was a concentrated sample of particles present in the picoplankton size range and the < 0.3 μm filtrate was defined operationally as the dissolved fraction. This entire process was repeated for triplicates and all samples were immediately put on ice, frozen at -20 °C within 8 hours of collection and remained frozen until processed.

Phycocyanin and phycoerythrin pigment levels were measured with a handheld fluorometer (Aquafluor, Turner Instruments Inc., San Jose, CA) on water fractions (WLW, < 50 μm, and < 0.3 μm) as well as the aerosol trap water. Fluorometry was not performed on the 0.3-2.0 μm water fraction as that fraction was collected on a filter (Figure 9). Aquafluor excitation wavelengths were 595 nm for phycocyanin and 545 nm for phycoerythrin. Phycocyanin, an accessory pigment to chlorophyll, is a blue-green protein pigment that is specific to cyanobacteria. Similarly, phycoerythrin is an accessory pigment that is specific to red protein pigment and commonly found in planktonic cyanobacteria.

All samples were subjected to a single freeze-thaw cycle and brought to room temperature (20-25C) before fluorometric measurements were taken (Haney & Leland, 2018). Phycocyanin and phycoerythrin in water and aerosol samples were measured prior to sample
concentration. Trap samples from June 01, 08, and 22, 2021 (Lower Mill Pond and Walkers Pond) were measured for pigments after concentration and results were adjusted for sample concentrations.

Figure 9. Flowchart showing the procedure for collecting water samples (WLW, < 50 μm, 0.3-2 μm, and < 0.3 μm) as described above. Created with BioRender.com.
Environmental Parameters

Temperature differential, light intensity, evaporation rate, wind velocity and wind direction, and humidity data were determined at all sites on each sampling date. Temperatures during the 4 h collection periods were measured every 10 min with HOBO data loggers (ONSET, Bourne, MA) attached to a stake directly next to the CLAM (Figure 10). The HOBO monitors were placed at three heights: 10 cm above water surface (HOBO A), on the water surface (HOBO B) and 10 cm below the water surface (HOBO C). The (air-water) temperature differential was calculated using the average air and below-surface HOBO temperatures.

The same HOBO data loggers also recorded light intensity at the same three heights. Three evaporation pans were set out next to the CLAM with ~50 g of water (Figure 11). Evaporation pans (10 cm x 10 cm) were weighed at the start and end of aerosol collection using a field balance (Fuzion Global Corp, Vaughan, Ontario). Evaporation rate (g/h$^1$/m$^2$) was calculated using the average difference between the two weights as well as the time elapsed between the two measurements (Watras et al., 2016). Wind speed was measured using a handheld digital wind speed anemometer (Lttsoyh, China).

General wind direction was recorded. To simplify and define prevailing wind, wind moving west to east meant wind coming from over the shore towards the CLAM at Walkers Pond. At Lower Mill Pond, wind moving west to east meant wind coming from over the lake towards the CLAM.

Relative humidity data were collected from Weather Underground (Weather Underground, 2021) which reported weather conditions at the Brewster, MA Oliver weather station (ID: KMABREWS41) located at 41.738° N, -70.106° W.
Figure 10. ONSET HOBO Data monitors attached to a stake to measure temperature and light intensity. Monitors were placed 10 cm above the water surface (A), at the water surface (B), and 10 cm below the surface of the water (C). Monitors were placed directly next to the aerosol collector and oriented to prevent shadows from being cast by monitors above.
Figure 11. Set of three evaporation pans set out next to the CLAM during aerosol collection. Pans were oriented so the CLAM did not cast a shadow over the pans during any of the collection period. Pans were covered by a clear mesh to prevent debris from falling into the pans without interfering with air flow or light exposure.

Toxin Analysis

All aerosol samples and water samples were tested for microcystins (MC), $\beta$-Methylamino-L-alanine (BMAA), and anatoxin-a (ATX) 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 to reach the limit of detection of the ELISA tests. Throughout processing and concentration, samples were repeatedly weighed to calculate final concentration factors. Final concentrations ranged from 10×-120×, depending on the type of sample.

Aerosol filters were processed by cutting each 25 mm diam filter individually into 12 equal slices. This was done using scissors and tweezers that were cleaned between each sample using 70% ethanol and Milli-Q water. Each sliced 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 any intracellular toxin. 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 sec (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 samples were 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 all filter debris, and placed into a new microcentrifuge tube. Repeating this step once more ensured the maximum volume of liquid sample was collected free of any remaining filter debris. Extracted toxin samples were then concentrated to 0.3 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.
The liquid aerosol trap samples were stored in 20 mL PET clear plastic vials as three individual traps all coming from the same trap system (one funnel, one filter, one pump). The trap samples went through three repetitions of the same FTVS cycle as described above. These samples were concentrated to 1 mL using the same speed vacuum systems as above. Each set of three individual trap samples (1 mL each, originating from the same trap system) were then combined to form a single, 3 mL, sample. Each sample was then further concentrated to a final volume of 0.3 mL resulting in a final concentration of 120x. Concentrated trap samples were stored in 1.5 mL microcentrifuge tubes at -20 °C until toxin analysis.

Water samples previously thawed for fluorometry subsamples to be taken were refrozen, thawed and mixed before 8 mL subsamples were taken and stored in 20 mL vials. These samples were processed with the three FTVS cycles described above. Water samples were then concentrated using the same speed vacuums from 8 mL to 1 mL. Samples were transferred into 1.5 mL microcentrifuge tubes and concentration was continued to a final volume of 0.4 mL or 20x 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 minimize solids in the ELISA test. All three ELISA tests 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 a 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. All toxin concentrations were based on the estimates from the standard curves. All standard curves had adjusted R² of >
Final toxin concentrations were adjusted for the SpeedVac concentrations and for the volume of air sampled.

**Statistical Analysis**

Data were recorded and organized 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 including two-way ANOVA and Tukey’s Post Hoc tests were used to determine the effects of fraction type and date on water toxicity. To correct for failed normality, toxin concentration values were log transformed and the two-way ANOVA tests were repeated on the transformed data. Those that continued to fail normality and/or equal variance were then rank transformed, two-way ANOVAs repeated. The results of all two-way ANOVAs on rank transformed data were analyzed and no deviations in the results between the transformed and untransformed data were found. Two-way ANOVAs on the untransformed data were reported.

Comparisons of aerosolized toxin among all dates in each lake individually were evaluated in SigmaPlot 12.5 using One-Way ANOVA and All Pairwise Multiple Comparison Procedures using Tukey’s Post Hoc tests. Parameters that failed normality or equal variance were reanalyzed using Kruskal-Wallis One-Way ANOVA on Ranks.

Standard t-tests and rank-sum comparisons performed in SigmaPlot 12.5 were used to determine significant differences in means between the two lakes. Parameters that failed normality and/or equal variance were reanalyzed using the Mann-Whitney Rank Sum Test.

Simple linear regressions performed in SigmaPlot 12.5 were used to determine the relationships between water toxin and air toxin, as well as air toxin and climatic variables. All linear regressions reported passed both normality and equal variance unless otherwise noted. When
outliers were present as determined by Cook’s distance, regressions were run both with and without the outlier data and regression results for both were reported. Additionally, possible reasons for an outlier or indicators of contamination were investigated.

Stepwise regressions were done using JMP to select the significant models through evaluation of Akaike information criterion (AICc) and BIC. After identifying and selecting significant models, JMP was used to retrieve the predicted values for each model which were then imported into SigmaPlot 12.5 to be graphed. Linear regressions were used to evaluate any covariation.
RESULTS

Seasonal Occurrence of Cyanotoxins in Water and Air

Water Microcystins

Microcystins (MC) in water fractions from the shore of Walkers Pond ranged from 0.35-6.00 ng MC L$^{-1}$ (Table 1). Water MCs at Walkers Pond varied depending on the fraction type and sampling date (two-way ANOVA, date × water fraction type interaction, p < 0.001) with the lowest MC concentrations for all fractions occurring in June (Figure 12). Microcystins in the 0.3-2.0 µm water fraction from Walkers Pond were significantly lower than other water fractions on all sampling dates from June 22nd- August 31st. All other water fractions (WLW, <50 µm and <0.3 µm) increased steadily throughout the sampling season until reaching a maximum on July 20th and August 3rd (Figure 12). MC concentrations did not change significantly in any size fraction between July 20th, August 3rd, and August 17th.

Microcystins in water fractions from the shore of Lower Mill Pond ranged from 0.38-3.78 ng MC L$^{-1}$ (Table 1). MCs in Lower Mill Pond differed significantly between size fractions and dates (two-way ANOVA, date × water fraction type interaction, p = 0.235, Figure 13). Microcystin concentrations were consistently lowest in the Pico (0.3-2.0 µm) water fraction, whereas other fractions did not have significant differences. There were no clear patterns for any of the water fractions throughout the sampling period although the MC concentrations were more variable in the early summer (Figure 13).

Walkers Pond had higher average concentrations of MC than Lower Mill Pond in all fractions (Table 1). However, these averages were not significantly different from each other in
the WLW fractions (Mann-Whitney Rank Sum Test, \( p = 0.071 \)), < 50 \( \mu m \) fractions (Mann-Whitney Rank Sum Test, \( p = 0.170 \)), or 0.3-2.0 \( \mu m \) fractions (two tailed t-test, \( p = 0.354 \), Figure 14). The average MC concentration in the dissolved (< 0.3 \( \mu m \)) fraction at Walkers Pond was significantly higher than that of Lower Mill Pond (Mann-Whitney Rank Sum Test, \( p = 0.021 \), Table 1, Figure 14). Both lakes reached a maximum concentration of MC in the Pico (0.3-2.0 \( \mu m \)) fraction on July 6th, suggesting similarities between seasonality.

Table 1. MC concentrations (ng MC L\(^{-1}\)) in all water fractions throughout the 2021 sampling season at Walkers Pond and Lower Mill Pond (Brewster, MA). Mean (± SE of the raw data), maximum, and minimum MCs in the WLW, < 50 \( \mu m \), 0.3-2.0 \( \mu m \), and < 0.3 \( \mu m \) fractions are reported in ng MC L\(^{-1}\). Bolded values represent means that are significantly different (\( p < 0.5 \)) between lakes.

<table>
<thead>
<tr>
<th>MC Concentrations (ng L(^{-1}))</th>
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<th>&lt; 50 ( \mu m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
</tr>
<tr>
<td></td>
<td>3.52 ± 0.39</td>
<td>2.50 ± 0.13</td>
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<tr>
<td>Max</td>
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<td>5.98</td>
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<tr>
<td>Min</td>
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<td>1.93</td>
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</table>

<table>
<thead>
<tr>
<th>MC Concentrations (ng L(^{-1}))</th>
<th>0.3-2.0 ( \mu m )</th>
<th>&lt; 0.3 ( \mu m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
</tr>
<tr>
<td></td>
<td>0.83 ± 0.07</td>
<td>0.73 ± 0.08</td>
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<td>Min</td>
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</tbody>
</table>
Figure 12. Microcystins (ng MC L$^{-1}$) in water fractions at Walkers Pond (Brewster, MA) during the 2021 sampling season. Water fractions include: WLW, < 50 µm, 0.3-2 µm, and < 0.3 µm. Error bars show ±SE. The effect of water fraction type on water MCs at Walkers Pond varied depending on the sampling date (two-way ANOVA, date x water fraction type interaction, p < 0.001).
Figure 13. Microcystins (ng MC L\(^{-1}\)) in water fractions at Lower Mill Pond (Brewster, MA) during the 2021 sampling season. Water fractions include: WLW, < 50 µm, 0.3-2.0 µm, and < 0.3 µm. Error bars show ± SE. There was a significant effect of fraction type on water MCs at Lower Mill Pond (\(p < 0.001\)) as well as an effect of sampling date on water MCs (\(p < 0.001\)) but no interaction between fraction type and sampling date was found (two-way ANOVA, date × water fraction type interaction, \(p = 0.235\)).
Figure 14. Average concentration of MC (ng MC L⁻¹) in all water fractions at Walkers Pond and Lower Mill Pond during the 2021 sampling season. Error bars show ± SE of the raw data. Average MC concentration of the < 0.3 µm fraction at Walkers Pond is significantly higher than that of Lower Mill Pond (Mann-Whitney Rank Sum Test, p = 0.021). No significant differences in MC concentrations of WLW (Mann-Whitney Rank Sum Test, p = 0.071), < 50 µm (Mann-Whitney Rank Sum Test, p = 0.170) and 0.3-2.0 µm fractions (t-tests, p = 0.354) were found between Walkers Pond and Lower Mill Pond.

**Aerosolized Microcystins**

Total aerosolized microcystins at Walkers Pond ranged from 330.00-710.19 pg MC m⁻³ (Table 2). On average, microcystin detected in the particulate form on the aerosol filters made up 20.35 ± 2.38% of the total aerosolized microcystin. At Walkers Pond, the highest concentration of aerosolized MC particulate was 165.90 pg MC m⁻³ (28.81% of total), on August 3rd (Figure 15). However, the highest concentration of total aerosolized MC (air filter MC + air trap MC) was recorded two weeks later (710.19 pg MC m⁻³, Figure 15). Total MC aerosol concentrations did not
differ significantly over all sampling dates at Walkers Pond (Kruskal-Wallis One-Way ANOVA on Ranks, \( p = 0.414 \), Figure 15).

Total aerosolized microcystins at Lower Mill Pond ranged from 256.61-500.76 pg MC m\(^{-3}\) (Table 2). On average, microcystin detected in the particulate form made up 16.53 \( \pm \) 2.25% of the total aerosolized microcystin. The highest concentration of aerosolized MC in particulate form (128.08 pg MC m\(^{-3}\)), as well as the highest percentage of particulate MC (29.22%) was at the end of the sampling season, on August 31st (Figure 16). However, the highest concentration of total aerosolized MC (500.76 pg MC m\(^{-3}\)) was recorded two weeks earlier on August 3rd. Total MC aerosol concentrations did not differ significantly over sampling dates (Kruskal-Wallis One-Way ANOVA on Ranks, \( p = 0.551 \), Figure 16).

Walkers Pond had higher average concentrations of aerosolized MC than Lower Mill Pond in all forms although these averages did not differ significantly (Table 2, Figure 17).

Table 2. Aerosolized MC concentrations (pg MC m\(^{-3}\)) throughout the 2021 sampling season at Walkers Pond and Lower Mill Pond (Brewster, MA). Mean (±SE of the raw data), maximum, and minimum MCs for the filters, water traps and total air are reported in pg MC m\(^{-3}\). No significant differences between lakes within each sample type (\( p > 0.5 \)).

<table>
<thead>
<tr>
<th>MC Concentrations (pg m(^{-3}))</th>
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<th>Water Trap</th>
<th>Combined (Total)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
<td>Walkers Pond</td>
</tr>
<tr>
<td>Mean ± SE</td>
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<td>Max</td>
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<tr>
<td>Min</td>
<td>57.39</td>
<td>51.51</td>
<td>272.62</td>
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Figure 15. Aerosolized microcystins (pg MC m^{-3}) at Walkers Pond (Brewster, MA) during the 2021 sampling season including the breakdown of filters in black and water traps in grey. Total MC aerosol concentrations did not differ significantly over all sampling dates at Walkers Pond (Kruskal-Wallis One-Way ANOVA on Ranks, p = 0.414).
Figure 16. Aerosolized microcystins (pg MC m$^{-3}$) at Lower Mill Pond (Brewster, MA) during the 2021 sampling season including the breakdown of filters in black and water traps in grey. Total MC aerosol concentrations did not differ significantly over sampling dates (Kruskal-Wallis One-Way ANOVA on Ranks, $p = 0.551$).
Figure 17. Aerosolized MC (pg MC m\(^{-3}\)) at Walkers Pond and Lower Mill Pond during the 2021 sampling season. Error bars are ±SE of the raw data. No significant differences in average MC concentrations on filters (t-test, \(p = 0.230\)), water traps (Mann-Whitney Rank Sum Test, \(p = 0.146\)), or total air (Mann-Whitney Rank Sum Test, \(p = 0.062\)) were found between the two lakes.

**Water BMAA**

\(\beta\)-Methylamino-L-alanine (BMAA) in water fractions from the shore of Walkers Pond ranged from below detectable limit (5000 ng L\(^{-1}\)) to 0.66 µg BMAA L\(^{-1}\) (Table 3). BMAA concentrations in the water varied between size fractions (\(p > 0.001\)) as well as between dates (\(p > 0.001\)) (two-way ANOVA, date × water fraction type interaction, \(p = 0.163\), Figure 18). Across all sampling dates, WLW BMAA did not differ significantly. The maximum BMAA concentration in the < 50 µm fraction (June 1\(^{st}\)) only differed significantly from the minimum
BMAA concentration in the < 50 µm fraction (August 31st). Otherwise, there was no significant differences between any < 50 µm BMAA across the sampling dates. The 0.3-2.0 µm fraction on each date had no significant differences in BMAA concentration when compared to all other dates. The < 0.3 µm fraction peaked on July 6th at 0.66 µg BMAA L⁻¹ (Figure 18). This concentration was significantly higher than those on June 1st, July 20th, and August 31st. The second highest BMAA concentration in the < 0.3 µm fraction was significantly higher than those on June 1st and August 31st (Figure 18).

BMAA in water fractions from the shore of Lower Mill Pond ranged from 0.05-2.07 µg BMAA L⁻¹ (Table 3). There was no significant effect of fraction type (p = 0.165) or sampling date (p = 0.757) on water BMAA at Lower Mill Pond (two-way ANOVA, date × water fraction type interaction, p = 0.711, Figure 19).

Walkers Pond had higher average concentrations of BMAA than Lower Mill Pond in all fractions except the dissolved (< 0.3 µm) fraction (Table 3, Figure 20). These averages were significantly different between lakes in all fractions with Walkers Pond having significantly more BMAA on average than Lower Mill Pond in the WLW fractions (Mann-Whitney Rank Sum Test, p = 0.031), < 50 µm fractions (two tailed t-test, p = 0.002), and 0.3-2.0 µm fractions (two tailed t-test, p < 0.001, Figure 20). The average MC concentration in the < 0.3 µm fraction at Lower Mill Pond was significantly higher than at Walkers Pond (Mann-Whitney Rank Sum Test, p = 0.002, Figure 20) and the maximum dissolved BMAA was approximately three times higher than the maximum in Walkers Pond.
Table 3. BMAA concentrations (µg BMAA L⁻¹) in all water fractions throughout the 2021 sampling season at Walkers Pond and Lower Mill Pond (Brewster, MA). Mean (± SE of the raw data), maximum, and minimum BMAA in the WLW, < 50 µm, 0.3-2.0 µm, and < 0.3 µm fractions are reported in µg BMAA L⁻¹. Bolded values represent means that are significantly different (p < 0.5) between lakes. BDL refers to concentrations below 5000 ng L⁻¹.

<table>
<thead>
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<td>Lower Mill Pond</td>
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<tr>
<td>Mean ± SE</td>
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<table>
<thead>
<tr>
<th>BMMA Concentrations (µg L⁻¹)</th>
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<th>&lt; 0.3 µm</th>
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</thead>
<tbody>
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<td>Lower Mill Pond</td>
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<td>Mean ± SE</td>
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<td>Mean ± SE</td>
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<td>Max</td>
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<tr>
<td>Min</td>
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<td>0.15</td>
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</tbody>
</table>
Figure 18. BMAA (µg BMAA L⁻¹) in water fractions at Walkers Pond (Brewster, MA) during the 2021 sampling season. Water fractions include: WLW, <50 µm, 0.3-2.0 µm, and <0.3 µm. Error bars are ± SE. There was a significant effect of fraction type on water BMAA at Walkers Pond (p < 0.001) as well as an effect of sampling date on water BMAA (p < 0.001) but no interaction between fraction type and sampling date was found (two-way ANOVA, date × water fraction type interaction, p = 0.163).
Figure 19. BMAA (µg BMAA L⁻¹) in water fractions at Lower Mill Pond (Brewster, MA) during the 2021 sampling season. Water fractions include: WLW, < 50 µm, 0.3-2.0 µm, and < 0.3 µm. Error bars are ± SE. No significant effect of fraction type or sampling date on water BMAA was found at Lower Mill Pond (two-way ANOVA, date × water fraction type interaction, p = 0.711).
Aerosolized BMAA

Total aerosolized BMAA at Walkers Pond ranged from 14.74–80.91 ng BMAA m⁻³ (Table 4). On average, BMAA detected in the particulate form made up 5.53 ± 1.40% of the total aerosolized BMAA. Total BMAA aerosol concentrations peaked on August 17th at 80.91 ng BMAA m⁻³ with 17.39% extracted in the particulate form (Figure 21). The concentration of aerosolized BMAA on the filters on August 17th was 14.07 ng BMAA m⁻³. Total BMAA aerosol concentrations did not differ significantly over sampling dates (Kruskal-Wallis One-Way ANOVA on Ranks, p = 0.782, Figure 21).
Total aerosolized BMAA at Lower Mill Pond ranged from 8.49- 50.16 ng BMAA m$^{-3}$ (Table 4). On average, BMAA detected in the particulate form made up 6.59 ± 4.30% of the total aerosolized BMAA. The highest concentration of aerosolized BMAA in the particulate form was 13.34 ng BMAA m$^{-3}$, on August 17th (Figure 22). The highest percent of total aerosolized BMAA extracted in the particulate form was 38.82%, on July 2nd. However, on August 3rd, the highest concentration of total aerosolized BMAA was recorded at 50.16 ng BMAA m$^{-3}$. Total BMAA aerosol concentrations did differ significantly over all sampling dates but no significant differences between specific groups were identified (One-Way ANOVA on Ranks, p = 0.037, Figure 22).

Walkers Pond had higher average concentrations of aerosolized BMAA than Lower Mill Pond both in the dissolved form and total combined but Lower Mill Pond had a higher average BMAA concentration in the particulate form (Table 4). However, these averages were not significantly different from each other in the particulate form (Mann-Whitney Rank Sum Test, p = 0.745), dissolved form (Mann-Whitney Rank Sum Test, p = 0.118), or both combined into total aerosolized toxin (Mann-Whitney Rank Sum Test, p = 0.433, Figure 23).
Table 4. Aerosolized BMAA concentrations (ng m\(^{-3}\)) throughout the 2021 sampling season at Walkers Pond and Lower Mill Pond (Brewster, MA). Mean (± SE of the raw data), maximum, and minimum BMAA for the filters, water traps and total are reported in ng BMAA m\(^{-3}\). BDL refers to concentrations below 5000 ng L\(^{-1}\). No significant differences between lakes within each sample type (p > 0.5).

<table>
<thead>
<tr>
<th>BMAA Concentrations (ng m(^{-3}))</th>
<th>Filter</th>
<th>Water Trap</th>
<th>Combined (Total)</th>
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<tbody>
<tr>
<td></td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
<td>Walkers Pond</td>
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<td>Mean ± SE</td>
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<td>42.25 ± 7.94</td>
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<tr>
<td>Max</td>
<td>14.07</td>
<td>13.34</td>
<td>66.84</td>
</tr>
<tr>
<td>Min</td>
<td>BDL</td>
<td>BDL</td>
<td>13.04</td>
</tr>
</tbody>
</table>

Figure 21. Aerosolized BMAA (ng BMAA m\(^{-3}\)) at Walkers Pond (Brewster, MA) during the 2021 sampling season including the breakdown of filters in black and water traps in grey. Total BMAA aerosol concentrations did not differ significantly over sampling dates (Kruskal-Wallis One-Way ANOVA on Ranks, p = 0.782).
Figure 22. Aerosolized BMAA (ng BMAA m⁻³) at Lower Mill Pond (Brewster, MA) during the 2021 sampling season including the breakdown of filters in black and water traps in grey. Total BMAA aerosol concentrations did differ significantly over all sampling dates but no significant differences between specific groups were identified (One-Way ANOVA on Ranks, p = 0.037).
Figure 23. Aerosolized BMAA (ng BMAA m⁻³) at Walkers Pond and Lower Mill Pond during the 2021 sampling season. Error bars are ± SE of the raw data. Mean BMAA concentrations on filters (Mann-Whitney Rank Sum Test, p = 0.745), water traps (Mann-Whitney Rank Sum Test, p = 0.118), or total air (Mann-Whitney Rank Sum Test, p = 0.433) did not differ significantly between the two lakes.

**Water Anatoxin-a**

Anatoxin-a (ATX) in water fractions from the shore of Walkers Pond ranged from 0.001-1.07 µg ATX L⁻¹ (Table 5). The effect of date on water ATX varied depending on the type of water fraction (two-way ANOVA, date × water fraction type interaction, p < 0.001, Figure 24). All water fractions on June 1st and 8th, did not differ significantly between fraction types. On June 22nd, July 6th, and July 20th, WLW and < 50 µm ATX were significantly higher than ATX concentrations in all other water fractions. After July 20th, WLW and < 50 µm ATX decline and reentered a similar range to the ATX concentrations in the other fractions (Figure 24). On Aug
3rd and 17th, water fractions did not differ (p > 0.05). The < 50 µm and < 0.3 µm fractions were significantly different only on August 31st.

ATX in water fractions from the shore of Lower Mill Pond ranged from 0.001-0.14 µg ATX L⁻¹ (Table 5). The effect of date on water ATX varied depending on the type of water fraction (two-way ANOVA, date × water fraction type interaction, p < 0.001, Figure 25). On July 20th, WLW and < 50 µm ATX were significantly higher than all other fractions on that date. WLW was also significantly higher than all other fractions on August 3rd. But < 50 µm was not significantly different than < 0.3 µm. There was a secondary peak of WLW and < 50 µm ATX concentration on August 31st, this time including the < 0.3 µm fraction as well (Figure 25). The Pico (0.3-2.0 µm) fraction was not always significantly lower than the other fractions, but it did not exhibit a peak comparable to the other fractions (Figure 25). Lower Mill Pond had higher average concentrations of ATX than Walkers Pond in all fractions except the Pico (0.3-2.0 µm) fraction (Table 5). These averages were not significantly different between lakes in all fractions including the WLW fractions (Mann-Whitney Rank Sum Test, p = 0.703), < 50 µm fractions (Mann-Whitney Rank Sum Test, p = 0.733), 0.3-2.0 µm fractions (Mann-Whitney Rank Sum Test, p = 0.345), and < 0.3 µm fractions (Mann-Whitney Rank Sum Test, p = 0.258, Figure 26).
Table 5. ATX concentrations (µg ATX L\(^{-1}\)) in all water fractions throughout the 2021 sampling season at Walkers Pond and Lower Mill Pond (Brewster, MA). Mean (± SE of the raw data), maximum, and minimum ATX in the WLW, < 50 µm, 0.3-2.0 µm, and < 0.3 µm fractions are reported in µg ATX L\(^{-1}\). No significant differences between lakes within each fraction (p > 0.5).

<table>
<thead>
<tr>
<th>ATX Concentrations</th>
<th>WLW</th>
<th>&lt; 50 µm</th>
<th>0.3-2.0 µm</th>
<th>&lt; 0.3 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µg L(^{-1}))</td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.40 ± 0.08</td>
<td>1.16 ± 0.37</td>
<td>0.38 ± 0.08</td>
<td>1.12 ± 0.38</td>
</tr>
<tr>
<td>Max</td>
<td>1.07</td>
<td>5.40</td>
<td>0.99</td>
<td>5.29</td>
</tr>
<tr>
<td>Min</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ATX Concentrations</th>
<th>0.3-2.0 µm</th>
<th>&lt; 0.3 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µg L(^{-1}))</td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.1</td>
</tr>
<tr>
<td>Max</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>Min</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 24. ATX (µg ATX L⁻¹) in water fractions at Walkers Pond (Brewster, MA) during the 2021 sampling season. Water fractions include: WLW, < 50 µm, 0.3-2.0 µm, and < 0.3 µm. Error bars are ± SE. The effect of date on water ATX varied depending on the type of water fraction (two-way ANOVA, date × water fraction type interaction, p < 0.001).
Figure 25. ATX (µg ATX L⁻¹) in water fractions at Lower Mill Pond (Brewster, MA) during the 2021 sampling season. Water fractions include: WLW, < 50 µm, 0.3-2.0 µm, and < 0.3 µm. Error bars are ± SE. The effect of date on water ATX varied depending on the type of water fraction (two-way ANOVA, date × water fraction type interaction, p < 0.001).
Figure 26. Average concentration of ATX (µg ATX L⁻¹) in all water fractions at Walkers Pond and Lower Mill Pond during the 2021 sampling season. Error bars are ± SE of the raw data. No significant difference was found between average ATX concentration between Walkers Pond and Lower Pond in all fractions including, the WLW fractions (Mann-Whitney Rank Sum Test, p = 0.703), < 50 µm fractions (Mann-Whitney Rank Sum Test, p = 0.733), 0.3-2.0 µm fractions (Mann-Whitney Rank Sum Test, p = 0.345), and < 0.3 µm fractions (Mann-Whitney Rank Sum Test, p = 0.258).

### Aerosolized Anatoxin-a

Total aerosolized ATX at Walkers Pond ranged from 2.64-8.85 ng ATX m⁻³ (Table 6). On average, ATX detected in the particulate form made up 1.57 ± 0.58% of the total aerosolized ATX. Total ATX aerosol concentrations peaked on August 3rd at 8.85 ng ATX m⁻³ with 7.08% extracted in the particulate form (Figure 27). August 3rd was also the date of maximum ATX concentration detected in the particulate form (0.63 ng ATX m⁻³). Total ATX aerosol concentrations did not differ significantly over sampling dates (One-Way ANOVA, p = 0.768, Figure 27).
Total aerosolized ATX at Lower Mill Pond ranged from 2.35-9.78 ng ATX m\(^{-3}\) (Table 6). There were no clear peaks of total aerosolized ATX on any of the dates except for July 6\(^{th}\). A large spike occurred with the total aerosolized ATX reaching 9.78 ng ATX m\(^{-3}\) (Figure 28). On average, ATX detected in the particulate form made up 1.88 ± 0.76% of the total aerosolized ATX. The highest concentration of aerosolized ATX in the particulate form was 0.32 ng ATX m\(^{-3}\), on August 31\(^{st}\) (Figure 28). The highest percent of total aerosolized ATX collected in the particulate form was 10.58%, on August 31\(^{st}\). Total ATX aerosol concentrations did not differ significantly over sampling dates (Kruskal-Wallis One-Way ANOVA On Ranks p = 0.492, Figure 28).

Walkers Pond had higher average concentrations of aerosolized ATX than Lower Mill Pond in all forms (Table 6). When compared statistically, there was a significant difference in average aerosolized ATX between each lake in the dissolved form (Mann-Whitney Rank Sum Test, p = 0.031) and total air (Mann-Whitney Rank Sum Test, p = 0.038, Figure 29). The difference between average ATX concentrations in the particulate form was not significant (Mann-Whitney Rank Sum Test, p = 0.967) between Walkers Pond and Lower Mill Pond (Figure 29).
Table 6. Aerosolized ATX concentrations (ng ATX m\(^{-3}\)) throughout the 2021 sampling season at Walkers Pond and Lower Mill Pond (Brewster, MA). Mean (± SE of the raw data), maximum, and minimum ATX for the filters, water traps and total are reported in ng ATX m\(^{-3}\). Significantly different averages between lakes are bolded (p < 0.05). BDL refers to concentrations below 0.15 ng ATX mL\(^{-1}\).

<table>
<thead>
<tr>
<th>ATX Concentrations (ng m(^{-3}))</th>
<th>Filter</th>
<th>Water Trap</th>
<th>Combined (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Walkers Pond</td>
<td>Lower Mill Pond</td>
<td>Walkers Pond</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.22 ± 0.08</td>
<td>0.18 ± 0.05</td>
<td>6.10 ± 0.81</td>
</tr>
<tr>
<td>Max</td>
<td>0.63</td>
<td>0.32</td>
<td>8.22</td>
</tr>
<tr>
<td>Min</td>
<td>BDL</td>
<td>BDL</td>
<td>2.64</td>
</tr>
</tbody>
</table>

Figure 27. Total aerosolized ATX (ng ATX m\(^{-3}\)) at Walkers Pond (Brewster, MA) during the 2021 sampling season including the breakdown of filters in black and water traps in grey. Total ATX aerosol concentrations did not differ significantly over sampling dates (One-Way ANOVA, p = 0.768).
Figure 28. Total aerosolized ATX (ng ATX m$^{-3}$) at Lower Mill Pond (Brewster, MA) during the 2021 sampling season including the breakdown of filters in black and water traps in grey. Total ATX aerosol concentrations did not differ significantly over sampling dates (Kruskal-Wallis One-Way ANOVA On Ranks, $p = 0.492$).
Figure 29. Aerosolized ATX (ng ATX m\(^{-3}\)) at Walkers Pond and Lower Mill Pond during the 2021 sampling season. Error bars show ± SE of the raw data. Walkers aerosolized ATX was significantly higher than that of Lower Mill Pond in the dissolved form (Mann-Whitney Rank Sum Test, \(p = 0.031\)) and total air (Mann-Whitney Rank Sum Test, \(p = 0.038\)). The difference between average ATX concentrations in the particulate form was not significant (Mann-Whitney Rank Sum Test, \(p = 0.967\)) between Walkers Pond and Lower Mill Pond.

Potential Drivers of Toxic Aerosol Production

**Biological Factors and Insignificant Climatic Factors**

There were no significant correlations between aerosol toxicity and pigment concentrations (PC and PE) used as a bioindicator. Additionally, no significant relationships were found between aerosol toxicity and light intensity or humidity regardless of location or toxin.
Microcystins at Walkers Pond

Surprisingly, in contrast to Lower Mill Pond the concentration of microcystins in the water at Walkers Pond was not an indicator of the concentration of MCs in the air. Aerosolized MC in filters and/or water traps were not significantly correlated with MCs in any water fraction. However, air-water temperature differential was the best climatic predictor of aerosolized microcystins (dissolved water trap MC) at Walkers Pond (p = 0.003, Adj. R²: 0.750, Figure 30). As the air became warmer relative to the water, the concentration of aerosolized microcystins decreased. The lack of significant relationship with the aerosolized filter MCs may be accounted for by the small n size i.e., several filters had MC concentrations too small to detect. No other climatic factors (evaporation rate, wind velocity, light intensity, and humidity) showed a significant relationship with aerosolized MC at Walkers Pond.

Examined individually, air temperature was negatively correlated with aerosolized MC (p = 0.047, Adj. R²: 0.428) but not correlated with water temperature (p = 0.203). The trend between air temperature and aerosolized MC was synonymous with that of temperature differential and aerosolized MC (Figure 30); the lowest concentrations of aerosolized MC occurred when air temperature was warmest.
Figure 30. Aerosolized MC in water traps at Walkers Pond (Brewster, MA) by air-water temperature differential (°C) (p = 0.003, Adj. R²: 0.750). Error bars are ± SE. Equation: Walkers Aerosolized MC in Water traps (pg MC m⁻³) = 536.186 - (17.073 * (Air – Water Temp (°C))).

Dissolved aerosolized microcystin at Walkers Pond was predicted well using a two-parameter linear model including both air and water temperature differential and MC concentration in the Pico (0.3-2.0 µm) size fraction (p = 0.004, Adj. R²: 0.839, Figure 31). The addition of MC in the Pico (0.3-2.0 µm) fraction strengthened the air-water temperature relationship (Figure 30) accounting for roughly 84% of the variability in the aerosolized MCs (Figure 31). When evaluated as a single parameter, Pico (0.3-2.0 µm) fraction MCs in the water did not predict aerosolized MCs at Walkers Pond (p = 0.369).
Figure 31. Walkers Pond predicted aerosolized MC in water traps (pg MC m\(^{-3}\)) vs. actual aerosolized MC measured in water traps (pg MC m\(^{-3}\)). This two-parameter model includes difference between air and water temperature (effect \(p = 0.002\)) and the 0.3-2.0 µm fraction MC (effect \(p = 0.092\)). Dotted red lines are confidence intervals (99%). Predicted Aerosolized MC in Water traps (pg MC m\(^{-3}\)) = 632.162 - 119.078 \* 0.3-2.0 µm Fraction MC (ng MC L\(^{-1}\)) - 16.735 \* (Air-Water Temperature (°C)).

Microcystins at Lower Mill Pond

Aerosolized microcystins captured via air filter, water traps, or both combined were not correlated with MCs in any water fraction at Lower Mill Pond. In contrast to Walkers Pond, recorded climatic factors singly or in combination were not correlated with aerosolized MCs in Lower Mill Pond. No combination of environmental parameters accurately predicted any form of aerosolized MCs at Lower Mill Pond. The air-water temperature parameter significant at
Walkers Pond (Figure 30) was not significant at Lower Mill Pond ($p = 0.868$, Adj. $R^2$: 0.00, Figure 32).

![Graph](image_url)

*Figure 32. Aerosolized MC in water traps at Lower Mill Pond (Brewster, MA) by air-water temperature differential ($^\circ$C) ($p = 0.868$, Adj. $R^2$: 0.00). Error bars are ±SE.*

**BMAA at Walkers Pond**

Aerosolized BMAA (filter, trap, or total) was not correlated with BMAA concentrations in any water fractions at Walkers Pond. None of the forms of aerosolized BMAA correlated with any singular climatic parameter. However, BMAA aerosolized in the dissolved form was predicted by a three-parameter model including BMAA concentration in the $< 50$ µm fraction, air-water temperature differential, and evaporation rate ($p = 0.023$, Adj. $R^2$: 0.884, Figure 33). BMAA in the $< 50$ µm fraction, air-water temperature differential, and evaporation rate all contributed significantly to the model ($p = 0.0065$, $p = 0.035$, and $p = 0.0175$, respectively).
However, individually these factors were not significant predictors ($p > 0.05$) of aerosolized BMAA.

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**Figure 33.** Walkers Pond predicted aerosolized BMAA in water traps vs. actual aerosolized BMAA in water traps. This three-parameter model includes BMAA in the $< 50 \, \mu m$ fraction (effect $p = 0.0065$), difference between air and water temperature (effect $p = 0.035$), and evaporation rate (effect $p = 0.0175$). Dotted red lines are confidence intervals (99%). Predicted Aerosolized BMAA in Water traps (ng BMAA m$^{-3}$) = $-85.973 - (2.076 \times \text{Air - Water Temperature (°C)}) + (0.0552 \times \text{Evaporation Rate (g$^{-1}$ hr$^{-1}$ m$^{-2}$))} + (241.072 \times <50 \mu m$ fraction BMAA ($\mu g$ BMAA L$^{-1}$)).

**BMAA at Lower Mill Pond**

At Lower Mill Pond aerosolized dissolved BMAA was positively correlated with BMAA concentration in the Pico (0.3-2.0 $\mu m$) fraction ($p = 0.014$, Adj. $R^2$: 0.605, Figure 34). However, this correlation was influenced by a single outlier (Cook’s D: 0.802, Figure 34). Aerosolized BMAA on the filters (particulate form) and the combined total, were not correlated with BMAA concentration in any water fraction or with single or combined climatic parameters at Lower Mill Pond.
Figure 34. Aerosolized BMAA in water traps (ng BMAA m$^{-3}$) at Lower Mill Pond (Brewster, MA) by BMAA concentration in the 0.3-2.0 µm fraction (µg BMAA L$^{-1}$) including outlier ($p = 0.014$, Adj. $R^2$: 0.605). Error bars are ± SE. Two data points represent an average of two replicates and two data points represent a single observation due to replicates being BDL. These data points do not have SE bars for this reason. Equation: Walkers Pond Aerosolized BMAA in Water traps (ng BMAA m$^{-3}$) = 19.2-39 + (505.195 * 0.3-2.0 µm fraction (µg BMAA L$^{-1}$)). The regression is dependent of the outlier (0.133, 50.158) (Cook’s D: 0.802). The relationship was evaluated without this data point as well and no significant relationship between the aerosolized dissolved BMAA and BMAA in the Pico (0.3-2.0 µm) fraction ($p = 0.354$, Adj. $R^2$: 0.007).

**ATX at Walkers Pond**

Particulate, dissolved, or total aerosolized ATX at Walkers Pond could not be predicted by any singular or combination of environmental parameters. Included in the tested parameters were evaporation rate and wind velocity as both were significant parameters at Lower Mill Pond (Figure 37, Figure 38). However, neither evaporation rate ($p = 0.273$, Figure 35) nor wind velocity ($p = 0.899$, Figure 36) were correlated with aerosolized dissolved ATX at Walkers Pond.
Figure 35. Aerosolized ATX in water traps (ng ATX m\(^{-3}\)) at Walkers Pond (Brewster, MA) by evaporation rate (g\(^{-1}\) hr\(^{-1}\) m\(^{-2}\)) (\(p = 0.273\), Adj. \(R^2\): 0.078). Error bars are ± SE.

Figure 36. Aerosolized ATX in water traps (ng ATX m\(^{-3}\)) at Walkers Pond (Brewster, MA) by wind velocity (m\(^{-1}\) s\(^{-1}\)) (\(p = 0.899\), Adj. \(R^2\): 0.00). Error bars are ± SE.
**ATX at Lower Mill Pond**

Aerosolized dissolved ATX at Lower Mill Pond was positively correlated with evaporation rate \( p = 0.006, \text{ Adj. } R^2: 0.76, \text{ Figure } 37 \). This relationship, however, is dependent on a singular outlier, \((1086.96, 9.774)\), recorded on July 6\(^{th}\) (Cook’s D: 42.49). Without the outlier, there was no significant relationship between the aerosolized ATX in the water traps and evaporation rate \( p = 0.465, \text{ Adj. } R^2: 0.000 \).

Wind velocity was also positively correlated with aerosolized dissolved ATX \( p = 0.021, \text{ Adj. } R^2: 0.555, \text{ Figure } 38 \), i.e., ATX in the water traps tended to be highest on the days when average wind velocity was relatively higher. Again, this relationship is dependent on an outlier recorded on July 6\(^{th}\), \((3.344, 9.774)\) (Cook’s D: 25.45). Without the outlier, there was no significant relationship between the aerosolized ATX in the water traps and wind velocity \( p = 0.550, \text{ Adj. } R^2: 0.000 \).

When evaporation and wind velocity were combined, a significant two-parameter model predicted aerosolized ATX in the water traps \( p = 0.020, \text{ Adj. } R^2: 0.789 \). However, this model is unreliable due to covariance between evaporation rate and wind velocity \( p = 0.038, \text{ Adj. } R^2: 0.5232 \). There were no other individual or combination of environmental parameters that correlated with particulate, dissolved, or total aerosolized ATX.
Figure 37. Aerosolized ATX in water traps (ng ATX m\(^{-3}\)) at Lower Mill Pond (Brewster, MA) by evaporation rate (g\(^{-1}\) hr\(^{-1}\) m\(^{-2}\)) including outlier (p = 0.006, Adj. R\(^2\): 0.764). Error bars are ± SE. Equation: Lower Mill Pond Aerosolized ATX in Water traps (ng ATX m\(^{-3}\)) = -0.674 + (0.00882 * Evaporation Rate (g\(^{-1}\) hr\(^{-1}\) m\(^{-2}\))). This relationship is dependent on a singular outlier, (1086.96, 9.774), recorded on July 6th (Cook’s D: 42.49). Without the outlier, there was no significant relationship between the aerosolized ATX in the water traps and evaporation rate (p = 0.465, Adj. R\(^2\): 0.000).
Aerosolized ATX in water traps (ng ATX m$^{-3}$) at Lower Mill Pond (Brewster, MA) by wind velocity (m$^{-1}$ s$^{-1}$) including outlier (p = 0.021, Adj. R$^2$: 0.555). Error bars are ± SE. Equation: Lower Mill Pond Aerosolized ATX in Water traps (ng ATX m$^{-3}$) = 1.162 + (1.978 * Wind velocity (m$^{-1}$ s$^{-1}$)). This relationship is dependent on an outlier recorded on July 6th, (3.344, 9.774) (Cook’s D: 25.45). Without the outlier, there was no significant relationship between the aerosolized ATX in the water traps and wind velocity (p = 0.550, Adj. R$^2$: 0.000).

Summary of Potential Drivers of Toxin Aerosol Production:

- Aerosolized MC (in water traps) at Walkers Pond were correlated with temperature differential (p = 0.003, Adj. R$^2$: 0.750, Figure 30), air temp (p = 0.047, Adj. R$^2$: 0.428), and 2), and a two-parameter model including temperature differential and the Pico (0.3-2.0 µm) fraction MC (p = 0.004, Adj. R$^2$: 0.839, Figure 31).
- Aerosolized BMAA (in water traps) at Walkers Pond were predicted by a three-parameter model including < 50 µm fraction BMAA, evaporation rate and temperature differential (p = 0.023, Adj. R²: 0.884, Figure 33).

- Aerosolized BMAA (in water traps) at Lower Mill Pond were correlated with the Pico (0.3-2.0 µm) fraction BMAA (p = 0.014, Adj. R²: 0.605, Figure 34).

- Aerosolized ATX (in water traps) at Lower Mill Pond were correlated with evaporation rate (p = 0.006, Adj. R²: 0.760, Figure 37) and wind velocity (p = 0.021, Adj. R²: 0.555, Figure 38).
DISSCUSSION

Aerosolized Toxin

Although there has been considerable research on the importance of aerosolized marine toxins, such as brevetoxin (red tide) (Cheng et al., 2005; Pierce et al., 2003, 2005; Tesson et al., 2016), aerosolization of cyanobacteria in lakes is an understudied field despite its important health implications. Studies have presented evidence of aerosolization of microcystins (MCs), β-Methylamino-L-alanine (BMAA), and anatoxin (ATX). However, these studies are limited and do not use methods that include a water trap to collect dissolved toxin. Concentrations of aerosolized cyanotoxin detected in this study suggest that lack of liquid trap to collect dissolved toxin results in underestimation of total aerosolized toxin concentrations.

Microcystin Concentrations

Total aerosolized microcystins (MCs) ranged from 330.00-710.19 pg MC m$^{-3}$ with a mean of 498.79 ± 64.94 pg MC m$^{-3}$ at Walkers Pond. At Lower Mill Pond, total aerosolized microcystins ranged from 256.61-500.7 pg MC m$^{-3}$ with a mean of 357.38 ± 25.33 pg MC m$^{-3}$ (Table 2). While these values were generally higher than similar studies (Backer, 2008; Cheng et al., 2007; Langley, 2019; Murby & Haney, 2015; Wood & Dietrich, 2011), my study measured both particulate and dissolved toxin whereas these comparative studies measured particulate toxin only (relied solely on filters for aerosol collection). When comparing concentrations of aerosolized particulate (filter) microcystins, the range of values recorded at Walkers Pond (57.39-165.90 pg MC m$^{-3}$, mean of 91.81 ± 11.46 pg MC m$^{-3}$) and Lower Mill Pond (51.51-128.08 pg MC m$^{-3}$, mean of 73.27 ± 8.75 pg MC m$^{-3}$) (Table 2), were comparable to reported
concentrations at New Hampshire lakes (13-384 pg MC m$^{-3}$) (Murby & Haney, 2015). Dissolved toxin component (water trap) was a significant contributor to aerosolized toxin contributing to an average of 79.65-98.45% of total aerosolized toxin (Figure 15, Figure 16), suggesting that concentrations detected in my study could be a better representation of total aerosolized toxin when compared to studies that only used air filters and, as a result, likely underestimated total aerosolized toxin.

**BMAA Concentrations**

Total aerosolized $\beta$-Methylamino-$L$-alanine (BMAA) ranged from 14.74-80.91 ng BMAA m$^{-3}$ at Walkers Pond with a mean of 45.13 ± 8.88 ng BMAA m$^{-3}$. At Lower Mill Pond, total aerosolized BMAA ranged from 8.49-50.16 ng BMAA m$^{-3}$ with a mean of 24.66 ± 4.32 ng BMAA m$^{-3}$ (Table 4). Concentrations in the air at these Cape Cod lakes were comparable to aerosols detected in a South African lake using a liquid sampler with a design similar to that of the CLAM water trap (6-39 ng BMAA m$^{-3}$) (Scott & Downing, 2018). However, the upper range BMAA levels were two times higher in my study, with 80 ng BMAA m$^{-3}$ at Walkers Pond. Generally, levels of BMAA in water fractions in my study (0.05-2.07 µg L$^{-1}$, Table 3) were similar to those found in comparative studies (0.015-25.3 µg L$^{-1}$), though on the lower end (Abbes et al., 2022; Al-Sammak et al., 2014; Lance et al., 2018; Pip et al., 2016).

**Anatoxin-a Concentrations**

Total aerosolized anatoxin (ATX) ranged from 2.64-8.85 ng ATX m$^{-3}$ with a mean of 6.20 ± 0.81 ng ATX m$^{-3}$ at Walkers Pond (Table 6). At Lower Mill Pond, total aerosolized ATX ranged from 2.35-9.78 ng ATX m$^{-3}$ with a mean of 3.95 ± 0.59 ng ATX m$^{-3}$ (Table 6). The range of aerosolized particulate ATX detected at Walkers Pond was (0.22 ± 0.08 ng ATX m$^{-3}$) and
Lower Mill Pond (0.18 ± 0.05 ng ATX m\(^{-3}\), Table 6). There are no known comparable studies that quantify aerosolized ATX in the field.

Whole lake water (WLW) detection of ATX ranged from 0.01-1.07 µg ATX L\(^{-1}\) at Walkers Pond and 0.01-5.40 µg ATX L\(^{-1}\) at Lower Mill Pond (Table 5). The range of ATX concentrations in the Brewster lakes water was on the lower end of the typical range of ATX concentrations seen in similar studies (0.006-35.0 ng L\(^{-1}\)) (Aguilera et al., 2018; Al-Sammak et al., 2014; Hilborn et al., 2014).

Potential Environmental Drivers of Aerosolized Cyanotoxins

The majority of toxic aerosolization studies focus on marine systems. It is widely believed that wave action and wind are a significant driver of aerosolized toxin in marine systems (O’Dowd & de Leeuw, 2007; Zhang & Du, 2017). Unfortunately, there are very few studies that explore the possible drivers of cyanotoxin aerosolization in freshwater lakes. Because of this, mechanisms of cyanotoxin aerosolization are largely unexplained. This study identifies specific environmental factors that correlate to cyanotoxin aerosolization, advancing the understanding of cyanotoxin aerosolization mechanisms. Interestingly, environmental factors influencing toxic aerosol collection appear to differ between different toxins as well as between lakes. The absence of correlation between aerosol toxicity and biological factors (PC and PE) was surprising and may suggest the majority of aerosolized toxin measured was independent of cyanobacterial cells. This aligns with the finding that on average, 79.65-98.45% of the total aerosolized toxins were collected in the dissolved form.
**Potential Environmental Drivers of Aerosolized Microcystins**

Without the addition of climatic parameters, MC in the water fractions did not predict aerosolized MCs at either lake. Air-water temperature differential predicted aerosolized dissolved MCs at Walkers Pond with aerosol toxicity decreasing as air became warmer relative to the water ($p = 0.003$, Adj. $R^2$: 0.750, Figure 30).

The air-water temperature model was improved with the addition of MCs in the Pico (0.3-2.0 µm) water fraction ($p = 0.004$, Adj. $R^2$: 0.939, Figure 31), accounting for 94% of the variability in aerosolized dissolved MCs. This suggests aerosolized MCs at Walkers Pond were generated by the Pico (0.3-2.0 µm) water fraction. The Pico fraction may have aerosolized more easily compared to the large, bloom-forming cyanobacteria due to its small size (Lewandowska et al., 2017). This phenomenon could be due to the smaller surface area and weight, implying smaller cells require less energy to aerosolize. The importance of picocyanobacteria for aerosolization was consistent with Langley’s 2019 study which recognized the importance of picocyanobacteria (defined as 0.22-2.0 µm in Langley’s study), stating, “[aerosolized] picocyanobacteria were generally equal to or more numerous than larger aerosolized cells” (Langley, 2019). The picocyanobacteria discussed in Langley’s study were based on cells counted with epifluorescence on the aerosol filters, whereas the Pico fraction in my study refers to the concentration of MC in the 0.3-2.0 µm water fraction.

In both studies, air-water temperature differential was also found to be an important driver of aerosolization. Importantly, however, the indirect relationship (decrease in toxic aerosol concentration as air temperature became warmer relative to the water) identified in my study was the opposite of Langley’s findings (Langley, 2019) (Figure 30). This could be due to the
difference in aerosol form between the two studies. The relationship in my study refers to aerosolized toxin concentration in the dissolved form decreasing as temperature differential increases whereas, Langley’s relationship was based on the number of cells detected on air filters (Langley, 2019). So, while the two studies found opposite patterns, the relationships were based on different forms of aerosols. It is possible that when temperature differential is low (water cooler relative to air) less cyanobacterial cells aerosolize but, more dissolved toxin aerosolizes, thus suggesting the possibility of different mechanisms of aerosolization depending on form of toxin.

Differences between the two Cape Cod lakes were striking. There were no correlations between aerosolized MC at Lower Mill Pond and any individual or combination of parameters. The absence of correlation between any water fraction MC and aerosolized MC suggests the water fraction from which aerosolized MCs derived from was dynamic throughout the sampling season. This may have also affected the mechanism of MC aerosolization meaning that the climatic factors driving aerosolization changed as the water fraction aerosolizing changed. The inconsistency between both the source of aerosolized toxins as well as the climatic drivers suggests mechanisms of aerosolization differ between source of aerosolized toxin. It is also possible that a climatic parameter or combination of climatic parameters not recorded in this study were drivers of MC aerosolization at Lower Mill Pond.

The importance of temperature differential and the Pico fraction seen at Walkers Pond was absent at Lower Mill Pond, suggesting that fluctuation of water MCs and proportions of water MCs in each fraction throughout the sampling season may be due to changes in the cyanobacterial community composition or population growth stage across different dates.
Changes in proportions of water MCs between each fraction could affect relative concentration of aerosolized MC derived from each water fraction.

At Lower Mill Pond, *Dolichospermum* was the dominant bloom-forming cyanobacteria (BFC) observed during the majority of the sampling period except for a complete shift in early July to *Microcystis* before a new population of *Dolichospermum* regained dominance and remained dominant through the end of the sampling season (Association to Preserve Cape Cod & Brewster Ponds Coalition, 2021). There were no clear patterns in MC concentration in the water fractions at Lower Mill Pond throughout the sampling season (Figure 13). The sporadic concentrations of MCs may be related to the shifts in dominance. For example, the spike in MC in the Pico (0.3-2.0 µm) fraction on July 6th (Figure 13) could have been related to the dominance of *Microcystis* at this time. Additionally, smaller populations of *Woronichinia* and *Oscillatoria* present at times (Association to Preserve Cape Cod & Brewster Ponds Coalition, 2021) could have been producing and aerosolizing MCs at different rates, masking the effect of potential drivers on aerosolization of a singular species. All Lower Mill Pond composition and dominance data (Association to Preserve Cape Cod & Brewster Ponds Coalition, 2021) should be treated with discretion as data reflects the composition of the large BFC size fraction and community structure may vary between fractions. Additionally, BFC samples were not sampled directly at the sight of the aerosol collector. BFC samples were collected both from the shore and at the deepest point in Lower Mill Pond. Future studies should gather composition data on the Pico (0.3-2.0 µm) fraction rather than only the BFCs. Determining the composition of both the Pico fraction and the aerosols through genetic testing would lead to better understanding of which species are present and which are aerosolizing. This would allow for better resolution of how aerosolized cyanotoxins may be affected due to change in water composition.
Additionally, with shifts in dominance comes cycles of population growth and decay. The stage of the cyanobacteria population can affect the concentration of toxin in the water, possibly impacting the aerosolization potential of that water. However, the impact of growth stages on aerosol production has not been evaluated. When the population is in decay, cells break apart and once intracellular (cell-bound) toxin is released into the water. Both intracellular and extracellular microbes and molecules have been shown to aerosolize (Evans et al., 2019), suggesting that both intracellular and extracellular toxins can aerosolize. However, these two forms of aerosols may be regulated by different factors, may degrade at different rates once aerosolized and likely impact proportion of toxins present in each water fraction.

*Potential Environmental Drivers of Aerosolized BMAA*

When predicting aerosolized dissolved BMAA at Walkers Pond, the < 50 µm fraction on its own did not predict aerosolized BMAA. However, the < 50 µm BMAA was able to predict aerosolized BMAA with the inclusion of both temperature differential and evaporation rate in the model (p = 0.023, Adj. R²: 0.884, Figure 33). At Lower Mill Pond, toxin concentration in the Pico (0.3-2.0 µm) water fraction was an important factor in predicting aerosolized BMAA without the inclusion of any climatic factors (p = 0.014, Adj. R²: 0.605, Figure 34). This suggests that at Walkers Pond, aerosolized BMAA is most often aerosolizing from the < 50 µm fraction whereas at Lower Mill Pond, the BMAA present in the Pico (0.3-2.0 µm) fraction was the source of BMAA in aerosols. This could be due to a range of different factors including difference in community composition between the lakes.

At Walkers Pond, the importance of the environmental variables (< 50 µm BMAA, temperature differential, and evaporation rate) in the model predicting aerosolized BMAA
(Figure 33) suggests that the mechanism of aerosolization remained consistent throughout the sampling seasons as well as the main source of aerosolized toxin (in this case, the < 50 µm water fraction). The consistency between both the source of aerosolized toxins as well as the climatic driver suggests that mechanisms of aerosolization remain constant within fractions. Additionally, Langley identified a similar model in 2019 predicting aerosol production using the < 50 µm water fraction and temperature differential to predict cyanobacteria aerosolization (p < 0.0001, Adj. R²: 0.98) (Langley, 2019). Although my model is predicting aerosolized toxins whereas Langley’s predicted aerosolized cells, the coherence in parameters suggest that the < 50 µm fraction is aerosolized via a mechanism relating to temperature differential.

At Lower Mill Pond, no climatic factors strengthened the relationship of Pico (0.3-2.0 µm) fraction BMAA and aerosolized BMAA. This could be a result of the sampling location at Lower Mill Pond. At Lower Mill Pond sampling took place on a dock that was shaded and partially protected from the wind by overhead trees and surrounding brush (Figure 6). This protected location led to a small range of values in environmental parameters. For example, aerosolized MC at Walkers Pond was best predicted by Pico (0.3-2.0 µm) fraction MC and temperature differential which had a range of -1.38-15.41(C). At Lower Mill Pond, temperature differential values ranged from -2.50-7.16 (C). The relationships between aerosolized toxin and climatic parameters may not be sensitive enough to show a significant relationship with a range of independent variable values as small as Lower Mill Pond. This explanation could be further explored by sampling multiple locations simultaneously on one lake with a large range of temperature differential values. In this study, proximity of sampling sites would be important to reduce the variability due to composition differences. It is also possible that other, unknown,
climatic parameters excluded from this study influence aerosolization of BMAA at Lower Mill Pond.

*Potential Environmental Drivers of Aerosolized ATX*

At Walkers Pond, no parameters or combination of parameters predicted aerosolized ATX suggesting that the water fraction aerosolizing and mechanisms of ATX aerosolization was variable depending on the date (Figure 35, Figure 36). The inconsistency between both the source of aerosolized toxins as well as the climatic drivers suggest mechanisms of aerosolization differ between water fractions. This could be due to influence of environmental parameters not recorded in this study or changes in community composition and life stage across different dates.

Throughout the sampling season, dominance of the cyanobacterial community shifted from *Woronichinia* in early June to *Dolichospermum*. Composition also consisted of varying populations of *Microcystis* and *Aphanizomenon* (Association to Preserve Cape Cod & Brewster Pond Coalition, 2021). ATX in the WLW and < 50 µm fractions experienced a large spike directly after the shift of dominance from *Woronichinia* to *Dolichospermum* (Figure 24). These ATX concentrations remained high until the dominance experienced a shift from *Dolichospermum*, to a mix of *Microcystis, Woronichinia*, and *Dolichospermum*. During August, ATX concentrations in the WLW and < 50 µm fractions remained relatively low as *Dolichospermum* regained dominance. ATX production in water fractions was impacted by shifts in community composition, which likely affected ATX aerosol potential and may explain why the recorded environmental parameters had no correlation with aerosolized ATX. All Walkers Pond composition and dominance data (Association to Preserve Cape Cod & Brewster Ponds Coalition, 2021) should be treated with discretion as BFC samples were not sampled directly at
the sight of the aerosol collector. BFC samples were collected both from the shore and at the deepest part of Walkers Pond. Additionally, data reflect the composition of the BFC size fraction which may vary between fractions. To gain insight on the composition of the Pico fraction, future studies should determine species composition of the Pico fraction and the aerosols through genetic testing. This would lead to a better understanding of which species are present in the water and which are aerosolizing. This would allow for better resolution of how aerosolized cyanotoxins may be affected due to change in water composition.

As mentioned in “Potential Environmental Drivers of Aerosolized Microcystins” (Page 68), shifts in dominance create cycles of population growth and decay, possibly impacting toxin levels and forms of toxin in the water. It is conceivable that a change in the toxin profile of the water could impact aerosolization potential of that water. However, this hypothesis has not been tested.

At Lower Mill Pond, ATX aerosolization was driven by evaporation rate and wind velocity (p = 0.006, Adj. R²: 0.760, Figure 37, and p = 0.021, Adj. R²: 0.555, Figure 38) but did not appear to be correlated with any water fraction. This is the only instance in both lakes across all toxins were a correlation between climatic factors and aerosolized toxin could not be strengthened with the inclusion of toxin presence in water fractions. This suggests that the source of aerosolized ATX was variable throughout sampling season but the mechanism of ATX aerosolization remained constant. Unfortunately, this relationship between aerosolized ATX at Lower Mill Pond and both wind velocity and evaporation rate were dependent on outliers. However, these outliers followed the same patterns as the regression. This illustrates the need for a wider range of values in the data set to be able to properly evaluate this potential relationship.
The correlation between aerosolized ATX at Lower Mill Pond and evaporation rate suggests simple evaporation, a passive mechanism, was the primary mechanism for aerosolization of ATX. At the same time, the correlation between aerosolized ATX and wind velocity at Lower Mill Ponds suggests wind driven, active aerosolization could have also occurred. However, evaporation rate and wind velocity correlated with each other \((p = 0.038, \text{ Adj. } R^2: 0.532)\). This suggests wind velocity influenced evaporation rates and either mechanism of aerosolization could have occurred exclusively or both simultaneously.

In alliance with my findings, multiple studies have identified the possibility of wind driven aerosols, the majority describe a direct relationship between wind speed and aerosol production (Dueker et al., 2011, 2017; D. J. Smith et al., 2013). However, Langley’s 2019 study found an inverse relationship suggesting aerosol production is highest when wind speed is slowest (Langley, 2019). A discrepancy in aerosol production and aerosol collection could be culpable for these findings. The hypothesis that aerosol production increases with increased wind speed and surface disturbance could be true in all studies mentioned above, including Langley’s study. However, direction of wind flow could impact the method of aerosol collection. If wind flow moved from the center of lake towards the location of aerosol collection on the shore, this wind could contain higher relative levels of aerosolized toxin than if the wind moved from the shore towards the site of aerosol collection. If the wind moving through the aerosol collection site contained lower levels of toxin than the true site, the sample collected may have been diluted and vice versa.

To test this hypothesis, I included wind direction. By including direction, source of wind could be determined and used to potentially explain discrepancies in relationship between wind
speed and aerosol toxicity. However, at both lakes on all dates, wind blew towards the shore from the center of the lake. Without variation in source of wind, this hypothesis could not be tested but the direct relationship between wind velocity and aerosolized toxin did align with the dilution hypothesis.

**Summary and Comparison of Potential Environmental Drivers Between Aerosolized Toxins**

The environmental drivers, both climatic and presence of water toxin, have an impact on toxic aerosol production. However, these effects are variable depending on the lake and toxin being examined. Differences in the aerosolization of cyanotoxins at the two lakes was striking, despite their close proximity and general similarities in size and trophic condition. Differences in the size-structure of the plankton communities and lake morphometry were likely contributing factors. Also unexpected were the differences in the factors regulating aerosol production of each toxin. Comparison of the differences in drivers between the lakes and toxins have suggested that mechanisms of aerosolization and therefore the climatic drivers may depend on the water fraction source of aerosolized material. For example, MC in the Pico (0.3-2.0 µm) fraction at Walkers Pond was the source of aerosolized MC (Figure 31). The aerosolization of this fraction appears to be driven by temperature differential which suggests Pico (0.3-2.0 µm) fraction toxin aerosolizes via a specific mechanism effected by temperature differential. This relationship is likely dynamic between different toxins, locations, and compositions. This hypothesis cannot be examined with the data collected in this study as there were no two instances between toxins and/or lakes where the water fractions related to aerosol production were equivalent and both relationships were strengthened by climatic parameters. This hypothesis assumes that different mechanisms of aerosolization are correlated with varying climatic factors. However, mechanisms
of aerosolization were not directly determined in this study so the connection cannot be evaluated.

A major conclusion of this evaluation is that while the concentration of aerosolized toxin does differ depending on certain climatic factors, these climatic factors may change depending on toxin, location and which water fraction is the source of the aerosols.

Mechanisms of Aerosolization

A variety of aerosolization mechanisms have been proposed previously including wind driven aerosolization (surface disturbance and sprays), evaporation, and micro-bubble bursting (Blanchard & Syzdek, 1972; Cheng et al., 2007; Dueker et al., 2011; Medina-Pérez et al., 2021; Murby & Haney, 2015; Sahu et al., 2015). My study found only a direct correlation between wind velocity and aerosolization for ATX in Walkers Pond suggesting wind-generated aerosolization (physical surface disturbance) occurred (Figure 38), but only in one lake and with one of the three toxins. Direct correlation of aerosolized toxin with evaporation and temperature differential suggests passive aerosolization, (aerosolization without a physical disturbance i.e., evaporation), is the more dominant mechanism in this study (Figure 30, Figure 33, Figure 37). It is likely that these contrasting mechanisms of aerosolization may occur simultaneously, as suggested by the aerosolized ATX at Lower Mill Pond that was correlated with both wind velocity and evaporation rate. On the other hand, the persistence of aerosolization rates under calm conditions in the day (this study), at night (Langley, 2019) and in laboratory flasks without wind (Murby & Haney, 2015) indicate that wind is not a prerequisite for lake aerosolization to occur and that passive, evaporative-related aerosolization is a dominant mechanism of creating toxic aerosols in small lakes.
Results this study showed that multiple cyanotoxins aerosolize simultaneously (Figure 17, Figure 23, Figure 29). Aerosolized toxins were correlated with different environmental factors, suggesting the possibility of dual aerosolization mechanisms between different toxins (Figure 31 and Figure 33).

**Evaluation of the Compact Lake Aerosol Monitor**

The method of aerosol collection used in this study has benefits in understanding total aerosolization of these cyanotoxins. The trap method reveals that a major fraction of cyanotoxins was dissolved; this is a significant component when comparing against the filter methods used in similar studies (Dueker et al., 2011; Langley, 2019; Medina-Pérez et al., 2021; Murby & Haney, 2015). During initial testing of the water traps, data suggested that while this method of aerosol collection is more efficient than a filter only method, it still may not be 100% efficient. This potential inefficiency would mean that concentrations measured in this study may be an underestimation despite being higher than comparable studies. A limitation of the water traps is the porosity of the diffusers resulting in restricted air flow. The air pumps used in this study were not strong enough to pull more than 1 L air min\(^{-1}\) through the diffusers due to resistance. This flow rate limited the total volume of air passing through the collector and resulted in many filters having below detectable toxin concentrations. This prevented the evaluation of the impact environmental parameters had on aerosolized particulate toxin.

The inclusion of the water traps in the Compact Lake Aerosol Monitor (CLAM) allowed for the collection of aerosolized toxins that would have otherwise been excluded. These toxins were operationally defined as dissolved toxin due to their ability to pass through the 0.3 µm GFF filter. The use of the 0.3 µm GFF filter before the water traps allowed for the collection of toxin...
that was operationally defined as cell-bound (intracellular) toxin due its inability to pass through the 0.3 µm GFF filter (Langley, 2019). The distinction between cell-bound and dissolved toxin allowed for data collection on total aerosolized toxin as well as cell-bound toxin exclusively to better compare with studies that did not include water traps. While the distinction between toxic aerosol form provided higher resolution data to interpret, the operational definitions of cell-bound and dissolved toxin have not been tested. It is conceivable that a percentage of moisture containing dissolved toxin was retained on the filter, contributing to what was interpreted as cell-bound toxin. Furthermore, the water traps could have captured any cell debris or cells that were damaged due to air pressure on the GFF filters and, as a result, were able to pass through the GFF filter. This could be evaluated in future studies by passing collected trap samples through a 0.1 µm filter and analyzing the filter for particulate.

Although the distinction between aerosolized cell-bound and dissolved toxin provided insight into potential mechanisms of aerosolization, these interpretations should be treated with discretion as this method does not provide insight into whether aerosolized dissolved toxins captured were in the dissolved form before aerosolization, because of the mechanism of aerosolization, or because of cell damage during sample collection. For example, an aerosolized particle could be degraded by sunlight and effect the form of toxin at time of collection (Edinger et al., 1968; Lacey & West, 2006). Future studies should investigate the effects of UV exposure on toxin molecules once aerosolized.

The CLAM is designed to collect three replicate samples in the same location during each collection. As a result, this set of replicates can be used to assess variation in data due to collection method but cannot assess variation in data due to location on the lake. It is likely that
aerosol production on a singular lake varies due to spatial variation in cyanobacterial communities and varying levels of protection from climatic elements. A follow up study using CLAMs to simultaneously collect aerosols from various locations on a singular lake would allow for analysis of aerosol toxicity variation due to differences in community composition in the microenvironment. Contrastingly, manipulation experiments with two adjoining CLAMs could test variation in aerosol production due to individual climatic factors without the influence of biotic factors or other climatic factors (e.g., construct a wind barrier around one CLAM).

**Atmospheric Loading and Health Implications**

There is increasing interest in aerosols as a mode of transfer of cyanotoxins to humans (Stommel et al., 2013). Aerosolized cyanotoxins could help explain the epidemiological studies that indicate a significant influence of cyanobacteria in lakes on the incidence of non-alcoholic liver disease (Zhang et al., 2015) and amyotrophic lateral sclerosis, ALS, (Torbick et al., 2017). The presence of cyanobacteria cells in the lungs of patients who died of ALS further indicates the potential risk of breathing toxic aerosols (Facciponte et al., 2018).

The World Health Organization (WHO) set provisional tolerable daily intake of microcystin-LR at 0.04 µg kg⁻¹ d⁻¹ for food (WHO et al., 2021). Based on this guideline, an adult who weighs 60 kg (132.2 lbs.), would have a tolerable daily intake through food of 5.29 µg MC (5290 ng MC). While this limit is higher than the estimated exposure at Walkers Pond (9.98 ng MC) and Lower Mill Pond (7.15 ng MC), these potential exposure estimates should not be considered insignificant considering this guideline is based on exposure via ingestion. Exposure through inhalation is estimated to be ~10x more toxic due to a more direct route of absorption in the body (Wood & Dietrich, 2011). Due to the deviations in mode of action of MC once in the
body, Wood & Dietrich suggest a maximum daily inhalation concentration of 200 ng MC day\(^{-1}\) for a 60 kg adult (Wood & Dietrich, 2011). Also, it is fair to assume that inhalation is only one of the sources for human consumption of MC, since MC is prevalent in many foods, such as seafoods (Ibelings & Chorus, 2007) and vegetables (Machado et al., 2017). There are no comparable guidelines for daily intake of BMAA or ATX.

Estimations of potential daily inhalation at Walkers Pond and Lower Mill Pond cannot be used to determine whether daily intake is below the recommended limit due to the extensive assumptions included in these calculations. These calculations are based on the assumed inhalation of 20 m\(^3\) of air per day. This volume of air is variable due to deviations in age, sex, body size, rate of breathing, level of exercise and generally ranges from 10-30 m\(^3\) of air per day for adults. (EPA, 2011; EPA Office of Emergency and Remedial Response, 1989). For example, inhalation may be several times higher for a persons engaged in active sports or lower when sleeping (EPA, 2011; EPA Office of Emergency and Remedial Response, 1989).

Daily inhalation of aerosolized toxins was calculated by extrapolating the average total aerosolized concentration of each toxin. It was estimated that on average, an adult human on the shore of Walkers Pond had the potential to inhale 10.0 ng MC, 902.6 ng BMAA, and 124.0 ng ATX per day (Table 7). On the shore of Lower Mill Pond these estimated averages of MC are 7.2 ng MC, 493.2 ng BMAA and 79.0 ng ATX per day (Table 7). These exposure estimates only account for exposure through inhalation while exposure to cyanotoxins through direct consumption may increase total daily exposure. Additionally, cyanotoxins have been found to have potentially have synergistic effects, meaning an increase in toxicity when multiple
cyanotoxins are present (Chia et al., 2019; Metcalf & Codd, 2020). Future studies should further examine the potential for synergistic effects to allow for accurate risk assessments.

Table 7. Estimations of total daily inhalation of MC, BMAA, and ATX (ng day\(^{-1}\)) at Walkers Pond and Lower Mill Pond. Calculations are based on mean toxin concentrations over the entire 2021 sampling season. These values were calculated using the mean toxin concentrations and extrapolating to represent human inhalation potential. These calculations assume an inhalation rate of 20 m\(^3\) of air per day, consistent exposure, and no deviations in toxin concentrations directly above water surface and toxin concentration during inhalation.

<table>
<thead>
<tr>
<th></th>
<th>MC (ng day(^{-1}))</th>
<th>BMAA (ng day(^{-1}))</th>
<th>ATX (ng day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walkers Pond</td>
<td>10.0</td>
<td>902.6</td>
<td>124.0</td>
</tr>
<tr>
<td>Lower Mill Pond</td>
<td>7.2</td>
<td>493.2</td>
<td>79.0</td>
</tr>
</tbody>
</table>

We often think of lakes as isolated aquatic ecosystems with well-defined boundaries. However, we have seen in this study that molecules produced by cyanobacteria in a lake can be rapidly transferred into the atmosphere as aerosols, representing a loading of the atmosphere with toxins. Total daily atmospheric loading of these toxins may be dependent on the specific environment. For example, two otherwise identical lakes could vary in aerosol production due to variations in elevation or latitude. A lake located in a low elevation, tropical region might be expected to have a relatively lower average air-water temperature differential than a lake located at a high elevation due to a reduction in air temperature during diel cycles at higher elevations (Edinger et al., 1968; Liu et al., 2009; Paaijmans et al., 2008). Based on my study, all other things equal, a lake in the tropics should show increased toxic aerosol production when compared to a high-altitude lake, as concentrations of aerosolized toxins in Brewster, MA were highest when air-water temperature differential was relatively low as it likely would be in the tropics. As demonstrated by Walkers Pond and Lower Mill Pond (Table 8), even connected lakes
in close proximity can vary dramatically in lake characteristics, composition, and toxic aerosol production.

Total daily atmospheric loading of MC, BMAA, and ATX from each lake was also calculated by extrapolating the average total aerosolized concentration of each toxin (Table 8). It was estimated that on average, total atmospheric loading of MC, BMAA and ATX per day by Walkers Pond was 11,900 µg, 1,080,503 µg, and 148,440 µg, respectively. At Lower Mill Pond average total atmospheric loading of MC, BMAA and ATX per day was estimated at 4,230 µg, 291,772 µg, and 46,736 µg, respectively. Total atmospheric loading of each toxin per day was larger at Walkers Pond due to a consistently higher rate of aerosol production as well having about twice the surface area compared to Lower Mill Pond (Table 8). While these values estimate total toxic aerosol production in a single day, future studies should further explore the pathway of cyanotoxins after aerosolization. For examples, molecules are more exposed to meteorological effects such as wind and sunlight that can affect potential for aerosols to travel long distances, degradation, and deposition (Rosas et al., 1989; and “The Aerobiology Pathway,” 2006).
Table 8. Estimations of total daily atmospheric loading of MC, BMAA, and ATX (µg day\(^{-1}\)) at Walkers Pond and Lower Mill Pond. Calculations are based on mean toxin concentrations over the entire 2021 sampling season. These values were calculated by estimating the mean aerosolization rate per hour and applying it to the entire surface area of the lakes. Calculations are based on the assumption that there is no variation in aerosol production rate due to spatial variation, time of day or date. Additionally, these calculations assume that all aerosol collections in this study had exactly 240 L of air filtered during the collection period. In actuality this value differed slightly between collections due to small discrepancies in pump flow rates and duration of collection. Lastly, these calculations do not factor in any degradation of toxins after aerosolization.

<table>
<thead>
<tr>
<th>Daily toxin release rates</th>
<th>MC (µg day(^{-1}))</th>
<th>BMAA (µg day(^{-1}))</th>
<th>ATX (µg day(^{-1}))</th>
</tr>
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<tr>
<td>Walkers Pond</td>
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<td>4,230</td>
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</tbody>
</table>

This study provided valuable information regarding the aerosolization of cyanotoxins microcystins (MC), β-Methylamino-L-alanine (BMAA), and anatoxin-a (ATX). One of the biggest accomplishments of this study was confirmation of the presence of all three targeted cyanotoxins in aerosols simultaneously. The insight gained regarding potential drivers and therefore possible mechanisms further develops the limited knowledge base of cyanotoxin aerosolization which is a consequence of relatively few published studies addressing the topic. Considering the implications of this research in terms of potential health consequences, the continuation of this research is essential. A further improved and standardized method of aerosol collection would allow for more beneficial comparisons between studies and more efficient experimentation. The direct exploration of possible mechanisms of aerosolization and their relation to climatic factors would provide insight into the hypothesis that these mechanisms occur simultaneously, and the rate of each mechanism can change individually. Future studies designed to expand the knowledge on cyanotoxin aerosolization would encourage a more robust understanding of aerosolization mechanisms and ultimately expedite the goal of developing accurate human risk assessments.
Major Findings:

1. Microcystins (MC), \(\beta\)-Methylamino-\(L\)-alanine (BMAA), and anatoxin-a (ATX) can aerosolize simultaneously.

2. Environmental factors (wind, evaporation rate, temperature differential) correlate with aerosol toxicity and indicate there are multiple mechanisms of cyanotoxin aerosolization.

3. Water toxin does not always predict aerosolized toxin, but the Pico (0.3-2.0 \(\mu\)m) fraction may be more readily aerosolized than BFCs. This varies between toxin and lake.
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