ENVIRONMENTAL REGULATION OF CYANOBACTERIA AEROSOLS FROM LOW PRODUCTIVITY LAKES

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ABSTRACT

ENVIRONMENTAL REGULATION OF CYANOBACTERIA AEROSOLS FROM LOW PRODUCTIVITY LAKES

By

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University of New Hampshire, September 2019

Cyanobacteria produce a range of toxins harmful to both humans and wildlife. Microcystins (MCs) are common and potent cyanotoxins that inhibit protein phosphatases in the liver. Chronic exposures can result in tumor promotion and cancer. Toxicity primarily follows ingestion of MCs, however inhalation may be an important secondary and compounding route of exposure to cyanotoxins. The LD$_{50}$ of intratracheally applied MC-LR is 50x lower than the oral LD$_{50}$. While MCs are well documented in water, little is known about cyanobacteria and cyanotoxins in aerosols and the factors that regulate the movement of cells and toxins into the air. To begin characterizing cyanobacteria presence in the air, lake-generated aerosols were measured from eight New England lakes of varying productivity. Air samples were collected with a portable on-lake aerosol monitor for nine-hour periods during the day, and nine-hour periods directly following day sampling at night. Corresponding water samples were taken at the beginning and end of each aerosol sampling period. Environmental and water quality factors were collected simultaneously with aerosols.

In this first study documenting MCs aerosols generated from low-productivity lakes, microcystin concentrations ranged from below detectable levels to 3.79 pg MCs m$^{-3}$ from lakes with a range of 4 to 90 ng MCs L$^{-1}$ in the near surface lake water. The presence of MCs in aerosols from low productivity lakes demonstrates the wide prevalence of aerosolized
cyanobacteria. A combination of techniques, including sample concentration and the use of epifluorescence, were employed to quantify low levels of toxins and cyanobacteria cells in the aerosols. Aerosolized MCs were correlated with total aerosolized cells at night (p = 0.008, Adj R²: 0.66), but not during the day, potentially indicating differences in aerosolization mechanisms and drivers based on time of day. Total aerosolized cyanobacteria ranged from $3.5 \times 10^4$ to $1.9 \times 10^5$ cells m$^{-3}$. On average, 56.9 ± 4.23% of total cells are picocyanobacteria (0.22 – 2 µm), and 99.2 ± 0.14% of cells were smaller than 10 µm. Emission of larger cells (>2 – 40 µm) varied based on the lake. No colonies were detected in aerosols.

The community composition of cyanobacteria in lake water has an influence on the aerosol cell and toxin composition as not all cyanobacteria are equally as likely to become aerosolized (as seen by the dominance of small cells in aerosols). Despite nine times higher MCs toxicity in the large net cyanobacteria compared to all other sampled lakes, Lake Attitash did not emit significantly higher levels of aerosolized MCs. Lower productivity lakes with low water MCs had disproportionately higher aerosolized toxins and cyanobacteria cells (power function linear regression, p <0.0001, Adj R²: 0.85). A probable explanation is that low MCs lakes are dominated by small cyanobacteria that produce low levels of toxin but are more likely to enter the air, while high MCs lakes are dominated by large colonial forms that are more likely to remain in the water.

At night, cyanobacteria cells became aerosolized in more predictable patterns than during the day. Though the time of day effect on aerosolization varied between lakes, aerosolized cell concentrations were more strongly correlated with environmental factors at night across all lakes. Toxicity in the water and the temperature differential between the air and water were the two most important environmental drivers of cyanobacteria cell aerosolization. Wind was
surprisingly negatively correlated to total and larger cells (both parameters log10 transformed: total cells $p = 0.033$, Adj $R^2$: 0.235; larger cells $p = 0.015$, Adj $R^2$: 0.306). Although the human health effects of MCs in aerosols have not been defined, the levels of both cyanobacteria cells and microcystins described in this study suggest possible chronic effects on humans and wildlife. The dominance of picocyanobacteria also indicates the potential for cells to travel deep into lung tissue. Building an understanding of aerosolized MCs and the sizes of aerosolized cyanobacteria cells emitted from varying lake types, and the factors regulating this process, could lead to better estimates of cyanotoxin exposure.
BACKGROUND

Cyanobacteria and Cyanotoxins

Cyanobacteria are ancient, gram-negative prokaryotes that appeared 3.5 billion years ago (Taylor and Taylor 1993). They are responsible for introducing chlorophyll-a based photosynthesis, which eventually led to the oxygenation of the planet (Marais et al. 1992). Through forming endosymbiotic relationships with the ancestors of eukaryotic cells, cyanobacteria initiated the evolutionary path towards the development of chloroplasts (Margulis 1973). Today, cyanobacteria are among the most common prokaryotic groups on earth and have a cosmopolitan distribution. They inhabit terrestrial environments, as well as marine, brackish, and fresh waters. These habitats range in extremity from deserts, tropics, hot springs, and polar regions (Jonasson et al. 2008; Liyanage et al. 2016). Cyanobacteria exist in such a wide variety of conditions and environments because of specialized competitive advantages.

Cyanobacteria in freshwater have several adaptations that make them successful competitors for resources. Many species can fix atmospheric nitrogen, thereby having an important role in biogeochemical cycling processes (Boopathi and Ki 2014). They are also able to solubilize phosphorus and sequester iron (Boopathi and Ki 2014). This enables cyanobacteria to inhabit freshwater environments with low nutrient availability. Another advantage is their ability to harvest light wavelengths that most photosynthetic organisms are unable to use (Aráoz, Molgó, and Tandeau de Marsac 2010; Osswald et al. 2007). Special accessory pigments on their chlorophyll called phycobilins enable them to harvest this additional light energy. Cyanobacteria are also able to regulate their buoyancy in water columns, allowing them to migrate toward optimal light and nutrient conditions (De Figueiredo et al. 2004; Liyanage et al. 2016). Because
of these advantages, when the balance of lake ecosystems is disrupted, cyanobacteria often dominate over other phytoplankton.

Increases in human populations around water bodies, intensification of agriculture, and at times a lack of sufficient water management has led to eutrophication of many freshwater bodies. This increased nutrient loading, along with global climate change, is leading to more frequent and severe toxic cyanobacteria bloom events worldwide (De Figueiredo et al. 2004; Paerl and Otten 2013; Paerl, Hall, and Calandrino 2011; F. Zhang et al. 2015). Often referred to as freshwater “harmful algal blooms”, these events poise a risk to ecosystem, human, and wildlife health (Aráoz, Molgó, and Tandeau de Marsac 2010; Paerl, Hall, and Calandrino 2011).

Cyanobacteria blooms can have a drastic impact on the ecosystem, causing hypoxia and disrupting the food web (Paerl and Otten 2013). Many genera of cyanobacteria also produce a range of secondary metabolites broadly referred to as cyanotoxins. Cyanobacteria can produce one or a combination of toxins, classified by their mode of toxicity. These cyanotoxins include dermatoxins, hepatotoxins, or neurotoxins (De Figueiredo et al. 2004; Boopathi and Ki 2014; Y.-S. Cheng et al. 2007). While not all cyanobacteria produce toxins, the effect of those that do range from mild to fatal (Geoffrey A. Codd, Morrison, and Metcalf 2005). Concentrated levels of cyanotoxin are a public health issue threatening our water sources, food supplies, and recreational activities (Y.-S. Cheng et al. 2007; Liyanage et al. 2016).

Microcystins

Microcystins (MCs) are a group of cyclic heptapeptides and are the most frequently occurring, widespread, and diverse group of cyanotoxins (Žegura, Štraser, and Filipič 2011). These molecules contain seven different amino acids: three common D-amino acids, two novel D-amino acids, and two variable L-amino acids. Different variants arise through combinations of
L-amino acids (De Figueiredo et al. 2004). Though new variants are frequently discovered, there are currently around 100 identified congeners (Environmental Protection Agency, USEPA 2015). The most common and toxic congener is microcystin-LR, with the variable amino acids leucine and arginine (De Figueiredo et al. 2004; Wu et al. 2017).

Microcystins are produced by many different cyanobacterial genera, including *Microcystis*. There are no conclusive studies as to why cyanobacteria produce MCs. It has been suggested that microcystins are internally used in cyanobacteria cells to regulate endogenous protein phosphatases and helping reserve nitrogen (De Figueiredo et al. 2004). Externally, MCs production can benefit cyanobacteria by negatively impacting competing phytoplankton. Generally, this is done by reducing growth rates in competing phytoplankton (Kearns and Hunter 2001). In a more specific case, MC-LR can paralyze typically mobile *Chlamydomonas reinhardtii*, a green alga (Kearns and Hunter 2001). Production of these toxins can give cyanobacteria a competitive advantage for resources by inhibiting competition.

Beyond direct competition with other phytoplankton, MCs may act as a chemical defense against herbivory. *Daphnia* are primary grazers of phytoplankton. They are not selective eaters and ingest cyanobacteria that produce MCs when cyanobacteria are in higher abundance than their preferred food sources (Rohrlack et al. 2001). One study showed a direct correlation of *Daphnia* death with amount of *Microcystis* ingested (Rohrlack et al. 2001). Unlike *Daphnia*, calanoid copepods are selective grazers and have been shown to actively avoid ingestion of MCs containing cyanobacteria (De Figueiredo et al. 2004). The toxic effect of MCs continues beyond primary grazers through the lake food web.

Microcystins have shown a range of toxic effects in many fish species. Microcystin toxicity in rainbow trout leads to hepatotoxicosis, causing changes in cellular morphology,
protein phosphatase inhibition, and liver necrosis (De Figueiredo et al. 2004). Some fish, such as the zebrafish, show behavioral changes in association with lower exposure to MCs. After sublethal doses of MC-LR in zebrafish, fish showed reduced motility, increased rates of activity at night, reduced spawning efforts and decreased eating (Chorus 2001). MCs has also caused deaths in a variety of wild birds around the world (Chen et al. 2009; Krienitz et al. 2003; Murphy et al. 2003) Nishizawa et al. 2015). There have also been many documented deaths of field and domestic animals from MCs exposure. These animals include cattle, sheep, horses, pigs and canines (Carmichael 1992; L. C. Backer et al. 2013). Animals are more likely to swim in contaminated water bodies and drink from these water sources than humans. In addition to this increased chance of exposure, many animals groom their feathers or fur after swimming, resulting in further exposure (L. C. Backer et al. 2013). In some cases animals actively seek out dried cyanobacteria crusts or mats (G A Codd et al. 1992). Beyond animals, MCs is also toxic to plants, acting as a growth inhibitor in potato shoots and mustard seedlings (McElhiney, Lawton, and Leifert 2001).

Despite the challenge in diagnosing human toxicity to MCs, there have been documented cases of cyanotoxicity ranging from mild with passing symptoms, to development of chronic health issues, or fatality. Human symptoms to MCs toxicity are similar to other animals and can include skin and eye irritation, rashes and blisters around the mouth and nose, blisters in the mouth, sore throat, stomach cramps, vomiting, nausea, diarrhea, fever, headache, muscle and joint pain (Liyanage et al. 2016). While the epidemiological evidence for cyanobacterial toxicity in humans is not thorough, exposures spanning from 1960 to present day have been documented (Svirčev et al. 2017). In addition to reports of individual cases, a study performed across the contiguous U.S. found a statistically significant relationship between cyanobacteria blooms and
nonalcoholic liver disease (F. Zhang et al. 2015). This is a potential indicator of the widespread issue of human exposure to MCs.

**Routes of Exposure**

Humans who interact more frequently with aquatic environments that host the species of MCs producing cyanobacteria are at greater risk of exposure to cyanotoxins. This could mean people who work on the water, recreational lake users, or lakeside residents. MC-LR is very stable in water, resistant to pH extremes and temperatures up to 300 C (WHO 1998). Though subject to photodegradation and microbial decay, MCs can persist for months or years in natural water and in the dark (Liyanage et al. 2016). As a result, exposure to MCs can occur even when the cells that produce them have died. Exposure to MCs can occur through several routes.

Ingestion of cyanotoxins is the primary route of exposure. This can occur through contaminated drinking water, food, or dietary supplements. Consuming contaminated drinking water is a problem worldwide, in both industrialized and economically emerging countries including Argentina, Australia, Bangladesh, Canada, Czech Republic, China, Finland, France, Germany, Latvia, Poland, Thailand, Turkey, Spain, Switzerland, the United Kingdom and the United States (Hoeger, Hitzfeld, and Dietrich 2005). Microcystin levels reported in these drinking water supplies ranged from 1 µg L⁻¹ to 8 µg L⁻¹ in raw water (Hoeger, Hitzfeld, and Dietrich 2005). Monitoring of MCs is not been required in the United States, so records of concentrations in drinking water sources are incomplete. One survey of Florida drinking water sources conducted in 2000 found a range of below detectable to 12.5 µg L⁻¹ in finished drinking water (USEPA 2015).

The World Health Organization has set a provisional guideline of 1.0 µg L⁻¹ MC-LR in drinking water (WHO 1998). Microcystins are on the United States EPA candidate contaminant
list. They currently have a health advisory in place to help regulate exposure to MCs. This guideline aims to prevent non-carcinogenic health effects over a ten-day exposure to MCs in drinking water. For bottle fed infants and pre-school children, they suggest 0.3 \( \mu g \text{ L}^{-1} \) should not be exceeded, and for school age children through adults, intake of 1.6 \( \mu g \text{ L}^{-1} \) should not be exceeded in a 10 day time frame (USEPA 2015). Due to the chronic health effects of MCs, contact with cyanobacteria should be as limited as much as possible.

Beyond consumption of cyanotoxins from drinking water, humans can also ingest MCs through food sources. Mussels, crayfish, and fish can accumulate MCs in their tissue (USEPA 2015). Crop plants irrigated with MCs contaminated water can accumulate the toxins, potentially transferring to humans via intake of these vegetables (De Figueiredo et al. 2004). One study showed accumulation of MCs in spray irrigated \textit{Lactuca sativa}, a commonly used salad lettuce (Geoffrey A. Codd, Metcalf, and Beattie 1999).

Outside of more traditional food sources, concentrated cyanobacteria are commonly sold as a health supplement through the health food market. The proposed benefits of eating the \textit{Spirulina} or \textit{Aphanizomenon} supplements are that they will help with weight loss, increase alertness and energy, act as an anti-depressant, and treat attention deficit hyperactivity disorders in children (Žegura, Štraser, and Filipič 2011). These dietary supplements are not regulated, so they could have variable toxin levels (Liyanage et al. 2016). Eight products out of 18 surveyed in a study had cyanotoxin levels higher than the tolerable daily limits (Roy-Lachapelle et al. 2017). In one infamous case, an employee of a company selling the “blue-green algae” supplements died of liver failure after regularly taking their product (Žegura, Štraser, and Filipič 2011). The popularity of dietary supplements and the potential risk they pose is a serious concern.
Though an unusual route of exposure, human cases of intravenous exposure to MCs occurred in Caruaru, Brazil, at a dialysis clinic. Patients experienced visual disturbances, nausea, vomiting, and muscle weakness following a routine hemodialysis treatment. Out of 131 patients, 100 developed acute liver failure (Azevedo et al. 2002). Of the 100 afflicted patients, 52 died (Azevedo et al. 2002). After inspection of the water used for the treatment, they found high concentrations of picocyanobacteria. Following this incidence, the Brazilian Health Ministry have begun monitoring cyanotoxins as part of their quality control protocol (Azevedo et al. 2002).

Recreation such as swimming, sailing, jet skiing, canoeing, or fishing, on bodies of water with toxic cyanobacteria blooms could result in MCs contacting the nasal membranes, or inhalation. Inhalation could also occur if lake-side residents use untreated lake water for washing dishes and showering (USEPA 2015). A widely-used home water treatment technique is to boil water to remove potential pathogens, however MCs is not destroyed by boiling (Rao et al. 2002) and the process may aerosolize the toxin, increasing exposure. Respiratory symptoms similar to pneumonia have been reported in people canoeing in freshwater reservoirs containing Microcystis blooms in the United Kingdom (Turner et al. 1990). Despite reports of symptoms occurring following respiratory exposure, little is known about the extent of exposure to cyanotoxins via inhalation. It is often thought to be driven by wind and wave action that create a spray of coarse aerosols and small water droplets. Though most likely a low-level exposure, inhalation of aerosolized cyanobacteria cells and dissolved cyanotoxins from the lake surface could be a constant and unavoidable route of exposure.

While there are many routes of exposure to MCs, oral exposure through food and water is the primary route. Despite being the most frequent type of exposure, acute toxicity via ingestion
requires a higher amount of MCs than other routes of exposure (De Figueiredo et al. 2004; Ito, Kondo, and Harada 2000; Benson et al. 2008). The ingestion 50% lethal dose ($LD_{50}$) of MC-LR in mice is 5000 µg kg$^{-1}$, while the intraperitoneal $LD_{50}$ for mice is between 50 and 158 µg kg$^{-1}$ (WHO 1998; De Figueiredo et al. 2004). The $LD_{50}$ for mice exposed to MC-LR intratracheally is around 100 µg kg$^{-1}$, a level that corresponds with the intraperitoneal $LD_{50}$ (Ito, Kondo, and Harada 2000). This means respiratory absorption caused death at about one-fiftieth of the oral dose. Even though inhalation of MCs may be a secondary route of exposure, the low $LD_{50}$ indicates the importance of understanding this type of contact.

**Mode of Action: Oral and Inhalational Exposure**

The liver is the primary target organ for MCs in humans, birds, wild animals, livestock and fish (Xie et al. 2005). When orally ingested, MCs are not hydrolyzed by stomach peptidases (Dow and Swoboda 2000). Instead, MCs are transported across the ileum and into the bloodstream via membrane-bound bile-acid transporters (De Figueiredo et al. 2004). These Organic Acid Transporter polypeptide (OATp) transport proteins are produced in the gastrointestinal tract, kidney, liver, brain and other tissues (USEPA 2015). The OATp receptor family typically facilitate the cellular, sodium-independent uptake and export of amphipathic compounds such as steroids, drugs, bile salts, and peptides (X. Cheng et al. 2005). OATp uptake of MCs into the liver is limited due to competitive binding when the amphipathic compounds are present (USEPA 2015). Once in the bloodstream, MCs primarily accumulate in the liver where they enter hepatocytes, but also accumulate in the kidney and intestine (WHO 1998). There is evidence that MCs are detoxified in the liver into more water-soluble products and excreted in the urine and feces (WHO 1998).
Though not thoroughly investigated, there are two proposed pathways of MCs movement through the body when entering via the respiratory system. Fitzgeorge et al. (1994) found that intranasal instillation to MC-LR caused necrosis in olfactory epithelium (following repeated sub-lethal doses), leading to the destruction of large areas of the mucus membrane. This developed into deep bleeding in the nasal vessels, resulting in openings where further inhaled toxin can enter the blood stream. It is suggested that this could explain the higher toxicity of MCs following respiratory exposure compared to ingestion (Fitzgeorge, Clark, and Keevil 1994). Following this route of exposure, lesions were observed in the liver, but not in the lung, or the pancreas, spleen, lymph nodes or kidney. Ito et al. (2000) found that intratracheally-applied MC-LR in mice enters into the lung from the alveoli where it is absorbed into blood capillaries. When the dose was high enough, this exposure resulted in bleeding in the liver, but had no effect on the lung, pancreas, spleen, lymph nodes or kidney.

Regardless of the route of exposure, once MCs enter the liver via the bloodstream, they inhibit protein phosphatase types 1 and 2A by covalently binding to them (WHO 1998). When functioning properly, phosphatases help keep the balance between phosphorylation and dephosphorylation of key cellular proteins that control metabolic processes, gene regulation, cell cycle control, transport, secretory processes, cytoskeleton structure, and cell adhesion (USEPA 2015). The result is an accumulation of phosphorylated proteins in the liver (Boopathi and Ki 2014) and destabilization of the cytoskeleton (USEPA 2015). This leads to altered cell function, followed by cellular apoptosis, and necrosis (Boopathi and Ki 2014). Microcystins also bind to ATP synthetase, potentially leading to cell apoptosis (De Figueiredo et al. 2004). In a high enough dose, this can result in intrahepatic bleeding, and eventually hemorrhagic shock and liver failure, however lower doses can result in chronic toxicity (Liyanage et al. 2016).
Acute and Chronic Toxicity

Microcystins can cause both acute and chronic toxicity depending on the dose and duration of exposure. There are few cases describing acute toxic responses of humans in the literature. Because there is a wide range of clinical signs of toxicity, diagnosis is more difficult. The most infamous case of acute toxicity in humans was the exposure of patients at a dialysis clinic to water with MCs (Azevedo et al. 2002). As an example of recreational exposure to MCs, a single swimmer in Argentina was immersed in a *Microcystis* bloom, with concentrations of MCs around 48.6 µg/L (Giannuzzi et al. 2011). Four hours after exposure, the patient had a fever, nausea, and abdominal pain and three days later presented dyspnea and respiratory distress and was diagnosed with atypical pneumonia. A week after exposure the patient developed hepatotoxicosis and after 20 days of varied health complications, completely recovered. Though it can occur, humans are not exposed to the level of MCs needed to develop acute toxicity as often as wildlife and domestic animals are.

Chronic exposure to MCs can lead to tumor promotion and cancer (De Figueiredo et al. 2004; USEPA 2015; Emiko et al. 1997). MCs inhibit protein phosphatases 1 and 2A, both important enzymes involved in tumor suppression (De Figueiredo et al. 2004). In addition to inhibiting tumor suppressors, MCs themselves are also suspected to be tumor promoters. In studies that expose animals to MC-LR and tumor initiators, the combination of the two (and not tumor initiators alone), increase the presence of tumor formation indicators (glutathione S-transferase placental form-positive foci; Zhou, Yu, and Chen 2002). Although the mechanism isn’t clear, MCs also induce damage to the DNA. A proposed pathway of genotoxicity is through the induction of reactive oxygen species (ROS) formation, which can cause breaks in DNA strands and mutagenic oxidative DNA lesions (Žegura, Štraser, and Filipić 2011). Additionally,
MC-LR inhibits two pathways of DNA repair, which is also a key factor involved in carcinogenesis (Žegura, Štraser, and Filipič 2011). Several studies from China have shown the relationship between liver and colon cancer and regular ingestion of drinking water containing cyanobacteria (Zhou, Yu, and Chen 2002; Ueno et al. 1996). Some populations may be at greater risk to the chronic effects of MCs, including the elderly and children, and hepatitis-B patients (Chorus 2001). Regular inhalation of MCs in addition to other routes could result in chronic exposure.

Aerosols

Lake-generated aerosols are fine liquid droplets or particles suspended in air that occur through natural emission from lake surfaces. Typically unseen, relatively little is known about aerosols in general, and even less about aerosols generated from freshwater. Aerosols can carry a diverse assemblage of microbial life, chemical content, nutrients and pollutants from a lake surface into the air (Dueker et al. 2011; Dueker, O’Mullan, Weathers, et al. 2012). Darwin was among the first to acknowledge and document the presence of microbial life in aerosols, recording the presence of freshwater aquatic organisms in aerosols deposited on his ship as he traveled along the coast of Africa (Darwin 1846). Since then, an array of microbial life has been identified in aerosols, including bacteria, fungi, viruses, protozoa, and algae (Sahu and Tangutur 2015; Murby and Haney 2015). Secondary metabolites of aquatic organisms, such as toxins can also become aerosolized. Aerosols act as a connection between ecosystems, uniting lake health with the health of surrounding environments, wildlife, and humans (Dueker et al. 2011; Dueker, O’Mullan, Weathers, et al. 2012; K. Sharma, K. Rai, and Singh 2006; Nishizawa et al. 2015; Sahu and Tangutur 2015). The movement of aerosolized lake material provides an avenue for large scale ecosystem transfer.
Aerosolization of microbes include both viable and dead or damaged cells (Dueker, O’Mullan, Weathers, et al. 2012; Dueker, O’Mullan, Juhl, et al. 2012). The viability of cells affects their ecology as they move between ecosystems. Viable cells have the potential to disperse to new environments and colonize novel areas. Aerosolized organisms can travel long distances from their originating environments before they are deposited through gravitational settling, surface interruption, or inhalation (Dueker, O’Mullan, Juhl, et al. 2012). This could result in the contamination of drinking water or the stimulation of a bloom event when deposited in an aquatic ecosystem (K. Sharma, K. Rai, and Singh 2006; Sahu and Tangutur 2015). Because of this dispersal and their ability to remain viable through this process, aerosols are thought to play a role in the cosmopolitan distribution of some bacterial and algal species (Dueker et al. 2011; K. Sharma, K. Rai, and Singh 2006).

Aerosols can connect seemingly isolated ecosystems, such as deserts and oceans, lakes and urban areas, and allow for genetic exchange between microbiomes (Dueker, O’Mullan, Weathers, et al. 2012). Algae and waterborne organisms from the mainland have been discovered on the volcanic island Surtsey, 32 km off the south coast of Iceland (K. Sharma, K. Rai, and Singh 2006), in Antarctica (F. Zhang et al. 2015), and can inhabit clouds (Sahu and Tangutur 2015). There is growing evidence that suggests microbial aerosols impact the climate by serving as fog, cloud, ice and rain nucleators (Dueker, O’Mullan, Weathers, et al. 2012; K. Sharma, K. Rai, and Singh 2006). In addition to wide dispersal, it is also estimated that aerosols contain a vast amount of biological material as well. While difficult to quantify, it has been proposed that the current bacterial concentration in the atmosphere ranges from $1 \times 10^4$ to $1 \times 10^6$ cells m$^{-3}$, depending on the ecosystem type (Dueker, O’Mullan, Weathers, et al. 2012). One study even
suggested that the aerial environment carries around half of the global microbial diversity (Sahu and Tangutur 2015).

The composition of microbial life in air has wide reaching implications, but the effect of aerosolized material on a local level is equally worthy of exploration. Multiple studies have shown that the local content of airborne bacteria is dependent on the location and proximity to the sources originally hosting the microbial life (K. Sharma, K. Rai, and Singh 2006; Dueker, O’Mullan, Juhl, et al. 2012; Sahu and Tangutur 2015). The overlap in community composition of surface water and aerosolized bacteria in the case of water reservoirs in Varanasi City, India (K. Sharma, K. Rai, and Singh 2006), superfund sites in Newtown Creek, New York (Dueker, O’Mullan, Juhl, et al. 2012), and in lakes in Nelson, New Zealand (Wood and Dietrich 2011) show how freshwater systems are significant contributors to local aerosol composition. Despite knowing this, we do not know what environmental factors control or influence the abundance, viability, and diversity of microbial aerosols from lake ecosystems (Dueker, O’Mullan, Weathers, et al. 2012; Sahu and Tangutur 2015; Dueker, O’Mullan, Juhl, et al. 2012).

Understanding the production of locally generated aerosols is important when considering the non-viable cells that become aerosolized from lakes as well. Though non-viable cells would not have ecosystem effects, the toxins, allergens or pathogens these cells carry could still be present and even more available. Biological products such as toxins and viruses may be aerosolized independent of cells (Sharoni et al. 2015), having consequences for human and wildlife health on a local level. Bevetoxin (commonly referred to as red tide) is a potent neurotoxin created by the dinoflagellate Karenia brevis that is known to become aerosolized, travel to shore and result in both acute and chronic health effects in humans following inhalation (Fleming, Backer, and Baden 2005). Similar to the production of toxins in the marine world, the
toxin produced by cyanobacteria in freshwater ecosystems can also become aerosolized. Because of the ecosystem and human health related consequences of this phenomenon, a detailed understanding of cyanobacteria and cyanotoxins in lake-generated aerosols and how this material gets there is especially important.

It is generally believed that aerosols with biological material occur through a wind-driven, bubble-bursting process (Duncan C Blanchard and Syzdek 1972). Bubbles formed by trapped air gradually rise to the surface of water where they catch bacteria, algae, and toxins concentrated at the water surface. As the bubbles burst into the air, the concentrated biological material is ejected and carried into the air by the droplets (D C Blanchard and Woodcock 1956). This same process is thought to be responsible for moving salts, and other organic material from water into the air (D C Blanchard and Woodcock 1956), and it has been documented that this is also how brevetoxins enter the air (Fleming, Backer, and Baden 2005).

**Aerosolized Cyanotoxins**

The photosynthetic components of aerosols are often overlooked. As a result, not much is known about the extent, driving factors, distribution and consequences of aerosolized photosynthetic organisms – especially in the case of cyanobacteria (Dueker et al. 2011; Sahu and Tangutur 2015; Dueker, O’Mullan, Juhl, et al. 2012). A greater understanding of aerosolized cyanobacteria has become more important in light of the suggestion that exposure to the cyanobacteria neurotoxin BMAA (beta-Methylamino-l-alanine) via aerosols may be contributing to the development of neurodegenerative disease (Stommel, Field, and Caller 2012). Several studies have documented the presence of cyanobacteria cells and cyanotoxins in aerosols and begun to quantify environmental exposure to aerosolized cyanobacteria.
Cheng et al. established that cyanotoxins can move into the air via the bubble-bursting process, documenting the generation of aerosols with two dominant sizes around 1.4 and 27.8 µm in a lab setting, and 0.4 and 6.5 µm in the field (Y.-S. Cheng et al. 2007). In the field they measured MCs levels of 0.023 to 0.057 ng m\(^{-3}\) from a lake with around 1 µg MCs L\(^{-1}\) (Y.-S. Cheng et al. 2007). Using the same techniques, <0.1 (below detection limit) – 2.89 ng MCs m\(^{-3}\) were measured in aerosols from the Midwestern United States, and two lakes in California with water concentrations of 2 – 500 µg MCs L\(^{-1}\) (L. C. Backer et al. 2010; L. Backer et al. 2008). In New Zealand, Dietrich and Wood documented 1.8 pg m\(^{-3}\) in aerosols from lakes with up to five different variants of MCs with levels up to 700 µg MCs L\(^{-1}\) total. Collectively, the levels of measured MCs are not high. Despite this, further investigation of aerosol output is needed as the chronic threat posed by these levels of inhaled MCs is unknown. Additionally, the focus of these studies was to characterize the concentrations of aerosols that humans may be exposed to while recreating (aerosols were collected on boats and through personal air samplers on lake-goers), instead of characterizing the direct output of aerosolized cyanotoxins from the lakes.

Using a novel collection technique that captures air directly above the lake surface, estimates of aerosolized MCs from New Hampshire lakes ranged from <13 (below detectable limits) to 384 pg MCs m\(^{-3}\) (Murby & Haney, 2016). The advantage of this methodology is it allows for a more direct measurement of cyanobacteria aerosol released from waterbodies, minimizing the effect of dispersal from the source, or contamination with air from other sources. Though perhaps less indicative of human exposure while recreating on shore, it allows one to better understand the ecological phenomenon behind cyanobacteria aerosolization. The study also demonstrated that cyanotoxins can become aerosolized under controlled laboratory conditions without the artificial generation of bubbles, suggesting that evaporative processes
could be responsible for movement into the air in the field as well (Murby & Haney, 2016). My study aims to use this aerosol collection technique on a range of New England lakes in order to broaden our understanding of the drivers behind cyanotoxin aerosolization. This could ultimately lead to the creation of better-informed management of exposure to cyanotoxins via aerosols.
INTRODUCTION

Cyanobacteria are becoming an increasing problem worldwide in freshwater systems due to global climate change and increased nutrient loading (Paerl, Hall, and Calandrino 2011). In addition to causing ecological damage, cyanobacteria produce a range of toxins harmful to humans and wildlife. The most widespread cyanotoxins are microcystins (MCs), potent hepatotoxins with around 100 different congeners. At high levels, MCs can cause acute liver failure and death (WHO 1998). Chronic exposure to lower concentrations is more common in humans and can lead to tumor promotion and cancer (USEPA 2015). The primary route of exposure to cyanotoxins is through drinking water. As a result, the USEPA has developed advisory drinking water guidelines. Over a ten day time frame, bottle-fed infants and young children should not exceed $0.3 \mu g \ L^{-1}$, and intake for school age children and adults should not exceed $1.6 \mu g \ L^{-1}$ (USEPA 2015). While these guidelines begin to manage exposure to MCs, they do not consider the additional intake of cyanotoxins via other routes. Inhalation may be an important secondary and compounding route of exposure to MCs that is not currently considered.

Inhalation of larger coarse aerosols containing cyanotoxins can occur during recreational activities such as water or jet skiing. Aerosolization can also occur without turbulence, thus cyanotoxins could be inhaled during swimming, canoeing or recreating on a lake shore. Lakeside residents who have contaminated well water or use lake water in their houses could inhale cyanotoxins while showering or washing dishes (USEPA 2015). Respiratory symptoms in humans have been associated with inhalational exposure to cyanobacteria. Reported symptoms of exposure range from allergy-like to the development of pneumonia (Stewart et al. 2006; Philipp and Bates 1992; Turner et al. 1990). The effect of inhaling MCs is difficult to isolate because it is often coupled with other forms of exposure. Despite the challenge of documenting
inhalation effects in the field, several lab studies have validated the risk of MCs inhalation under controlled conditions.

Lab studies indicate that inhalation is an extremely effective route of exposure to MCs. The 50% lethal dose (LD$_{50}$) of ingested MC-LR (the first identified and a common form of MC) is 5000 µg kg$^{-1}$ of body weight in mice (WHO 1998). Intratracheally administered MC-LR has an LD$_{50}$ of 100 µg kg$^{-1}$, approximately 50 times lower than when ingested (Ito, Kondo, and Harada 2000). The inhalational LD$_{50}$ corresponds more closely with the intraperitoneal LD$_{50}$ of 50 – 158 µg kg$^{-1}$ (WHO 1998; De Figueiredo et al. 2004). Although ingestion may be the most common route of exposure, inhalation is a more potent form of contact based on these studies.

Fitzgeorge et al. (1994) found that intranasal instillation to MC-LR caused necrosis in olfactory epithelium, leading to the destruction of large areas of the mucus membrane. The cuts in nasal vessels caused by MCs exposure potentially facilitated further movement of MCs into the bloodstream that ultimately caused lesions in the liver (Fitzgeorge, Clark, and Keevil 1994). Ito et al. (2000) found intratracheally applied MC-LR in mice entered the body from alveoli where it was absorbed into blood capillaries. At high doses, this exposure resulted in hemorrhaging in the liver. These studies suggest that compared to the oral route, where some detoxification can occur (USEPA 2015), there are fewer barriers to MCs when inhaled (Ito, Kondo, and Harada 2000).

Environmental studies in New Hampshire and New Zealand have documented aerosolized MCs ranging from 0.2 to 384 pg MCs m$^{-3}$ above lakes and on lake shores (Murby and Haney 2015; Wood and Dietrich 2011). Levels ranging from below detectable level (0.1 ng m$^{-3}$) to 2,890 pg m$^{-3}$ were measured in personal samplers and in samplers stationed on boats or along the shore of lakes in the Midwestern United States and in California (L. C. Backer et al.
While these studies have not documented levels of aerosolized microcystins that pose concern for acute toxicity, there are still many facets of this phenomenon that are not well documented or understood. Though in many cases aerosolized cyanobacteria may not be an immediate threat, it is possible that constant inhalational exposure to low levels of MCs could result in chronic toxicity. 

Furthering our understanding of cyanobacteria aerosolization will help us better comprehend the risk of inhalational exposure to cyanotoxins. Studies of aerosolized cyanotoxins so far have been performed on lakes with high levels of toxin production in hopes of maximizing detectable levels in the air. However, the size of cyanobacteria that have the potential to become aerosolized has not been thoroughly investigated. Though highly concentrated cyanobacteria blooms have the potential to produce more toxin, and therefore generate greater public interest, smaller picocyanobacteria (0.2 – 2 µm) most likely have a higher chance of becoming aerosolized (Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017).

Autotrophic picoplankton, including picocyanobacteria, are among the most numerous photosynthetic organisms in the world (Callieri and Stockner 2002; Callieri 2007), and are capable of toxin production (Jakubowska and Szeląg-Wasielewska 2015; Blaha and Marsalek 1999). A recent study evaluating the presence of aerosolized microalgae and cyanobacteria originating from the Baltic Sea discovered that picocyanobacteria were the most frequent component in aerosolized material (Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017). In addition to small cells, it is believed that MCs could be aerosolized independent of cells in the free or “dissolved” form. Due to their stable nature, it is thought that these free toxins could persist in the air without degrading (Wood and Dietrich 2011). Aerosolized free MCs
could result in a more severe toxic effect when inhaled. The size and form of aerosolized cyanobacteria cells and cyanotoxins have direct consequences for human health.

Another gap in our knowledge is the influence of meteorological factors (including day night differences) and water quality on the production of aerosolized cyanobacteria. The three proposed mechanisms behind aerosolization of biotic material from lake surfaces include: micro-bubble-bursting (D C Blanchard and Woodcock 1956; Y.-S. Cheng et al. 2007), wind (Dueker et al. 2011; Sahu and Tangutur 2015; Smith 1973), and evaporation (Murby and Haney 2015). While the focus of this study was not on these mechanisms, I measured several factors that could influence these processes. The measured possible environmental drivers are water and air temperature, the temperature differential between air and water, air humidity, wind, nitrogen, phosphorus, and the MCs in different water fractions. These include both indirect and direct drivers that influence the growth of cyanobacteria in the water, and drivers more directly influencing the releasing processes. It is important to gain a greater understanding of these drivers on a local scale, as there is often a significant overlap of aerosolized microbes above sources such as water bodies (K. Sharma, K. Rai, and Singh 2006; Dueker, O’Mullan, Juhl, et al. 2012).

Several studies have linked the health of surrounding human populations to local bodies of water. A study of freshwater across the contiguous United States showed a correlation with water quality and incidence of non-alcoholic liver disease (F. Zhang et al. 2015). Using satellite imagery of New England lakes, Torbick et al. (2017) found a relationship between lake phycocyanin levels (a pigment specific to cyanobacteria) and “hotspots” of Amyotrophic Lateral Sclerosis (ALS). It is believed ALS is linked to the exposure of BMAA, another cyanotoxin that acts as a neurotoxin (Cox, Banack, and Murch 2003). While the contribution of exposure via
inhalation was not determined in these studies, it is believed that this is an unavoidable and constant route of contact for populations surrounding lakes and could help explain the correlation (Stommel, Field, and Caller 2012; Y. L. Zhang et al. 2015; Torbick et al. 2017; Caller et al. 2009). A greater understanding of local-scale drivers of aerosolization could lead to better-informed management of exposure for lake-side communities.

The goals of this study are to 1) characterize cyanobacteria presence in aerosols collected directly above the lake surface and 2) gain a better understanding of ecological drivers behind cyanobacteria aerosolization (meteorological, time of day, and water quality). To achieve this, aerosols were sampled using a modified version of the collection technique described by Murby and Haney (2016). The present study serves to enhance our understanding of the process of cyanobacteria aerosolization as a step for future development of exposure estimates to MCs via inhalation on a local level.
METHODS

Study Sites

Eight lakes with varying trophic status were selected to encompass a range of natural cyanobacteria levels. Lakes varied in geographical location from northern New Hampshire to northern Massachusetts (Figure 1) and have a range of basic morphometric parameters (Table 1). Sampling started in the beginning of August and extended into the end of October in 2016 (Table 1). Three of the lakes included in this study typically have low concentrations of cyanobacteria: Christine Lake (Stark, NH), Lake Cochichewick (North Andover, MA), and Willand Pond (Dover, NH). Two lakes typically have mid-range levels of cyanobacteria: Old Durham Reservoir (Durham, NH), and Baboosic Lake (Amherst, NH). The final three lakes typically have elevated levels of cyanobacteria: Lake Attitash (Amesbury, MA), York Pond (Milan, NH), and Nippo Pond (Barrington, NH). To characterize sampled lakes, basic water quality parameters were recorded from the deep site of each lake (Table 3). Based on this assessment, mean phycocyanin levels ranged from 0.83 µg L⁻¹ in Christine Lake, to 81.3 µg L⁻¹ in York Pond. The Trophic State Index (TSI; Carlson, 1977) based on phosphorus, chlorophyll and secchi disk was calculated (Table 2), and generalized trophic status was assigned based on the guidelines established in A Coordinator’s Guide to Volunteer Lake Monitoring Methods (Carlson and Simpson 1996). Based on this assessment, Christine and Willand are oligotrophic; Cochichewick, Baboosic and Nippo are mesotrophic; ODR, York and Attitash are eutrophic.
Figure 1. Study lake locations, six in New Hampshire and two in Northern Massachusetts. Eutrophic lakes are labeled green, mesotrophic are turquoise, and oligotrophic are dark blue. Trophic status determined using Carlson’s Trophic State Index.
Table 1. Lake location (center of lakes), sampling date and basic morphometric data. New Hampshire lakes information from Department of Environmental Services Water Quality Database. Lake Attitash information from the EPA and Lake Cochichewick from town government websites. Morphometric data could not be found for Old Durham Reservoir.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Lake Location</th>
<th>Latitude °N</th>
<th>Longitude °W</th>
<th>Sampling Date</th>
<th>Watershed Area (Ha)</th>
<th>Mean Depth (m)</th>
<th>Max Depth (m)</th>
<th>Volume (Ha-m)</th>
<th>Surface Area (Ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitash</td>
<td>Amesbury, MA</td>
<td>42.852620</td>
<td>-70.978107</td>
<td>8-Aug</td>
<td>1010.1</td>
<td>3.7</td>
<td>9.8</td>
<td>621.7</td>
<td>155.4</td>
</tr>
<tr>
<td>Baboosic</td>
<td>Amherst, NH</td>
<td>42.882254</td>
<td>-71.574690</td>
<td>19-Aug</td>
<td>787.8</td>
<td>4.1</td>
<td>8.8</td>
<td>368.2</td>
<td>89.8</td>
</tr>
<tr>
<td>Christine</td>
<td>Stark, NH</td>
<td>44.627980</td>
<td>-71.392023</td>
<td>27-Aug</td>
<td>1165.5</td>
<td>6.9</td>
<td>19.5</td>
<td>474.7</td>
<td>68.8</td>
</tr>
<tr>
<td>Cochichewick</td>
<td>North Andover, MA Barrington, NH</td>
<td>42.687815</td>
<td>-71.100591</td>
<td>19-Oct</td>
<td>1295.0</td>
<td>13.7</td>
<td>23.0</td>
<td>1627.7</td>
<td>228.2</td>
</tr>
<tr>
<td>Nippo</td>
<td>North Andover, MA Barrington, NH</td>
<td>43.217436</td>
<td>-71.085349</td>
<td>2-Aug</td>
<td>173.8</td>
<td>7.1</td>
<td>15.8</td>
<td>244.9</td>
<td>34.5</td>
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<tr>
<td>Old Durham Reservoir</td>
<td>Durham, NH</td>
<td>43.144814</td>
<td>-70.939679</td>
<td>17-Oct</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Willand</td>
<td>Somersworth, NH</td>
<td>43.228592</td>
<td>-70.895305</td>
<td>26-Oct</td>
<td>116.5</td>
<td>4.7</td>
<td>11.2</td>
<td>162.7</td>
<td>34.8</td>
</tr>
<tr>
<td>York</td>
<td>Milan, NH</td>
<td>44.504996</td>
<td>-71.338778</td>
<td>15-Sep</td>
<td>90.4</td>
<td>2.8</td>
<td>5.2</td>
<td>23.5</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Table 2. Phosphorus, Chlorophyll a, Secchi Disk Depth values and calculated Trophic State Index. All samples taken from the deep site of the lake, and from an integrated tube of the top 3 m of epilimnion, error is ±1 standard error.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Trophic Status</th>
<th>Total Phosphorus (µg L⁻¹)</th>
<th>Phos TSI</th>
<th>Chlorophyll a (µg L⁻¹)</th>
<th>Chl TSI</th>
<th>Secchi Disk Depth (m)</th>
<th>SDD TSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitash</td>
<td>Eutrophic</td>
<td>23.63 ± 0.38</td>
<td>50</td>
<td>16.83 ± 0.42</td>
<td>58.27</td>
<td>1.43 ± 0.03</td>
<td>54.84</td>
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<tr>
<td>Old Durham Reservoir</td>
<td>Eutrophic</td>
<td>14.5 ± 0.23</td>
<td>54</td>
<td>5.42 ± 0.09</td>
<td>61.23</td>
<td>3.9 ± 0.61</td>
<td>NA</td>
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<tr>
<td>York</td>
<td>Eutrophic</td>
<td>4.83 ± 0.48</td>
<td>52</td>
<td>1.73 ± 0.03</td>
<td>50.24</td>
<td>9.17 ± 0.02</td>
<td>66.21</td>
</tr>
<tr>
<td>Baboosic</td>
<td>Mesotrophic</td>
<td>23.7 ± 0.10</td>
<td>43</td>
<td>5.58 ± 0.11</td>
<td>47.15</td>
<td>2.38 ± 0.01</td>
<td>40.37</td>
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<tr>
<td>Cochichewick</td>
<td>Mesotrophic</td>
<td>12.50</td>
<td>50</td>
<td>37.43 ± 0.93</td>
<td>47.43</td>
<td>1.58 ± 0.02</td>
<td>47.49</td>
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<tr>
<td>Nippo</td>
<td>Mesotrophic</td>
<td>32.57 ± 0.77</td>
<td>41</td>
<td>22.77 ± 0.44</td>
<td>66.11</td>
<td>-</td>
<td>53.40</td>
</tr>
<tr>
<td>Christine</td>
<td>Oligotrophic</td>
<td>7.13 ± 0.39</td>
<td>27</td>
<td>1.51 ± 0.01</td>
<td>35.95</td>
<td>7.08 ± 0.20</td>
<td>28.04</td>
</tr>
<tr>
<td>Willand</td>
<td>Oligotrophic</td>
<td>27.33 ± 0.33</td>
<td>32</td>
<td>7.42 ± 0.34</td>
<td>34.62</td>
<td>0.65 ± 0.08</td>
<td>31.76</td>
</tr>
</tbody>
</table>
Aerosol Collection

Aerosol samples were collected around 2-3 m from the shore of lakes in the littoral zone, over approximately 1 m water depth (Figure 2, orange stars). Aerosol samples were collected using a modified version of the newly developed portable cyanobacteria aerosol collection system (Murby and Haney 2015). The modified version is identical in basic design but combines three pumps and three connected collection systems in one unit or Compact Lake Aerosol Monitor (CLAM, Figure 3).

The CLAM uses a battery-powered vacuum pump to collect aerosols as they naturally emerge from the lake onto a filter (Figure 3). The pumps draw air at a rate of 2.5 liters per minute (LPM). A screen attached the collector funnel reduces large horizontal movements of aerosolized material. Air is drawn up the collector funnel through 2 mm diameter Tygon tubing and passes through a Whatman GFF 25 mm diameter glass fiber filter to trap water vapor and aerosolized particles. Before use, filters were pre-filtered with 15 mL of distilled water and combusted at 500 C for 1 hour to sterilize and remove cells and other organic matter prior to aerosol collection. Combustion also reduces the effective pore size of the filter from 0.6 µm to approximately 0.3 µm (Nayar and Chou 2003).

One CLAM was deployed at each sampling site; the samples collected aerosols in triplicate for both epifluorescence and toxin analysis. Nine-hour collections of aerosols were made during the day and again at night. Night sampling began at sunset directly following day sampling. When switching between day and night sampling, the GFF filters from day sampling were removed and stored on ice in a cooler. Night air samples were collected on a new set of filters.
Figure 2. Aerosol (orange star) and deep site (yellow star) sampling locations at each lake. Eutrophic lakes are dark green, mesotrophic lakes are turquoise, and eutrophic are blue. New Hampshire lakes have a darker blue background, Massachusetts lakes have a lighter blue background.
Figure 3. Diagram of important Compact Lake Aerosol Monitor (CLAM) components. Image shows an example of one collection system for simplicity, actual units have three combined collection systems in one.

Water Sampling

Surface water samples were taken in triplicate. Water samples were taken next to aerosol collectors to get the best representation of the water interacting with the air in that location. The top 10 cm of lake water was sampled using a 500 mL PETG Nalgene bottle. This sample of surface water was mixed, and a subsample of 120 mL was saved. Water samples were taken at the beginning and end of aerosol collection. The aggregated 240 mL of sample from the beginning and end of sampling is considered to be an average surface water condition during the sampling period. A separate set of start and finish water samples were taken for the night sampling.

The combined sample from each day or night period was fractionated into four 30-mL subsamples to characterize the cyanobacteria toxins present in various size fractions (whole lake water, <50 µm, <2 µm, and <0.22 µm). Unfiltered whole lake water (WLW) represents all organisms in the water. The <50 µm fraction was collected by pouring WLW through a ring net...
with a 53 µm Nitex mesh. Using a 60 mL syringe, the <2 µm fraction was separated by passing lake water through 2 µm TTTP Isopore membrane filters (MilliporeSigma, Burlington MA) to isolate and concentrate picocyanobacteria for toxin concentration. The dissolved toxin fraction was separated using a second 60 mL syringe and 0.22 µm nylon syringe filters (Fox Scientific Inc, Alvarado TX). Fractionation was done immediately after collecting the final water sample. Fractionated samples were kept on ice in a dark cooler, then transferred to a freezer for storage until they were prepared for toxin analysis.

Climatic Factors

Two HOBO data loggers (ONSET, Bourne, MA) were deployed during aerosol collection. One logger (Onset ProV2) was placed on a platform next to the aerosol collectors and recorded air temperature and relative humidity every 10 min. The second logger (Onset Pendant) was placed underneath this platform, suspended around 10 cm below the surface of the water, and recorded water temperature every 10 min. The day and night average of these three parameters is presented. A handheld anemometer (Extech, Nashua, NH) was used to record wind speeds at the beginning and end of aerosol sampling during the day and night. Estimates of evaporation were made for this study using Linacre’s formula (1977) based on temperature (adapted from Penman’s evaporation over water surface (Penman 1978)) and estimating relative dew point as described by Lawrence (2004).

Deep-site Sampling

Vertical profiles and integrated tube sampling were also collected at the deep-site of each lake to characterize the system (Figure 2, yellow stars). A multi-parameter sonde (YSI, EXO II, Yellow Springs, OH), coupled with a data logger (YSI, Handheld, Yellow Springs, OH) was lowered through the water column to rapidly record the lake water-quality parameters in 3-
second intervals. A range of physical and chemical properties were recorded (Table 3), including depth, temperature, dissolved oxygen (concentration and percent oxygen), pH, specific conductance, oxidation reduction potential (pH corrected to get the E7 values), chlorophyll a fluorescence (excitation wavelength 470 ± 15 nm), and phycocyanin fluorescence (excitation wavelength 590 ± 15 nm).

Table 3. Summary of lake water quality parameters measured at deep site of lakes using the EXO II multiparameter probe. Values represent an average over the top 3 m, and error is ±1 standard error.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Temperature (°C)</th>
<th>Phycocyanin (µg L⁻¹)</th>
<th>Chlorophyll a (µg L⁻¹)</th>
<th>Oxygen (%)</th>
<th>Oxygen (mg L⁻¹)</th>
<th>pH</th>
<th>Specific Conductance (µS cm⁻¹)</th>
<th>E7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitash</td>
<td>27.78 ± 0.04</td>
<td>17.15 ± 0.45</td>
<td>16.83 ± 0.42</td>
<td>111.58 ± 0.27</td>
<td>8.76 ± 0.02</td>
<td>8.18 ± 0.02</td>
<td>202.38 ± 0.03</td>
<td>202.12 ± 1.11</td>
</tr>
<tr>
<td>Baboosic</td>
<td>26.85 ± 0.02</td>
<td>5.25 ± 0.06</td>
<td>5.42 ± 0.09</td>
<td>100.45 ± 0.23</td>
<td>8.02 ± 0.02</td>
<td>8.09 ± 0.04</td>
<td>146.01 ± 0.09</td>
<td>195.66 ± 3.28</td>
</tr>
<tr>
<td>Christine</td>
<td>22.01 ± 0.01</td>
<td>0.83 ± 0.03</td>
<td>1.73 ± 0.03</td>
<td>97.49 ± 0.02</td>
<td>8.52 ± 0.00</td>
<td>7.9 ± 0.00</td>
<td>23.62 ± 0.01</td>
<td>219.43 ± 0.31</td>
</tr>
<tr>
<td>Cochichewick</td>
<td>16.69 ± 0.02</td>
<td>4.16 ± 0.06</td>
<td>5.58 ± 0.11</td>
<td>101.88 ± 0.17</td>
<td>9.91 ± 0.02</td>
<td>7.66 ± 0.00</td>
<td>300.75 ± 0.05</td>
<td>89.52 ± 14.22</td>
</tr>
<tr>
<td>Nippo</td>
<td>25.53 ± 0.00</td>
<td>8.49 ± 0.13</td>
<td>37.43 ± 0.93</td>
<td>109.06 ± 0.03</td>
<td>8.92 ± 0.00</td>
<td>7.64 ± 0.03</td>
<td>101.78 ± 0.03</td>
<td>182.52 ± 0.67</td>
</tr>
<tr>
<td>Old Durham Reservoir</td>
<td>14.38 ± 0.12</td>
<td>9.81 ± 0.13</td>
<td>22.77 ± 0.44</td>
<td>82.27 ± 0.79</td>
<td>8.39 ± 0.06</td>
<td>7.48 ± 0.00</td>
<td>259.65 ± 0.09</td>
<td>80.91 ± 0.57</td>
</tr>
<tr>
<td>Willand</td>
<td>26.31 ± 0.02</td>
<td>4.69 ± 0.05</td>
<td>1.51 ± 0.01</td>
<td>102.22 ± 0.04</td>
<td>8.24 ± 0.00</td>
<td>6.97 ± 0.00</td>
<td>264.33 ± 1.12</td>
<td>271.05 ± 0.96</td>
</tr>
<tr>
<td>York</td>
<td>18.53 ± 0.06</td>
<td>81.3 ± 4.10</td>
<td>7.42 ± 0.34</td>
<td>68.28 ± 4.06</td>
<td>6.36 ± 0.38</td>
<td>8.67 ± 0.02</td>
<td>25.68 ± 0.53</td>
<td>206.82 ± 7.47</td>
</tr>
</tbody>
</table>

An integrated tube (3 m depth, 19 cm inner diam Tygon tubing) was used to collect a sample of epilimnetic water at the same location as the deep-site profile. These samples were taken in triplicate for each lake and were frozen until they were analyzed for total phosphorus and total nitrogen. Total phosphorus was determined with the ammonium molybdate colorimetric method (USEPA Method 365.3), total nitrogen with the Kjeldahl method; methods followed the standard operating procedure of the New Hampshire Lakes Lay Monitoring Program (UNH, Durham, NH).
Epifluorescence Microscopy

Aerosolized material collected on filters was examined with epifluorescence microscopy (Olympus BX41, Olympus DP72 camera) with an automated stage (Model type ES222/G, Prior Scientific Instruments, Fulbourn Cambridgeshire, ENG) and an X-Cite series 120Q Lumen Dynamics mercury light (120-watt, Excelitas Technologies, Waltham MA) to enumerate cyanobacteria cells. Two narrow band excitation wavelengths of 435 nm (CHL cube) and 572 nm (PC cube) discriminate between cyanobacteria and other photosynthetic cells (Figure 4). The 435 nm wavelength excites a broad window of chlorophyll pigments, while the 572 nm more specifically excites phycocyanin / phycobilin pigments accessory to chlorophyll. While chlorophylls are found in all phytoplankton, the phycocyanin / phycobilin pigments are found primarily in cyanobacteria. CellSens Dimension imaging software (Olympus, Chelsmford, MA) digitally creates a dark field image where individual cells can be clearly visualized and counted on the surface of the glass fiber filters.

Figure 4. Epifluorescence images of aerosol filters, left image shows autofluorescence of aerosolized phytoplankton under the chlorophyll excitation 435 nm, right image is the same location under phycocyanin excitation at 572 nm. Cyanobacteria appear red under the chlorophyll excitation while the phycocyanin cube only excites cyanobacteria.
A rectangle in the center of the filter was determined using the cellSense program (Figure 5). The x-y-boundaries of this rectangle were entered into Excel, and a random number generator was used to pair an x and y location within the defined rectangle. Using this method, 20 random locations were selected per filter, and entered into the cellSense program. The z-depth for the filter was determined by scanning through the filter at 10 other locations and determining the maximum and minimum z-locations where fluorescing cells could be found while using the PC cube (Figure 5). A 200 µm z-depth was established by taking the average depth where cells were found and adding 100 µm in each direction. The program was then set to take an image at every 3 µm within the established 200 µm z-stack range. As a result, 68 images were taken per location. Images were taken with a 530.8 ms exposure time. The 68 images per location were compressed into one complied image, producing a maximum projection image. By creating one image per location, all fluorescing cyanobacteria cells from the 200 µm range can be seen in one image.

![Diagram of random location and depth determination for epifluorescence imaging on aerosol filters](image)

Cyanobacteria were counted using the Count and Measure option in the CellSense program. Six size bins were created and the number of cells in each size category was counted for each image, then summed between all 20 images to determine totals for the filter. The six size categories, based on equivalent spherical diameter (ESD) were: 0.2-2 µm, 2-4 µm, 4-10 µm, 10-20 µm, 20-40 µm, and larger. The smallest size category of cell corresponds with the size of
picocyanobacteria, the middle three size categories represent the nano (2-20 µm) sized cyanobacteria, while the largest size category and anything larger represent cells in the micro sized range of cyanobacteria. The number of cells per area counted was extrapolated to the effective filter area (21 mm) of the 25 mm diameter filter. These counts were then adjusted for air flow rate and duration of sampling to calculate the cells m⁻³ of air sampled. Biovolume of aerosolized cells was estimated by using the measured radius of each cell counted and assuming a sphere shape. Biovolume followed the same patterns as cell count, so the results are not included. Following microscopy, air filters were frozen until processed for MCs.

**Toxin analysis**

The concentrations of microcystins were measured using the High Sensitivity QuantiPlate ELISA (enzyme-linked immunosorbent assay) technique for MCs (EP-022, Enviro Logix Inc, Portland ME) in aerosol samples, as well as the <0.22 µm, <2 µm, <50 µm, and WLW fractions. Subsamples of the water fractions were subjected to three cycles of rapid freeze-thaw, vortex, and sonication. Samples were frozen at -80 C, thawed in a warm water bath at 40 C, vortexed for 10 s using the Vari-Whirl Mixer, level 6 speed (VWR Scientific, Radnor PA), and sonicated for 3 min in the Ultrasonic Bath CPX/CPXH series (ThermoFisher Scientific, Waltham MA). This process disrupts cells and releases toxins for detection.

To increase detection sensitivity of the ELISA, water samples were concentrated in the RC10.10 speed-vacuum (Jouan Inc., Winchester VA). Depending on the type of sample and the anticipated toxin levels, samples were concentrated 5x, 10x or 20x. This was achieved by using multiple mini centrifuge vials per sample and a larger initial sample of water for higher concentrations. Ultimately the volume was reduced, and samples were combined into one vial with a final sample volume around 0.3 mL. Specific concentration factors were determined for
each sample by the weight difference pre and post speed-vac. Concentrated samples were stored frozen at -20 C.

Toxins were also extracted from aerosol filters. Filters were cut into 12 pieces using clean scissors and tweezers, then placed in a 2.0 mL centrifuge tube. Between filters, scissors and tweezers used to hold the filters while cutting were rinsed in ethanol (70%) and Milli-Q three times, dried with a KimWipe, then rinsed in Milli-Q another time and dried again. Toxins were extracted from aerosol filters by adding 1.8 mL of Milli-Q to the filter and performing three freeze-thaw, sonicate, and vortex cycles as described above.

After the standard freeze-thaw cycles, as much liquid material as possible was removed from the vial and transferred into another vial. This transfer includes the dissolved toxin collected on the air filter and some fibrous glass fiber material that has disintegrated and become suspended in the Milli-Q water. These samples were centrifuged for 3 min at 10,000 RPM in the Gusto Mini Centrifuge (Heathrow Scientific, Vernon Hills IL). The supernatant was removed, careful to avoid getting any of the filter material now concentrated at the bottom of the vial. This step was repeated, further separating filter material from the sample. The collected supernatant, now free of glass fiber filter material, was concentrated by speed-vacuuming as described to 0.07 mL. All samples were stored frozen at -20 C.

Prior to toxin analysis samples were thawed and centrifuged at 10,000 RPM for 1 min 30 s to avoid particulate contamination, then run following the ELISA procedure. ELISA plate absorbances were measured using the 800TS Microplate Reader and Gen 5 Microplate Reader and Imager Software (BioTek, Winooski VT) with the primary wavelength at 450 nm, and the reference wavelength at 630 nm. Toxin concentrations were derived from standard curves fitted with a four-parameter logistic equation. All standard curves had an $R^2 > 99\%$. 
Microcystin levels from the ELISA were adjusted for SpeedVac concentration to calculate toxicity as ng MCs L\(^{-1}\). Toxicity was evaluated in four filtrate fractions: WLW, <50 µm, <2 µm and dissolved (<0.22 µm). Two additional calculated fractions are evaluated to determine the toxicity contribution from specific sized organisms: net plankton and particulate toxicity. Calculations were done by subtracting unmanipulated fractions from each other. Net plankton are greater than 50 µm and derived by subtracting the MCs in the <50 µm from the WLW. The total particulate toxicity is derived by subtracting the MCs in the dissolved fraction from the WLW. For aerosolized cyanobacteria, the toxicity per filter was adjusted for flow rate and duration of sampling to determine the MCs toxicity in pg m\(^{-3}\) of air sampled.

**Statistical Analysis**

Data were organized and stored in Excel and all graphs were generated using SigmaPlot 12.5 (SYSTAT Software Inc., Chicago IL). Analyses including two-way ANOVAs and Tukey’s Post Hoc tests were used to determine the relationship between time of day and lake for water toxicity and aerosolized cyanobacteria material, and to evaluate if there was an interaction between time of day and lake. MCs in water fractions, aerosolized MCs and aerosolized cells failed normality in the two-way ANOVAs. All but the <2 µm, dissolved, net plankton and aerosolized MCs passed equal variance. In order to address the issue of equal variance, those parameters that did not pass were log transformed, and the two-way ANOVAs were performed on transformed data. The <2 µm fraction continued to fail equal variance after log transformation. The parameter was rank transformed and the results of the two-way ANOVA were compared to the results of the untransformed data (Conover and Iman 1981). There were no deviations in results, so the results from the two-way ANOVA on the untransformed data are presented. A two-way ANOVA was also done on the temperature difference between air and
water to evaluate time of day and lake differences. Temperature difference was treated the same way as the <2 µm fraction. When there was a significant interaction between time of day and lake, the differences between lakes were presented during the day separately from the night.

Linear regressions were conducted in SigmaPlot and were performed on untransformed data. When indicated, the data were log transformed to meet the assumptions of normality and constant variance.

Relationships between aerosol cell counts and aerosol toxicity, climatic factors, and water quality were examined with stepwise regressions performed in JMP 14.0 (SAS Institute Inc., Cary NC). All-possible models were generated, and the models with the lowest Akaike information criterion (AICc) were selected. AIC is an indicator of the relative quality of a statistical model in relation to other models generated for a given set of data. AICc adjusts for small sample sizes. In the case where a two-parameter model was selected, the predicted values were exported to graph the model in SigmaPlot.

The influence of single lakes on the models generated by the stepwise regressions was determined using three different measures in JMP: hat values, studentized residuals and Cook’s D values. Hat values were used as a measure of leverage, evaluating the impact with respect to the independent variables. Because of the small samples size, values above $2(k+1)/n$ where k is the number of predictors and n is the sample size was used (Systemworks, n.d.). Studentized residuals were used to determine unequal residuals, after adjusting for different leverage. Studentized residuals greater than two, data that lie outside the 95% confidence interval, were considered high (Systemworks, n.d.). Cook’s Distance test evaluates the influence of a single observation based on the total changes in all residuals when the observation is deleted from the estimation process, values above one were considered high influence (Systemworks, n.d.).
Observations with high influence by any of these measures were noted, but the relationships with the full data set were always presented because the sample size was too small to justify removing any of the data.

In addition to this formal analysis of outliers, there are three lakes with measures that had exceptionally high variability. These measurements are <2 µm MCs in Lake Attitash at night, large aerosolized cells in Old Durham Reservoir at night and aerosolized MCs in York Pond during the day. Because the sample size for each measurement is small (n = 3), I could not justify the removal of the outlying data points. Each analysis was performed without these outliers to determine how they affected the outcomes of tests. The major conclusions did not change with their removal, so all data were included in the analysis.

It is best to look at aerosolized MCs during both the day and night because the combination alleviates some influence issues that occur when the time of day is evaluated independently. A consistent difficulty in statistically evaluating this dataset is the high level of toxin present in Lake Attitash compared to the other lakes. Though I selected lakes based on nutrient status and prior bloom history in an attempt to sample lakes across a range of toxicity, most lakes had low toxicity, compared to the high toxicity of Lake Attitash.
RESULTS

Water Microcystins

Microcystins in whole lake water (WLW) from the shore of the eight lakes ranged from 2.80 to 88.86 ng L\(^{-1}\) across both day and night (Figure 6). Lake Attitash had significantly higher levels of MCs toxin compared to the other lakes, while the other lakes had more similar MCs. York Pond had the second highest level of toxin, but was significantly lower than Lake Attitash during both the day and night (Figure 6). The effect of time of day on WLW MCs varied depending on the lake (two-way ANOVA, time of day × lake interaction p <0.001). Five of the eight sampled lakes have higher levels of WLW toxicity during the day compared to the night, though only Lake Attitash had significantly higher MCs in the WLW during the day compared to the night (p <0.001, Figure 6). MCs did not statistically differ in the WLW based on season, however the general trend was a decrease in toxicity as sampling date progressed from Summer into Fall (sampling date vs. log WLW MCs was used to meet the assumptions of normality and constant variance, p = 0.2086, Adj R\(^2\): 0.1233).
Lakes with the highest MCs in the WLW typically had proportionally high levels of MCs in the <50 µm fraction (Figure 7). Similar to the WLW, Lake Attitash had the highest concentration of MCs followed by York Pond, with a similar lower level found in the rest of the lakes during both the day and night sampling (Figure 7). The toxicity in the <50 µm fraction varied from 2.37 to 34.88 ng L⁻¹ during the day (with all but Attitash between 2.37 and 13.51 ng L⁻¹), and was more similar across lakes at night only varying 2.75 to 22.12 ng L⁻¹ (with all but Attitash between 2.75 and 10.02 ng L⁻¹). The effect of time of day on MCs in the <50 µm fraction varied depending on the lake and time of day (two-way ANOVA, time of day × lake interaction p <0.001). Lake Attitash (p <0.001), York Pond (p = 0.004), Willand Pond (p =
0.340) and Old Durham Reservoir had higher (p = 0.10) toxicity during the day, while the remaining lakes had very little difference in toxicity depending on time of day (Figure 7).

![Bar chart showing Microcystins toxicity in the <50 µm fraction during day and night. Lake Attitash has much higher levels of toxicity compared to the rest the lakes. Other lakes have similar levels of toxin in the <50 µm fraction. Time of day differences are lake-specific (two-way ANOVA time of day × lake interaction: p <0.001). Half of the lakes have higher toxin levels in this fraction during the day, but this difference is only significant in Lake Attitash (p <0.001) and York Pond (p = 0.004), indicated by an asterisk. Statistical differences between lakes during the day are indicated by letters A-E, and differences between lakes at night are indicated by w-z (p <0.05). Error bars are ±1 standard error.](image)

Figure 7. Microcystins toxicity in the <50 µm fraction during day and night. Lake Attitash has much higher levels of toxicity compared to the rest the lakes. Other lakes have similar levels of toxin in the <50 µm fraction. Time of day differences are lake-specific (two-way ANOVA time of day × lake interaction: p <0.001). Half of the lakes have higher toxin levels in this fraction during the day, but this difference is only significant in Lake Attitash (p <0.001) and York Pond (p = 0.004), indicated by an asterisk. Statistical differences between lakes during the day are indicated by letters A-E, and differences between lakes at night are indicated by w-z (p <0.05). Error bars are ±1 standard error.

Different patterns in the levels of MCs emerge for plankton in the <2 µm fraction compared to the WLW (Figure 8). Lake Attitash continues to have a significantly higher level of toxin compared to the other lakes, but Baboosic Lake and Willand Pond have the second highest level of toxicity in the <2 µm fraction while York Pond has the fourth highest level. The remaining lakes had lower and similar toxicities in this fraction. Compared to the toxicity in the larger fractions, the range of MCs was much smaller in the <2 µm fraction from 1.98 ng L\(^{-1}\) at the lowest in Christine Lake during the day, to the highest of 14.61 ng L\(^{-1}\) in Lake Attitash at night. This is a difference of 12.63 ng L\(^{-1}\), while the difference in the range of MCs in WLW is
86.06 ng L$^{-1}$. The level of MCs in the <2 μm fraction did not vary by the time of day ($p = 0.49$) and the changes in MCs from day to night did not vary based on the lake (two-way ANOVA, time of day × lake interaction $p = 0.99$).

Similar to the <2 μm fraction, there is a much narrower range of MCs levels across lakes in the dissolved fraction compared to the MCs in larger fractions (Figure 9). Lake Attitash continued to have a significantly higher concentration of toxin compared to the other lakes. York Pond, Baboosic Lake and Willand Pond had the second highest levels (Figure 9). Overall, the MCs in the dissolved fraction of water did not vary greatly between lakes, ranging from 2.50 ng L$^{-1}$ in Christine Lake during the day to 13.68 ng L$^{-1}$ in Lake Attitash during the day. Even though
Lake Attitash had a higher level of WLW MCs compared to the other lakes, the level of MCs in the dissolved fraction in Lake Attitash was similar to the other lakes.

Dissolved toxins did not vary consistently with time of day in all lakes (two-way ANOVA, time of day × lake interaction p < 0.001), although dissolved MCs varied significantly with time of day in more lakes than any other MCs size fraction. For example, Lake Attitash had significantly more dissolved MCs during the day than night (p < 0.001), whereas Nippo Pond (p = 0.001), Lake Cochichewick (p = 0.012) and Christine Lake (p < 0.001) had significantly higher levels of dissolved MCs during the night (Figure 9).

![Figure 9. Time of day differences in the toxicity in the dissolved fraction (<0.22 µm) across lakes. Three lakes had higher levels of dissolved microcystins during the day. Lake Attitash was the only one of these lakes to have significantly more microcystins during the day. The remaining five lakes had higher levels of dissolved toxins during the night. Of these lakes Nippo Pond, Lake Cochichewick and Christine Lake have significantly higher levels of dissolved microcystins during the day compared to night, significant differences indicated by asterisks. The effect of time of day on the dissolved fraction significantly varied by lake (two-way ANOVA time of day × lake interaction p < 0.001). Statistical differences between lakes during the day are indicated by letters A-E, and differences between lakes at night are indicated by w-z (p < 0.05). Error bars are ±1 standard error.](image-url)
High MCs in the net plankton (>50 mm) can indicate the presence of large forms of MCs producing cyanobacteria. Lake Attitash, York Pond and Nippo Pond have the largest presence of MCs producing net cyanobacteria (Figure 10). The remaining lakes all have less than 4.43 ng L\(^{-1}\) MCs (Baboosic day net MCs) in these large forms of cyanobacteria (Figure 10). The concentration of MCs in the net plankton did not differ significantly between day and night (p = 0.70) and the relationship between time of day differences did not vary by lake (two-way ANOVA time of day \(\times\) lake interaction p = 0.488).

![Graph showing microcystins toxicity in net plankton (>50 µm) across day and night.](image)

**Figure 10.** Microcystins toxicity in net plankton (>50 µm) across day and night. Toxicity in this fraction follows similar patterns to the whole lake water toxicity. MCs in the net plankton did not significantly vary based on the time of day (p = 0.701) and there was no interaction between time of day and lake (two-way ANOVA time of day \(\times\) lake interaction p = 0.488). Statistical differences between lakes are indicated by letters A-D (p < 0.05). Error bars are ±1 standard error, except Nippo Pond values are max/min.

Particulate MCs, calculated as the difference between the dissolved fraction (<0.22 µm) and the WLW toxicity, represents the MCs bound within cells in contrast to MCs that is “free” or dissolved in the lake water. The differences in MCs in this fraction vary between lakes in a
similar pattern as the net plankton (Figure 11). Lake Attitash had the highest concentration of particulate toxicity. York and Nippo Ponds had similar levels to each other, though significantly higher levels than the remaining lakes (Figure 11). There was little variation in the MCs in the particulate fraction across the remaining lakes. Changes in MCs in the net plankton from day to night did vary in different ways depending on the lake (two-way ANOVA time of day × lake interaction p <0.001). Lake Attitash was the only lake with a significantly higher concentration of toxicity during the day compared to night (p <0.001, Figure 11). York Pond, Nippo Pond, and Old Durham Reservoir had higher particulate MCs levels during the day than at night (though not statistically different), while the remaining lakes had little variation in MCs levels in the particulate fraction based on time of day (Figure 11).

Figure 11. Particulate toxicity, microcystins contained within cells, defined as toxicity in the whole lake water after subtracting the dissolved toxin. The MCs in this fraction follows similar patterns to the whole lake water and net plankton. Particulate toxicity varied from day to night in different ways across lakes (two-way ANOVA time of day × lake interaction p <0.001). Half the lakes have higher particulate toxin during the day, while the other lakes show little deviation in particulate toxicity from day to night. Attitash has significantly more toxin in the particulate toxin during the day, compared to the night (p <0.001) and is indicated by an asterisk. Statistical differences between lakes during the day are indicated by letters A–C, and differences between lakes at night are indicated by w–z (p <0.05). Error bars are ±1 standard error except Nippo Pond are max/min.
Aerosolized Microcystins

Aerosolized microcystins from all eight lakes varied from below detectable levels, to the lowest recorded level of 0.93 pg m\(^{-3}\) in Nippo Pond at night to 3.79 pg m\(^{-3}\) in York Pond during the day (Figure 12). There was no clear diel pattern, as half of the lakes had higher toxicity during the day, and half had higher toxicity at night (\(p = 0.711\)). The effect of time of day changes on aerosolized MCs did not vary between lakes (two-way ANOVA time of day × lake interaction \(p = 0.335\)).

Figure 12. Aerosolized microcystins measured above lake water. Lakes are ordered on the x-axis by average aerosolized microcystins across day and night. There was no significant effect of time of day on aerosolized MCs (\(p = 0.711\)) and the time of day effect did not differ between lakes (two-way ANOVA time of day × lake interaction \(p = 0.335\)). Error bars are ±1 standard error, except for York night, ODR day, Christine day and night and Willand day which are max/min values. Nippo Pond night only had one replicate above the detection limit. Statistical differences between lakes are indicated by letters A and B (\(p < 0.05\)).
Aerosolized Cyanobacteria Cells

Aerosolized cyanobacteria cells collected on the filters were categorized by cell size. Small cells in the 0.2 – 2 µm size range were often the most dominant size category of cells found on filters making up an average of around 56.9 ± 4.23% of total cells counted (Figure 13). Approximately 99.2 ± 0.14% of the cyanobacteria cells found on filters were below 10 µm; cells larger than 10 µm were rarely found. The relative abundance of cyanobacteria cells of each size class varied greatly depending on lake. For example, while Willand Pond primarily had picocyanobacteria in aerosols, Old Durham Reservoir aerosols were dominated by cells larger than 2 µm (Figure 13). There also appear to be different relationships between the abundance of aerosolized material in each size categories during the day compared to at night on a lake specific basis. For instance, there are comparable levels of picocyanobacteria in aerosols during the day and night in Lake Cochichewick, but at night there are more cells in the 2-4 µm and 4-10 µm categories (Figure 13). Further analysis considered the total cell count or the total cell count as two size categories: picocyanobacteria (0.2 – 2 µm) or larger cells (2 – 40 µm).
Aerosolized cyanobacteria were detected on filters at all lakes. Concentrations of total aerosolized cyanobacteria cells ranged 10-fold, from a minimum of 19,507 cells m\(^{-3}\) in aerosols at York Pond to a maximum of 194,705 cells m\(^{-3}\) in aerosols at Lake Attitash (Figure 14). During the day, Old Durham Reservoir had significantly higher levels of total aerosolized cyanobacteria cells than York Pond (\(p = 0.046\)). There was little variation in total aerosolized cyanobacteria across the other lakes during the day (Figure 14). At night, only Lake Attitash and Old Durham Reservoir had significantly higher levels of total aerosolized cyanobacteria cells, while the other lakes did not statistically differ from each other.

Total aerosolized cyanobacteria cells varied based on time of day differently depending on the lake (two-way ANOVA time of day × lake interaction \(p < 0.001\)). Six of the eight lakes had higher total aerosolized cells during the night compared to the day (Figure 14). Lake Attitash
and Old Durham Reservoir had higher concentrations of aerosolized cyanobacteria at night compared to the day (p <0.001 and 0.002 respectively, Figure 14). Though some lakes produce higher concentrations of total cyanobacteria in aerosols during the day, across all lakes total aerosolized cyanobacteria cells increase on the average by 84 ± 37% at night.

![Figure 14. Total aerosolized cyanobacteria cells enumerated through epifluorescence. ODR has the most aerosolized cyanobacteria during the day, and Attitash has the most during the night. Lake Attitash and Old Durham reservoir had significantly more aerosolized cells at night compared to the day, statistical significance between day and night values within a lake are indicated with asterisks. Except for Nippo and Willand, all lakes have higher levels of aerosolized cells during the night. Significant differences between lakes are indicated by letters A and B for day, and w-x for night (p <0.05). Error bars are ±1 standard error.](image)

Aerosolized picocyanobacteria follow the same general trend across lakes and based on time of day as total aerosolized cyanobacteria, but there are some differences (Figure 15). The minimum concentration of aerosolized picocyanobacteria cells was 7,360 cells m⁻³ from York Pond, and the maximum aerosolized picocyanobacteria cells was 152,140 cells m⁻³ from Lake Attitash (Figure 15). During the day, Willand Pond generated the highest concentration of aerosolized picocyanobacteria, but is only statistically higher than York Pond (p = 0.008). Beyond this difference there is little variation in day-time aerosolized picocyanobacteria. At
night, Lake Attitash produces a significantly higher level of picocyanobacteria compared to all other lakes (Figure 15).

Aerosolized picocyanobacteria either increase or decrease based on time of day depending on the lake (two-way ANOVA time of day × lake interaction p <0.001). Lake Attitash (p <0.001), Old Durham Reservoir (p = 0.01), Baboosic Lake (p = 0.01), and York Pond (p > 0.05) have a higher concentration of picocyanobacteria cells aerosolized during the night, compared to the day. Lake Cochichewick and Nippo Pond had comparable day and night aerosolized picocyanobacteria, while Christine Lake and Willand Pond had higher levels during the day (p > 0.05).

![Figure 15. Picocyanobacteria (0.2 – 2 µm) aerosolized from lakes and time of day differences. Picocyanobacteria show different trends than the total aerosolized cyanobacteria. Five lakes had higher levels of aerosolized picocyanobacteria at night. This difference was statistically significant in Lake Attitash, Old Durham Reservoir and Baboosic Lake. Error bars are ± 1 standard error. The effect of time of day on aerosolized picocyanobacteria varied depending on the lake (two-way ANOVA time of day × lake interaction p < 0.001). Significant differences between lakes are indicated by letters A - C for day, and w-y for night (p < 0.05). Significant differences between day and night within a lake are indicated with asterisks.](image-url)
The larger aerosolized cyanobacteria cells had different trends than the total and the picocyanobacteria (Figure 16). Aerosolization of larger cyanobacteria cells was higher at night compared to the day except for Nippo and Willand Ponds, though the rate of aerosolization of larger cyanobacteria cells during the day versus the night did not vary significantly depending on the lake (two-way ANOVA time of day × lake interaction p = 0.157). This increase in aerosolized larger cyanobacteria cells at night was only significant for Lake Attitash (p = 0.025) and Old Durham Reservoir (p = 0.002). Across lakes, Old Durham Reservoir had a significantly higher level of aerosolized larger cells, but there were no significant differences in the output of aerosolized larger cells among the other lakes.

Figure 16. Large (2 - 40 µm) aerosolized cyanobacteria cells. The trends in larger aerosolized cyanobacteria cells are different from both the total and pico-sized cells. Old Durham Reservoir alone had significantly higher levels of large aerosolized cells compared to the other lakes. Lake Attitash and Old Durham Reservoir had significantly higher levels at night. Differences between day and night levels of aerosolized larger cells within a lake are indicated with asterisks. Significant differences between lakes are indicated by letters A and B (p <0.05). Error bars are ± 1 standard error.
**Relationship Between Aerosolized Cyanobacteria Material**

During the day, differences in aerosolized MCs are not explained by any of the aerosolized cyanobacteria cell types (total, pico or larger). At night however, the total cell count explained 67% of the variability in aerosolized MCs ($p = 0.008$, Adj $R^2$: 0.67; Figure 17). As the two different methods for evaluating cyanobacteria in aerosols (toxins or cell counts) are correlated during at night, but not during the day, there may be different driving forces and mechanisms behind the process of their aerosolization.

![Graph showing the relationship between aerosolized cyanobacteria cells and aerosolized microcystins measured by ELISA at night.](image)

**Figure 17.** Relationship between aerosolized cyanobacteria cells enumerated through epifluorescence and aerosolized microcystins measured by ELISA at night. Aerosolized cells and toxins did not have a significant linear relationship during the day. Standard error bars are ±1. Equation: Aerosolized MCs (pg MCs m$^{-3}$) = 1.13 (± 0.24) + 9.61 $E^{-006}$ (± 2.49 $E^{-006}$) * total aerosolized cyanobacteria (cells m$^{-3}$). Lakes are colored as following: York dark red, Willand light red, Old Durham Reservoir orange, Nippo yellow, Cochichewick green, Christine turquoise, Baboosic dark blue, Attitash purple.

**Important Water Quality and Climatic Variables Drivers for Lake Aerosols**

Total nitrogen and phosphorus levels were measured from integrated tube samples from the epilimnion of lakes at the deep sampling locations. Levels of these nutrients varied across
lakes (Table 5) with Christine Lake having the lowest levels of nitrogen (177.33 ± 3.18) and phosphorus (4.83 ± 0.48) and Old Durham Reservoir had the highest nitrogen (786.33 ± 11.92) and phosphorous (32.57 ± 0.77). Climatic and water quality parameters varied by sampling location and between day and night measurements within one sampling location (Table 4 and 5). Evaporation estimated from this model did not appear as a significant driver of aerosolization. However, there are likely inaccuracies in evaporation estimated from simplified models. Future work should directly measure evaporation using the pan-evaporation technique (Linacre 1977) to determine if this is an important factor in day-time aerosolization.

Air and water temperature differential (air temperature – water temperature) had the most significant effect on the aerosolization of cyanobacteria cells of all parameters measured. During the day, the temperature differential was most often negative and was always negative during the night (Figure 18). At night there was generally a larger difference between air and water temperatures. The differential between atmosphere and water temperatures during the day vs. night differed between lakes (two-way ANOVA time of day × lake interaction p <0.001). The air and water temperature differed significantly between day and night in all lakes (Figure 18). As the temperature differential between air and water becomes more negative, humidity increases when day and night are evaluated together (p = 0.0261, Adj R²: 0.26). Wind was negatively correlated with total and larger aerosolized cells when both parameters were log10 transformed (total cells p = 0.033, Adj R²: 0.235; larger cells p = 0.015, Adj R²: 0.306) as a negative power function.
Table 4. Total nitrogen and total phosphorus measured at the deep site of each lake. Samples from integrated tubes taken from the top 3 m of the lake. Standard error is ±1 is included with mean of triplicate samples. Nippo Pond samples were collected on 14 July.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Total Nitrogen (µg L⁻¹)</th>
<th>Total Phosphorus (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitash</td>
<td>537.33 ± 16.83</td>
<td>23.63 ± 0.38</td>
</tr>
<tr>
<td>Baboosic</td>
<td>380 ± 7.51</td>
<td>14.5 ± 0.23</td>
</tr>
<tr>
<td>Christine</td>
<td>177.33 ± 3.18</td>
<td>4.83 ± 0.48</td>
</tr>
<tr>
<td>Cochichewick</td>
<td>488.67 ± 26.91</td>
<td>23.7 ± 0.10</td>
</tr>
<tr>
<td>Nippo</td>
<td>297.00</td>
<td>12.50</td>
</tr>
<tr>
<td>Old Durham Reservoir</td>
<td>786.33 ± 11.92</td>
<td>32.57 ± 0.77</td>
</tr>
<tr>
<td>Willand</td>
<td>237.33 ± 1.33</td>
<td>7.13 ± 0.39</td>
</tr>
<tr>
<td>York</td>
<td>494.04 ± 7.98</td>
<td>27.33 ± 0.33</td>
</tr>
</tbody>
</table>
Table 5. Climate factors measured during the day and night throughout aerosol collections. Averages for air and water temperatures, temperature differential, and relative humidity are from every 10 min during the day or night sampling. Wind measurements were taken at the start and finish of day and night sampling. Standard error is ±1.

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Lake</th>
<th>Air Temp (°C)</th>
<th>Water Temp (°C)</th>
<th>Temp Differential (°C)</th>
<th>Relative Humidity (%)</th>
<th>Wind (m s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td>Attitash</td>
<td>64.71 ± 0.49</td>
<td>64.54 ± 0.16</td>
<td>0.17 ± 0.46</td>
<td>55.52 ± 2.20</td>
<td>0.44 (0.87, 0.00)</td>
</tr>
<tr>
<td></td>
<td>Baboosic</td>
<td>64.31 ± 0.34</td>
<td>55.76 ± 0.13</td>
<td>-1.45 ± 0.33</td>
<td>84.91 ± 1.00</td>
<td>0.33 (0.67, 0.00)</td>
</tr>
<tr>
<td></td>
<td>Christine</td>
<td>57.79 ± 0.45</td>
<td>59.52 ± 0.40</td>
<td>-1.73 ± 0.26</td>
<td>70.34 ± 1.42</td>
<td>1.4 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Cochichewick</td>
<td>54.51 ± 0.40</td>
<td>42.02 ± 0.19</td>
<td>9.49 ± 0.32</td>
<td>65.31 ± 2.16</td>
<td>0.70 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>Nippo</td>
<td>57.64 ± 0.30</td>
<td>63.88 ± 0.15</td>
<td>-6.24 ± 0.26</td>
<td>63.89 ± 0.86</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Old Durham Resevoir</td>
<td>53.51 ± 0.83</td>
<td>43.36 ± 0.29</td>
<td>10.15 ± 0.65</td>
<td>67.89 ± 2.11</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Willand</td>
<td>33.65 ± 0.86</td>
<td>35.46 ± 0.06</td>
<td>-1.81 ± 0.81</td>
<td>45.32 ± 0.92</td>
<td>0.60 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>York</td>
<td>43.78 ± 0.43</td>
<td>52.42 ± 0.24</td>
<td>-8.64 ± 0.30</td>
<td>51.81 ± 1.15</td>
<td>0.49 ± 0.19</td>
</tr>
<tr>
<td><strong>Night</strong></td>
<td>Attitash</td>
<td>50.14 ± 0.29</td>
<td>61.65 ± 0.05</td>
<td>-11.51 ± 0.25</td>
<td>88.72 ± 0.57</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Baboosic</td>
<td>50.62 ± 0.37</td>
<td>63.50 ± 0.08</td>
<td>-12.88 ± 0.29</td>
<td>95.55 ± 0.21</td>
<td>0.09 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Christine</td>
<td>43.75 ± 0.21</td>
<td>54.35 ± 0.13</td>
<td>-11.2 ± 0.10</td>
<td>89.71 ± 0.23</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Cochichewick</td>
<td>37.82 ± 0.65</td>
<td>45.20 ± 0.09</td>
<td>-7.38 ± 0.57</td>
<td>89.95 ± 1.33</td>
<td>0.63 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>Nippo</td>
<td>42.32 ± 0.45</td>
<td>60.27 ± 0.16</td>
<td>-17.95 ± 0.30</td>
<td>90.58 ± 0.46</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Old Durham Resevoir</td>
<td>41.91 ± 0.22</td>
<td>42.86 ± 0.09</td>
<td>-0.95 ± 0.17</td>
<td>87.02 ± 0.52</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Willand</td>
<td>14.21 ± 0.62</td>
<td>34.47 ± 0.06</td>
<td>-20.26 ± 0.59</td>
<td>84.94 ± 1.86</td>
<td>1.11 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>York</td>
<td>25.56 ± 0.36</td>
<td>48.49 ± 0.12</td>
<td>-22.93 ± 0.25</td>
<td>93.08 ± 0.16</td>
<td>0.13 (0.26, 0.00)</td>
</tr>
</tbody>
</table>
Figure 18. Temperature differential between air and surface water. Measurements of water and air temperature were taken every ten minutes throughout the duration of day and night sampling at each lake location. Averages across the full eight-hour aerosol collection times are presented here. Error bars are ±1 standard error. Significance groupings for the day are represented by A-E, and groupings for the night are u-z (p < 0.05). All lakes have significant differences between day and night temperature differentials, indicated by asterisks.

Diel Drivers of Aerosolized Toxicity and Time of Day Differences

When day and night values of aerosolized toxicity are combined, the <50 µm fraction of MCs best predicts aerosolized MCs caught on the GFF filters (p = 0.006, Adj R²: 0.39, Figure 19). The WLW MCs model was almost as strong of a predictor as the <50 µm MCs. It is helpful to consider this relationship with both day and night values as it reduces the influence of single lakes that are most likely due to a lake effect, a consequence of not having lakes with mid-range MCs toxicity. Despite this there are some potential influence issues with this relationship. Aerosolized MCs from York Pond during the day has one replicate that was much higher than the other two replicates (seen in the large standard error). If this high replicate is removed, the average of the remaining values drops the value directly onto the regression line shown in Figure
19. York Pond day and Lake Attitash day are outliers, each based on one of the three outlier assessments (York studentized residual 2.42, and Attitash hat value 0.68 (cut off 0.25)).

![Graph showing relationship between microcystins in the <50 µm fraction of lake water compared to aerosolized microcystins collected on the GFF fine filters.](image)

**Figure 19.** Relationship between microcystins in the <50 µm fraction of lake water compared to aerosolized microcystins collected on the GFF fine filters. The MCs collected on air filters increases linearly with level of microcystins in the <50 µm fraction of water. Day values are indicated by circles, night values with triangles. Error bars are ±1 standard error. Refer to Figure 17 for color key. Equation: Aerosolized MCs (pg MCs m⁻³) = 1.38 (± 0.26) + 0.07 (± 0.02) * <50 µm MCs (ng L⁻¹).

**Drivers of Aerosolized Cyanobacteria Cells and Time of Day Differences**

The total aerosolized cells during the day are best predicted by the differential between air and water temperature (p = 0.0278, Adj R²: 0.511, Figure 20). As the air temperature becomes warmer than the water temperature, aerosolization of total cells increases. At night the total aerosolized cells are best explained in a two-parameter model including the MCs toxicity in the WLW and the air and water temperature differential (Figure 21). This relationship is influenced by Lake Attitash and ODR, as these lakes had the greatest variability in total aerosolized cyanobacteria cells compared to the other lakes (Attitash Cook’s D: 2.68, hat value: 0.94 (cut off 0.75); ODR Cook’s D value: 1.29). If these influential lakes are removed from the
model, the combination of MCs in WLW and the temperature differential no longer significantly predict the total aerosolized cells. It is only in combination that these two parameters can predict total aerosolized cells. When considered separately, neither the WLW MCs nor the air and water temperature differential significantly predict the aerosolization of total cyanobacteria cells (WLW p = 0.08, temperature differential p = 0.17).

Figure 20. Total aerosolized cyanobacteria cells during the day predicted by the temperature differential between air and water. As the air becomes increasingly warmer than the water, there are increasingly more aerosolized cyanobacteria. Error bars are ± 1 standard error. Refer to Figure 17 for color key. Equation: Total Aerosolized Cyanobacteria (cells m⁻³) = 46472.76 (± 4133.26) + 1900.57 (± 658.62) * Air-Water Temperature Differential (C).
During the day, none of the measured variables explained the aerosolization of picocyanobacteria cells. At night, microcystins in the <50 µm fraction and the temperature differential can be used to predict aerosolized picocyanobacteria in a two-parameter model (Figure 22). Lake Attitash was flagged as an outlier (Cook’s D: 12.63, studentized residuals: 2.05), however removing it had little effect on the p-value, slope or R² of the relationship (p = 0.001, Adj R²: 0.95 without Attitash). When the two parameters are evaluated separately, the <50 µm MCs significantly predict aerosolized picocyanobacteria (p = 0.003 Adj R²: 0.76), but the temperature differential does not (p = 0.57).
Figure 22. Predicted aerosolized picocyanobacteria vs. actual aerosolized picocyanobacteria. Model includes the microcystins in the <50 µm fraction (effect p = 0.00001), and the temperature differential between air and water (effect p = 0.0005). Lake Attitash (purple) extends the scope of this relationship, however the model does not lose predictive power if it is removed. Red dotted lines are confidence interval (99%). Refer to Figure 17 for color key. Predicted aerosolized picocyanobacteria (cells m$^{-3}$) = 18810.57 (± 5750.02) + 7273.74 (± 419.78) * <50 µm MCs (ng L$^{-1}$) + 2763.80 (± 351.51) * Air-Water Temperature Differential (°C).

During the day when looking at drivers of larger cells, a single parameter model using nitrogen has the lowest AICc (p = 0.03, Adj R$^2$: 0.50, Figure 23). This relationship, however, is completely dependent on ODR (Cook’s D: 4.37, studentized residual: 2.34), and when it is removed nitrogen no longer predicts aerosolized larger cells. The next best model for predicting aerosolized larger cells during the day is the temperature differential between the air and water (p = 0.05, Adj R$^2$: 0.41, Figure 23), however this relationship is also entirely driven by ODR (Cook’s D: 2.37, studentized residual: 2.40), and when ODR is removed the relationship is not maintained.
Figure 23. Nitrogen (left) and temperature differential (right) as predictors for daytime aerosolized large cyanobacteria cells. Nitrogen is the best model according to the AICc however it is largely driven by Old Durham Reservoir (yellow), and there is no relationship when this lake is removed. The temperature differential is the second-best model based on the AICc, however it too is largely driven by Old Durham Reservoir, and again when the lake is removed there is no longer a significant relationship. Error bars are ±1 standard error. Nippo Pond does not have error bars for nitrogen because it is only one value. Refer to Figure 17 for color key.

Similar patterns are seen in drivers of aerosolized large cyanobacteria cells during the night, but they account for more variability than during the day and are not dependent on one lake. The best parameter to predict night aerosolized large cyanobacteria cells is nitrogen ($p = 0.003$, Adj $R^2$: 0.75, Figure 24). Christine Lake and ODR are identified as an influential data points (Christine Cook’s D: 1.37 and studentized residual: 2.23; ODR Cook’s D: 2.71), however, nitrogen becomes an even stronger predictor of aerosolized larger cells when they’re removed ($p < 0.0001$, Adj $R^2$: 0.99). If water quality is not considered in the step wise regression, the temperature differential is the best predictor of aerosolized large cells at night ($p = 0.03$, Adj $R^2$: 0.52, Figure 24). ODR and York Pond are influential in this relationship (ODR Cook’s D: 2.18, studentized residual: 1.93; York Cook’s D: 1.26, studentized residual: 1.97), but the model improves if they are removed.
Figure 24. At night, large aerosolized cyanobacteria cells are best predicted by nitrogen (left). The temperature differential is the next best predictor (right). Both relationships remain significant when Old Durham Reservoir (yellow) is removed. Refer to Figure 17 for color key. Equations: Larger Aerosolized Cyanobacteria (cells m$^{-3}$) = $-14628.65 \pm 11602.94 + 117.96 \pm 25.09$ * Nitrogen (µg L$^{-1}$). Larger Aerosolized Cyanobacteria (cells m$^{-3}$) = $72197.48 \pm 13951.19 + 2796.32 \pm 946.62$ * Air-Water Temperature Differential (C).

Water Toxicity and Aerosolized Toxicity

The propensity of a lake to produce toxic aerosols could be defined as the relationship between total MCs toxicity in lake water and the potential for it to become aerosolized. Surprisingly, as the toxicity in a lake increases, the percent of total toxin that becomes aerosolized decreases, i.e. lakes with less total toxicity have a higher percent of total toxicity entering the air (power function linear regression, $p < 0.0001$, Adj $R^2$: 0.85, Figure 25). For example, in this study, the most oligotrophic lake with the lowest MCs concentration in the water (Christine Lake) had roughly 15 times more of its total water toxicity aerosolized than the most eutrophic lake with the highest MCs concentration in the water (Lake Attitash).
Figure 25. Percent of aerosolized microcystins out of total microcystins, explained by whole lake water microcystins. Both day (circle) and night (triangle) values are shown. X and Y axes are log10 transformed. Lakes with higher toxicity have a smaller percent of their total toxicity entering the air, while lakes with lower toxicity in their water have a higher percent of their total MCs toxicity entering the air. Refer to Figure 17 for color key. Equations: log10 Aerosolized MCs of Water MCs (%) = -3.87 (± 0.11) + -0.86 (± 0.09) * log10 WLW MCs (ng L⁻¹).
DISCUSSION

Aerosolized Microcystins

This is the first study to document the presence of microcystins in aerosols generated from low-toxin lakes. Measured aerosolized toxin levels varied from below detection to 3.79 pg MCs m$^{-3}$ from lakes with a range of 4 – 90 ng MCs L$^{-1}$ in lake water. While this water toxicity is representative of New England lakes (Haney and Ikawa 2000), it is low compared to levels found globally (Chorus and Bartram 1999; Chorus 2001). Past studies performed in New Zealand, California and the Midwest U.S. evaluated cyanobacteria aerosols from eutrophic lakes with levels of water MCs ranging from 1 to 700 µg L$^{-1}$. Despite this large difference in water toxicity and methodology, the aerosolized levels I found were very similar to the range of 0.2-16.2 pg MCs m$^{-3}$ found for eutrophic lakes in New Zealand (Wood and Dietrich 2011). Previous levels of aerosolized MCs in New Hampshire, ranging from 13 to 384 pg m$^{-3}$ are comparable though somewhat higher than what I found (Murby and Haney 2015). In highly eutrophic lakes in Michigan and California, levels of aerosolized MCs were also higher and ranged from 23 pg m$^{-3}$ to 2,890 pg MCs m$^{-3}$ (Backer et al. 2010; Backer et al. 2008; Cheng et al. 2007).

Aerosolized Cyanobacteria Cells

Depending on the ecosystem type, total bacterial concentrations in the atmosphere are reported to range from $1 \times 10^4$ to $6 \times 10^6$ cells m$^{-3}$ (Dueker, O’Mullan, Weathers, et al. 2012). Total aerosolized cyanobacteria in the present study ranged from $3.5 \times 10^4$ to $1.9 \times 10^5$ cells m$^{-3}$, comparable to concentrations found by Murby and Haney (2015; $8 \times 10^3$ to $1.6 \times 10^5$ cells m$^{-3}$ in the field, and $2.3 \times 10^4$ to $3.6 \times 10^5$ cells m$^{-3}$ in the laboratory) using very similar collection and enumeration techniques to those used in this study. The slightly higher levels I documented in the field compared to Murby and Haney were likely the result of using GFF filters that allowed
for higher volume sampling; the hay-stack nature of the glass fiber filters catches cells on multiple levels, preventing saturation and clogging of filter pores. My study employed epifluorescence to directly observe aerosolized cells from depths within the filter, allowing me to quantify picocyanobacteria. Epifluorescence is one of the few specific microscopy techniques that allows for the enumeration of cells in the picocyanobacteria range, and uses specific wavelengths to isolate the autofluorescence of only cyanobacteria (Callieri and Stockner 2002), a technique essential for estimating total aerosolized cyanobacterial load.

Total aerosol cell counts were divided into picocyanobacteria cells and larger cells. The picocyanobacteria category encompasses only the smallest cyanobacteria cells and comprises a narrower range of cell sizes (0.22 – 2 µm) compared to the larger cell category (>2 – 40 µm). Despite this, picocyanobacteria were generally equal to or more numerous than larger aerosolized cells (picocyanobacteria were more abundant in 10 out of the 16 collection periods, Figure 13). Similarly, picocyanobacteria were also the dominant biotic material in Baltic Sea aerosols collected over both land and sea (Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017).

In the present study it was rare to find cells larger than 10 µm, and no full colonies were found in aerosols. There was occasional evidence of microcolonies consisting of up to five picocyanobacteria cells, although it was not possible to distinguish these potential colonies from cells that clumped on the filter by chance. In lakes, microcolonies comprised of 5 to 50 pico-sized cells are considered transitional forms between single celled and colonial morphotypes, and may be a response to a change in nutrients or zooplankton grazing (Callieri 2010). Lewandowska et al. (2017) found Baltic Sea aerosols contained picocyanobacteria-sized single cells of *Synechococcus, Synechocystis, Aphanocapse, Aphanothece, Microcystis, Merismopedia,*
Woronichinia and Cyanodictyon. Many of these genera commonly occur as colonies, suggesting that larger aerosolized cells were also likely derived from colonial forms in the water. Cells can be released as colonies break apart from colonies as colonies age and disintegrate, or are broken apart by zooplankton grazing (Porter 2017; Deason 1980). In a large body water such as the Baltic Sea, colonies may also be disrupted by wave action and water turbulence, though this is less likely in the small lakes in the current study.

Dominance of picocyanobacteria in lake aerosols is most likely related to their relatively high abundance in lakes. Surface layers of small lakes can have pico-cell concentrations ranging from a few hundred to a few hundred thousand (Jakubowska and Szeląg-Wasielewska 2015; Szeląg-Wasielewska 2013) or several million cells per mL of water (Ning 2000). Both live and dead bacteria cells accumulate in the surface microlayer (defined as the top 200-400 µm of surface water) of marine waters (Aller et al. 2005). This accumulation of bacteria material greatly influences the local aerosol environment (Aller et al. 2005), since the surface microlayer is the interface with the atmosphere. Picocyanobacteria are ubiquitous in all lakes, but are especially important and dominant in low nutrient oligotrophic systems (Callieri 2007). Many of the lakes in my study were low in nutrients as well as MCs, indicating the higher likelihood of being dominated by picocyanobacteria.

Another possible explanation for the dominance of picocyanobacteria in aerosols is the ease in which they can enter the air (Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017). One might expect that a relatively low energy input is required for small cells to be picked up by wind, freed from the surface as a result of micro-bubble bursting, or through evaporation from the lake surface, while larger cells or colonies would require much more energy to become airborne by any of these processes. In addition to the higher likelihood of entering the air, it is
possible for picocyanobacteria to be independently mobile (Brahamsha 1999). Despite not having flagella, the rapid movement of some *Synechococcus* species has been documented (Brahamsha 1999), this phenomenon may assist in cells crossing the air-water boundary.

This study demonstrated the importance of picocyanobacteria in aerosols. Though the MCs production within picocyanobacteria is not well documented (Jakubowska and Szeląg-Wasielewska 2015), it has been suggested that some species produce MCs (Blaha and Marsalek 1999). Cell size can also potentially inform us about persistence in aerosols, and travel distances. While larger cells have the capacity to carry a higher toxin load per cell into the atmosphere than picocyanobacteria, exposure to picocyanobacteria is probably more likely. Larger bacterial cells do not persist as long in aerosols compared to smaller cells because of gravitational settling (Dueker, O’Mullan, Weathers, et al. 2012; Dueker et al. 2011). The small size probably allows picocyanobacteria to persist longer in aerosols, permitting them to travel farther, which along with the abundance of picocyanobacteria in aerosols increases the likelihood of exposure to picocyanobacteria over larger cells.

**Environmental Drivers of Cyanobacteria Aerosolization**

Overall, cell counts rather than toxins may provide better insights into the mechanisms behind aerosolization. In this study cell counts enabled me to explain more variation and with more confidence than aerosolized MCs. This may be due to the variability in the amount of toxin per aerosolized cell. Not only is there variability in the toxicity per unit biomass between different species of cyanobacteria (Liyanage et al. 2016), but the level of toxin production within a cell can vary based on the physiological state of the cell (age, metabolic activity), and many environmental factors including light and nutrients (Deblois and Juneau 2010). Though we may ultimately care about the consequences associated with exposure to aerosolized cyanotoxins,
focusing on the movement of cyanobacteria cells into the aerial environment may help us develop a clearer understanding of the phenomenon by eliminating the variability in toxicity per cell.

*Water Microcystins Toxicity and Cyanobacteria Community Composition*

The level of MCs in the water was an important predictor of both aerosolized MCs and aerosolized cells. Though I attempted to include other measures of cyanobacteria presence in the lake water through fluorescence using a handheld fluorometer, pigment levels in the size fractions were often near or below the limit of detection. Therefore, in this study MCs were the only indicator of cyanobacteria in the lake water. Determining the MCs in different size fractions of water allowed me to evaluate which fraction of water, and thus what sized MC-producing cyanobacteria most directly interact with the atmosphere.

When day and night aerosolized MCs were combined, the <50 µm fraction of MCs in lake water was the best single environmental predictor of aerosolized MCs (Adj R²: 0.39, Figure 19). Despite potential variability in the cell quota of toxins, the WLW toxicity was the most important MCs variable for total cells (Figure 21), while the <50 µm fraction was the most important fraction for picocyanobacteria in the air (Figure 22). Together, these findings suggest that while the majority of aerosolized cells were picocyanobacteria, larger cells greater than 2 µm were important contributors to aerosolized toxicity and cells. This is also consistent with the hypothesis that colonies with higher levels of toxin per cell may be contributing single cells into the air, potentially following colony disintegration. The net plankton was never a significant predictor of aerosolized material.

The water fractions reveal that even though some lakes may have more microcystins than others, if the toxin is packaged within larger colonial forms of cyanobacteria, it is less likely to
enter the air. The MCs in eutrophic lakes are mostly present in the >50 µm fraction (Figure 10, see Attitash and York). The toxicity in this fraction is produced by large colonial forms of cyanobacteria. Colonial cyanobacteria have higher MCs content (Leland and Haney 2018), longer lifespans (Reynolds, Oliver, and Walsby 1987) and are not as grazable by zooplankton (Lampert 1987). The consequences of these life history traits are that bloom-forming cyanobacteria accumulate and retain their toxins for longer periods of time, decreasing the mobility of toxins both within and out of the lake ecosystem. By contrast, oligotrophic lakes primarily have MCs in the dissolved fraction (Figure 9). This fraction of toxin is likely generated by picocyanobacteria that have high turnover rates, regularly shifting MCs into the dissolved fraction, possibly via grazer induced damage or through excretion by zooplankton.

The largest variability in toxicity among the sampled lakes was in the larger fractions, while the level of toxicity in the <2 µm and dissolved fractions was consistent across lakes (Figure 8, Figure 9). This presents an interesting dichotomy: Lakes with the lowest overall toxicity have the highest proportion of total toxicity in the dissolved fraction. Within my study lakes, Lake Attitash has nine times the MCs toxicity in the large net plankton cyanobacteria compared to all other sampled lakes (Figure 10). Despite this, the aerosolized MCs emitted from Lake Attitash did not differ significantly from the levels aerosolized from lakes with much lower toxicity in the water (Figure 12). These data support the assertion that the community composition of cyanobacteria in lakes has a large influence on aerosolized material because not all cyanobacteria are as likely to interact with the aerosol environment.

A higher percentage of total toxin is transferred into the air from relatively clean lakes, than from lakes with high toxin (Figure 25). This does not mean that lower toxin lakes are producing more toxic aerosols but demonstrates the importance of how the MCs are “packaged”
within cells. For example, based on my study lakes, around 30x more of the toxin from a lake with 1 ng MCs L\(^{-1}\) will become aerosolized compared to a lake with 50 ng MCs L\(^{-1}\). Assuming this relationship is maintained for higher toxin lakes, a lake with 300 ng L\(^{-1}\) in the WLW would produce around 300 pg MCs m\(^{-3}\) in the air. The eutrophic lakes evaluated in previous studies (Wood and Dietrich 2011; Backer et al. 2010; Backer et al. 2008; Cheng et al. 2007; Murby and Haney 2015) measuring water MCs in the microgram per liter range (in comparison to the nanogram per liter range I measured) likely had high abundances of large, bloom-forming cyanobacteria.

**Air and Water Temperature Differential as an Important Environmental Driver**

In addition to water toxicity, the temperature differential between air and water appears to be the most important environmental influence on the aerosolization of cyanobacteria cells. In combination with water toxicity at night, the temperature differential helps explain the total and pico-sized aerosolized cells very well (total Adj R\(^2\): 0.92, pico Adj R\(^2\): 0.98, both two-parameter models including water MCs). As the air above lakes becomes cooler than the water (Figure 18), cyanobacteria are less likely to be aerosolized. Fog may explain part of this temperature differential effect. When a cold and dry air front moves over a warm waterbody, water molecules evaporate from the water surface into the colder air (Willett 1929). High humidity water vapor condenses in the cold air, producing fog (Willett 1929). It is common to see this formation during summer and early fall when the water temperature in lakes is warm, but the air temperature fluctuates to lower temperatures faster than the body of water. Although I noted high fog conditions during night sampling when there was a large temperature differential, I did not attempt to quantify its presence.
While there was an increase in humidity at night across all sampling dates, humidity alone was not significantly correlated with either aerosolized toxins or cells. High humidity is an indicator of high water-vapor conditions, but the formation of fog is most dependent on the air temperature being colder than the water. While humidity may indicate when fog is present (as air and water temperature differential gets more negative, humidity increases $p = 0.0261$, Adj $R^2$: 0.26), high humidity can occur independently from fog. Fog has been shown to have a distinct effect on microbial aerosols by altering composition, deposition rates, and viability of biotic material (Dueker, O’Mullan, Weathers, et al. 2012; Dueker et al. 2011; Evans et al. 2019). Fog increases particle size in aerosols, increasing the relative abundance of coarse aerosol droplets with a diameter $>2$ µm, increasing gravitational settling rates and decreasing transportation distances (Dueker, O’Mullan, Weathers, et al. 2012; Dueker et al. 2011; Evans et al. 2019; Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017).

**Different Drivers of Aerosolization Based on Cell Size**

The temperature differential between air and water is also important for the aerosolization of larger cells (Figure 23 and Figure 24). However, if water quality parameters (nitrogen and phosphorus) are included in the stepwise regressions, larger aerosolized cyanobacteria cells are best predicted by nitrogen levels in the water. Though there was a continued importance of temperature differential for the aerosolization of both large and picocyanobacteria, some drivers appear cell-size dependent. Picocyanobacteria are particularly adapted to thriving in low nutrient conditions (Callieri 2007), while larger forms of cyanobacteria proliferate in water bodies that have more nutrients (Paerl, Otten, and Kudela 2018). Thus, nitrogen may indicate conditions are optimal for larger cyanobacteria, resulting in greater probability of large cells in the air.
Time of Day Differences in Aerosolization and Potential Mechanisms

Smith (1973) examined time of day differences in aerosols, and found peaks in algae and protozoa abundance in aerosols collected on top of buildings between the hours of 2 and 6 PM, 8 PM and 4 AM, and 8 AM and noon. I found day-night differences in aerosol production but no consistent pattern, levels of aerosolization appear to be more source dependent, and vary lake by lake. With the exception of Willand and Nippo Ponds, there were greater cell emissions at night compared to the day (Figure 14). Though aerosolized levels of cyanobacteria are not statistically higher at night across all lakes, I was able to predict the variation in aerosolized material with greater certainty for night aerosols.

During the day, total aerosolized cells were best explained (51% of the variability) by the temperature difference between air and water (Figure 20). At night, the total aerosolized cells were predicted by the MCs in the WLW and the air and water temperature differential (Adj $R^2$: 0.92, Figure 21). Variations in daytime aerosolization of picocyanobacteria were not explained by any of the measured parameters. At night, by contrast, 92% of the variability in aerosolized picocyanobacteria can be predicted with a two-parameter model including MCs in the <50 µm fraction as well as the air and water temperature differential (Figure 22). Aerosolization of larger cells is explained best by nitrogen regardless of time of day, however at night nitrogen accounts for 15% more variability (day Adj $R^2$: 0.50, Figure 23; night Adj $R^2$: 0.75, Figure 24).

Time of day differences may also help explain the relationship between aerosolized cells and aerosolized MCs. Interestingly, these two parameters were not always correlated. There was no correlation between total aerosolized cells and aerosolized MCs during the day, but there was at night ($p = 0.008$, Adj $R^2$: 0.67; Figure 17). Similarly, daytime cyanobacteria aerosol studies performed on New Hampshire lakes did not find a correlation between aerosolized cells and
aerosolized MCs (Murby and Haney 2015). There may be different mechanisms behind the process of aerosolization at night that are not accounted for during the day.

Perhaps the simplest explanation for time of day differences are the typically calmer environmental conditions at night. Wind action over lakes is often solar driven, with higher wind speeds and turbulent surface water conditions during the day. When day and night values were combined, both total aerosolized cells and larger cells had negative linear correlations with increasing wind speed (both parameters log10 transformed, total cells p = 0.033, Adj R²: 0.235; larger cells p = 0.015, Adj R²: 0.306). Other studies have found that wind speeds increase aerosolization of biotic material, but the direction of wind also affects this relationship (Dueker, O’Mullan, Weathers, et al. 2012; K. Sharma, K. Rai, and Singh 2006; Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017; Smith 1973). My results may contradict other studies because of methodological differences. In my case, increased wind speeds and air turbulence during the day (Table 4) may dilute the air coming directly from the lake reducing the effective cell concentrations sampled by the CLAMs. Thus, wind would tend to both reduce concentrations of lake-generated toxins and cells, in addition to adding variability to the aerosol estimates. This would be reflected in greater variability in daytime data and poorer relationships with environmental variables. With less lateral movement of air at night, the aerosolized cells would be more consistently collected by the CLAMs, providing clearer regression relationships. Wind was recorded in this study only at the beginning and end of sampling and may not fully represent the wind conditions over the entire 8 h collection. Future studies should record wind speed continuously throughout aerosol sampling.

In addition to these diel wind effects, there are also diel cycles in growth and metabolic functions of cyanobacteria (Welkie et al. 2019). A study on the day to night changes in
picocyanobacteria division and growth revealed the highest percentage of dead cells occurred at the beginning of night, and the smallest percentage of dead cells in the morning (Llabrés, Agustí, and Herndl 2011). The percent dead cells increase throughout the day as picocyanobacteria succumb to environmental stressors including ultraviolet radiation, oxidative stress, and nutrient deprivation (Llabrés, Agustí, and Herndl 2011). Freshly divided cells and dead cells likely have different features that may increase or decrease the likelihood of aerosolization and could contribute to the observed differences in day to night aerosolized cyanobacteria.

Though the numbers of live cells may build during the night, I did not see a consistent increase of toxin in the water at night across lakes. Recent work has shown cyanobacteria primarily synthesize toxin at night (Davenport 2016), so the highest toxin levels possibly had not built-up until sunrise. My study reveals differences in total water toxicity based on time of day, however the methodology of mixing start- and stop- time water samples could have muted a diurnal signal. In higher toxin lakes, the level of MCs appears to decrease at night, while the opposite is true for low toxin lakes that tend to have higher MCs levels at night (Figure 6). These differences could be caused by varying behavior of the dominant forms of cyanobacteria. Higher toxin lakes are dominated by larger colonial forms of cyanobacteria that may move lower in the water column at night (Richardson and Castenholz 1987; Reynolds, Oliver, and Walsby 1987). Lower toxin lakes are dominated by picocyanobacteria that are highly grazed on at night (Jakubowska and Szelag-Wasielewska 2015; Llabrés, Agustí, and Herndl 2011), releasing toxins or altering cells in ways that could increase their aerosolization.

Zooplankton grazing on cyanobacteria could be an important additional factor behind aerosolization. Grazing rates are typically higher at night when zooplankton migrate to the surface of the lake to feed at elevated rates to minimize fish predation (Jeppesen et al. 1997;
Haney and Hall 1975). Copepods, cladocerans and rotifers are all known to graze on cyanobacteria, including picocyanobacteria (Motwani and Gorokhova 2013; Wilson and Steinberg 2010). Some of these zooplankton are known to be sloppy feeders (Lampert W. 1978), breaking apart colonies and fragmenting individual cells as they graze. This process could be changing the surface chemistry of cells or releasing toxins. The effects of grazing are likely complicated and depend on the composition of phytoplankton and zooplankton. Despite this, one result may be an increase in dissolved toxins and cells that are more likely to become aerosolized. This avenue is worthy of further investigation.

**Evaluation of Aerosol Collection and Analysis**

The lower water toxicity in the present study coupled with low though comparable aerosolization levels indicate the importance of detecting low concentrations of microcystins in both water and aerosols. The observed concentrations of MCs were detectable because of newly developed techniques that enabled me to observe the presence of toxins that would be below the limit of detection for most previous studies. This finer resolution provided me with the opportunity for more detailed exploration into the factors controlling aerosolization.

Using GFF/Fine filters, combusted to reduce the effective pore size to around 0.3 µm (Nayar and Chou 2003), was important for detection of low toxin levels. The two advantages of using this filter are first, the small pore size allows for the collection of picocyanobacteria that are not caught on filters with larger pore size and secondly, because of the nature of the fibrous filter, aerosolized cyanobacteria material becomes embedded throughout the filter. This minimizes filter “clogging”, allowing for larger air volume collection. Another advantage is the placement of air intake for the CLAM directly above the lake surface, providing a more direct estimate of the rate of cyanobacteria aerosolization, minimizing the effect of wind. This
technique enabled me to focus on the drivers and the process of lake aerosolization itself rather than on human exposure to the toxins.

Also, steps taken during the toxin extraction process increased sensitivity to MCs detection. The use of small (25 mm diam) filters allowed for MCs extraction from the entire filter with a small volume (1 mL) of water. Freeze-thaw-vortex-sonicate cycles in triplicate helped remove cells from the filter and break cells apart to free MCs for detection in the ELISA. Another important step that enhances the detection of MCs is the concentration of extracted aerosol material using a SpeedVac. Samples were concentrated approximately 9x via speed vacuuming, increasing detection to very low levels. The high sensitivity ELISA kit (limit of detection 100 ng L\(^{-1}\), EnviroLogix Inc, Portland ME) also increased low-level toxin detection. The concentration technique was also used to estimate MCs in water fractions, resulting in a better understanding of the relationship between water and aerosolized MCs.

There are many advantages to quantifying aerosolized cyanobacteria using the adapted epifluorescence microscopy technique developed by Murby and Haney (2015), such as easier and more accurate enumeration of cells on multiple levels of the glass fiber matrix inherent to GFF filters. At each random location on the filter, 68 images were taken to capture cells at multiple depths. The combined single image per location allows for representation at all depths, while simultaneously preventing counting the same cell multiple times at different levels (a phenomenon caused by strong fluorescence). The CellSens software program allowed me to count cells and easily classify them based on selected size categories. Enumerating the aerosolized cyanobacteria cells gives me an estimate of cyanobacteria aerosolization independent from the multitude of factors that influence toxin production.
In addition to the advantages to using epifluorescence there are also disadvantages, such as the time required for cell counts. Despite automation that allowed for greater examination of total filter area it is still a very time intensive technique. Other challenges of this technique that make total automation difficult include light rings around fluorescing cells, clumping of cells, and dilution of fluorescence from larger non-biological particles.

For efficiency in aerosol collection, I used each aerosol filter first for epifluorescence microscopy, followed by MCs extraction. Although MCs may be subject to photodegradation (Liyanage et al. 2016) it is not known whether there was any measurable degradation of MCs during the brief exposure to specific wavelengths during epifluorescence. To avoid this uncertainty, when possible, it would be best to use separate filters for toxin extraction and epifluorescence. Another unknown is how aerosolized dissolved microcystins from lakes interact with filters. While it is presumed that some of this toxin is retained on the filters due to their matrix of glass fibers, it is also possible that some free MCs may pass through the filter. This could also help explain why aerosolized cells and MCs were not correlated during the day. Dissolved toxin may vary by lake and season (Bláhová et al. 2007; Zheng et al. 2004; Aboal and Puig 2005). It is conceivable that environmental conditions differ from the movement of dissolved MCs and the propulsion of cells into the air. Unfortunately, in this study I was not able to distinguish between dissolved toxin and cell-bound toxins on the filters, and the efficiency of dissolved MCs collection on filters is not yet resolved.

**Consequences for Human Health**

Acute exposure to microcystins primarily results in intrahepatic bleeding, hemorrhagic shock and liver failure (Liyanage et al. 2016). While the liver is considered the primary target organ for MCs, several studies suggest the respiratory system could be an additional target.
Intraperitoneal injections in mice result in alveolar collapse and inflammatory response (Soares et al. 2007). Lung tissue may be susceptible to MCs toxicity because similar to the liver, lung tissue also has OATPs transporters (Roth, Obaidat, and Hagenbuch 2012). This effect on lung tissues is not only a result of acute exposure, but is seen at sub-chronic (Carvalho et al. 2016) and chronic low-dose exposures to MCs (Wang et al. 2016; Oliveira et al. 2015). Alveolar collapse and lung cell apoptosis can negatively impact the process of gas exchange in the lung, resulting in compounding issues in the respiratory system and systematically (Wang et al. 2016). MC-LR travels to the lungs in the blood following oral exposure (Wang et al. 2016). Studies have also suggested damaged livers are capable of releasing inflammatory mediators that can result in secondary injuries in the lung (Massey et al. 2018). To the best of my knowledge, no studies to date have directly addressed the effects of combined oral and inhalation exposures to MCs. If lung function is reduced from ingested MCs, the effect of MCs entering via the respiratory system may greater, or vice versa. Another unknown are the possible synergistic effects of multiple toxins in the body.

The deposition location of cyanobacteria cells or the dissolved form of MCs within the lungs likely influences this interaction and highlights the importance of evaluating aerosolized cyanobacteria cells in addition to aerosolized toxins. Cells 2 µm and smaller can travel deep into the lungs, whereas larger cells are more likely to be caught in the upper respiratory tract (Lewandowska, Śliwińska-Wilczewska, and Woźniczka 2017; Hussain, Madl P, and Khan 2011). Cells and dissolved toxins that reach the alveoli have a more direct interface with the blood stream. This increases the chance of exerting a toxic effect. Facciponte et al. (2018) recently documented the presence of cyanobacteria cells in nasal cavities as well as in lung tissue using epifluorescence. Cells deposited in lungs already suffering from the toxic effect induced by
ingested MCs may cause enhanced exposure. The average cell diameter found in the lungs (via bronchoalveolar lavage) was 3.17 µm (Facciponte et al. 2018). This confirms the ability of picocyanobacteria cells to travel into lung tissue, signifying the importance of understanding the cell sizes of aerosolized cyanobacteria material.

Wood and Dietrich (2011) adjusted the WHO drinking water guidelines for the increased toxicity of inhalation vs oral ingestion. On the assumption that an average adult inhales 30.2 L min\(^{-1}\) over 24 h, Wood and Dietrich (2011) suggested that the concentration of aerosolized MCs should not exceed 4.58 ng MC-LR m\(^{-3}\). My observed levels of aerosolized MCs (average of 2 pg m\(^{-3}\)) are around 2000x lower than the calculated daily allowable limit via inhalation. There are several considerations important for evaluating the significance of the observed aerosol concentrations. First, the suggested concentration limit is based on the average ventilation rates of adults. Any variation due to relative amounts of exercise, gender, age, or illness will affect inhalation rates (Allan and Richardson 1998), thus changing the acceptable aerosolized concentrations. Second, it is also probable that inhalation is only one route of exposure, compounding the primary ingestion route (Stommel, Field, and Caller 2012). Lastly, there may also be unknown consequences associated with chronic exposure from the multiple routes, a possibility supported by the correlation between non-alcoholic liver disease and cyanobacteria blooms across the United States (Zhang et al. 2015).

An understanding of aerosolized MCs and the sizes of aerosolized cyanobacteria cells emitted from varying lake types could lead to better estimates of cyanotoxin exposure. In the future, these techniques for collecting aerosols should be applied to a wider range of lakes in order to further test what environmental factors regulate aerosols. Continued collection of cyanotoxins in aerosols including the neurotoxin BMAA could lead to the development of
improved exposure models of aerosolized material with less reliance on field collections. Such models may allow one to predict when aerosolization will be highest and advise the public to avoid exposure via lake aerosols. As populations of cyanobacteria expand in our freshwater systems due to anthropogenic causes, understanding all routes of exposure will be important for mitigating cyanotoxin exposure.
APPENDIX: EXPLORATION INTO THE EFFECT OF ZOOPLANKTON GRAZING ON CYANOBACTERIA AEROSOLIZATION IN ROCK POOLS AT THE ISLE OF SHOALS

INTRODUCTION

Cyanobacteria are ubiquitous in all environments, inhabiting deserts, tropics, hot springs, and even arctic regions (Jonasson et al. 2010; Liyanage et al. 2016). Cyanobacteria have many adaptations that make them strong competitors in freshwater systems. Consequently, they are an increasing problem worldwide as they take advantage of nutrient loading and rising temperatures (Paerl et al. 2018). In addition to the ecological damage they can cause, many cyanobacteria genera produce harmful secondary metabolites toxic to both humans and wildlife (Carmichael 1992). Understanding how these organisms move through and beyond their freshwater habitats is essential to limiting human exposure to cyanotoxins. The primary route of human exposure to cyanotoxins is via ingestion through contaminated drinking water or food. Inhalation of aerosolized cyanobacteria cells and from freshwater systems is a less understood route of exposure and under investigation. While initial studies have established the presence of cyanobacteria and cyanotoxins in aerosols (Wood and Dietrich 2011; Y.-S. Cheng et al. 2007; L. C. Backer et al. 2010; Murby and Haney 2015), there are still many gaps in our understanding of the mechanisms behind the process and types of aquatic systems that emit aerosols.

The primary mechanism of aerosolization is proposed to be related to a process of micro-bubble bursting (Blanchard and Syzdek 1972). Bubbles form from trapped air, and gradually rise to the water surface. At the surface, they burst, transporting the concentrated biological material into the air in tiny droplets (D C Blanchard and Woodcock 1956). Wind and evaporation are also considered important mechanisms that regulate aerosolization by providing the force needed to carry material from the surface of the water into the air (Dueker 2012; Murby and Haney 2015).
Other emerging direct and indirect factors influencing aerosolization include air and water temperature differentials, nutrient conditions, and time of day (Langley MS thesis). To date, most studies of aerosolization have focused on abiotic factors, and have largely ignored the possibility of biological interactions. I hypothesize that an important factor driving cyanobacteria aerosolization, and possibly a factor that could help explain time of day differences, is zooplankton grazing.

Zooplankton are the primary grazers of phytoplankton ranging in size from 1 - 50 µm in freshwater ecosystems (Burns 1968). Herbivorous zooplankters include rotifers, cladocera, and calanoid copepods. Because of their small size, picocyanobacteria are a primary food source for nanoplanktonic protozoans (Jakubowska and Szeląg-Wasielewska 2015). They have also been found in marine mesozooplankton guts (Motwani and Gorokhova 2013; S. E. Wilson and Steinberg 2010) and freshwater *Daphnia* (Callieri et al. 2004). Larger phytoplankton are mechanically difficult to eat, and typically have physical features that prevent digestion (Brooks and Dodson 1965). Despite this, zooplankton can sometimes ingest smaller colonies, pieces of colonies (Porter 2017), or exert a mechanical effect on the structure of colonies (Deason 1980). The close interactions between grazers and phytoplankton may affect cyanobacteria in multiple ways. It is possible that the process of zooplankton grazing is physically changing cells and colonies in a way that may be affecting the process of cyanobacteria aerosolization. I hypothesize that zooplankton grazing will increase aerosolized cyanobacteria, and this increase will be more present at night as grazing rates are typically higher at night (Haney and Hall 1975).

To better understand the effects of zooplankton grazing on phytoplankton aerosolization, I measured aerosols above rock pools with contrasting plankton on Appledore Island in the Gulf of Maine. Rain-fed freshwater pools provide a unique opportunity to study contrasting micro-
systems exposed to similar environmental variables, often developing extreme conditions, such as high densities of phytoplankton and zooplankton. This was a first step in understanding the biological influence of zooplankton grazing on the process of cyanobacteria aerosolization.

METHODS

Study Site

Appledore Island (42.9891 N, 70.6142 W) is one of nine small islands in the Isles of Shoals, located 10 km off the coast in the Gulf of Maine, USA. The 38.5 ha island is dominated by vegetation in the middle, and ringed by a rocky coast (Sirianni 2017). There are around 4,000 freshwater rock pools along the perimeter of the island, ranging in size from 1 to 30,000 L (Sirianni 2017; Simonis 2013). These pools have a patchy distribution above the high-tide line, and are closed rainwater systems (Sirianni 2017; Simonis 2013), some receiving airborne salt during storms. Two rock pools near each other on the north east side of the island (between Siren’s Cove and Broad Cove) were chosen for this experiment. One rock pool (RP 300), was clear and had visibly high concentrations of zooplankton grazers, while the other (RP 312) was green and had visibly high concentrations of phytoplankton (Figure 26). Rock pools were also chosen because of similarity in depth and exposure to the same environmental factors (weather and marine influence) due to their proximity (Table 6).
Figure 26. Freshwater rock pool sampling locations on Appledore Island. Left image is RP 312, dominated by phytoplankton. Right image is RP 300, dominated by zooplankton.

Table 6. Location, basic morphometric description, and climate factors of the two study freshwater rock pools on Appledore Island in the Isle of Shoals.

<table>
<thead>
<tr>
<th>Rock Pool</th>
<th>Time of Day</th>
<th>Latitude °N</th>
<th>Longitude °W</th>
<th>Max Depth (m)</th>
<th>Air Temp (C)</th>
<th>Relative Humidity (%)</th>
<th>Wind (m s⁻¹)</th>
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<td>70.61288</td>
<td>0.3925</td>
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<td>51</td>
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<td>(zooplankton dominant)</td>
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<td></td>
<td></td>
<td>68</td>
<td>54.1</td>
<td>0.77</td>
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<tr>
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<td>(phytoplankton dominant)</td>
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</table>

Aerosol Collection

A multi-parameter sonde (YSI, EXO II, Yellow Springs, OH), coupled with a data logger (YSI, Handheld, Yellow Springs, OH) recorded a range of physical and chemical properties in each pool at the beginning of the sampling period during both day and night. Fluorometry was performed on water samples after they were frozen, then thawed and warmed to room temperature. The AquaFluor Handheld Fluorometer (Turner Designs, San Jose, CA) was used,
measuring chlorophyll at an excitation of 395/130 nm, and phycocyanin at an excitation wavelength of \( \leq 595 \) nm. Air temperature and relative humidity were recorded with a handheld recorder (AcuRite, Lake Geneva, Wisconsin) and wind speed was recorded using a handheld anemometer (Extech, Nashua, NH) at the beginning of aerosol sampling for both day and night. Night sampling was performed from 9:00 PM on 19 July 2016 to 6:00 AM on July 20\(^{th}\). Day sampling was performed from 6:15 AM to 3:16 PM on 20 July 2016.

Aerosol samples were collected using the Compact Lake Aerosol Monitor (CLAM), a modified protocol established by Murby and Haney (2015). Modifications and a description of the aerosol collection protocol can be found in detail in Langley MS thesis. CLAMs were positioned over the rock pools, suspended by boards spanning the distance of the pools (Figure 26). Aerosols were collected directly over both pools simultaneously for 9 h under identical environmental conditions. Filters were changed between day and night collections.

**Aerosol Analysis**

Aerosolized material collected on the filters was examined with epifluorescence microscopy (Olympus BX41, Olympus DP72 camera) with an automated stage (Model type ES222/G, Prior Scientific Instruments, Fulbourn Cambridgeshire, ENG) and an X-Cite series 120Q Lumen Dynamics mercury light (120-watt, Excelitas Technologies, Waltham MA) to enumerate cyanobacteria cells. Two narrow band excitation wavelengths of 435 nm (CHL cube) and 572 nm (PC cube) discriminate between cyanobacteria and other photosynthetic cells. The 435 nm wavelength excites a broad window of chlorophyll pigments, while the 572 nm more specifically excites phycocyanin, phycobilin, pigments accessory to chlorophyll. While chlorophyll is found in all phytoplankton, phycocyanin is found primarily in cyanobacteria.
CellSens Dimension imaging software (Olympus, Chelmsford, MA) digitally creates a dark field image where individual cells can be visualized clearly and counted on the surface of the glass fiber filters. Imaging and counting procedures are described in detail in Langley MS thesis. Water grab samples from each pool were evaluated for MCs using an ELISA (QuantiPlate High Sensitivity, EnviroLogix Inc, Portland ME) following the extraction and concentration protocol described in Langley MS thesis, however there were no detectable toxins, so aerosol samples were not tested for MCs.

Data were organized and stored in Excel. All graphs and analyses were generated using SigmaPlot 12.5 (SYSTAT Software Inc., Chicago IL). Aerosolized cyanobacteria cells were categorized into size classes based on equivalent spherical diameter (ESD): 0.2-2 µm, 2-4 µm, 4-10 µm, 10-20 µm, 20-40 µm, and larger. Beyond the initial description of aerosolized material, these size categories are simplified into total cell count, picocyanobacteria (0.2 – 2 µm), and larger cells (2 – 40 µm), for ease of analysis and understanding trends. The relationship between time of day and rock pool for aerosolized cells (total, pico and larger), was determined using two-way ANOVAs. Day and night values were combined to evaluate differences between rock pools via a t-test.

RESULTS

Rock pool 300 ("zooplankton pool") was dominated by high densities of Daphnia pulex with active grazing occurring during the day and night. Rock pool 312 ("phytoplankton pool") had a dense layer of suspended phytoplankton throughout the pool; the phytoplankton growth was not just a surface scum. The chlorophyll values were around 10 µg L⁻¹ higher in the phytoplankton pool compared to the zooplankton pool (Table 7). Phycocyanin levels were
around eight times higher in the phytoplankton pool compared to the zooplankton pool (Table 7), indicating the highest levels of cyanobacteria in the pool without *Daphnia*. The specific conductance was around 23 times higher, salinity 28 times higher, and dissolved oxygen around three times higher in the phytoplankton pool compared to the zooplankton pool (Table 7).

**Table 7.** Physical and chemical properties recorded using the multi-parameter sonde (YSI, EXO II, Yellow Springs, OH) at each pool. Chlorophyll a and phycocyanin fluorescence were measured using a handheld fluorometer (AquaFluor, Turner Designs, San Jose, CA). The phytoplankton dominant pool appears to have a stronger marine influence than the zooplankton pool.

<table>
<thead>
<tr>
<th>Rock Pool</th>
<th>Time of Day</th>
<th>Temperature (°C)</th>
<th>Phycocyanin (μg L⁻¹)</th>
<th>Chlorophyll a (μg L⁻¹)</th>
<th>Oxygen (%)</th>
<th>Oxygen (mg L⁻¹)</th>
<th>pH</th>
<th>Specific Conductance (µS cm⁻¹)</th>
<th>Salinity (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 (zooplankton dominant)</td>
<td>Day</td>
<td>21.98</td>
<td>3.26</td>
<td>51.97</td>
<td>50.1</td>
<td>4.44</td>
<td>8.55</td>
<td>922.6</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>22.662</td>
<td>2.47</td>
<td>49.43</td>
<td>46.6</td>
<td>4</td>
<td>8.47</td>
<td>883.9</td>
<td>0.43</td>
</tr>
<tr>
<td>312 (phytoplankton dominant)</td>
<td>Day</td>
<td>21.096</td>
<td>19.16</td>
<td>68.07</td>
<td>162.9</td>
<td>13.34</td>
<td>8.65</td>
<td>20788.5</td>
<td>12.42</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>22.662</td>
<td>25.74</td>
<td>62.78</td>
<td>101.8</td>
<td>8.23</td>
<td>8.75</td>
<td>20604.9</td>
<td>12.29</td>
</tr>
</tbody>
</table>

**Figure 27.** Aerosolized cyanobacteria cells identified and counted using epifluorescence. Error bars represent ±1 standard error for the total cell counts on filters. Night values are dotted. A two-way ANOVA was performed to evaluate the differences between time of day, and rock pool. There was no interaction between the factors, and no statistical differences between rock pools, or time of day.
Aerosolized cyanobacteria (of all cell sizes) are around 1.5x higher from the zooplankton pool compared to the phytoplankton pool, but is not significantly different due to large variation and small sample size (n = 3, total p = 0.13, pico cells p = 0.20, larger cells p = 0.05). When day and night values are combined, the zooplankton pool had significantly higher concentration of larger cells than the phytoplankton pool (t-test, p = 0.03), supporting the hypothesis that zooplankton grazing increases aerosolized cyanobacteria. Total aerosolized cyanobacteria in aerosols varied from $3.6 \times 10^4$ to $5.1 \times 10^4$ cells m$^{-3}$. Aerosols were dominated by picocyanobacteria cells (sizes 0.2-2 µm), and almost all cells were below 10 µm (Figure 27). Picocyanobacteria and larger cells follow the same trends of total cells across rock pools and between day and night (Table 8).

The ratio of night:day aerosolized cells in the zooplankton pool was 1.15, contrasted with only 0.76 (higher daytime aerosolized cells) in the phytoplankton pool. Because of large variations in aerosol cell concentrations, the day-night differences were not significant at p > 0.05 (total p = 0.917, pico p = 0.98, larger p = 0.63), and the effect of time of day did not vary between the two rock pools for any of the size fractions of aerosolized material. Though these differences were not statistically significant, the data weakly support the hypothesis that nocturnal grazing effect may cause an increase in aerosolized cyanobacteria at night.
Table 8. Total aerosolized cells, picocyanobacteria and larger cells aerosolized above contrasting rock water pools. Aerosols are dominated by picocyanobacteria, and the aerosols above rock pools do not vary significantly, and there is no effect of time of day.

<table>
<thead>
<tr>
<th>Rock Pool</th>
<th>Time of Day</th>
<th>Total Cyanobacteria</th>
<th>Picocyanobacteria</th>
<th>Larger Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 (zooplankton)</td>
<td>Day</td>
<td>44285.37 ± 2456.86</td>
<td>27619.03 ± 3121.63</td>
<td>16666.34 ± 770.26</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>51005.18 ± 17186.75</td>
<td>33402.99 ± 12983.61</td>
<td>17550.72 ± 4279.31</td>
</tr>
<tr>
<td>312 (phytoplankton)</td>
<td>Day</td>
<td>36594.03 ± 4235.13</td>
<td>23469.59 ± 2859.62</td>
<td>13124.44 ± 1802.86</td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>27895.87 ± 4280.24</td>
<td>18116.88 ± 2700.01</td>
<td>9779.00 ± 1598.01</td>
</tr>
</tbody>
</table>

DISCUSSION

The pattern of aerosol production in the two rock pools with and without grazing is consistent with the proposed hypotheses that zooplankton grazing should increase the concentration of aerosolized cyanobacteria cells. Based on phycocyanin concentration, the zooplankton pool had a lower concentration of cyanobacteria, but despite this had very similar aerosolized cyanobacteria concentrations to the phytoplankton pool (Table 7). Day-night differences could not be ascertained with only three replicates and the large variance between samples. This single experiment supports the hypothesis that zooplankton grazing may increase aerosols despite the phytoplankton pool having a greater concentration of potentially aerosolizable cells.

Environmental factors could not explain differences in aerosolized material between rock pools as they were exposed to the same fluctuations in environmental conditions (Table 6), and any minor difference were not sufficient to account for the discrepancies in aerosolization between the two pools. Differences in composition of cyanobacteria between pools, however, may influence the aerosolized material. If the cyanobacteria in the phytoplankton dominant pool are large and colonial, they are less likely to enter the air (Langley MS thesis). However, these cyanobacteria are more likely to be single celled than colonial. Zooplankton grazing drives
populations towards colonial-dominant compositions (Haney 1987). In the absence of grazing, phytoplankton do not require the protection provided by colonial massing, and similar to being in culture, they remain as single cells. Based on previous surveys of the Appledore rock pools, rock pools lacking Daphnia are dominated by single-cells phytoplankton (Haney, unpubl).

Diel patterns of aerosols also supported the concept of heightened nocturnal grazing effects on aerosol production. Though not statistically different, night aerosolization was greater in the zooplankton pool than during the day (while levels were higher during the day in the phytoplankton pool). Such a diel effect might be expected if high levels of photosynthesis during the day produce conditions of supersaturation of dissolved oxygen, increasing aerosols through an abundance of microbubbles. This condition would be absent in the zooplankton pool because of lower concentrations of phytoplankton and the added consumption of oxygen by grazers.

The effect of time of day on the grazing hypothesis may be muted because of several differences in the rock pool habitat compared to a lake. Though zooplankton migrate down during the day in lakes to avoid fish predation (Jeppesen et al. 1997), there are no predators to initiate that behavior in this pool. However, zooplankton in clear systems move down during the day to avoid UV light. Because the depth of Pool 300 is <30 cm, there is little place for zooplankton to take refuge. During the day, Daphnia in Pool 300 were observed clustering in shadows caused by small overhangs in the pool, potentially avoiding direct UV light. To some degree, grazing may be reduced during the day as zooplankton take refuge from UV radiation (Rautio and Tartarotti 2014). Despite this, the concentration of grazers overall was so high, many grazers were visibly active throughout the pool during the day. Continuous grazing throughout the day in a spatially confined volume likely explains the lack of more pronounced diel differences in aerosolization.
Differences in water quality between the two pools appear to both be influenced by and influence organism composition. The high salinity in rock pool 312 may prevent zooplankton establishment (Schallenberg, Hall, and Burns 2003). I did not formally quantify the *Daphnia pulex* in RP 300, but they were observed swimming in very dense patches of approximately 100-200 individuals per liter. While salinity may affect the composition of phytoplankton, it is clearly not inhibiting phytoplankton growth. Chlorophyll and phycocyanin pigments in rock pool 300 are both around 14 times higher than the average chlorophyll level of 4.26 µg L⁻¹ found in New England lakes (Haney and Ikawa 2000). Both rock pools provide a stark contrast to each other, especially in terms of phycocyanin levels, that are around eight times higher in the phytoplankton pool than the zooplankton pool. The very high phycocyanin:chlorophyll ratio (5.9 day, 10.4 night) indicates a dominance of cyanobacteria in the pool, although no attempt was made to identify the specific taxa present.

Total aerosolized cell counts were low compared to average total cyanobacteria concentration in aerosols measured above New England lakes (Langley MS thesis). However, the relative proportion of picocyanobacteria compared to larger material in aerosols is similar to that seen over lakes (Langley MS thesis). Picocyanobacteria and larger cells above the rock pools followed similar trends to the total aerosolized cells when evaluating differences between the pools and changes between day and night within the pools (Table 8). The concentration of larger cyanobacteria cells in the air above the two rock pools was statistically higher in the zooplankton pool, suggesting that grazing should be a factor considered as an influence on the composition and concentration of lake aerosols.

To promote ideas and future research on zooplankton-aerosol interactions, I postulate four possible pathways zooplankton grazing may influence aerosolization of cyanobacteria:
1.) Colony disruption as a result of post abdominal rejections (Deason 1980). The size of large cyanobacteria colonies provides defense against herbivory, as they cannot easily be handled. While copepods are more selective grazers as they are able to avoid certain particles based on size and taste, filter feeding cladocerans are not able to do this (Lampert 1987). Filaments cause mechanical interference, reducing filtering efficiency in grazers (Haney 1987). Cladocera can narrow their carapace gape to avoid ingesting larger filaments, but smaller filaments and colonies continue to pass into the food groove. Any unwanted ingested particles are removed through post-abdominal rejections (Lampert 1987). As the animals scrape unwanted material from their filtering appendages, colonies can be fragmented into more easily aerosolized smaller pieces or single cells.

2.) Partial zooplankton digestion of small colonies. While the gelatinous sheath around ingested colonies may be digested, entire colonies and viable cells can be egested (Porter 2017). Unprotected smaller groupings of cells are more susceptible to further damage. The resulting small clumps of cells or individual cells freed from colonies are more likely to become aerosolized. This is supported by the observed composition of aerosolized cells; most of them are single cells under 10 µm, and no colonies were seen in aerosols (Figure 27).

3.) Sloppy feeding of zooplankton. Picocyanobacteria and single cells in the nanoplankton size range are most commonly grazed by copepods, cladocerans and rotifers (Motwani and Gorokhova 2013; S. E. Wilson and Steinberg 2010). Daphnia are sloppy feeders, inefficiently breaking apart cells and releasing cell contents into the water (Lampert 1978). This process could be fragmenting cells into smaller pieces that are more likely to become aerosolized or release dissolved cyanotoxins into the water that later become aerosolized.
4.) Chemical changes to picocyanobacteria following zooplankton egestion entire cells. There is evidence that picocyanobacteria can pass through the zooplankton gut and remain intact and viable (S. E. Wilson and Steinberg 2010). While still a functioning cell, passage through the gut may alter the physical and chemical properties of the surface of the cell, similar to the changes colonies go through. Egested picocyanobacteria cells may behave differently, increasing their chance of aerosolization. For example, hydrophobic phytoplankton are more likely to accumulate in the surface microlayer of oceans, thus increasing their aerosolization (Michaud et al. 2018).

While this is a cursory study, the large contrast in pool composition and density of plankton indicates that these small and easily manipulated rock pools would be useful systems to examine the effects of biotic factors on aerosolization of cyanobacteria. These preliminary results encourage further testing of the aerosol grazing hypotheses and indicate that experimental design needs to include a larger number of replicate aerosol samples in order to have a more sensitive test of these hypotheses. Future work should also evaluate the influence of grazing pressure on cyanotoxins, as there are likely even more pronounced changes in cyanotoxicity aerosolization under grazing pressure. While the rock pools allowed me to compare aerosolization rates under heavy grazing and in the absence of grazing, more specific laboratory studies will be needed to examine the validity of the proposed pathways in detail. Though only a simplified examination of the effect of zooplankton grazing on cyanobacteria aerosolization, this study suggests that zooplankton may be exerting influence on the aerosolization process.
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