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Using Wetlands to Prevent the Surface Accumulation of *Aphanizomenon flos-aquae* from
Upper Klamath Lake

by

Arick Christopher Rouhe

A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Environmental Sciences and Resources

Dissertation Committee:

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Abstract

The ability to regulate buoyancy (sinking and floating) using cellular gas vesicles is a unique characteristic that allows many common bloom-forming cyanobacteria to accumulate at water surfaces and dominate systems. Typical control and management strategies include nutrient manipulation and phosphorus reduction, which are effective but do not reduce the advantage of buoyancy control. Since buoyancy control is based upon a mechanism that is driven by photosynthesis along with environmental conditions that trigger vesicle formation and ion exchange, buoyancy regulation can be influenced by manipulating extracellular conditions. In this study I manipulated extracellular conditions using wetland water and additions of potassium, sodium, and calcium in small-scale lab experiments and larger scale, near-lake containers with *Aphanizomenon flos-aquae* from Upper Klamath Lake, Oregon. The results indicate a target mixture of 10% wetland water reduces surface accumulation, increases cellular turgor pressure (a measure of the ability of gas vesicle forming cells to control buoyancy), and leads to fewer rafts at the surface of the water column. By adding ions at the same concentration as the target wetland mixture, similar results were found. This research represents the basis of a possible strategy for mitigating surface blooms of buoyant cyanobacteria in lakes using wetland water and/or ion additions that could be used in tandem with nutrient manipulation and phosphorus reduction.

For Monica

Thank you for all of your patience and support through this long process

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Table of Contents

Abstract	i
Dedication	ii
Acknowledgements	iii
List of Tables	v
List of Figures	vii
Chapter 1: Introduction	1
Chapter 2: Buoyancy Loss Methodology	27
Chapter 3: Small Scale Treatments	62
Chapter 4: Large Scale Treatments	87
Chapter 5: Study Conclusions	124
References	132
Appendix A: 2-day trial <i>in situ</i> water chemistry	144
Appendix B: 2-day trial nutrient concentrations	146
Appendix C: 2-day trial ion concentrations	148
Appendix D: GK-12 Fellowship report	150

List of Tables

2.1 Average applied pressure for each intensity preconditioning treatment category	39
2.2 Summary of results for comparisons of the treatment levels from the light intensity preconditioning treatments using the applied external pressure measurements	42
2.3 Average applied pressure for each duration preconditioning treatment category	45
2.4 Summary of results for comparisons of the treatment levels from the light duration preconditioning treatments using the applied external pressure measurements	49
2.5 Summary of results for the applied external pressure analysis from potassium additions	54
3.1 Concentration of Sodium, Potassium, and Calcium in UKL lake water and low, mid, and high treatments after additions	66
3.2 Summary of results for the analysis of the average applied external pressure to cause the surface rafts to lose buoyancy after the wetland water treatments	69
3.3 Summary of results for the percent surface raft analysis	70
3.4 Summary of results for the analysis of average suspension position	70
3.5 Summary of results ion addition treatments	76
4.1 Summary of water chemistry methods	93
4.2. Average suspended raft depth for each 2-day trial treatment	98
4.3. Percentage of surface rafts for each 2-day trial treatment	99
4.4. <i>In situ</i> water chemistry for Agency Lake, Putnam's Point, and Wood River Wetland	109
4.5. Average <i>in situ</i> water chemistry for 2-day trials	110
4.6. <i>In situ</i> water chemistry for 8-day trial	111

4.7. Nutrients data for Agency Lake, Putnam's Point, and Wood River Wetland	112
4.8. Nutrient means for 2-day trial treatment replicates	113
4.9. Nutrient data for 8-day trial	113
4.10. Mean and variance of sodium, potassium, and calcium concentrations measured from samples taken on each day that water was collected from Putnam's Point, Wood River Wetland, and Agency Lake	114
4.11. Mean sodium, potassium, and calcium concentrations for each 2-day wetland water treatment and control container	115

List of Figures

1.1 Images from Walsby (1994) to show gas vesicles and gas vacuoles	5
1.2 Map of the Upper Klamath Lakes system showing key features and study locations	15
1.3 Images of <i>A. flos-aquae</i>	16
1.4 Map of UKL and Agency lake showing drained and undrained wetlands from Bradbury et al. (2004)	19
2.1 Bergeon 5555/98 pressure chamber and small-scale experimental set-up	32
2.2 Average buoyancy loss curve for each light-intensity treatment	38
2.3 Buoyancy loss curves of each replicate for each of the light intensity preconditioning treatments	40
2.4 Boxplots of data from each light intensity preconditioning group	41
2.5 Average buoyancy loss curve for each light-duration preconditioning treatment	44
2.6 Buoyancy loss curves of each replicate for each of the light duration preconditioning treatments	46
2.7 Boxplots of data from each light duration treatment group for the pressure at which the bottom rafts lost buoyancy	48
2.8 Average buoyancy loss curve for each potassium addition treatment	51
2.9 Boxplots of data from each potassium addition group	53
2.10 Comparison of pressure collapse curves from the original work by Walsby (1971) and the research presented here using buoyancy loss curves	56
2.11 Comparison of pressure collapse curves from Thomas and Walsby (1985) and the research presented here using buoyancy loss curves	57
3.1 Buoyancy loss in <i>A. flos-aquae</i> rafts after wetland water treatments	68
3.2 Average of percent rafts floating at the surface after the 10 minute dark-adapted period	71

3.3 Profile images of representative replicates from each group after 10-minute dark-adapted period	72
3.4 Buoyancy loss in <i>A. flos-aquae</i> rafts after ion addition treatments	74
3.5 Average of percent rafts floating at the surface after ion addition treatments	77
4.1 Images of the large-scale study site and set-up	89
4.2. Source water and 2-day trial water temperature	96
4.3. Boxplots of the percent surface rafts and average suspension depth	100
4.4. Buoyancy loss curves of rafts in large-scale treatments after 24hrs	101
4.5. Buoyancy loss curves of rafts in large-scale treatments after 48hrs	103
4.6. Average buoyancy loss curves of treatments from all three large-scale, 2-day trials	105
4.7. Average externally applied pressure to cause the last rafts to lose buoyancy	106
4.8. 8-day trial buoyancy loss curves	108
4.9. Biplot of the 2-day trial principle component analysis	116

Chapter 1. Introduction

Prevalent management of cyanobacteria in lakes

Two classic studies have cemented the importance of cyanobacteria control by nutrient manipulation in lakes. Smith (1983) published an article in *Science* detailing his analysis of cyanobacteria in regards to the ratio of total nitrogen to total phosphorus (TN:TP) in 17 lakes around the world. Using proportion of cyanobacteria cell volume per water volume during the growing season, Smith (1983) showed a distinct boundary ratio of TN:TP (by weight) at 29:1. Below the ratio of 29:1, the included lakes showed varying phytoplankton percentages of cyanobacteria from close to zero all the way up to 100. Above the ratio of 29, no lakes existed with more than 20 percent of the phytoplankton assemblage consisting of cyanobacteria. This analysis by Smith (1983) was based on whole-lake fertilization experiments led by David Schindler from 1969-1975. Schindler's results, published in a series of papers in the early 1970's, were summed up in a seminal paper (Schindler, 1977). The results indicated that cyanobacteria dominance could be manipulated by changing the ratio of nitrogen to phosphorus. At an N:P ratio of 5:1, cyanobacteria dominated the percent biomass of phytoplankton in the experimental lakes. When an N:P ratio of at least 14:1 was applied to the experimental lakes, green algae dominated the phytoplankton.

The two articles described above (Schindler, 1977; Smith, 1983) have shaped cyanobacteria management since the time of their publication because they are based upon a sound physiological advantage. The advantage is as follows: nitrogen fixing cyanobacteria absorb nitrate and ammonium when those nitrogen species are present in the surrounding water, but switch to nitrogen fixation when nitrate and ammonium

concentrations become the limiting nutrient. Thus, nitrogen fixation gives nitrogen fixing cyanobacteria a competitive advantage when nitrate and ammonium concentrations are low (limiting) in the surrounding waters. The N:P ratio is a way of measuring when nitrogen or phosphorus is the limiting nutrient. The N:P ratios of 14:1 and 29:1 described by Schindler and Smith support the limiting nutrient theory but, as Schindler and Smith also explain, each ratio is not a magic number. Other studies cited by Schindler and Smith (Pearsall, 1932; Hutchinson, 1944; Leonardson and Ripl, 1980) and studies that cite Schindler and Smith (Elser et al., 2009; Paerl et al., 2011; Harris et al., 2014) use different boundary ratios. Even with slightly different ratios in each of these studies, the theory is still supported by the fact that when nitrate and/or ammonium levels in lake waters are increased in comparison to phosphorus, the advantage of nitrogen fixation is diminished and nitrogen-fixing bacteria species do not tend to dominate the phytoplankton. The sheer number of supporting studies for nutrient manipulation make this a close to irrefutable limnological theory. Schindler (1977) and Smith (1983) have been cited over 1500 and 800 times respectively, and have made nutrient ratio manipulations a common method for managing cyanobacteria in lakes.

Tilman et al. (1982) took the ratio of Schindler (1977) one step further by adding that the ratio is important but varies for each species and should only apply when light is not the limiting variable and when the other species present are better competitors for phosphorus. The research of Tilman and others (Titman and Kilham, 1976; Tilman, 1977; Tilman et al., 1982) shows that nutrient manipulation has its limitations because it is only based on competition for nutrients and the advantage of nitrogen fixation. In Tilman et al. (1982), the theory of N:P ratio is supported when diatom and green algae species are

present that can out-compete cyanobacteria species for phosphorus. This study also shows that the N:P ratio is not as important when light is the limiting factor. Zevenboom and Mur (1980) also showed that low light intensity can negate the advantage of nitrogen fixation. Their experiments revealed that cyanobacteria out-compete other taxa at mid and high light intensity only when nitrogen is the limiting nutrient. At low light intensity, cyanobacteria can out-compete other taxa at all nitrogen concentrations. The results of these studies indicate that nutrient manipulation is a successful management tool, but not under conditions in which light is a limiting factor.

Other than nitrogen fixation, advantages possessed by cyanobacteria include: rapid inorganic carbon uptake (Smith et al., 1987), accumulation of cellular nitrogen stores in the form of phycocyanin (Fogg et al., 1973), accumulation of cellular phosphorus stores (Stewart and Alexander, 1971), filament clumps as a deterrent to predation (Kalff and Knoechel, 1978; Burns, 1968), and buoyancy regulation via gas vesicles and ballast (Oliver, 1994). While all of these can be factors that provide an advantage for cyanobacteria, the factor that is most relevant to the conditions under which nutrient manipulation is limited or not applicable as a management tool for reducing cyanobacteria (during light limitation), is buoyancy regulation via gas vesicles and ballast.

Background on cellular gas vesicles in cyanobacteria

Buoyant cyanobacteria regulate their position in the water column through a mechanism that involves the formation of gas vesicles (Walsby, 1987). Cyanobacteria are the only phytoplankton group to regulate their buoyancy using gas vesicles (Reynolds, 2006) and the sole purpose of gas vesicles in cyanobacteria is for buoyancy

regulation (Walsby, 1972). Gas vesicles are small, gas-filled structures that are found in the cytosol of cyanobacteria and non-photosynthesizing bacteria. Gas vesicles can exist as single structures in the cytosol (right side of Figure 1.1), but in many species they are connected together into larger groups called gas vacuoles (Oliver, 1994). Vacuoles can be composed of hundreds of gas vesicles that work like an internal scaffolding to provide the physical structure of the vacuole (Walsby, 1981). When viewed in cross section, the vesicles give the vacuole a honeycomb-like appearance (left side of Figure 1.1).

Gas vesicles are formed in the cytosol from a homologous protein (Walker et al., 1984) into thin, mesh-like walls about two nm in thickness and arranged in ribs running at right angles (Walsby and Armstrong, 1979) with pore sizes up to 0.63nm (Walsby, 1984). The pores allow the vesicles to fill with gases (H_2 , N_2 , O_2 , CO_2 , CO , CH_4 , and Ar) that diffuse from a dissolved state in cell solutions into the empty space of a gas vesicle as it is being formed (Walsby, 1994). The inner surface of gas vesicles is highly hydrophobic, keeping water from passing through the pore spaces in the mesh wall (Walsby, 1969; Hayes et al., 1986). Vesicles are cylindrical in shape and enclosed on each end by a cone (Walsby, 1981). After formation, the small gas vesicles are attached together, creating a larger structure called a gas vacuole (Bowen and Jensen 1965).

Figure 1.1. Images from Walsby 1994 to show gas vesicles and gas vacuoles. On left, transverse section of *Microcystis* sp. Gas vacuoles are large, light colored areas with an internal honeycomb-like structure. Gas vesicles are the smaller “honeycomb” structures in the larger gas vacuole. On right, freeze fracture of *Prosthecomycobium pneumaticum* to show single gas vesicles (not stacked into gas vacuoles) with straight-sided cone structure.

Gas vacuoles are important for cyanobacteria buoyancy regulation because they create large, low-density fractions of cell volume that reduce overall cell density. However, gas vesicle structure has been shown to be more important to buoyancy regulation than the larger gas vacuole structure because the ability of cyanobacteria to regulate buoyancy (described later in this section) is related to the width of individual gas vesicles within gas vacuoles and not to the structure of the larger gas vacuoles (Walsby, 1994). Gas vesicle width between individuals of a species does vary, but variation within species is small due to the homology of gas vesicle proteins (Hayes et al., 1986; Walker et al. 1984). Variations in gas vesicle width between species are greater than variations in gas vesicle width within species, leading to differences in the hydrostatic conditions

under which each species can regulate buoyancy efficiently (Walsby, 1994; Hayes and Walsby, 1986).

Due to the inverse relationship between gas vesicle width and gas vesicle strength, species with narrow vesicles can remain buoyant under a wider range of hydrostatic pressure conditions than species with wider gas vesicles (Hayes and Walsby, 1986). For example, species such as *Trichodesmium thiebautii*, found in the ocean, can face high external pressure conditions because of very narrow gas vesicles (45nm) that are difficult to collapse so that they withstand mixing to several hundred meters of depth and retain buoyancy. Freshwater taxa do not face such extreme hydrostatic pressure conditions, so their vesicles are much wider. Among freshwater taxa, *Microcystis aeruginosa* and *Oscillatoria agardhii* have narrow, hard to collapse vesicles with widths of 67nm and 64nm respectively. *Dolichospermum flos-aquae* (84nm) and *Aphanizomenon flos-aque* (78nm) are considered moderate fresh water taxa in regard to vesicle width, making their vesicles more susceptible to collapse than species of *Microcystis* and *Planktothrix* (Oliver, 1994; Hayes and Walsby, 1986). The width and environmental pressure conditions for each species are important for gas vesicles because they can collapse under external environmental pressures due to depth and, most importantly for freshwater taxa, under cellular conditions that increase turgor pressure (the role of cellular turgor pressure in the collapse of gas vesicles to cause sinking will be explained in the next section).

Background on buoyancy regulation in cyanobacteria

Cyanobacteria control positive buoyancy (a.k.a. floating) through the formation of gas vesicles, which decrease cell density. In general, biological cells are not positively

buoyant because the chemical components that form cell structures have a higher density than water (998 kg m^{-3}). Protein, carbohydrates, and nucleic acids have average densities of $1.3 \times 10^3 \text{ kg m}^{-3}$, $1.5 \times 10^3 \text{ kg m}^{-3}$, $1.7 \times 10^3 \text{ kg m}^{-3}$ respectively (Walsby and Reynolds, 1980). The only major cell component with a lower density than water, lipids (860 kg m^{-3}), does not typically constitute a high enough percentage of cell volume to reduce the overall cell density below 998 kg m^{-3} (Sargent, 1976). While some phytoplankton taxa increase lipid production and storage in order to lower cell density near 998 kg m^{-3} , cyanobacteria typically do not (Reynolds, 2006). Cell density for cyanobacteria, in the absence of gas vesicles, ranges from $1.025 \times 10^3 \text{ kg m}^{-3}$ for *Dolichospermum flos-aquae* to $1.093 \times 10^3 \text{ kg m}^{-3}$ for filaments of *Planktothrix agardhii* (Walsby, 1994). Typical gas vesicle density ranges from 162 kg m^{-3} for taxa with wide gas vesicles to 210 kg m^{-3} for taxa with narrow gas vesicles (Walsby, 1972). With gas vesicles, cyanobacteria cell density can be reduced to 942 kg m^{-3} (Walsby et al., 1992).

Gas vesicle forming cyanobacteria can control negative buoyancy (a.k.a. sinking) by increasing cell density through three mechanisms: increase in ballast, decrease in vesicle volume to cell volume, or collapse of vesicles. Cyanobacteria can increase density through the build-up of carbohydrates as ballast (Utkilen et al., 1985; Oliver and Walsby, 1984). When buoyant cyanobacteria rise to the surface due to gas vesicle formation, they are exposed to higher light intensities, leading to increased synthesis and accumulation of carbohydrates (Konopka and Schnur, 1980). Increase in cell density and loss of buoyancy from carbohydrate accumulation is considered to be ubiquitous among gas vesicle forming cyanobacteria and has been shown to occur through direct measurements in many cyanobacteria taxa including *Aphanizomenon* (Kromkamp et al.,

1986), *Microcystis* (Thomas and Walsby, 1985), and *Dolichospermum* (Oliver and Walsby, 1984).

Another method by which cyanobacteria increase cell density is through gas vesicle dilution. Cellular processes that lead to vesicle dilution include: cell growth without change in vesicle volume or number (Reynolds, 1972; Thomas and Walsby, 1985; Utkilen et al., 1985), repression of gas vesicle protein production during periods of growth at high light intensity (Pfeifer, 2012), repression of gas vesicle construction during periods of high light intensity (Thomas and Walsby, 1985), cell division without a change in absolute gas vesicle volume (Pfeifer, 2012), and increased cell volume due to carbohydrate accumulation and ion exchange during photosynthesis (Oliver, 1994; Thomas and Walsby, 1985). Carbohydrate accumulation and ion exchange during photosynthesis are also the contributors to cellular turgor pressure increases that are measured during exposure of cyanobacteria cells to high light intensity (Oliver, 1994; Kromkamp et al., 1986; Thomas and Walsby, 1985; Oliver and Walsby, 1984; Allison and Walsby, 1981; Konopka et al., 1978).

Increased turgor pressure can ultimately lead to gas vesicle collapse, which is the third method by which buoyant cyanobacteria increase cell density. Vesicle collapse due to turgor build up from exposure to high light intensity is also considered to be ubiquitous in gas vesicle forming cyanobacteria (Oliver, 1994; Walsby, 1994). However, gas vesicle collapse depends on the width of the individual gas vesicles and taxa with narrow vesicle width require higher turgor pressures for gas vesicle collapse than taxa that contain wide vesicles. Because of this, *Microcystis*, a narrow vesicle-width taxa, requires a longer exposure to high light intensities in order to build up enough turgor pressure for vesicle

collapse when compared to wider-vesicle taxa like *Aphanizomenon*, *Dolichospermum*, and *Gloeotrichia* (Oliver, 1994; Walsby, 1994). Due to gas vesicle width and strength, studies indicate that gas vesicle collapse may not play a role in the process of buoyancy regulation for *Microcystis* in some natural systems (Zohary and Breen, 1989; Thomas and Walsby, 1985) but it plays a major role in buoyancy regulation for species of *Aphanizomenon* (Kromkamp et al., 1986) and *Dolichospermum* (Oliver and Walsby, 1984) in natural systems. While the ability to induce negative buoyancy does not provide buoyant cyanobacteria an implicit advantage over non-buoyant phytoplankton taxa (that also sink because they are more dense than water), it does allow cyanobacteria to regulate water column position in order to pick up nutrients from the metalimnion or the sediment surface (Reynolds and Walsby, 1975; Fogg et al., 1973)

Buoyant cyanobacteria use positive and negative buoyancy to migrate vertically in the water column in order to optimize conditions. Reynolds and Walsby (1975) summarize this movement with the following sequence. Gas vesicle formation leads to a decrease in cell density and positive buoyancy. As the cells float upwards in the water column, they are exposed to higher light intensity, leading to an increase in photosynthesis. Photosynthesis at higher light intensity triggers three processes that increase density: cell growth, carbohydrate accumulate, and turgor pressure. Timing of these three processes can begin to effect density within two to five hours of exposure to high light intensity (Kromkamp et al., 1986) and once density increases above 998 kg m^{-3} , the cells sink to a lower position in the water column. At a lower position in the water column, the decrease in light intensity leads to a reduction in turgor pressure, a reduction in cell growth rate, and an increase in the rate of gas vesicle formation. These

three processes decrease cell density and lead to positive buoyancy over a time frame of 12-24hrs (Walsby 1994). While this sequence can vary in time due to cellular conditions, environmental conditions, and water column depth, it is generally considered a diel migration that occurs daily in lakes (Reynolds, 2006; Oliver, 1994).

Factors that influence buoyancy regulation in gas vesicle forming cyanobacteria

The ability of buoyant cyanobacteria to regulate cell density and control both positive and negative buoyancy is influenced by many factors. The essential factors for the research presented here are light attenuation, ion uptake, and available phosphorus. Light attenuation in the water column is an important factor for buoyancy regulation via gas vesicle production because of its direct effect on photon irradiance. As explained earlier, high photon irradiance reduces gas vesicle production and low photon irradiance triggers gas vesicle production (Oliver, 1994). Thus, light attenuation has a negative effect on photon irradiance, carbohydrate production, turgor pressure, and should diminish the effect of the reduction in gas vesicles under high irradiance.

Available phosphate also has a direct effect on the formation of gas vesicles. Many studies show that buoyancy of cyanobacteria decreases when phosphorus is limiting and buoyancy increases when phosphorus concentration increases (Oliver, 1994). A major reason for this is that cell metabolism increases with readily available phosphorus, leading to the synthesis of cell polymers that obviate the accumulation of carbohydrate ballast (Klemer, 1991). Specific studies of gas vesicle synthesis also indicate that gas vesicle production is inhibited when phosphorus is limiting. Konopka et al. (1987) showed that gas vesicle content in phosphate limited cultures of *Aphanizomenon flos-aquae* remained low, even when light intensity was high. When

phosphate concentration was increased, vesicle content also increased. Kromkamp et al. (1989) showed the same relationship between phosphorus and gas vesicles for cultures of *Microcystis aeruginosa* when phosphorus was limiting and when phosphorus concentrations were increased. These studies confirm that gas vesicle production (and positive buoyancy) is inhibited when phosphorus is limiting and facilitated when phosphorus is abundant.

The third factor to be highlighted here is ion exchange. Ion exchange is an essential component of cyanobacteria photosynthesis for the uptake of carbon during photosynthesis and the efflux of hydrogen ions in order to maintain intracellular pH (Kaplan et al., 1989). Studies by Kaplan et al. (1989) show that sodium ions are taken up by *Synechococcus* cells during high light when hydrogen ions are released from cells during photosynthesis. Scherer et al. (1988) showed that the same results of sodium ion uptake during hydrogen ion efflux for *Dolichospermum*. Cyanobacteria release hydrogen ions from cells during photosynthesis in order to alleviate pH imbalance through a Na^+/H^+ antiporter (Blumwald et al., 1984). Cyanobacteria also bring sodium ions into cells through a symport protein along with bicarbonate ions for carbon accumulation for photosynthesis (Kaplan et al., 1984). Both of these processes led to the accumulation of sodium ions in cyanobacteria cells. In their study on the contribution of carbohydrate accumulation from photosynthesis in *Dolichospermum*, Grant and Walsby (1977) found that low molecular weight carbohydrates only accounted for part of the turgor pressure necessary to collapse gas vesicles and that the other part was accounted for by inorganic ion uptake. Allison and Walsby (1981) demonstrated that potassium ions were the inorganic ions most responsible for the increased turgor pressure in *Dolichospermum*

under high light conditions. The study by Allison and Walsby (1981) also demonstrated that the turgor pressure in *Dolichospermum* cells could be increased by elevating the external concentrations of potassium ions. All together, these research studies indicates that turgor pressure is directly linked to ion exchange through symport and antiport proteins during photosynthesis and that changes in the external ion concentration can influence the magnitude of the intracellular turgor pressure in cyanobacteria cells.

The research gap that provides the context for this dissertation

Using gas vesicles to control water column position, buoyant cyanobacteria taxa can take advantage of low light conditions as well as accumulate at the surface and create low light conditions via shading as a competitive advantage. In a review of cyanobacteria taxa that create harmful algal blooms (CyanoHABS), Paerl and Otten (2013) provide a detailed description of the worldwide problem of toxic and nuisance cyanobacteria blooms. In their review, Paerl and Otten show that the most common toxin producing and problem-causing, phytoplankton cyanobacteria genera worldwide are *Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Nodularia*, *Oscillatoria*, *Trichodesmium*, *Microcystis*, and *Planktothrix*. While many of these can fix nitrogen and are candidates for control via nutrient manipulation, not all of them are nitrogen fixers. The main characteristic these genera have in common is buoyancy regulation via gas vesicle formation. Based upon this relationship, a management tool that utilizes conditions under which buoyancy regulation can be reduced, inhibited, disrupted, or suppressed would be a beneficial addition to nutrient manipulation as a cyanobacteria management technique. However, buoyancy inhibition as a management technique represents a large gap in published literature. This is not to say that buoyancy

regulation research is lacking. Published studies of buoyancy regulation and the mechanisms behind buoyancy regulation have a long history that includes hundreds of peer-reviewed publications. It is the application of buoyancy regulation research in order to obviate CyanoHABS that is lacking.

Study System Description

The Upper Klamath Lake system is located in a volcanic rock dominated graben on the eastern side of the Cascade Range in southern Oregon (Snyder and Morace, 1997). The main water body in this system is Upper Klamath Lake (UKL). At full pool, UKL has a total surface area of 232 km², an average depth of 2.8 m, and a maximum depth of 15.2 m (Wood et al., 2008, Johnson et al., 1985). UKL has a shallow average depth compared to its maximum depth due to the fact that the deepest portion of the lake is confined to a narrow trench that stretches along the western shore of the lake south from Eagle Point to Buck Island (Eldridge et al., 2013). Outside of the trench, over 90% of the UKL is less than 4 m in depth (Hoilman et al., 2008). Just north of UKL in the Upper Klamath Lake system is Agency Lake. Agency Lake is much smaller than UKL with a surface area of 38 km², a maximum depth of 3 m, and an average depth of 0.9 m (Hoilman et al., 2008). Until 2007, UKL and Agency Lake were connected by Agency Straits, a narrow passage between the Klamath Wildlife refuge and the drained wetland area of Tulana Farms. In 2007, sections of the levees around Tulana Farms and the Williamson River Delta were removed, flooding an area of ~30 km². Completed in 2009, this reclamation project connected UKL and Agency Lake, forming one contiguous hydrologic system that consists of UKL, Agency Lake, and many littoral wetlands for a

total area of 305 km² and an average depth of 2.6 m (Eldridge et al., 2013, Wood et al., 2013).

The Upper Klamath Lake system drains an area of 9,842 km² from three main tributaries and multiple artesian springs (Wood et al., 2013; Lindeberg and Wood, 2013; Hoilman et al., 2008). One of the main tributaries, the Wood River, flows into the north end of Agency Lake through the Wood River Marsh (Lindenberg and Wood, 2009). The other main tributaries, the Williamson and Sprague rivers, form a confluence just north of UKL and flow into UKL as the Williamson River through the Williamson River Delta (Lindenberg and Wood, 2009). Water from artesian springs enters the lake from many sources and locations, with the largest input entering the lake through Pelican Bay in the northwest corner of UKL (Lindenberg and Wood, 2009). The main outflow from the system occurs through the Link River Dam located at the southern end of UKL (Lindenberg and Wood, 2009). The Link River Dam was completed in 1921, allowing for water level regulation of UKL (an existing, natural lake) both above and below pre-dam levels (Snyder and Morace, 1997). From the Link River Dam, water flows into the short Link River, ending in Lake Ewauna, which forms the headwaters of the Klamath River. The main features of the lake described above, can be found on the map of the Upper Klamath system in Figure 1.2.

Figure 1.2. Map of the Upper Klamath Lakes system showing key features and study locations

Taxa of Focus For This Study

Aphanizomenon flos-aque (from this point on *A. flos-aquae*) is a filamentous, gas-vesicle-forming, diazotrophic cyanobacteria taxa in the order Nostacales (Reynolds, 2006). In many natural waters (including the study area of this thesis) the microscopic filaments (right side of Figure 1.3) cluster together into larger, macroscopic structures referred to as flakes, clusters, or rafts (middle and left side of Figure 1.3). *A. flos-aquae* produces gas vesicles inside individual vegetative cells and fixes nitrogen in heterocysts (Reynolds, 2006). Heterocysts are the specialized, spherical cells that are slightly larger than the vegetative cells (the larger, oval shaped cells in the left image of Figure 1.3). In the study area for this research, filaments of *A. flos-aquae* form akinetes as conditions change during the fall months, which sink to the sediment and remain there through the winter months. In the study site for this research, *A. flos-aquae* has been identified specifically as *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* Aph K-2 (Li et al., 2000).

Figure 1.3. Images of *A. flos-aquae* collected from Upper Klamath Lake. *A. flos-aquae* rafts in Upper Klamath Lake from klamathvalley.com (left). *A. flos-aquae* rafts under 4X (middle) and 100X (right) magnification from photomacrography.net.

History of A. flos-aquae, Land Use, and Wetlands in Upper Klamath Lake

Since the formation of the lakes in the Klamath Basin over the last 10,000 years, wetlands have been an integral part of the Upper Klamath Lake system (Dicken, 1980; Snyder and Morace, 1997). During the Pleistocene, a much larger and deeper lake existed over much of the basin. Known as Lake Modoc, its extent was much larger than the lakes in the upper basin today. It had an area of 2,839 km² and with a surface level 30 meters higher than the existing Upper Klamath Lake system (Dicken, 1980). In the late Pleistocene it began to shrink, forming many shallow lakes and wetlands in the Klamath basin. Since that time, reed-sedge wetlands and lake fringe areas have been integral to the ecology of the system. Analysis of soil cores from the Upper Klamath Basin show that reed-sedge type peat exists extensively throughout the region, having been formed through thousands of years of plant accumulation in soils that were saturated or under shallow water (Snyder and Morace, 1997). Analysis of lake sediment cores shows that until ~200 years ago, the UKL system was dominated by small benthic diatoms that are synonymous with systems dominated by wetland water (Bradbury et al., 2004; Coleman et al., 2004; Eilers et al. 2004). Altogether, these core sample studies indicate that reed-sedge wetlands had a major influence on water chemistry, nutrient uptake and release, and ecology in the waters of the Upper Klamath Lake system until the 19th century.

Starting in the 1800's, land use practices began to change in the Upper Klamath Basin, leading to drastic changes in lake ecology. With the establishment of settlements and a trade route through region during the mid 1800's, land in the region began to be used for agriculture (Snyder and Morace, 1997) and near-lake wetlands began to be used for wild hay harvest and cattle grazing (Akins, 1970). During this same time period, core

samples analyzed in the studies by Bradbury et al. (2004) and Eilers et al. (2004) show that the UKL diatom community began to shift from small, benthic diatoms to dominance by planktonic diatom taxa that are indicative of a system with increased nutrient input and turbidity (Bradbury et al., 2004). By the late 1800's, large areas of wetlands began to be drained (see figure 1.4) for agriculture and livestock, a practice that continued through much of the first half of the 20th century (Bradbury et al., 2004; Snyder and Morace, 1997). During this time period of large-scale wetland draining, the diatom assemblage shifted incrementally from taxa indicative of wetland dominated waters to taxa indicative of increased phosphorus and silica inputs (Bradbury et al., 2004, Eilers et al., 2004). As the diatom assemblage shifted in the first half of the 20th century, the cyanobacteria taxa *A. flos-aquae*, absent before the 1850's, slowly increased in abundance (Bradbury et al., 2004). By the mid 20th century, *A. flos-aquae* dominated the phytoplankton assemblage in Upper Klamath Lake (Bradbury et al., 2004; Eilers et al., 2004; Phinney and Peak, 1961). This major shift in the phytoplankton assemblage to *A. flos-aquae* directly relates to the time in which the major wetlands adjacent to UKL were drained for agriculture and livestock (Bradbury et al., 2004; Eilers et al. 2004). By the early 1970's, 64% of the wetlands around UKL and Agency Lake had been drained for agriculture and livestock use (Snyder and Morace, 1997) and *A. flos-aquae* was the dominant phytoplankton taxa in UKL and Agency Lake during the summer months (Sanville et al., 1974; Bradbury et al., 2004).

Figure 1.4. Map of UKL and Agency lake showing drained and undrained wetlands. Drained wetlands are accompanied by the year(s) in which they were drained (from Bradbury et al. 2004 who adapted it from Snyder and Morace 1997). Core site indicates location of core sample used in the Bradbury et al. 2004 study.

Quantitative measurements of *A. flos-aquae* in UKL were first reported in the mid 1900's. Bonell and Mote (1942) measured *A. flos-aquae* concentrations to be 20,000,000 cells per cubic meter while studying midge larvae in UKL and Phinney and Peak (1961) describe the results of measurements taken throughout the mid 1950's with *A. flos aquae* counts ~200,000 filaments per milliliter. Subsequent reports show that UKL and Agency Lake consistently exhibited similarly high concentrations of *A. flos-aquae* throughout the

latter half of the 1900's (Kann, 1998; Snyder and Morace, 1997; Sanville et al., 1974).

These reports also indicate that the UKL lake system has also exhibited high nutrient concentrations over that same time period, leading to the current classification as hypereutrophic (Bradbury et al., 2004; Eilers et al., 2004; Hoilman et al., 2008; Eldridge et al., 2013).

While the abundance of *A. flos-aquae* was not reported or quantified until the mid 1900's, the UKL lake system is considered to have been historically productive. Paleolimnological studies of UKL indicate that the system, while dominated by diatoms, had high levels of production for hundreds of years prior to the 1900's (Eilers et al., 2004; Bradbury et al., 2004). Observational accounts also indicate the historical productivity of the system prior to *A. flos-aquae* being identified as the dominant taxa. In a study of fish in the lakes of the great basin, Cope (1883) described the system as “more prolific in animal life than any body of water known to me.” His description includes dense accumulations of mollusks and crustaceans as well as “waters full of vegetable impurities.” Bureau of reclamation reports from 1906 also describe the UKL as dense with organic matter that gives the lake a “decided green appearance” (Phinney and Peak, 1961). Together, the paleolimnological data and the observational accounts indicate that the UKL lake system was historically productive, even before the time of large-scale wetland draining and the dominance of *A. flos-aquae*.

Improving water quality in the Upper Klamath Lake system

Reducing *A. flos-aquae*, in order to improve water quality, has long been a focus in the Upper Klamath Lake system (Snyder and Morace, 1997). However, the unique characteristics of UKL and Agency Lake have made this a difficult task. Both lakes have

extremely high levels of nutrients, shallow depth, variably high winds, and a unique hydrologic regime. The shallow depth and variable high winds lead to internal loading of nutrients from the sediment due to the mixing of the water column all the way to the sediment surface by wind (Kann and Welch, 2005). This makes increasing the concentration of nitrogen to raise the N:P ratio an impractical option because at a high N:P ratio, phosphorus would still not be a limiting nutrient in the system. Removing enough phosphorus from the system to raise the N:P ratio to an acceptable level in UKL would also not be a practical option. TMDL analysis of the system has indicated that even decades long removal of 40% of external inputs of phosphorus would reduce phosphorus levels to ~150 ppb, but the phosphorus levels in the system would still be above eutrophic levels due to internal loading (Wood et al., 2013). Sequestration of phosphorus in the sediment, a common nutrient manipulation technique using aluminum sulfate, would also prove to be difficult due to the size of the system and the hydrologic regime. Since nutrient manipulation alone (in order to reduce the advantage of nitrogen fixation by *A. flos-aquae*) is not a practical option for the UKL system, reducing *A. flos-aquae* dominance by limiting the advantage of buoyancy could be a viable option.

In 2005, Geiger et al. submitted the results of a study to the Klamath Basin Ecosystem Restoration Office in which it was noted that clusters of *A. flos-aquae* were not observed in or next to in-lake wetlands of UKL or wetlands adjacent to the UKL. There are many theories about the low abundance of cyanobacteria in wetland phytoplankton assemblages (*i.e.* shading, resource competition, iron limitation). A key component of these theories is the presence of aquatic humic substances (AHS). AHS are small and large sized organic acids that are derived from the oxidative degradation of

organic material from allochthonous and autochthonous sources in all natural waters (McKnight and Aiken 1998, Perdue 1998). AHS are particularly associated with wetland waters due to the abundance of plant matter and microorganism activity, leading to the characteristic brown or yellow color of waters containing high concentrations of AHS (McKnight and Aiken 1998).

There are many characteristics of waters with high concentrations of AHS that influence phytoplankton abundance, but the most important characteristics for cyanobacteria buoyancy are light attenuation, cation binding, and high conductivity. Other than phytoplankton, AHS are the principle light-absorbing component of natural waters, significantly attenuating photosynthetically active radiation (Lean, 1998). Based upon the conditions presented earlier in this dissertation, on factors that influence buoyancy regulation, wetland waters should not inhibit cyanobacteria buoyancy due to changes in available light. Light attenuation should decrease the available light and trigger vesicle formation in order to decrease cell density and float to the surface. However, the degradation of plant products to form AHS leads to the accumulation of small, organic acids that increase conductivity and lead to osmotic stress. High conductivity is common in the wetlands around UKL (Lindenberg and Wood, 2009), and the osmotic stress that this creates should lead to higher turgor pressure and the inhibition of buoyancy in cyanobacterial cells. Additionally, AHS can also alter ion species availability when the accumulations of small, organic acids form large, cation-binding aggregate structures. (Tipping, 2002; Imai et al., 1999; Perdue, 1998). The cation-binding aggregates have been shown to remove iron from bioavailability (Imai et al., 1999), have been implicated in removing phosphate from bioavailability (Steinberg,

2003), and can change the bioavailability of many monovalent and divalent cations (Tipping, 2002; Perdue, 1998). Thus, the cation binding properties of AHS in wetland waters could reduce ion availability and lower turgor pressure due to a lack of ions for exchange during photosynthesis.

Cation binding is important to consider for buoyant cyanobacteria because cations play key roles in cell turgor pressure, density, and gas vesicle collapse. Allison and Walsby (1981) showed that small increases in potassium (<10mM) to the culture media of buoyant cyanobacteria (*Dolichospermum flos-aquae*) lead to large changes in internal potassium and loss of buoyancy when exposed to high and low light. Kaplan et al. (1989) showed that *Synechococcus* and *Dolichospermum* preferably exchange hydrogen ions within cells with sodium ions outside cells to alleviate the drop in cellular pH due to the uptake of carbon dioxide. Therefore, a change in the availability of cations from lake water to wetland water could lead to changes in buoyancy behavior of cyanobacteria by altering the turgor pressure of cells that contain gas vesicles. Based upon cation binding properties of AHS, increased buoyancy due to a lack of available ions for exchange could possibly keep turgor pressure from increasing. Another possibility is that cation-binding properties of wetland water coupled with the high cation exchange capacity of wetland soils (Mitsch and Gosslink, 2007) make wetland water a viable option for increasing cellular turgor and buoyancy regulation in cyanobacteria due to an increase in wetland water ion content. This creates another gap in the published literature about buoyancy control of cyanobacteria in natural waters that contain high levels of AHS from wetlands. While there are many studies published about the characteristics of AHS in wetland

water, studies of the effects of AHS from wetlands on buoyancy in CyanoHABs are lacking.

Research Rationale

A reduction of buoyancy control and surface accumulation could be a valuable management tool that would complement current cyanobacteria management strategies. Reducing surface accumulations of buoyancy regulating cyanobacteria would allow more light to penetrate the water column, diminishing the advantage of superior cyanobacteria growth in low light (Huisman et al. 1999, Klausmeier and Litchman 2001). Preventing or reducing cyanobacteria surface accumulation would require conditions that lead to an increase in cell density and/or reduction in gas vesicle number and effectiveness. This can be accomplished by establishing conditions that lead to an increase in cellular turgor pressure. Turgor pressure in cells of buoyant cyanobacteria naturally develops as cells accumulate carbohydrates and exchange ions during photosynthesis. As turgor pressure accrues, gas vesicles begin to collapse under the increasing pressure. This process is considered ubiquitous among gas vesicle forming cyanobacteria and has been shown to play a major role in buoyancy regulation of *Aphanizomenon*.

A crucial aspect of increasing cell turgor pressure in order to collapse gas vesicles for management of buoyant cyanobacteria is that turgor pressure can be induced and accelerated. As shown throughout the 1970's and 1980's, Anthony Walsby designed and fabricated a specialized instrument that applied incremental external pressure to a small chamber containing cyanobacteria from which vesicle collapse was determined due to changes in the refractive index. By changing the conditions under which cyanobacteria

were grown or preconditioned, this instrument allowed the calculation and comparison of turgor pressure changes and gas vesicle collapse related to preconditioning. Using this instrument and method, it was shown that increases in light intensity (Walsby, 1971; Oliver and Walsby, 1984) and duration of light exposure (Grant and Walsby, 1977; Thomas and Walsby, 1985) led to an increase in cellular turgor pressure in *Microcystis* and *Anabaena*. These same relationships between light duration, light intensity, and turgor pressure were also shown for *Aphanizomenon flos-aquae* by Kromkamp et al. (1986) using a similar method. Induction and acceleration of cellular turgor pressure and gas vesicle collapse has to occur due to physical and chemical changes to the conditions in which buoyant cyanobacteria are exposed. Addition of potassium ions led to an increase in turgor pressure and vesicle collapse, while addition of a hypertonic sucrose solution led to a decrease in turgor pressure and vesicle collapse (Walsby 1971, Dinsdale and Walsby 1972, Grant and Walsby 1977, Thomas and Walsby 1985). Thus, cellular turgor pressure in buoyant cyanobacteria can be manipulated by altering water chemistry, light exposure, and light intensity, making the disruption of buoyancy regulation and reduction surface accumulation a viable strategy. While induction of turgor pressure by manipulating light intensity and/or duration of light exposure is not a practical management strategy for most systems, altering water chemistry conditions in lakes through the manipulation of ions or wetland water that increase cellular turgor pressure may be feasible for lake-wide management.

In this dissertation, I investigated the influence of wetland water, potassium, sodium, and calcium on cellular turgor pressure and surface accumulation of *A. flos-aquae* collected from UKL using small scale, short-term lab trials and larger scale, near-

lake containers. The main focus in this study was the effect of humic-rich wetland water. I investigated humic-rich wetland water here based upon the observations by Geiger et al. 2005 regarding a lack of *A. flos-aquae* rafts near or in wetlands around UKL, from a study on the link between humic conditions and a reduction in *A. flos-aquae* from UKL using small-scale treatments (Haggard et al. 2013), the characteristics of AHS in wetland water, and from my own preliminary study that showed an increase in turgor pressure when rafts of *A. flos-aquae* from UKL were exposed to water from an adjacent wetland. I also investigated the effect of potassium, sodium, and calcium ion additions because of their involvement in the mechanisms for cation exchange during photosynthesis and previous studies that have linked them to the regulation of buoyancy in cyanobacteria.

To compare the turgor pressure between treatments I developed a method based on Walsby (1971), but used a commercially available pressure chamber for external pressure application and recorded the buoyancy loss of *A. flos-aquae* instead of vesicle collapse. In chapter two, I describe the adapted method and show the results of my laboratory tests that capture the same trend as Walsby's method for manipulations of light intensity, duration of light exposure, and potassium. In chapter three, I describe the small-scale laboratory trials using wetland water and ions. In chapter four, I describe the experiments and results of larger scale near-lake wetland water trials. The final chapter consists of conclusions, additional questions generated by this research, and future areas of research. Hypotheses will be listed at the beginning of each research chapter.

Chapter 2. Buoyancy Loss Methodology

The data in this chapter, along with the data in chapter 3, has been accepted for publication as one submission in *Lake and Reservoir Management*. It has not been published yet, so there is not a full citation. The general citation is listed below:

Rouhe AR and Rueter JG (2018). Reducing surface accumulation of *Aphanizomenon flos-aquae* using wetland water and cations to increase cellular turgor pressure and interfere with buoyancy regulation. *Lake and Reservoir Management*.

Some of the earliest studies on gas vacuoles in cyanobacteria were published by Heinrich Klebahn in a series of articles from 1895 to 1922 (Walsby, 1969; Fogg, 1941). Klebahn determined that vacuoles were filled with gas, that they disappeared when two to three atmospheres of pressure were rapidly applied externally, and that the disappearance was either due to the walls collapsing or cellular fluid rushing in through cracks in the walls (Walsby, 1971; Bowen and Jensen, 1965; Fogg 1941). In Klebahn's experiments, cells were subjected to sudden external pressure that was powerful enough (2-3 atmospheres of applied pressure with a hammer and cork bottle) to collapse all of the vesicles. The incremental pressure application developed by Walsby (1971) allowed for slow and measured pressure increases, making it possible for critical pressures to be determined, which was not a possibility with the classic hammer and cork bottle method

of Klebahn. Incremental pressure application and determination of critical pressures was possible in experiments by Walsby due to a specially designed apparatus with a pressurized cell and a colorimeter to measure the change in scatter as the vesicles collapsed. Gas vesicles have a small refractive index compared to cell solutions, allowing for determination of vesicle content by using a colorimeter (Walsby 1971). Using this apparatus and detection method, percent vesicle collapse could be calculated at each applied pressure increment due to changes in the refractive index.

While the method of applying external pressure in order to compare turgor pressure using pressure collapse curves was effective, its reliance on a uniquely constructed apparatus made its use and application limited. Almost all of the cited literature on this topic is authored by Walsby, either as the first author or as a coauthor. This apparatus must be assembled manually, using parts that are not all commercially available, or not easily adapted from commercially available materials, and consequently has not been used widely by other researchers.

For the experiments in this thesis, I wanted to compare turgor pressure in cyanobacterial cells using externally applied pressure after exposure to various conditions using a simple apparatus that could be more widely applied to lake research and monitoring than the one designed by Walsby. Furthermore, I wanted to be able to visually analyze the position in the water column of the *A. flos-aquae* rafts as the pressure was being applied and to record the pressure at which the *A. flos-aquae* rafts lost buoyancy. You cannot see the position of the algae in the cuvette in Walsby's apparatus. While percent vesicle collapse does relate to water column position, percent vesicle collapse does not directly indicate the pressure at which rafts begin sinking from the

surface, are suspended in the middle of the water column, or are resting on the bottom of the container. To accomplish this type of data collection, I used a commercially available pressure chamber in which I could see and record the position of the rafts at each pressure increment.

The hypotheses of this part of the study were as follows:

1. As light intensity increases, the external pressure required to cause *A. flos-aquae* rafts to sink will decrease and the buoyancy loss curve will occur at lower applied pressures.
2. As the light exposure time increases, the external pressure required to cause *A. flos-aquae* rafts to sink will decrease and the buoyancy loss curve will occur at lower applied pressures.
3. As the external concentration of potassium ions increases, the external pressure required to cause *A. flos-aquae* rafts to sink will decrease and the buoyancy loss curve will occur at lower applied pressures.

Methods

External Pressure Application and the pressure collapse curve

External pressure was applied using a Bergeon 5555/98, a commercially available pressure chamber (shown on right side of the photo in Figure 2.1). Prior to the external pressure application, *A. flos-aquae* rafts, in 70 mL of filtered lake water, were mixed in 100 mL Pyrex beakers under differing conditions (See sections below on preconditioning conditions). After the preconditioning period, the contents of each beaker were placed into the chamber for pressure application. However, the chamber of the Bergeon 5555/98

is small and a 100 mL Pyrex beaker is a very tight fit. To avoid spills and loss of surface rafts while inserting the beaker into the chamber, the contents of each preconditioning breaker were transferred to a slightly smaller 70 mL cuvette that fit easily into the chamber. Transfer was completed by pouring most of the contents of the well-mixed beaker into the cuvette, followed by transfer of any remaining rafts with a transfer pipette. Once the cuvette containing *A. flos-aquae* rafts after preconditioning was placed into the chamber, pressure was applied in 0.2 atm increments.

The buoyancy loss curve for each preconditioning treatment replicate was plotted from counts of the buoyant *A. flos-aquae* rafts (rafts at the surface and suspended in the water column) and rafts on the bottom of the cuvette at each pressure increment. These counts were then used to create a plot of percent buoyant rafts at each pressure. In the experiments by Walsby (1971), percent vesicles were determined using the refractive index of the solution containing cyanobacteria cells after each pressure increment and the refractive index for each applied pressure was then used to calculate the percent vesicles collapsed. Walsby (1971) created pressure collapse curves by plotting the percent collapsed vesicles at each applied pressure. The pressure collapse curve experimental method established the relationship between applied external pressure, turgor pressure, and gas vesicle collapse. The pressure collapse curve method did not relate applied pressure to changes in overall buoyancy of cells or clusters of cells. Because the rafts used in the study presented here were macroscopic and easily seen with the naked eye, external pressure was applied and rafts were counted visually. Gas vesicle collapse and cellular turgor pressure were not directly measured or calculated here because the relationship between applied external pressure, turgor pressure, and gas vesicle collapse

has already been demonstrated for vesicle forming cyanobacteria (Walsby, 1971; Walsby, 1982; Oliver, 1994) and specifically for *A. flos-aquae* (Konopka et al., 1978; Kromkamp et al., 1986). Here, I simply counted the number of rafts that had lost buoyancy and the number of rafts that were still suspended in the water column or at the surface of the water in the cuvette after each pressure increment was applied. That data was then used to plot percent buoyant rafts at each applied pressure to create a buoyancy loss curve. Thus, the buoyancy loss curves in this study represent a practical adaptation of the pressure collapse curves created by Walsby (1971) that simply show the percent of buoyant rafts remaining after each increment pressure.

Preconditioning protocol

Rafts were collected from UKL (see Figure 1.4 for collection site location) at sundown in five gallon opaque buckets, sealed with a lid, transported to the lab, and stored at room temperature overnight. *A. flos-aquae* rafts were mixed in 100 mL beakers that contained a total volume 70 mL. Replicates consisted of 68 mL of treatment water and 2 mL of raw lake water containing *A. flos-aquae* rafts. For the light intensity and duration preconditioning, treatment water consisted of lake water filtered through Whatman GF/F glass microfiber filters. For the external potassium ion concentration preconditioning, treatment water consisted of lake water filtered through Whatman GF/F glass microfiber filters with additions of KCl (See external ion concentration preconditioning section below for volumes and concentrations of additions).

For preconditioning, rafts were transferred to each beaker with a disposable transfer pipette. Each replicate was continually mixed with a Spinwedge® and a stir plate during the entire preconditioning time, which provided very slow vertical and horizontal

movement (due to the triangular shape of the Spinwedge[®]) to fully mix the volume of water in each treatment replicate. Beakers were suspended in a water bath at $20\text{ C} \pm 0.1\text{ C}$. Each beaker was individually illuminated from above with a 20 W 12 V halogen lamp at a distance of about 15 cm, but adjusted accordingly for each lamp in order to achieve the desired surface water light intensity for each replicate. Surface water light intensity was measured for each lamp with a LI-COR 1400 flat sensor. The set-up described here is shown in the image on the left side of Figure 2.1.

Figure 2.1. Left side: Picture of set-up used for preconditioning of *A. flos-aquae*. Water bath constructed of plexi-glass, suspended above stir plates, with individual halogen bulbs above each replicate. Right side: Bergeon pressure chamber used to create the external pressure for analysis of the buoyancy collapse curves.

After preconditioning was complete, each beaker was removed from the water bath, the contents were transferred to a 70 mL cuvette, and dark adapted for 10 min to halt active photosynthesis that would result in turgor pressure changes during external pressure application (MacIntyre et al., 1997). Following the dark-adapted period, the vertical positions of the rafts in the cuvette were recorded using a digital camera in minimal light for determination of surface accumulation and water column position.

Finally, each cuvette was placed into the pressure chamber for external pressure application.

Preconditioning for light intensity and duration

To determine the effects of light intensity, rafts of *A. flos-aquae* were mixed for two hours at intensities of 90, 150, 300, and 500 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$. The two-hour time limit was chosen to ensure that carbohydrate accumulation had little/no effect on water column position (per Walsby 1994, carbohydrate accumulation does not begin to effect buoyancy before two hours, with the onset of buoyancy altering accumulation initializing after two to five hours), and due to results of time manipulations in preliminary studies (data not shown). In preliminary studies, when rafts were mixed for greater than two hours, the size and shape of the rafts observably changed, most likely due to the rafts slowly breaking apart and losing filaments from the mixing water. At times shorter than two hours, rafts required more external pressure to be applied, requiring much more time to complete each pressure test. Therefore, two hours was determined to be the best preconditioning time length for the light intensity treatments. Six replicates were performed for each light intensity treatment.

To determine the effects of the duration of light exposure, rafts of *A. flos-aquae* were mixed at 300 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ for 30, 60, 120, and 180 minutes. In preliminary studies, light intensities below 300 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ led to a very slow response to external pressure application after two hours and light intensities above 300 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ led to very rapid responses, with many rafts already on the bottom of the container after preconditioning. The light intensity that elicited the most consistent response over all four light duration time periods in preliminary studies (data not shown) was 300 μmol

photon $\text{m}^{-2} \text{s}^{-1}$ so it was chosen for this experiment. Six replicates were performed for each light duration time period.

Preconditioning for external ion concentration

To determine the effects of external potassium concentration, rafts of *A. flos-aquae* were mixed for two hours at 300 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. The target potassium ion concentrations of the treatment mixtures were based upon the ion concentration of the most effective wetland water treatment group, which contained 10% wetland water by volume (see the wetland water treatments in chapter three of this dissertation for a full explanation). Concentrations of K^+ in the lake water and the 10% wetland treatments were determined during the wetland water addition experiments in 2015 using a Dionex ICS-5000 ion chromatography system fitted with an Ionpac[®] CS12A analytical column. Lake concentration was determined to be 1.3 mg/L and the 10% wetland water treatment was determined at 2.5 mg/L. To prepare the ion treatment replicates, Hanna Instruments 0.1 M Certified stock solutions of K^+ were added to filtered UKL water. Target concentrations for the low, mid, and high treatment levels were 1.9, 2.5, and 3.1 mg/L respectively to reach target concentrations. Ion experiments were performed in 2016 and potassium concentrations were assumed to be stable from 2015 analysis. Stock K^+ solutions were added to filtered lake water in order to create treatments in 0.6 mg/L K^+ increments. The mid-level treatment concentration was targeted to be equivalent to the 10% wetland concentration.

Curve comparison and data analysis

Buoyancy loss curves were compared graphically by plotting applied pressure on the x-axis and percent floating rafts on the y-axis. Individual replicates were plotted in

order to visualize variability and averages of each preconditioning treatment were plotted to compare treatment levels. Conclusions about differences between treatment replicates and trends between treatment levels were determined from the buoyancy loss curves based upon relative proximity of each curve on the plot to the other treatments (i.e. curves to the left of other curves were considered to have higher turgor pressure and to have had a higher effect from the treatment) and whether or not each curve line was distinct. Buoyancy loss curves were not compared statistically as a whole (see below for description of statistical analysis of points along the curve), they were simply compared graphically to assess congruity with the trends. The precedent of non-statistical curve analysis was established for pressure collapse curves by multiple authors and studies (Walsby, 1971; Walsby, 1975; Thomas and Walsby, 1985; Reed and Walsby, 1985; Walsby and Allison, 1981; Konopka et al., 1978; Kromkamp et al., 1986). These studies constitute a comprehensive list of the comparison studies for the buoyancy loss method described here. Due to the fact that none of the comparison studies for this research analyzed pressure collapse curves statistically, I decided not to analyze the buoyancy loss curves statistically when comparing them to previous studies results. When comparing them within this study, I used the statistical method described below.

To compare the results of external pressure application statistically, three points along the curve were chosen: the applied pressure required to cause the first raft(s) to lose buoyancy and sink to the bottom of the container, the applied pressure required to cause half (at least 50%) of the rafts to lose buoyancy, and the pressure required to cause the last rafts to lose buoyancy. During this research it was observed that after the dark-adapted period, rafts were spread out vertically and horizontally throughout the water

column. Some were floating at the water surface, but many were neutrally buoyant throughout the middle of the pressure container and neutrally buoyant near the bottom of the pressure container. As pressure was applied, the first rafts to lose buoyancy were always the rafts suspended near the bottom of the container. The next rafts to lose buoyancy were the rafts in the center of the water column and the last rafts to lose buoyancy were the rafts floating at the surface after the dark-adapted period. This observation led to the description of these buoyancy loss groups as bottom rafts, middle rafts, and surface rafts. The bottom rafts group indicated the average applied pressure at which the first rafts lost buoyancy. It was used to compare the turgor pressure of the rafts at the bottom of each treatment after the dark-adapted period. The middle group indicated average applied pressure at which 50% of the rafts had lost buoyancy in each treatment. It was used to compare the rafts suspended in the middle of the pressure chamber after the dark-adapted period. While this was a useful comparison of cell turgor pressure between the rafts suspended 0.5-3 centimeters below the water surface in each of the treatments, there were no clear boundaries in the middle of the container (i.e. the top or bottom of the water column) that allowed for this measurement to be easily applied to rafts from a specific location. It was a measurement that applied generally to the turgor pressure of the rafts suspended in the middle of the water column after the dark-adapted period. The surface rafts group indicated the average applied pressure at which the last raft lost buoyancy in each treatment. Due to the fact that the last raft to lose buoyancy in every treatment replicate was at the surface of the container after the dark-adapted period, this pressure was used to compare turgor pressure in the surface rafts. It specifically represented the highest turgor pressure in the surface raft(s) of each treatment replicate.

In the analysis of the curves, these groups were called the bottom (first rafts to lose buoyancy), middle, and surface (the last rafts to lose buoyancy). Statistical comparisons of these three groups indicated key differences between the curves and between the state of the turgor pressure in the cells of the rafts throughout the cuvette after preconditioning.

Graphical representation of buoyancy loss curves and statistical data analysis was conducted using R (R core team 2014). Data was checked for normality using the Shapiro-Wilk test and each data group was compared to a normal distribution using the Kolmogorov-Smirnov test (`ks.test` function in R). Equal variance for each group was compared using the `var.test` function. When assumptions for normality and equal variance were not violated, groups were compared by one-way ANOVA using the functions `aov` and `lm`. The linear model (`lm`) function was used to compare all the groups together and the ANOVA function (`aov`) was used to compare each treatment group with the other treatment groups and the control group (filtered lake water only for ion treatments) individually. When assumptions for normality and equal variance were violated, groups were compared by the Kruskal-Wallis rank sum test using the `kruskal.test` function.

Results

Light Intensity Treatments

The results of the light intensity treatments indicate a direct relationship between light intensity and turgor pressure in the cells of the *A. flos-aquae* rafts in this study. This relationship is supported from the data by the trend of the average buoyancy loss curves (Figure 2.2) and from the trend of the average pressure to cause rafts to lose buoyancy for each light treatment level (Table 2.1). The trend of the buoyancy loss curves was for each average curve to occur at lower applied pressures as the light intensity increased.

Figure 2.2 shows that each curve was distinct for each successive light intensity treatment, so it is clear that less applied external pressure was required to cause buoyancy loss at each successive light increase.

Figure 2.2. Average buoyancy loss curve for each light-intensity treatment

The same trend can also be seen in the average applied pressure required to cause the rafts at the bottom, middle, and surface of each treatment to lose buoyancy. Table 2.1 shows the average pressure to cause the bottom, middle, and surface rafts to lose buoyancy. In each group, as light intensity increased, the average externally applied pressure necessary that caused the rafts to lose buoyancy decreased. The trend of both of these data sets indicates that the increased light intensity in the treatments led to an increase in the turgor pressure of the cells in the *A. flos-aquae* rafts because the amount of external pressure required to cause the rafts to lose buoyancy decreased as light intensity increased, as shown by the pressure collapse curves and each of the three buoyancy loss comparison groups.

Table 2.1. Average applied pressure for each intensity preconditioning treatment category. Pressure has units of atm. Intensity has units of $\mu\text{mol photon m}^{-2} \text{s}^{-1}$.

Intensity	Bottom	Middle	Surface
90	1.40	2.20	2.77
150	1.03	1.97	2.57
300	0.76	1.73	2.23
500	0.23	1.60	2.07

While the averages explained above show a clear trend, it is important to note the variability in each treatment group. Figure 2.3 shows the raw buoyancy loss curve for each trial of each treatment group. By stacking the plots, the variability of each treatment can be visually compared between groups. The low intensity treatment (Figure 2.3A) appears to have the highest variability, and the high intensity treatment (Figure 2.3D) appears to have the lowest variability. Each plot in Figure 2.3 contains a replicate or two that appears to be similar to trials in consecutive treatments, but the overall trend of buoyancy loss at lower applied pressures can still be discerned from the general shift to lower applied pressure as light level increases.

The variability between the average applied pressure for the bottom, middle, and surface raft groups is shown on the boxplots in Figure 2.4. Many of these intensity groups show variability that is distinct from surrounding groups. However, intensity 300 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ for the middle and surface raft groups as well as intensity 90 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ for group surface raft group are highly variable, greatly overlapping with consecutive groups for intensities of 150 and 500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. This variability overlap is exhibited by the lack of significance between these groups when all groups were compared statistically (Table 2.2).

Figure 2.3. Buoyancy loss curves of each replicate for each of the light intensity preconditioning treatments. Plots show replicates at 90 (A), 150 (B), 300 (C), and 500 mol photon m⁻² s⁻¹ (D).

Figure 2.4. Boxplots of data from each light intensity preconditioning group for bottom (A), middle (B), and surface (C) raft groups. Intensity measured in units of $\text{mol photon m}^{-2} \text{s}^{-1}$

Table 2.2 Summary of results for comparisons of the treatment levels from the light intensity preconditioning treatments using the applied external pressure measurements. All groups were compared using ANOVA unless the comparison groups violated equal variance and/or normality assumptions, in which case they were compared using the Kruskal-Wallis rank sum test. Chi-Squared values denoted by χ^2 . Statistical significance at $p < 0.05$ denoted by *.

		Bottom rafts		Middle rafts		Surface rafts	
Intensity	Intensity	F	p-value	F	p-value	F	p-value
90	150	20.86	0.0011*	5.2412 χ^2	0.0221*	2.195	0.169
90	300	34.06	0.0002*	7.8204 χ^2	0.0052*	8.312	0.0163*
90	500	115.6	8.16e ⁻⁷ *	9.8589 χ^2	0.0017*	30.2	0.0002*
150	300	9.412	0.0119*	2.8097 χ^2	0.0937	3.846	0.0783
150	500	84.71	3.34e ⁻⁶ *	9.6585 χ^2	0.0019*	22.96	0.0007*
300	500	22.07	0.0008*	1.3134 χ^2	0.2518	1.033	0.3333

Table 2.2 shows the summary results of the statistical comparisons between each light intensity treatment for the bottom, middle, and surface raft groups. For the bottom rafts comparison, the variability in each treatment was low and there was very little overlap between any of the groups (Figure 2.4). This led to statistical significance ($p < 0.05$) between each light intensity treatment for the bottom rafts. However, not all light intensity treatments led to a statistically significant difference for the middle and surface rafts. The high variability seen in the boxplots for the 300 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ light intensity treatments for both the middle and surface raft groups resulted in a lack of statistical significance ($p < 0.05$) between the 150 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ light intensity treatments for both the middle and surface raft groups as well as the 500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ treatments for both the middle and surface raft groups. The high variability of the 90 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ treatment in the surface raft group also led to its lack of significance ($p < 0.05$) from the 150 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ treatment. However, it is important to note here that the highest light intensity and the lowest light intensity groups were statistically different for bottom, middle, and surface rafts.

The first hypothesis for this study was that as the exposure light intensity increased, the external pressure required to cause *A. flos-aquae* rafts to sink would decrease and the buoyancy loss curves would occur at lower applied pressures. This hypothesis was supported by the results of this study. The average pressure to cause buoyancy loss decreased in all groups (bottom, middle, and surface rafts) as light intensity increased and the average buoyancy loss curves occurred at progressively lower applied pressures as light intensity increased. While there was overlap in the variation between the average applied pressure to cause *A. flos-aquae* rafts to lose buoyancy for each treatment in each group and between the pressure collapse curves for each group, the overall trend was evident from the boxplots (Figure 2.4) and the replicate buoyancy loss curves (Figure 2.3). This data, together with the statistical comparison that showed significance between almost every group (Table 2.2), supports the hypothesis that buoyancy loss would occur at lower applied pressures and that buoyancy loss curves would occur at lower applied pressures in this study when light intensity increased.

Light Duration Treatments

The data of the light duration treatments indicated a direct relationship between exposure time and turgor pressure in the cells of the *A. flos-aquae* rafts. This relationship was supported by the trend of the data from the average buoyancy loss curves (Figure 2.5) and the average pressure to cause rafts to lose buoyancy for each light treatment level (Table 2.3). For the average buoyancy loss curves, the rafts lost buoyancy at lower externally applied pressure as duration of light exposure increased. Figure 2.5 shows that the average buoyancy loss curve for the longest duration time treatment (180 minutes) occurred at the lowest applied pressure, the average curve for the shortest duration time

(30 minutes) occurred the highest applied pressure, and the average curves for the 60 and 120 minute duration treatments occurred at successively intermediate applied pressures.

Figure 2.5. Average buoyancy loss curve for each light-duration preconditioning treatment.

For the average applied pressure to cause the rafts to lose buoyancy the same trend also occurred. The bottom, middle, and surface raft groups decreased as light duration increased in all groups with one exception: middle rafts for 180 minutes. In this case, the average pressure that caused loss of buoyancy for the 180 minute treatment was slightly higher than the 120 minute treatment, which did not follow the general trend. This is thought to be due to the excessive length of the 180 minute treatment. Preliminary studies showed that the rafts in each treatment over 120 minutes began to change in shape and size. Since the 180 treatment is an hour past the boundary limit for physical changes in the rafts, each group (bottom, middle, and surface) showed high variability for the 180 minute treatment (Figure 2.7). The variability did not change the average trend in the surface and bottom groups, but it did change the trend for the middle group due the greater range of water column depth over which the middle group rafts are spread.

However, the difference between the 120 and 180 minute treatments for the middle raft group was so small (0.03 atm), much smaller than the applied pressure increments used in this study, that these values were considered the same for this analysis. The rest of the treatments from each group followed the general trend and required less applied pressure to cause buoyancy loss as light duration increased. Since only the middle rafts in the 180 minute treatment did not fit the general trend but all of the other treatments did, the duration treatment data as a whole was considered to have supported trend of requiring less applied pressure to lose buoyancy with longer treatment time.

Table 2.3. Average applied pressure for each duration preconditioning treatment category. Pressure has units of atm. Time has units of minutes.

Duration	Bottom	Middle	Surface
30	1.03	2.10	2.80
60	1.00	1.93	2.50
120	0.86	1.77	2.40
180	0.47	1.80	2.27

The variability between replicates in each light duration treatment group was not high for the buoyancy loss curves. The stacked replicate plots (Figure 2.6) showed the replicates of each buoyancy loss curve to be similar for each light duration treatment length. While there was variability within each treatment level, the difference between each replicate curve was not large. The 30 and 60 minute treatment replicates showed the lowest variability, most similar results for each replicate curve, and the 120 minute treatments showed the highest variability.

Figure 2.6. Buoyancy loss curves of each replicate for each of the light duration preconditioning treatments. Plots show curves of replicates exposed to $300 \mu \text{mol photon m}^{-2} \text{s}^{-1}$ for 30 (A), 60 (B), 120 (C), and 180 (D) minutes.

Even though the overall trend of buoyancy loss at lower applied pressure as duration of exposure time increased, successive treatments did not always occur at distinctly lower applied pressures. For example, if plotted together, some of the 30 minute and 60 minute curves would be indistinguishable. One of the replicates from the 120 minute treatment would also be indistinguishable from many of the 30 minute and 60 minute curves if plotted together. However, many treatment replicates were distinct. All of the other replicates from the 120 minute treatments were distinct from the 30 minute treatments, all of the 180 minute treatment replicates were distinct from the 30 minute treatments, and all but one of the 180 treatment replicates were distinct from the 60 minute treatments. This indicated that duration time did show an effect on buoyancy loss here, despite the similarity between some intensity treatments, mainly due to clear distinction between the 30 minute (shortest) and 180 minute (longest) treatments.

Boxplots of the duration time treatments (Figure 2.7) showed high variability and crossover between bottom, middle and surface raft groups for many duration time treatment levels. Some groups were virtually identical between treatment levels (30 and 60 minute treatment levels for the bottom raft group). Other groups had high variability, with a great deal of crossover with other groups. The high variability of some treatment levels and the lack of distinction between other groups, made the overall trend of requiring less applied pressure to lose buoyancy with longer treatment time not clear across all of the boxplots. However, the difference between the lowest treatment level (30 minutes) and the highest treatment level (180 minutes) was significantly different ($p < 0.05$), for all three groups (bottom, middle, and surface). Thus, the boxplots of the duration treatments showed high variability, but still support the overall trend.

Figure 2.7. Boxplots of data from each light duration treatment group for the pressure at which the bottom rafts lost buoyancy (A), middle rafts lost buoyancy (B), and the surface rafts lost buoyancy (C). Time has units of minutes.

The high variability of some groups and the lack distinction between many treatments for bottom, middle, and surface rafts was quantitatively confirmed by the statistical analysis. Many of the treatment levels for the bottom, middle, and surface raft groups were not statistically different (Table 2.4). The bottom rafts, virtually identical for three of the four treatment levels, only showed a statistical difference ($p < 0.05$) between the longest treatment time and the two shortest treatment times. The high variability in the 60 minute and 120 minute treatments of the middle rafts led to a lack of statistical difference ($p < 0.05$) between most middle raft treatment levels. The high variability in the 60, 120, and 180 minute treatment levels of the surface rafts, led to a lack of statistical difference ($p < 0.05$) between most surface raft treatment levels too. However, the statistical analysis (Table 2.4) confirmed that the 30 minute and 180 minute treatment were distinct. Each group (bottom, middle, and surface rafts) showed a statistical difference between the 30 and 180 treatment levels ($p < 0.05$). Thus, the statistical comparison of the duration treatments showed high variability, but still supported the overall trend.

Table 2.4. Summary of results for comparisons of the treatment levels from the light duration preconditioning treatments using the applied external pressure measurements. All groups were compared using ANOVA unless the comparison groups violated equal variance and/or normality assumptions, in which case they were compared using the Kruskal-Wallis rank sum test. Chi-Squared values denoted by χ^2 . Statistical significance at $p < 0.05$ denoted by *.

		Bottom Rafts		Middle Rafts		Surface Rafts	
Duration	Duration	F	p-value	F	p-value	F	p-value
30	60	0.028 χ^2	0.8671	4.31	0.0646	7.105	0.0237*
30	120	1.5805 χ^2	0.2087	19.23	0.0014*	10	0.0101*
30	180	4.5732 χ^2	0.0325*	19.29	0.0013*	16	0.0025*
60	120	1.3928 χ^2	0.2379	3.378	0.0959	0.556	0.473
60	180	4.5901 χ^2	0.0322*	2.5	0.145	2.753	0.128
120	180	2.0069 χ^2	0.1566	0.172	0.687	0.769	0.401

The second hypothesis for this study was that as the light exposure time increased, the external pressure required to cause *A. flos-aquae* rafts to sink would decrease and the buoyancy loss curves would occur at lower applied pressures. This hypothesis was supported by the results of this study. The average buoyancy loss curves occurred at progressively lower applied pressures as the time of exposure increased and the average pressure to cause buoyancy loss decreased in almost all groups (bottom, middle, and surface rafts) for each successive increase in time of exposure. The trend toward buoyancy loss at lower applied pressures was clearly visible on the treatment buoyancy loss curves plots and was supported by the significant difference between the pressure to lose buoyancy between the 30 minute treatments and the 180 minute treatments in each group.

Potassium Addition Treatments

The potassium addition treatments consisted of three treatment levels and a control group. All treatment replicates and controls were conducted using a uniform duration (two hours) and intensity ($300 \mu\text{mol photon m}^{-2} \text{s}^{-1}$). Treatments consisted of *A. flos-aquae* rafts suspended in filtered lake water with potassium additions at low, mid, and high treatment levels. The control group replicates consisted of *A. flos-aquae* rafts suspended in filtered lake water only, no potassium additions. Treatments and controls were compared using average buoyancy loss curves, boxplots for group variability analysis (bottom, middle, and surface rafts), and ANOVA for statistical comparison between treatments and controls for each group.

The average buoyancy loss curves of the potassium addition preconditioning treatments showed that the *A. flos-aquae* rafts from each treatment level lost buoyancy at

a lower applied pressure than the control group. All treatment level curves on Figure 2.8 were distinct from the control group curve, did not cross or contact the control curve at any point before all rafts lost buoyancy, and all occurred at lower externally applied pressure than the control curve. This indicated that the cells in the rafts of the treatment groups had a higher turgor pressure than the cells in the rafts of the control replicates. However, the expected trend of buoyancy loss curves occurring at progressively lower applied pressures as the treatment levels increased did not occur. The low and mid treatment curves (respectively labeled low.add and mid.add on Figure 2.8) were almost identical through the entire length of the curve and after 30 percent of the rafts lost buoyancy, the high treatment curve (labeled high.add on Figure 2.8) was virtually the same too. This indicated that the cells in the rafts of all potassium treatment levels did not exhibit an increasing effect in turgor pressure over the range of concentrations used in this study.

Figure 2.8. Average buoyancy loss curve for each potassium addition treatment.

The results of the bottom, middle, and surface raft group comparisons also showed that an increase in potassium concentration did not lead to an increasing effect in turgor pressure. The boxplots in Figure 2.9 for the bottom and middle groups showed very similar variation within each treatment level, making the treatments in these two groups almost indistinguishable from each other. In the surface raft group, the low addition treatment had a slightly higher average applied pressure, but the high variability in the low and high additions made these treatments nonsignificant.

The control groups of the bottom, middle, and surface raft groups had high variability, with a mean and variance that was similar to all of the treatment groups. This made each control group nonsignificant from all potassium treatment levels. The bottom raft controls (Figure 2.9A) had the highest variability, spanning a range from zero (no pressure added) to a level of 0.8 atm. Only one replicate from the mid-level treatment had a higher bottom raft buoyancy loss pressure. While the controls in the middle and surface raft groups (Figure 2.9B and 2.9C respectively) did not span a range that overlapped the treatments to the extent of the bottom raft controls, they overlapped all treatments in each of their respective groups by a large margin. Even though the variance was high in the control group for bottom, middle, and surface rafts, it is important to note that the average pressure to cause buoyancy loss in each control group was lower than each treatment group.

Figure 2.9. Boxplots of data from each potassium addition group. Plots show the range of pressures at which bottom (A), middle (B), and surface (C) rafts lost buoyancy for each treatment and the control (no.add).

Using ANOVA to compare the treatment levels, it was confirmed there was no statistical difference ($p < 0.05$) between any treatment levels for the bottom, middle, and surface raft groups (statistical analysis not shown). Comparison of the treatments to the controls for the bottom, middle, and surface raft groups using ANOVA showed that all groups required less applied pressure than the controls, but none were different enough to be considered significantly different at $p < 0.05$. Considering the average means on Table 2.5 together with the boxplots in Figure 2.9, it is clear that the overlapping variability between the controls and treatments in each group, and the overlapping variability between many of the treatment groups, made all groups and controls appear similar. However, the lower mean applied pressure for all treatment groups and the statistical significance of the mid and high treatments in the surface rafts group at $p < 0.10$ indicates that the addition of potassium did generally lead to higher turgor pressure in cells of *A. flos-aquae* rafts.

Table 2.5. Summary of results for the applied external pressure analysis from potassium additions. Group means represent the average pressure at which bottom rafts lost buoyancy, middle rafts lost buoyancy, and the pressure at which the surface rafts lost buoyancy. Treatment groups with potassium additions were compared to the control group (no ion additions). F-ratio and p-value only shown for the comparison between the control and the surface raft group. * Denotes statistical significance $p < 0.10$.

Potassium Addition Level	n	Bottom rafts (atm)	Middle rafts (atm)	Surface rafts (atm)	ANOVA Results Surface Rafts	
					F	p-value
no.add	8	0.25	1.225	2.00	NA	NA
k.low	8	0.10	0.95	1.85	0.797	0.387
k.mid	8	0.15	1.125	1.7 *	3.15	0.0977
k.high	8	0.10	1.10	1.725 *	3.544	0.0807

The third hypothesis for this study was that as the external concentration of potassium ions increased, the external pressure required to cause *A. flos-aquae* rafts to sink would decrease and the buoyancy loss curve would occur at lower applied pressures.

The expected results for each successive potassium treatment level increase was for the bottom, middle, and surface raft groups to show a successive decrease in the applied pressure required to cause sinking and for each buoyancy loss curve to occur at successively lower applied pressures. The data from the potassium treatments did not show these expected trends, so this hypothesis was not supported by the results. All group averages were below the average of the controls, as was expected (Table 2.5), but the averages for each treatment level were all very similar and did not show the expected progressive decrease in the pressure to cause buoyancy loss as potassium treatment level increased.

Discussion

The results of the light intensity treatments in this study showed similar results to previous studies that used pressure collapse curves to investigate the effects of increasing light intensity on buoyant cyanobacteria. The initial publication that used pressure collapse curves to compare cyanobacteria after exposure to different conditions, Walsby (1971), relied heavily on the comparison between cells in water and cells in a hypertonic sucrose solution. However, light intensity was also compared. The pressure collapse curves for that experiment can be seen on the left side of figure 2.10 (open circles are high light conditions in water, open squares are normal light conditions in water). Pressure collapse curves were also used by Kromkamp et al. (1986) to compare *Aphanizomenon flos-aquae* filaments from laboratory cultures grown in low light. In that study, filaments were exposed to a range of intensities, 75 to 200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, for either two hours or five hours. The results of Kromkamp et al. (1986) are not shown

here, but vesicle collapse and buoyancy loss occurred at lower applied pressure as light intensity increased. While the units of measure, intensity of exposure, and length of exposure are not uniform between Walsby (1971), Kromkamp et al. (1986), and the research presented in this dissertation, the trend was the same: less external pressure was required to collapse gas vesicles and cause buoyancy loss when buoyant cyanobacteria cells were exposed to higher light levels.

Figure 2.10. Comparison of pressure collapse curves from the original work by Walsby (1971) and the research presented here using buoyancy loss curves. Left plot: Walsby 1971 pressure collapse curves from buoyant cyanobacteria cells in low light (squares) and under normal light conditions (circles), solid symbols show results in sucrose solution, open symbols show results in water. Right plot: buoyancy loss curves from the research presented here.

The relationship between exposure duration and buoyancy loss shown in the results of this study have also been shown in previous studies on the effects of light duration on turgor pressure and buoyancy loss in cyanobacteria. Thomas and Walsby (1985) exposed *Micrcystis* cells to $160 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ between one hour and five hours. The resulting pressure collapse curves can be seen on the left side of Figure 2.11, which shows a pressure collapse curve that occurs at a lower applied pressure for the five hour exposure than for the one hour exposure. Kromkamp et al. (1986) also compared exposure time for laboratory cultures of *A. flos-aquae* grown in low light. In that study,

filaments were exposed to a range of intensities from 75 to 200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ for either two hours or five hours. When exposed to 75 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, there was almost no difference in the pressure collapse curves of vesicle collapse between the two and five hour treatments. However, treatments exposed to 100 and 200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ each showed that the pressure collapse curves of the five-hour treatments occurred at much lower externally applied pressures than the two hour treatments. While the units of measure, intensity of exposure, and length of exposure are not uniform between Walsby 1971, Kromkamp et al. 1986, and this study, the trend is the same: less external pressure is required to collapse gas vesicles and cause buoyancy loss when buoyant cyanobacteria cells are exposed to light for longer periods of time.

Figure 2.11. Comparison of pressure collapse curves from Thomas and Walsby (1985) and the research presented here using buoyancy loss curves. Left plot: Thomas and Walsby 1985 pressure collapse curves from buoyant cyanobacteria exposed to 160 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ for 1 hours (circles) and 5 hours (squares), solid symbols show results in sucrose solution, open symbols show results in water. Right plot: buoyancy loss curves from the research presented here.

The most important comparison study for the results presented here on potassium additions is Allison and Walsby (1981). Their results are consistent with the results presented in this thesis, showing buoyancy loss at lower externally applied pressures after additions of potassium (which corresponds to higher turgor pressure after potassium additions) and with buoyancy loss curves occurring at lower applied pressures. However,

Allison and Walsby did not show their pressure collapse curves of the low and high potassium additions in their main experiment, so it is not possible to compare the similarity in the treatment curves found here with that of Allison and Walsby (1981). This would be an important comparison because the results of this dissertation show that all levels of potassium addition resulted in similar buoyancy collapse curves and it would be insightful to know if Allison and Walsby (1981) found similar results using pressure collapse curves. They only show the pressure collapse curves for the controls and the potassium treatments in the secondary experiment. In that comparison, the control curve and the high addition curve are distinct, with the potassium addition curve occurring at much lower applied pressures, which is the same result as presented in this dissertation for the comparison of the potassium treatments with the controls.

While the results of this dissertation show that the *A. flos-aquae* cells in potassium addition treatments lost buoyancy at lower average pressures than the controls, the experiments did not show a graded result from treatment level increases as was expected or as was shown to be the case in the light intensity and light duration trials. This is most likely due to the existence of a concentration threshold that effects turgor pressure in *A. flos-aquae* cells, leaving two explanations for the results of the potassium addition treatments. The first explanation is that all of the concentrations of potassium used in this study were above the threshold and a graded effect would be seen at lower concentrations. The second explanation is that a graded effect may not exist at any concentrations below the threshold, resulting in turgor pressure similar to the controls at any concentration below the threshold. Replication of this experiment using lower concentration solutions and a finer scale of additions (more treatment levels with

narrower differences) could determine which explanation is occurring here. I did not perform these experiments as part of this dissertation because my goal was to add potassium to the level of the target concentration from the most effective wetland water treatment. The concentrations were chosen to mimic potassium concentration levels when wetland water from the Wood River wetland is mixed with UKL lake water to 10% wetland water by volume (aka a 9:1 ratio of lake:wetland water). The wetland water treatment levels will be explained in more detail in the next chapter, but it is important to note here that the range of potassium treatment levels was chosen here to span potassium levels that were measured in the 10% wetland water treatments because it was the most effective wetland water dose for increasing cellular turgor pressure and reducing pressures at which buoyancy loss curves occurred. When choosing these treatment concentrations, determination of a threshold was not the goal. However, this would be a good exploration for future research in order to determine thresholds of the influence of external potassium concentrations.

The final discussion point here has to do with the comparison pressures for bottom, middle, and surface rafts groups. This was an addition to the pressure collapse curve method that could not be completed using the specialized apparatus explained by Walsby (1971). It requires looking at macroscopic clusters while pressure is being applied and provides an important insight into the physiological state of each raft after the dark-adapted period. For this study, the best comparison group was the surface rafts group because the surface rafts can have a wide variety of turgor pressures in order to be buoyant enough to be at the surface, they just need to have a lower limit of turgor pressure in order to still have many gas vesicles intact. This allowed for variation in each

group due to the treatment and the treatment level. Therefore, the surface group allowed for a comparison of the effects of the treatment conditions and not just the turgor needed to maintain a certain depth in the water column. Since reduction of surface accumulation due to treatment conditions is one of the main goals of this study, this made the surface rafts group the most important applied pressure group for this study and was the main pressure group that was used to compare wetland water treatments and ion addition treatments.

Conclusion

The overall goal of the set of experiments presented in this chapter was to adapt the methods of Walsby (1971) for use with a commercially available apparatus and a methodology that could be more widely used, instead of relying on an exclusive apparatus that requires a method based upon a refractive index analysis that cannot be used in the field or easily applied to buoyancy position. Based upon the results of these experiments, I conclude that this adaptation was successful. The overall trends shown by Walsby for light intensity and duration were shown by my research here using the adapted method, which supports this method adaptation as a success and validates its use for further experiments. Furthermore, the experiments of Walsby contained very little replication for each treatment, but the results of this study relied on at least six replicates for each treatment, making the result of the buoyancy loss method more robust than the pressure collapse method.

In addition to the validation of this method using buoyancy loss curves in place of pressure collapse curves, this research also adds the pressure groups bottom rafts, middle

rafts, and surface rafts. While each of these is important for comparing the turgor pressure of cells in rafts at the bottom, middle and top of the water column after the dark-adapted period, the most important of these for the application of this research is the surface rafts group. An overall goal of this research was to be able to use this method to find conditions that will interfere with cyanobacteria buoyancy and reduce surface accumulation. Due to the fact that the surface rafts group directly applies to rafts at the surface after the dark adapted period, a comparison of this group between treatments will provide direct insight into the effect of any treatment turgor pressure of cells in rafts at the water surface.

Chapter 3. Small Scale Treatments

The data in this chapter, along with the data in chapter 2, has been accepted for publication as one submission in Lake and Reservoir Management. It has not been published yet, so there is not a full citation. The general citation is listed below:

Rouhe AR and Rueter JG (2018). Reducing surface accumulation of *Aphanizomenon flos-aquae* using wetland water and cations to increase cellular turgor pressure and interfere with buoyancy regulation. *Lake and Reservoir Management*.

Goal, purpose, and hypotheses

The goal of the small-scale treatments was to investigate the effects of wetland water and cations on cellular turgor pressure, buoyancy regulation, and surface accumulation of *A. flos-aquae* in 70 mL trials with a duration of two hours. The main focus was the effect of wetland water. The purpose of the wetland water laboratory trials was to demonstrate an effective dose for use in the larger scale trials. The cation laboratory trials consisted of potassium, sodium, and calcium additions to concentrations that mimicked the most effective dose of wetland water. The purpose of the cations treatments was to investigate the role of external concentration of potassium, sodium, and calcium in the accrual of turgor pressure, buoyancy regulation, and surface accumulation during the wetland trials. The hypotheses for these sets of experiments were as follows:

1. As the percentage of wetland water increases, the cellular turgor pressure of *A. flos-aquae* cells will increase, resulting in fewer surface rafts, a lower suspension

depth for non-surface rafts, and buoyancy loss curves that occur at lower applied pressures.

2. As the concentration of potassium, sodium, and calcium increases, the cellular turgor pressure of *A. flos-aquae* cells will increase, resulting in fewer surface rafts, a lower suspension depth for non-surface rafts, and buoyancy loss curves that occur at lower applied pressures

For each treatment type (wetland water, potassium, etc), the expectation was for a progressive increase in turgor pressure as the treatment level was increased, for a progressive decrease in surface accumulation, a progressive decrease in suspension depth for non-surface rafts, and a progressive decrease in the pressure at which buoyancy loss curves occurred as the treatment level increased.

Methods

Small-scale lab treatments

A. flos-aquae rafts were collected at the southern end of UKL, far from any influence of wetland water, on the end of Putnams Point (WGS84 coordinates 42.239029, -121.807728), a rocky shore ~0.5 km north of the Link River Dam. Rafts were collected from the surface in amber bottles each day of the study before sunrise to avoid the onset of photosynthesis. Lake water for each treatment was collected each morning of the study with *A. flos-aquae* rafts, filtered through GF/F glass microfiber filters, and stored at room temperature.

A. flos-aquae rafts were mixed in 100 mL beakers that contained a total volume 70 mL, made up of 68 mL of filtered treatment water and two mL of raw UKL lake water containing *A. flos-aquae* rafts (~30 rafts per two mL). Rafts were transferred to each beaker with a disposable transfer pipette. Each trial was continually mixed for two hours with a stir plate, which provided very slow vertical and horizontal movement to fully mix the volume of water in each treatment replicate. Beakers were suspended in a water bath at $20\text{ C} \pm 0.1\text{ C}$. Each beaker was individually illuminated from above with a 20 W 12V halogen lamp at a distance of about 15 cm. Lamp distance was adjusted to achieve $300\text{ }\mu\text{mol photon m}^{-2}\text{ s}^{-1}$ at the water surface. Surface water light intensity was measured for each lamp-beaker pair with a LI-COR 1400 flat sensor.

After the completion of each treatment, I followed an identical process that started when the beaker was removed from the water bath and dark-adapted for 10 minutes. The purpose of the dark-adapted period was to halt active photosynthesis that would result in turgor pressure changes during turgor pressure determination (MacIntyre et al. 1997). After the dark-adapted period, the vertical position of each raft in the beaker was recorded using a handheld digital camera in minimal light for determination of surface accumulation and depth of suspended rafts (see section below for a full description of determination of surface accumulation and water column position). Finally, the contents of each beaker were placed into the pressure chamber for turgor pressure determination.

Surface accumulation and depth of suspended rafts

Surface rafts were visually counted just before the first pressure increment was applied in the pressure container. This was completed by visually counting individual rafts and by verifying the visual counts using the images on the digital video taken after

each dark-adapted period. Rafts were considered to be surface rafts if any part of the raft was touching the surface lens of the water at the top of the container. Depth of suspended rafts was determined using the digital video and the tracking software Logger Pro[®] from Vernier Software and Technology. After digitally marking the center of each suspended raft that was not a surface raft, I calculated the average depth of suspended rafts in each replicate after treatment.

External pressure application and treatment comparison

Cellular turgor pressure was compared by applying external pressure at increments of 0.2 bars (atm) using a Bergeon 5555/98. After each pressure increment, *A. flos-aquae* rafts at the surface, suspended in the water column, and on the bottom of the chamber were counted. Due to the macroscopic size of the *A. flos-aquae* rafts and the transparent walls of the pressure chamber, counts were completed visually and in real time. Counts were then used to create a buoyancy loss curve of percent buoyant rafts after each applied pressure increment. This method was adapted from Walsby 1971, as explained in chapter two.

Wetland water treatments

For the wetland water treatments, rafts of *A. flos-aquae* were mixed with 3, 10, 33, and 100% wetland water by volume. Treatment mixtures consisted of wetland water and lake water filtered through GF/F glass microfiber filters. The control group consisted of *A. flos-aquae* rafts mixed with filtered lake water only. Wetland water was collected from the Wood River Wetland each day of the study at sundown, filtered, and stored at room temperature in amber bottles overnight. Replicates of the wetland water treatment levels and the lake water controls were conducted over two seasons. Twenty-four

replicates of each treatment and controls were performed in June 2014 and 10 more replicates in July 2015.

Ion addition treatments

Treatment mixtures for potassium, sodium, and calcium ions were performed in June 2016. The target ion concentrations of the treatment mixtures were based upon the 10% wetland water treatments from 2015 (the most effective wetland dose from 2014 and 2015). Concentrations of K^+ , Na^+ , and Ca^{2+} for filtered UKL water and the 10% wetland treatments were determined during the wetland water addition experiments in 2015.

Concentration of each ion was determined using a Dionex ICS-5000 ion chromatography system fitted with an Ionpac[®] CS12A analytical column. To prepare the ion treatment replicates, Hanna Instruments 0.1 M Certified stock solutions of K^+ , Na^+ , and Ca^{2+} were added to filtered UKL water to reach target concentrations. For each ion, *A. flos-aquae* rafts were mixed in three treatment levels: low, mid, and high (see Table 3.1 for concentrations). The mid-level concentration was targeted to be equivalent to the 10% wetland concentration for each ion type. To understand the combined effect of these ion types, a fourth combination mixture group was added. The combination mixtures consisted of low, mid, and high treatment levels from all three ions combined together for each treatment level (i.e. low combination treatment consisted of all three low addition treatments together).

Table 3.1. Concentration of Sodium, Potassium, and Calcium in UKL lake water and low, mid, and high treatments after additions. Units in mg/L.

	Lake	Low Add	Mid Add	High Add
Sodium	9.46	12.46	15.46	18.46
Potassium	2.06	2.62	3.22	3.82
Calcium	6.88	7.73	8.58	9.43

Data analysis

Data analysis was conducted in R (R core team 2014). Data was checked for normality using the Shapiro-Wilk test and each data group was compared to a normal distribution using the Kolmogorov-Smirnov test (`ks.test` function in R). Equal variance for each group was compared using the `var.test` function. Data transformation did not improve normality and equal variance for most data sets and groups in this study, so all data was analyzed without transformation. When assumptions for normality and equal variance were not violated, groups were compared by one-way ANOVA using the functions *aov* and *lm*. The linear model (*lm*) function was used to compare all the groups together and the ANOVA function (*aov*) was used to compare each treatment group with the control group individually. When assumptions for normality and equal variance were violated, groups were compared by the Kruskal-Wallis rank sum test using the `kruskal.test` function.

Results

Wetland Treatment Groups

The wetland water treatment results show that cellular turgor pressure in *A. flos-aquae* rafts was highest in the 10% treatment group. The surface rafts required less applied pressure to induce sinking compared to all other treatment groups in both years (Figure 3.1C, 3.1D) and significantly less ($p < 0.05$) pressure than the control group in both years (Table 3.2). The average depth of the suspended rafts was also the lowest for the 10% treatment (Figure 3.2C, 3.2D) and significantly lower ($p < 0.05$) than the control group in both years (Table 3.4). These two pressure measurements indicate that the

average cellular turgor pressure of rafts in the 10% treatment groups was higher than the rafts in all other treatment groups and the control group.

Figure 3.1. Buoyancy loss in *A. flos-aquae* rafts after wetland water treatments. Curves show average percent rafts still floating at each externally applied pressure for the wetland water treatments in 2014 (A) and 2015(B). Points show average applied pressure at which the last surface raft lost buoyancy after wetland water treatments in 2014 (C) and 2015(D). Error bars represent 95% confidence interval and * indicate statistically significant difference from the control group (0% wetland water) at $p < 0.05$.

While the 10% treatment curve in 2014 is similar to other treatment levels (Figure 3.1A) and the 2015 curve crosses over with other treatment levels (Figure 3.1B), curves are distinct from the controls over most of the curve in 2014 and the full length of the curve in 2015. The 10% treatment curves are also distinct from other treatments and the controls over the last part of the curve in both years. Due to the fact that the last part of the curve corresponds to the surface rafts, the buoyancy collapse curves verify the results of the surface raft pressure measurements, which show that the surface rafts in the 10% treatment group had a higher turgor pressure than the surface rafts in the other treatments and the control group.

The cellular turgor pressures in the rafts of the 3% and 33% treatments were similar, lower than the 10% treatment group, and higher than in control group. The applied pressures required to cause the surface rafts to sink in these two treatments were nearly identical in 2014 and very similar in 2015 (Table 3.2). The average suspension position was also almost identical in 2014 (Table 3.4). While the suspension position was not as similar in 2015 between these groups, the average depth of the rafts in the replicates of both groups that year was deeper than the control group and shallower than the 10% treatment group. Altogether, this data indicates that the 3% and 33% treatments induced a similar level of turgor pressure in the cells of the rafts *A. flos-aquae* rafts and that the induced turgor pressure was higher than cells of the rafts in the control group and lower than the 10% treatment.

Table 3.2. Summary of results for the analysis of the average applied external pressure to cause the surface rafts to lose buoyancy after the wetland water treatments. Groups means represent the pressure at which the last raft lost buoyancy in each treatment. Experimental groups with added wetland water were compared to the control group (0% wetland water). * Denote statistical significance at $p < 0.05$.

Wetland Water (%)	n	2014 trials			(n)	2015 trials		
		Mean pressure (atm)	Kruskal-Wallis Rank χ^2	Sum results p-value		Mean pressure (atm)	Kruskal-Wallis Rank χ^2	Sum results p-value
0	24	2.2833	NA	NA	8	2.3	NA	NA
3	24	2.1583	1.945	0.1631	8	2.14	3.6018	0.0577
10	24	2.0957*	5.9766	0.0145	8	1.92*	9.3299	0.0023
33	24	2.1391	3.34	0.0641	8	2.08*	5.5395	0.0186
100	24	2.4333	2.1536	0.1422	8	2.4	1.336	0.2477

Table 3.3. Summary of results of the percent surface raft analysis. Group means represent the average percent of rafts at the surface. Experimental groups with added wetland water were compared to the control group (0% wetland water). * Denote statistical significance at $p < 0.05$.

2014 trials					2015 trials			
Wetland Water (%)	n	Surface rafts (%)	ANOVA results		n	Surface rafts (%)	ANOVA results	
			F	p-val			F	p-val
0	23	23.6	NA	NA	12	34.6	NA	NA
3	22	21.0	0.809	0.373	8	24.8	1.368	0.257
10	22	17.5*	4.53	0.0391	8	18.9*	5.719	0.0279
33	23	21.9	0.364	0.549	8	35.2	0.075	0.787
100	21	27.8	0.923	0.342	8	51.8*	5.624	0.029

The cellular turgor pressure of the rafts in the 100% treatment group was consistently lower than all of other treatment groups and the control group in this study. This is most easily seen on the buoyancy loss curves (Figure 3.1A, 3.1B) where the 100% treatment group collapse curve occurred at distinctly higher externally applied pressures over the length of the curve in both years. This is also clearly evident from the pressure to cause surface raft buoyancy loss (Figure 3.1C, 3.1D, Table 3.2). While the surface raft buoyancy loss pressures were not statistically higher than the control group in either 2014 or 2015, they were higher, which indicated that turgor pressure was reduced by the 100% wetland water treatment.

Table 3.4. Summary of results of the analysis of average suspension position. Group means represent the average depth of rafts not at the surface. Experimental groups with added wetland water were compared to the control group (0% wetland water) *Denotes statistical significance at $p < 0.05$.

2014 trials					2015 trials			
Wetland Water (%)	n	Depth avg. (cm)	ANOVA results		n	Depth avg. (cm)	ANOVA results	
			F	p-val			F	p-val
0	24	2.43	NA	NA	8	2.35	NA	NA
3	24	2.66*	6.193	0.0165	8	2.53	0.583	0.458
10	24	2.83*	19.76	$5.5e^{-05}$	8	2.81*	7.679	0.015
33	24	2.69*	8.883	0.0046	8	2.67	2.738	0.12
100	24	2.49	0.531	0.47	8	2.55	0.675	0.425

The average depth of the suspended rafts was lower than the controls in both years (Figure 3.2, Table 3.4), which indicates that the turgor pressure was higher than in the controls of non-surface rafts of this treatment. Whether or not this treatment induced a lower turgor than the control group for all rafts in each replicate is not clear based upon these measurements, but it is clear for the surface rafts because they consistently sank at lower applied pressures than the control group. Also important here is that the turgor pressure induced by the 100% treatment was far lower than all of the other treatment groups (3%, 10%, and 33%) for all rafts (surface rafts and suspended rafts).

Figure 3.2. Average of percent rafts floating at the surface after the 10 minute dark-adapted period for 2014 (A) and 2015 (B) and average depth of rafts suspended in the water column (but not at the surface) for replicates in each group for 2014 (C) and 2015 (D). Depth shown in plots C and D have units of centimeters below the surface. Error bars represent 95% confidence interval for each group and * indicate statistically significant difference from the control group (0% wetland water) at $p < 0.05$.

The comparison of the surface raft accumulation showed the same overall trend as the turgor pressure comparisons for each treatment group (Figure 3.2A, 3.2B, Table 3.3). The 10% treatments had a lower percentage of rafts at the surface than all of the other treatments and had a significantly lower ($p<0.05$) percentage of surface rafts than the control group in both years. The 100% treatment group had a higher percentage of surface rafts than all of the other treatment groups in both years and was significantly higher ($p<0.05$) than the control group in 2015, with an average of 51.8%. The 3% and 33% treatments groups had a similar average percentage of surface rafts that were below the average of the 100% group in both years and above the average of the 10% group in both years. In 2014, the 3% and 33% treatment groups had an almost identical average percentage of rafts but in 2015 the percentage of surface rafts for the 33% group was much higher, showing a higher average than the control.

Figure 3.3. Profile images of representative replicates from each group after 10 minute dark-adapted period. Images show the very top of the water column and cut off just above the bottom of the buoyancy chamber. Grid lines = 0.6cm.

Overall, the surface comparison shows the 10% wetland water treatment was the most effective at reducing *A. flos-aquae* surface accumulation, the 100% treatment was the least effective, and the 3% and 33% treatments showed an effect between the 10% and 100% treatments. To visualize what this surface raft trend looked like in each treatment, Figure 3.3 includes images of typical replicates from each treatment showing low surface accumulation in the 10% treatment, high surface accumulation in the 100% treatment, and an intermediate level of surface accumulation in the 3% and 33% treatments.

Ion Addition Treatments

The results of the ion addition treatments indicate that all levels of ion additions induced an increase in cellular turgor pressure in *A. flos-aquae* rafts. Buoyancy loss curves for each ion treatment level occurred at distinctly lower applied pressures than the control group (Figure 3.4A, 3.4C, 3.4E, 3.4G). The externally applied pressure to cause surface raft buoyancy loss was lower, on average, than the control group for each ion addition treatment level (Figure 3.4B, 3.4D, 3.4F, 3.4H) and significantly lower ($p < 0.05$) for all combination treatment levels (Table 3.5). Also, the mean suspended raft depth was significantly lower ($p < 0.05$) than the control group for all treatment levels except for the mid-level sodium treatment, which was significant at $p < 0.10$. All of these results indicate that each ion addition treatment induced an increase in cellular turgor pressure.

Figure 3.4. Buoyancy loss in *A. flos-aquae* rafts after ion addition treatments. Curves show average percent rafts still floating at each externally applied pressure for the ion addition treatments of sodium (A), potassium (C), calcium (E), and the ion combination (G). Points show average applied pressure at which the last surface raft lost buoyancy after ion addition treatments of sodium (B), potassium (D), calcium (F), and the ion combination (H). Potassium concentration used for combination plot, but each combo treatment consisted of additions of all three ions. Error bars represent 95% confidence interval and * indicate statistically significant difference from the control group (lake water with no additions) at $p < 0.05$.

The ion concentrations chosen for this study did not result in an overall increasing effect on cellular turgor pressure with successive additions. The buoyancy loss curves for sodium and potassium were almost identical for all treatment levels indicating that their effect on turgor pressure was similar for all treatment levels (Figure 3.4A, 3.4C). The calcium buoyancy loss curves were distinct over most pressures with the highest treatment level occurring at the lowest applied pressures. However, the low-calcium treatment occurred at lower externally applied pressures than the mid-level treatment, and all treatment level curves were almost identical over the last section of the curve, which corresponds to the surface rafts (Figure 3.4E). The buoyancy loss curves for the combination treatment showed that the low treatment level occurred at the lowest applied pressures, which indicates that the low treatment was most effective at inducing turgor pressure. For the applied pressure to cause surface raft buoyancy loss, the mid-level addition was most effective for all ion treatment types except for calcium (Table 3.5). For the depth of suspended rafts, the low-level addition was the most effective for all ion treatment types except for sodium.

The comparison of percent surface rafts showed that for all treatment levels of each ion addition type there were significantly fewer ($p < 0.05$) surface rafts than in the control group (Table 3.5). However, similar to the applied external pressure analysis, there was not a progressive effect based upon the treatment level concentration. Each ion addition type showed similar results between each treatment level (Figure 3.5A, 3.5C, 3.5E, 3.5G). Even though the high-level treatment had the fewest surface rafts for the sodium, potassium, and combination additions, the mid-level treatment had the highest surface raft percent for the potassium and combination treatments and the high-level

treatment had the highest surface raft percent for the calcium treatment, so no overall trend based upon treatment level was evident. Each treatment level led to similar surface raft accumulations and was not significantly different than other treatments levels within each ion addition type (data not shown).

Table 3.5. Summary of results ion addition treatments. Treatment groups with ion additions were compared to the control group (no.add treatment). ANOVA F-ratio and p-value only shown for the surface raft sink group (a.k.a surface raft buoyancy loss). Surface raft (%) and mean raft depth data were analyzed using Kruskal-Wallis Rank Sum. • Denotes statistical significance $p < 0.10$. * Denotes statistical significance at $p < 0.05$.

ion addition treatment	n	surface raft (%)	mean raft depth (cm)	surface raft loss (atm)	ANOVA Results surface raft loss	
					F	p-value
no.add	8	16.11	2.31	2.00	NA	NA
na.low	8	6.64*	2.78*	1.85	1.2353	0.2851
na.mid	7	5.70*	2.58 •	1.7143 •	3.3333	0.0910
na.high	8	4.94*	2.96*	1.8	1.6471	0.2202
k.low	8	4.31*	3.02*	1.85	0.797	0.387
k.mid	8	7.41*	2.81*	1.7 •	3.15	0.0977
k.high	8	3.98*	2.76*	1.725 •	3.544	0.0807
ca.low	7	6.32*	2.75*	1.971	0.028	0.869
ca.mid	7	9.15*	2.67*	1.8286	1.023	0.33
ca.high	7	9.18*	2.73*	1.7429	1.623	0.225
combo.low	7	2.91*	3.01*	1.6857 *	5.519	0.0353
combo.mid	8	3.08*	2.74*	1.7 *	4.846	0.045
combo.high	8	2.74*	2.78*	1.65 *	6.236	0.0256

Even though the ion addition treatments were all similar, and not statistically different from each other, the most effective ion addition type in this study was the combination treatment. Furthermore, the most effect level of the combination additions was the low-level treatment. As a group, the combination treatments resulted in fewer surface rafts, a lower depth of suspended rafts, and loss of surface rafts at lower externally applied pressures when compared to the treatments consisting of only one ion

type (sodium, potassium, or calcium). Within the ion combination group, the low-level treatment showed the most consistent overall effect on *A. flos-aquae* raft buoyancy.

Figure 3.5. Average of percent rafts floating at the surface after ion addition treatments of sodium (A), potassium (C), calcium (E), ion combination (G) and average depth of rafts suspended in the water column after ion addition treatments of sodium (B), potassium (D), calcium (F), and ion combination (H). Depths shown in centimeters below the surface. Potassium concentration used for combination plot, but each combo treatment consisted of additions of all three ions. Error bars represent 95% confidence interval for each group and * indicate statistically significant difference from the control group (lake water with no additions) at $p < 0.05$.

The buoyancy loss curve for the low treatment level occurred at a lower applied pressure than any other ion type treatment level, the suspended rafts had a lower average depth (3.01cm) than all other ion type treatments except the low level potassium treatment (3.02cm), the surface raft percent (2.91%) was lower than all other treatments except the high combination treatment (2.74%), and the applied pressure to cause surface raft buoyancy loss (1.69 atm) was lower than all other treatment levels except the high combination treatment (1.65 atm). These results all indicate that the ion combination treatments induced higher turgor pressure and surface raft reduction more consistently than all other ion type additions and that among the combination treatment levels, the low treatment levels was the most consistently effective at inducing turgor pressure and reducing surface raft accumulation.

Discussion

Wetland Treatment Groups

The results of the small-scale wetland treatments showed that a mixture of wetland water and lake water from UKL containing *A. flos-aquae* rafts led to an increase in cellular turgor pressure, a decrease in raft suspension depth, and a reduction in raft surface accumulation. The most effective mixture was the 10% wetland water treatment. This was an unexpected result for this study because the 100% wetland water treatment was expected to be the most effective. The hypothesis for the wetland water treatments was for surface accumulation of rafts to decrease and for cellular turgor pressure to increase as the concentration of wetland water increased, with a maximum effect to occur in the 100% wetland water treatments. However, the effect of wetland water on *A. flos-*

aquae turgor pressure and surface accumulation did not show a cumulative effect and the 100% treatment group was the least effective, so the hypothesis for the wetland water treatments was not supported by the data.

The fewer surface rafts, lower depth of suspended rafts, and lower applied pressure to cause buoyancy collapse in the 10% treatments imply that the conditions created by the 10% wetland water treatment led to *A. flos-aquae* buoyancy optimization in the water column. Optimization through buoyancy regulation, floating and sinking to adjust water column position, is dependent on existing conditions and influenced by many factors (Walsby, 1994). One of those factors is phosphate availability because it has a direct effect on the formation of gas vesicles (Oliver, 1994). Konopka et al. (1987) showed that gas vesicle content in phosphate limited cultures of *A. flos-aquae* remained low, even when light intensity was high. When phosphorus concentration was increased, vesicle content also increased. Kromkamp et al. (1989) showed the same relationship between phosphorus and gas vesicles for cultures of *Microcystis aeruginosa* when phosphorus was limiting and when phosphorus concentrations were increased. This is important for the results presented here because the wetland water used for this study had a much higher content of soluble reactive phosphorus (SRP) than lake water at the collection site. Measurements of SRP from UKL at the collection site averaged 15.83 µg/L compared to an average of 197.1 µg/L for the 10% treatment and 1340 µg/L for the 100% wetland water treatment. I did not measure gas vesicle content or synthesis in this study, but this drastic difference in SRP between the lake water and wetland water indicates that gas vesicle formation could have been triggered by the addition of wetland water. However, the process of triggering and producing gas vesicles takes up to 24hrs to

take full effect (Walsby, 1994), so it is unlikely that this factor influenced buoyancy and water column position of *A. flos-aquae* rafts in this study due to the two hour length of each treatment.

Another factor that influences sinking and floating is light attenuation. Gas vesicle construction is repressed when light intensity is high (Thomas and Walsby, 1985) and triggered when light intensity is low (Oliver, 1994). This could be an important factor in this study because wetland water greatly attenuates light due to high concentrations of dissolved organic compounds (Eloranta, 1999) that can greatly increase the extinction coefficient and lower overall irradiance (Lean, 1998). Based upon this relationship, addition of wetland water to the treatments in this study led to an increase in light attenuation and a decrease in overall irradiance in the treatment beakers. Increased surface accumulation of *A. flos-aquae* rafts in the 100% wetland treatments of this study could have been influenced by this effect, but similar to the role of SRP in surface raft accumulation, the level of influence is unclear for this study due to the two hour length of time for each treatment. Triggering and constructing gas vesicles due to light attenuation can take up to 24 hours (same overall mechanism that is triggered by high phosphate), so the length of the treatments here makes it likely that other factors were more influential to the results of this study.

Reduced irradiance also influences sinking and floating by decreasing cellular turgor pressure (Walsby, 1994). Two main processes that lead to increases in turgor pressure in cells of cyanobacteria, carbohydrate accumulation and ion exchange, are both driven by photosynthesis (Kromkamp et al., 1986; Oliver, 1994). Reduction in overall irradiance has been shown to decrease cellular turgor pressure in many species of

buoyant cyanobacteria (Thomas and Walsby, 1985; Oliver, 1994) and specifically in *A. flos-aquae* (Kromkamp et al., 1986). For this study, the results support that a decrease in cellular turgor pressure due to reduced irradiance could have occurred in the 100% wetland treatments due to absorption by the humic substances in the dark brown water. Average cellular turgor pressure of rafts in the 100% treatment groups was lower than all other treatments and the controls for all turgor pressure analyses in 2014 and 2015. The results also show that this did not occur in the 3, 10, or 33% treatment groups because the average cellular turgor pressure for all of these groups was higher than controls in both years of the study. Due to the fact that changes in irradiance have been shown to influence cellular turgor pressure in *A. flos-aquae* in two hours in other studies (Kromkamp et al., 1986), it is likely that the reduction in irradiance due to additions of wetland water had an effect on turgor pressure in this study. The effect, however, is not definitive since there are other factors that also influenced cellular turgor pressure in this study.

The last factor to be discussed here, that influences cellular turgor pressure in cyanobacteria, is ion exchange. This is an essential component of cyanobacteria photosynthesis because it facilitates the uptake of carbon and the efflux of hydrogen ions (Kaplan et al., 1989). Cyanobacteria release hydrogen ions from cells during photosynthesis in order to alleviate pH imbalance through a Na^+/H^+ antiport protein (Blumwald et al., 1984). This exchange is considered to occur in all cyanobacteria taxa, and has been specifically measured for *Synechococcus* and *Dolichospermum* (Sherer et al., 1988; Kaplan et al., 1989). Cyanobacteria also bring monovalent ions (i.e. sodium and potassium) into cells through symport proteins along with bicarbonate ions in order to

supply carbon for photosynthesis (Kaplan et al., 1984). Both of these processes accumulate ions in cyanobacteria cells during active photosynthesis, leading to an increase in turgor pressure. These are a key processes for this study because they can be influenced by the external concentration of sodium and potassium ions and they can occur over two hours of time. Cellular turgor pressure has been shown to change due to manipulations of the extracellular concentration of these ions in experiments lasting two to five hours (Allison and Walsby, 1981; Reed and Walsby, 1985). Therefore, ion exchange is a likely influence on the increase in turgor pressure of *A. flos-aquae* cells in this study, making the results of the individual and combination ion treatments important to consider.

Ion Addition Treatments

The hypothesis for the ion addition treatments was for surface accumulation of rafts to decrease and for cellular turgor pressure to increase as the concentration of ion additions increased, showing a maximum effect in the high addition treatment for each ion type. Similar to the results of Allison and Walsby (1981), extracellular addition of cations led to an increase in cellular turgor pressure of *A. flos-aquae* in the ion addition treatments. This occurred for all of the individual ion additions (sodium, potassium, and calcium) and the combination treatments. However, turgor pressure did not increase progressively with ion concentration as was expected, so the hypothesis for the ion addition treatments was not supported by the results. Many of the ion types showed similar results for all treatment levels, despite an increase in concentration. This lack of a progressive trend is seen best in the pressure collapse curves of potassium and sodium (Figure 3.4A, 3.4C) which show a clear separation between the curves of each addition

group from the control group but not a clear separation between the curves of the treatment groups. The most distinct separation between treatment level pressure collapse curves occurred in the combination additions for this study, but the separation didn't follow the expecting trend either. The low-level addition showed the lowest average collapse pressure instead of the high-level addition. Taken all together, the low-level ion addition treatments showed similar results to the mid-level and high-level addition treatments, indicating that a threshold level may exist at or below the low-level concentration additions used in this study.

The ion addition treatments were included in this study to elucidate the mechanism that led to increased turgor pressure and decreased surface accumulation in the wetland water treatments. They are important for this study due to the likely influence of ion exchange on cellular turgor pressure in the wetland water treatments. The concentrations of sodium, potassium, and calcium in each of the low-level additions in this study were targeted to be half way between the lake water and the 10% wetland water treatment. As stated in the methods, the mid-level ion addition treatments were targeted to have the same concentrations as the 10% wetland water addition treatments from 2015. My calculations for the concentration of the additions were based upon concentrations determined from lake water at the collection site in July of 2015. Sample ion concentrations at the collection site were determined to be higher in 2016 than they were in 2015. This difference led to the concentration of the low-level treatments in the ion addition treatments to be more comparable than the mid-level treatment additions to the ion concentrations in the 10% wetland water additions in 2014 and 2015.

The proximity of sodium, potassium, and calcium concentrations between the low-level ion additions and the 10% wetland water mixture is important here because the low-level addition combination treatment and the 10% wetland treatments were the most effective treatments. When the buoyancy collapse curves between the 2015 wetland water treatments (Figure 3.1B) and the combination ion treatments (Figure 3.4G) are compared, the low-level combination treatment occurs at lower applied pressures and the mid-level and high-level treatments occur at slightly higher applied pressures. This is similar to the trend of the 10% and 33% treatments for the wetland water additions, so the concentration of sodium, potassium, and calcium ions most likely influenced the cellular turgor pressure and surface accumulation results of *A. flos-aquae* rafts in the wetland treatments of this study. However, the effect shown in the three ion addition treatments was not analogous to the effect shown across the wetland water treatments for the surface raft accumulation and surface raft turgor pressure. The combination treatments were virtually identical for surface raft percent and the pressure to cause surface raft buoyancy loss. This was different from the wetland water treatments, which showed a discernable difference between the 3, 10 and 33% wetland water treatments for surface raft accumulation and surface raft turgor pressure. This shows that ions play a role in the effectiveness of the 10% wetland water treatment but that there are more factors than just these three ions that are leading to an increase in turgor pressure and a reduction of surface raft accumulation.

Conclusion

The purpose of the wetland water laboratory trials was to determine the most effective dose for use in the larger scale trials. The buoyancy loss curves, the surface accumulation enumeration, and the depth of suspended *A. flos-aquae* rafts data all support the 10% wetland water treatment as the most effective dose. While this is an unexpected result for this study, due to the expectation that the 100% treatment would be the most effective dose, it represents a practical target dose for use in larger scale trials. However, from the data collected during the small-scale wetland treatments, the exact mechanism that led to this result is unclear. What is clear is that introducing wetland water changed the light environment and the external cation concentration of each treatment, both of which are known to directly influence turgor pressure in buoyancy regulating cyanobacteria cells.

The purpose of the cation treatments was to investigate the role of external concentration of potassium, sodium, and calcium in the accrual of turgor pressure during the wetland water treatments and its effect on buoyancy regulation and surface accumulation. The mid-level doses for each ion type were targeted to emulate the 10% wetland water treatment concentration and the low-level and high-level doses were targeted to be just above and just below the 10% wetland water treatment (but not targeted to emulate the 3% and 33% wetland water treatments). While the results of all treatment levels indicated that increasing the external concentration of each of the included ion types led to an increase in *A. flos-aquae* cellular turgor pressure, the results did not indicate that the effect was intensified as concentration increased. In fact most treatment levels exhibited similar results for all individual ion treatments. The treatment

level the showed the most consistent results was the low-level combination treatment.

Due to the fact that this treatment was the closest to emulating the 10% wetland water treatment for ion concentration, it indicates that a combination of these three ions influenced the accrual of turgor pressure in the wetland treatments. However, the results for surface raft accumulation and the average depth of suspended rafts in combination treatments did not reveal the same trend demonstrated in the wetland water treatments. If taken altogether, this data indicates that the ion concentration in the wetland water played an role in the buildup of turgor pressure *A. flos-aquae* cells in the 10% wetland water treatments in the small-scale treatments of this study, but the effects of ions alone does not explain the extent of the results that support the 10% wetland water treatment as the most effective dose. The relationship between ions and turgor pressure will be important to analyze in the larger scale trials.

Chapter 4. Large Scale Treatments

Goal and Purpose

The goal of the large-scale treatments was to investigate the effects of the wetland water treatment levels used in the small-scale treatments on *A. flos-aquae* rafts in larger-scale replicates that were closer to actual lake conditions. To accomplish this, treatments were put into containers that could hold 300-liters of water and placed next to the levee between Agency Lake and the Wood River Wetland. The treatment containers were not artificially mixed and not covered in order to capture the naturally fluctuating conditions experienced by *A. flos-aquae* rafts in Agency Lake. The purpose was to compare the buoyancy measurements of *A. flos-aquae* rafts taken for the small-scale treatments with the same measurements taken from *A. flos-aquae* rafts in large-scale treatments.

General approach to large-scale trials

The main focus was on the most effective treatments from the small-scale laboratory study with consideration of practical wetland water doses that could be applied on a large scale. Since the 100% treatment was the least effective treatment in small-scale laboratory study, and represents an impractical lake-wide treatment level, it was not used as a treatment level in the large-scale study. The 3% and the 33% treatment levels showed intermediate levels of effectiveness in the small-scale study, with similar results for most measurements. The 33% treatment represents an impractical lake-wide treatment level but a 3% lake-wide treatment level would be feasible, so the 3% treatment level was include in the large-scale treatments but the 33% treatment level was not. In order to understand the effects of wetland water on *A. flos-aquae* buoyancy on a finer scale, treatments of 5%, 8%, 12%, and 15% were included in the large-scale study. The

target treatment was 10% wetland water and the additional treatments were included to determine the effectiveness of treatments just below and above the 10% target. The hypotheses for this set of experiments were as follows:

1. The 10% wetland water treatment will have a maximum effect on the cellular turgor pressure of *A. flos-aquae* cells resulting in fewer surface rafts, a lower suspension depth for non-surface rafts, and buoyancy loss curves that occur at lower applied pressures.
2. The treatments of wetland water below 10% will have an increasing effect on the cellular turgor pressure of *A. flos-aquae* cells resulting in fewer surface rafts, lower suspension depths for non-surface rafts, and buoyancy loss curves that occur at lower applied pressures as wetland water increases.
3. The treatments of wetland water above 10% will have a decreasing effect on the cellular turgor pressure of *A. flos-aquae* cells resulting in an increase in surface rafts, shallower suspension depths for non-surface rafts, and buoyancy loss curves that occur at higher applied pressures as wetland water increases.
4. The 10% wetland water treatment will have an increasing effect on the cellular turgor pressure of *A. flos-aquae* cells over time in the long term trial of this study, resulting in buoyancy loss curves that occur at progressively lower externally applied pressures.

Methods

Large-scale treatments set-up

The large-scale treatments were conducted in a flat area between the southwest corner of the Wood River Wetland and the north shore of Agency Lake at WGS84 coordinates 42.582524, -121.970948. Three sets of 2-day trials were implemented over nine days in June 2016. The first trial was initiated on June 21 and completed on June 23. The second trial was initiated on June 24 and completed on June 26. The third trial was initiated on June 27 and completed on June 29. Along with the three 2-day trials, one 8-day trial was implemented on June 21 and completed on June 29. All trials were set up by 12:00 PM on the initial day and final collections were completed at 12:00 PM on the final day, for a duration of exactly 48 hours for each 2-day trial and 192 hours for the 8-day trial.

Figure 4.1. Images of the large-scale study site and set-up. View of bin orientation looking north with wetland directly behind the bins (left). Close up view wetland water collection site located a 20 meters to the north of the bins (middle). View of lake water collection site on the north end of Agency Lake, seen by looking south from the site of the bins (right).

Treatments were conducted in HDPE bins and organized into a 3x4 grid about one meter apart (Figure 4.1). Total treatment volume for each bin was 300 liters. Bins were drained, rinsed, and scrubbed between each trial. Wetland water for each wetland

water treatment was collected from the Wood River Wetland pump station platform (shown in middle image of Figure 4.1) in 19-liter buckets (a.k.a. 5-gallon buckets), carried to the bins, measured using a 3.8-liter graduated measuring cup, and added to each bin to meet the correct treatment percentage for a total volume of 300 liters. When added to each container, wetland water was poured through Nitex mesh (64 micrometer pore size) to remove zooplankton and debris. Water from UKL containing *A. flos-aquae* rafts was collected at the southern end of UKL, far from any influence of wetland water, on the end of Putnams Point at WGS84 coordinates 42.239029, -121.807728. UKL water with rafts was collected in 19-liter opaque buckets before sunrise on the first day of each trial, capped with an opaque lid, and transported to the study site. 38 liters (two, 5-gallon buckets) of UKL water with *A. flos-aquae* rafts was added to each treatment container and the 0% wetland water control container for each of the three trials. Lake water for each treatment and lake water control container was pumped from the north shore of Agency Lake (shown in the left image of Figure 5.1). When added to each container, water was poured through Nitex mesh (64 micrometer pore size) to remove zooplankton and debris.

Each 2-day trial consisted of *A. flos-aquae* rafts in wetland water treatments of 3, 5, 8, 10, and 12%. All three sets of 2-day trials was initially planned to include a 15% treatment container, but the drain plug broke on one of the containers after the first trial. With no backup or extra container, the 15% trial was excluded from trials two and three since it represented the least practical lake-wide treatment level. Included in each trial was a 0% wetland water treatment container that consisted of 38 liters of UKL lake water containing *A. flos-aquae* rafts and 262 liters of Agency Lake water. The 0% wetland

water treatment was the control group to which *A. flos-aquae* turgor pressure, surface accumulation, and suspension depth from the wetland water treatment containers were compared in order to measure the effects of each wetland water treatments on *A. flos-aquae* buoyancy. Also included in each trial was a wetland water control and a lake water control. The wetland water control container consisted of 300 liters of wetland water for comparison of changes in water chemistry and nutrients of the wetland water in the container with the wetland water in the Wood River Wetland. The lake water control container consisted of 300 liters of Agency Lake water for comparison of changes in water chemistry and nutrients of the lake water in the container with the lake water in Agency Lake. After each trial, the location of each treatment container was moved to a different row in the 3x4 grid so that each treatment was located on a different row along the N-S axis. This was done to minimize spatial effects due to shading and temperature that may have been artificially induced by the grid of containers. Each treatment consisted of one replicate per trial for this study, there was no treatment replication within each trial.

The 8-day trial consisted of only two treatment containers. A 0% wetland water treatment and a 10% wetland water treatment. Each of these treatments was set up in the same manner as the 0% and 10% treatments as described above for the 2-day trials. These two containers were not moved during the study as to not disturb the buoyancy behavior of the *A. flos-aquae* rafts. The 0% container was located on the northeast corner of the grid and the 10% container was located in the center of the southern row of the grid. The 8-day trial containers were set up with the first 2-day trial on June 21 and were completed at the same time as the third 2-day trial on June 29.

Nutrient sample and in situ data collection

For each day of the 2-day and 8-day trials, temperature, dissolved oxygen, conductivity, and pH were collected *in situ* using a Eureka Manta. The Eureka Manta was calibrated daily using pH standards of 7 and 10 and 131.3 μ Siemen conductivity standard. Dissolved oxygen was calibrated using the protocol from Eureka for the optical DO probe. *In situ* measurements were taken beginning at 12:00 PM and ending around 2:00 PM (about 10 minutes per container) a depth of 25 cm, the midpoint of the water column in each container.

Nutrient samples were collected from just below the surface in 0.5 L, wide-mouth, amber Nalgene bottles at a depth of 10 cm. Nutrient samples were collected at 12:00 PM, just before *in situ* water quality data was collected for each bin. Raw water samples for TN and TP determination were placed into 250-mL, wide-mouth, HDPE bottles and frozen. Water for all other measured water quality parameters were filtered using a Nalgene hand pump and Whatman GF/F glass microfiber filters. After filtration, samples to be determined for NH_4 , NO_2/NO_3 , and SRP, were put into 125-mL, wide-mouth, HDPE bottles and frozen. Samples for Ca^{2+} , Na^+ , and K^+ were placed into 80-mL wide-mouth, HDPE bottles after filtration and frozen. Samples for non-purgeable organic carbon (NPOC) were placed into 50-mL, glass vials, acidified to a level between pH 2-3 with concentrated HCl, covered with Parafilm, mixed to ensure even acidification, and refrigerated. Measured water chemistry parameters, preparation, and preservation method, and analysis method are summarized on Table 4.1.

Table 4.1. Summary of water chemistry methods used to analyze water samples.

Nutrient	Abr.	Preparation	Preservation	Method
Kejdahl Total Nitrogen	TN	Unfiltered	Freezing	EPA 351.2 version 2.0
Ammonia	NH ₄	Filtering	Freezing	EPA 350.1 version 2.0
Nitrate/Nitrite	NO ₂ /NO ₃	Filtering	Freezing	EPA 353.3 version 2.0
Total phosphorus	TP	Unfiltered	Freezing	Standard Methods 4500-P-F (18 th & 19 th ed.)
Soluble Reactive Phosphorus	SRP	Filtered	Freezing	Standard Methods 4500-P-F (18 th & 19 th ed.)
Dissolved Organic Carbon	NPOC	Filtered	Acidified and refrigerated	EPA 415.1, EPA 9060A, Standard Methods 5310B
Calcium	Ca ²⁺	Filtered	Frozen	ISO 14911:1998
Sodium	Na ⁺	Filtered	Frozen	ISO 14911:1998
Potassium	K ⁺	Filtered	Frozen	ISO 14911:1998

Nutrient determination

Nitrogen and phosphorus chemistry parameters (TN, NH₄, NO₂/NO₃, TP, and SRP) were determined using a Smartchem 170/200 Westco Spectrophotometer and autoanalyzer from Unity Scientific. Standards were prepared based upon Smartchem method specifications and the concentration of each sample was determined using a 7-point standard curve. Cations (Ca²⁺, Na⁺, and K⁺) were determined using a Dionex ICS-5000 ion chromatography system fitted with an Ionpac CS12A analytical column. Standards were prepared using Dionex six Cation-II standard from Thermo Scientific. Water samples were determined for cations using a 7-point curve. NPOC was determined using a Shimadzu TOC-V CSH/CSN total organic carbon analyzer connected to an ASI-V autosampler. Standards were prepared based upon Shimadzu method

specifications and the concentration of each water sample was determined using a 5-point standard curve. Collection and analysis methods listed on Table 4.1 are cited by each manufacturer as the basis for the method for each industry protocol.

A. flos-aquae raft collection and buoyancy analysis

Rafts for buoyancy analysis were collected each day of the study at 12:00 PM before *in situ* and water chemistry samples were collected. Rafts were collected from the center of each container at the surface in 0.5L, wide-mouth, amber, HDPE bottles. Rafts were kept in the dark until analysis. For buoyancy analysis, rafts were transferred to 70-ml square cuvettes and then measured for surface rafts, suspended rafts, and buoyancy loss. Protocol for surface raft, suspended rafts, and buoyancy loss measurements were identical to the steps followed for buoyancy measurements of the small-scale trials after the dark-adapted period (as explained in chapter 2).

Data analysis

Data analysis was conducted in R (R core team 2014). Data was checked for normality using the Shapiro-Wilk test and each data group was compared to a normal distribution using the Kolmogorov-Smirnov test (`ks.test` function in R). Equal variance for each group was compared using the `var.test` function. Data transformation did not improve normality and equal variance for most data sets and groups in this study, so treatment groups were compared for buoyancy and water chemistry using ANOVA or Kruskal-Wallis rank sum testing without transformation. When assumptions for normality and equal variance were not violated, groups were compared by one-way ANOVA using the functions `aov` and `lm`. The linear model (`lm`) function was used to compare all the groups together and the ANOVA function (`aov`) was used to compare

each treatment group with the control group individually. When assumptions for normality and equal variance were violated, groups were compared by the Kruskal-Wallis rank sum test using the `kruskal.test` function. For Principle Component Analysis (PCA) of treatments based upon water chemistry parameters, data was log transformed to match the log transformation of the pH data and standardized to account for different measurement units. Data was standardized using the *scale* function. The principle component analysis was performed using the function *prcomp* and plotted using the *biplot* function.

Results

The purpose of completing the 2-day trials consecutively was to minimize fluctuations in weather, temperature, and source water chemistry. However, the nine days of the study were quite variable for weather and temperature. The days leading up to the study were sunny with temperatures around 30°C. During trial 1, the first three days of the study, the weather was cloudy with a slight breeze from the north and light rain on day two (June 22). The containers did not receive much direct sunlight due to cloudy skies and the air temperature was in the low 20's (°C). During the second three days, trial two of the study, the weather shifted from cloudy and cool to sunny and warm. The containers received full sun throughout the last day of trial two and the air temperature was around 30°C. The last three days of the study, trial three, was very warm with full sun, temperature above 30°C each day, and no wind.

The differences in the weather during each of the three 2-day trials had a considerable effect on the temperature of the containers. Figure 4.2 is a summary of the

temperature of the containers and source water for each trial (all temperature data is listed in Appendix A). Due to the sunny days and higher temperatures before the first trial, the containers at the beginning of trial one were around 22 °C but dropped almost 2 °C per container over the length of the trial. Trial two showed the opposite trend, starting with lower temperature due to the cooler weather at the beginning and gaining up to 5°C over the 2-day trial. Trial three showed little or no temperature changed during the 2-day trial. Temperatures in trial three started high for each container and remained high throughout the length of the trial.

Figure 4.2. Source water and 2-day trial water temperature. (A) Temperature of source water at collection time for each trial. (B) Average water temperature in treatment containers at set-up. (C) Water temperature in treatment containers at the end of each 2-day trial. (D) Change in temperature for each treatment over the 2-day trial. Bars in each cluster represent trial 1 (open), trial 2 (hatched), and trial 3 (solid). Y-axis for (A), (B), and (C) is temperature in °C. Y-axis for (D) is change in °C.

Overall, the changes in weather and temperature during the nine days of this study created a different set of conditions for each of the three 2-day trials. During the first 2-day trial, the bins were mixed by a breeze, received little direct sunlight, and had consistent temperatures in the low 20's (°C). The second 2-day trial started with cloudy weather and low temperatures that drastically changed to direct sunlight and much higher temperatures in the second day. All treatments showed large changes in temperature between from start to finish. The third 2-day trial received constant directly sunlight during the day and high temperatures from start to finish. This treatment showed almost no temperature change from start to finish in all treatment bins.

Surface accumulation and suspension depth for the 2-day trials

Surface accumulation and suspension depth measurements were made from the 70 mL cuvettes used to measure buoyancy after the rafts were transferred from the amber collection bottles. Due to the variability in conditions between each trial, the trials were compared individually and then compared using treatment averages for all trials together. The visual analysis of the images from each trial showed that the surface accumulation and suspension depth varied with each trial. Images from trial one showed many rafts in the top half each cuvette for all treatments and the control, with a few large rafts suspended in the center of the 10% and 12% treatment cuvettes. After trial two, all treatments contained rafts suspended in the lower half of the cuvettes, with a few rafts suspended in the upper half of the cuvette for the 8%, and 10% treatments, and the control. The third trial images were much different than the first two trials, with 8% and 10% treatments with very few rafts suspended in the upper half of the cuvette while all other treatments and the control showed an even spread of rafts throughout each cuvette.

From the average suspension depth (Table 4.2), the 8% and 10% treatments are similar in all three trials and the control group was highly variable. In trial one, the 8% and 10% treatments had the deepest average, the control had the smallest average, and the 3, 5, and 12% treatments were between the control and the 8% treatment. After the second trial, the 5% and 12% treatments had a higher percentage of rafts near the surface than the 8%, 10% and the control, leading to a lower average suspension depth. After the third trial, the 5% treatment had the shallowest average suspension depth, the rafts in the 8% and 10% treatments had the deepest average, and the 3% and 12% treatments had very similar suspension depths that were shallower than the 8% and 10%. Overall, average suspension depth of rafts in the 10% treatment was the deepest in each trial (despite changes in temperature and weather conditions), the depth of the rafts in the 8% treatment was similar to the 10% treatment, and the rafts in the control showed the shallowest average depth but not the shallowest in all three trials.

Table 4.2. Average suspended raft depth for each 2-day trial treatment. Units in cm from surface

	0%	3%	5%	8%	10%	12%
Trial 1	1.19	1.80	2.24	2.45	2.53	2.27
Trial 2	2.70	2.80	2.03	2.77	2.89	2.27
Trial 3	2.27	2.76	2.16	3.80	3.81	2.78
Mean	2.05	2.46	2.14	3.01	3.08	2.44

Along with the suspension depth data, the percent surface raft data also supports the visual analysis of the treatments. The percent surface raft data for each trial are listed in Table 4.3. The data shows that the 10% treatment had the lowest mean across all three trials, but not the lowest surface percent for each trial. In trial one and three, the 10%

treatment had the lowest surface raft percent, but in trial two the 8% treatment and the control had the lowest surface percent. The control also had a very low surface percent after trial three, making the mean surface rafts for all three trials of 13.8% very misleading because none of the measured surface percentages for any of the controls were close to that value. The same inconsistency was seen for the 8% and 12% treatments, with a high trial one value and much lower trial two and three values. However, the 3%, 5%, and 10% treatments were consistent across all three trials. This consistency led to variances of 3.89, 8.66, and 4.22 for the 3%, 5%, and 10% treatments respectively, compared to variances of 136.63, 25.57, and 47.38 for the control, 8%, and 12 % treatments respectively.

Table 4.3. Percentage of surface rafts for each 2-day trial treatment.

	0%	3%	5%	8%	10%	12%
Trial 1	27.2	16.7	16.7	14.3	5.3	20.0
Trial 2	6.7	13.6	12.5	4.4	9.1	11.1
Trial 3	7.4	13.0	18.2	7.7	5.9	6.5
Mean	13.8	14.4	15.8	8.8	6.7	12.5

Boxplots of the percent surface rafts and the suspension depth (Figure 4.6) show the high variability of the control groups compared to the treatment groups. For the percent surface raft measurements, the range of the control group spanned almost the entire range of the all of all of the treatment groups. While the range of the percent surface rafts for the control did not completely span the range of the 8%, 10%, and 12% treatments, it greatly overlapped with their ranges. For the suspension depth, the control group also had high variability. However, it did not span the range of the treatments as completely as the surface raft control. For the suspension depth, the 8% and 10%

treatment range extended much lower than the control, but there was still a lot of overlap. Kruskal-Wallis of the surface raft analysis and the suspension depth analysis showed that no treatment was statistical different from the control.

Figure 4.3. Boxplots of the percent surface raft analysis (A) and the average suspension depth analysis (B).

Overall, the results of the percent surface raft and suspension depth analyses showed that the 10% treatment had the largest effect on cellular turgor pressure of *A. flos-aquae* rafts in the large-scale treatments, but was not statistically different than the rafts in the controls. The visual analysis indicated that there were fewer rafts at the surface of each 10% treatment and that the suspended rafts appeared lower in the water column for the 10% treatment when compare to other treatments. The average suspension depth was

the lowest in each trial and had the lowest mean across all trials. For the percent surface rafts, there were fewer surface rafts after the dark-adapted period in two of the three trials, the means of all three trials were the lowest, and the variability was also low.

Buoyancy loss curves for the 2-day trials

The variability seen in the percent surface accumulation analysis and the average suspension depth data due to the differing conditions between trials was also seen in the buoyancy loss curves. This variability led to the average buoyancy loss curve for each treatment to show the expected trend. In order to fully explain the average results for each treatment, buoyancy loss curves for each individual trial are shown in Figure 4.4.

Figure 4.4. Buoyancy loss curves of rafts in large-scale treatments after 24hrs for trial 1 (A), trial 2 (B), trial 3(C), and the average of each treatment across all three trials (D).

The buoyancy loss curves after 24 hours show large differences between each trial but the curves for each treatment level look very similar when the trials are averaged

together. In trial one (Figure 4.4A), the *A. flos-aquae* rafts in most of the treatments lost buoyancy before the rafts in the control group. The 5% and 10% treatments lost buoyancy the fastest. However, most treatments only showed separation from the control curve over the last half of the curve. This indicates that the surface rafts had higher turgor pressure than the control for most treatments. In trial two, *A. flos-aquae* rafts from all treatments lost buoyancy before the rafts in the control (Figure 4.4B). All treatment curves occurred at lower applied pressures than the controls, with the rafts in the 12% treatment losing buoyancy at the lowest applied pressures. In trial three, all treatments and the control lost buoyancy at about the same applied pressure (Figure 4.4C). Surface rafts in the 3%, 10%, and 12% treatments lost buoyancy at one pressure increment lower than the control, but the control curve occurred at lower applied pressures than the treatment curves at most pressures, indicating that the rafts in each treatment did not have a higher turgor pressure than the rafts in the control. Therefore, each trial showed very different results after 24 hours, but the rafts in most treatments did lose buoyancy at lower applied pressures than the rafts in the control. This was especially true for the surface rafts, which is revealed by the average buoyancy collapse curves in Figure 4.4D. All treatments occur at lower applied pressure than the control over the last half of the curve, with the last rafts in the 5%, 8%, and 10% curves losing buoyancy at lower applied pressure than the last rafts in the 12% and the control.

The buoyancy loss curves after 48 hours show trends similar to the curves after 24 hours, but the rafts in the 10% treatments consistently lost buoyancy at lower applied pressures than the rafts in the other treatments and the controls. For trial 1, the surface rafts for all treatments (except 3%) lost pressure and lower applied pressures than the

surface rafts of the control (Figure 4.5A) and the 10% and 12 % treatments lost all buoyancy at a lower applied pressure than all other treatments. For trial 2, most treatments curves occurred at lower applied pressures (except 3%) for almost the entire curve, with the rafts in the 12% treatment lost all buoyancy at the lowest applied pressure, followed by the rafts in the 10% treatment (Figure 4.5B). For trial 3, the rafts in all treatments lost all buoyancy at a lower applied pressure than the rafts in the control, but over the length of the curves most treatment curves occurred at lowered applied pressures than the control group (Figure 4.5C).

Figure 4.5. Buoyancy loss curves of rafts in large-scale treatments after 48hrs for trial 1 (A), trial 2 (B), trial 3(C), and the average of each treatment across all three trials (D).

Due to the fact that the rafts in most treatments also lost all buoyancy before the rafts in the control, the surface rafts in the treatments had a higher turgor pressure than

the surface rafts in the control. Of the treatments, the surface rafts in the 10% treatment had the highest turgor pressure. When all three trials were averaged together (Figure 4.5D), the control and most of the treatments were very similar. The only treatment that occurred regularly at a lower applied pressure than the control was the 10% treatment. All trials showed that after 48 hours the rafts in the 10% treatment lost buoyancy before almost all other treatments and well before the rafts in the control, indicating a higher cellular turgor pressure.

In order to reduce crowding in the plots and to be able to more clearly present the results of the treatment averages after 24 hours and 48 hours, Figure 4.6 shows plots with average low-level wetland water treatments and average high-level wetland water treatments on separate plots for both the 24 hours and the 48 hours analysis. The low-level treatments (3%, 5%, and 8%) after 24 hours showed that all treatment curves are similar and indicate a higher level of turgor pressure in surface rafts when compared to the control. The 10% and 12% curves showed the same trend, the last half of the curves occurred at lower applied pressures, but it is not as pronounced in the 10% and 12% curves as it is in the low-level curves.

Figure 4.6. Average buoyancy loss curves of treatments from all three large-scale, 2-day trials. (A) 3%, 5%, and 8% treatments after 24hrs. (B) 10% and 12% treatments after 24hrs. (C) 3%, 5%, and 8% treatments after 48hrs. (D) 10% and 12% treatments after 48 hrs.

For the 48 hour plots, the low-level treatment curves looked very similar to the control curve, except for the final buoyancy loss pressure. This indicates that most rafts in the low-level treatments had similar turgor pressure to the rafts in the control except for very last surface rafts to lose buoyancy. A similar result was shown for the 12% treatment after 48hrs. However, the 10% treatment consistently occurred at lower applied pressures than the control, indicating that the surface rafts and most of the other rafts in the 10% treatments had a higher turgor pressure than the rafts in the other treatments and the control.

In order to compare the surface raft turgor pressure in each treatment with the turgor pressure in the controls, I analyzed the average applied pressure that caused the

last raft to lose buoyancy for each treatment (Figure 4.7). For the 24 hour analysis, all of the treatments required less externally applied pressure to cause the last raft to lose buoyancy compared to the rafts in the controls, which lost buoyancy at an average applied pressure of 2.5 atm. Surface rafts in the 3%, 5%, 8%, and 12% lost buoyancy at 2.3, 2.2, 2.3, and 2.3 atm respectively, pressures that are all very similar with low variability. However, none of them were statistically different from the controls due to the high variability of the control data.

Figure 4.7. Average externally applied pressure to cause the last rafts to lose buoyancy after 24hrs (A) and 48hrs (B). Error bars represent SEM for each treatment.

The treatment that required the least externally applied pressure to cause the last raft to lose buoyancy after 24 hours was the 10% treatment. Rafts in the 10% treatment lost buoyancy at an average externally applied pressure of 1.8 atm, but had higher variability than the other groups. The high variability led to a lack of statistical difference (at $p < 0.05$) when compared to the control. For the 48-hour analysis, all of the treatments required less externally applied pressure to cause the last raft to lose buoyancy compared to the control. The control required 2.3 atm of pressure, on average, to cause the last raft to lose buoyancy and the 3%, 5%, and 8% treatments required 2.2, 2.1, and

2.2 atm of pressure respectively. The 10% and 12% treatments required much less pressure to cause the last rafts to lose buoyancy, 1.9 atm. However, none of the treatments were statistically different ($p > 0.05$) from the control.

Buoyancy loss curves for the 8-day trials

The results of the buoyancy loss curves from the 8-day trial show that the *A. flos-aquae* rafts in the 10% treatment had a greater increase in turgor pressure than the rafts in the 0% wetland control over the length of the trial. After the first 24 hours, most of the rafts in the control had a higher turgor pressure than the 10% treatment. However, both of the curves in Figure 4.8A show that the last raft to lose buoyancy in each treatment occurred at the same applied pressure, which indicates that the surface rafts had a similar turgor pressure at the end of the first day in both the control and the 10% treatment. As the trial progressed, this relationship slowly changed, as the rafts in the 10% treatment showed increasing levels of turgor pressure compared to the rafts in the control. This shift can be seen from the buoyancy loss curves in Figure 4.8B-D for day three, day five, and day eight. Each successive day of the trial, the rafts in the 10% treatment gained more turgor pressure than the rafts in the control, as shown by curves that occurred at lower applied pressures as the trial progressed.

Figure 4.8. 8-day trial buoyancy loss curves. (A) day 1, 24hrs after initial set-up. (B) Day 3. (C) Day 5. (D) Day 8.

In addition to the trend of the rafts in the 10% treatment gaining more turgor pressure than the rafts in the control, there are two other trends to note from the 8-day trial. The first is that rafts in both the 10% treatment and the 0% wetland water showed an increasing turgor pressure throughout the 8-day trial. Over the first three days of the trial, the buoyancy loss curves of rafts in both the 10% treatment and 0% wetland water occurred at progressively higher externally applied pressures, indicating a decrease in overall turgor pressure. This can be seen by comparing Figure 4.8A and 4.8B. However, over the rest of the trial, the buoyancy collapse curves occurred at progressively lower externally applied pressures, indicating an increase in overall turgor pressure. This can be seen by comparing Figure 4.8C and 4.8D. The end result was an overall increase in turgor pressure shown by the buoyancy loss curves that occurred at lower applied

pressures on days seven and eight when compared to the curves on day one. The second trend of note was that after the first day, the last rafts in the 10% treatment lost buoyancy at a lower applied pressure than the rafts in the control during each day of the trial. On the day one, the last rafts lost buoyancy at the same externally applied pressure in both the 10% treatment and the control. This indicates that the surface rafts had an equal turgor pressure after day one, but the surface rafts in the 10% treatment had a greater turgor pressure over the rest of the 8-day trial.

In situ chemistry

The temperature in the source water for the treatment containers varied slightly over the eight days of this study, but the other *in situ* chemistry parameters were relatively stable (Table 4.4). Putnams point was stable across all three dates for all three parameters, except for DO between June 21 and June 24. Agency Lake was also stable for all three parameters, except for an increase in pH between June 21 and June 24. Wood river wetland, while also stable across all three parameters, had very low DO and extremely high conductivity each day of the study.

Table 4.4. In situ water chemistry for Agency Lake, Putnam's Point, and Wood River Wetland collected on the morning of the beginning of each trial.

	Putnams Point			Wood River Wetland			Agency Lake		
	6/21	6/24	6/27	6/21	6/24	6/27	6/21	6/24	6/27
pH	9.77	9.87	9.8	7.16	7.47	7.53	7.62	8.84	8.84
Conductivity (µS)	115	114	116	333	336	332	94	95	90
DO (mg)	9.75	10.26	10.86	1.41	1.34	1.45	8.13	8.49	8.64

The *in situ* chemistry measurements of the 2-day trials showed that all of the treatments and controls were very similar for pH and dissolved oxygen, but had very different levels of conductivity due to the amount of wetland water in each treatment.

The wetland water used for this study had a specific conductance between 332 and 336 μS (Table 4.5) and the main lake water used for the trials was from Agency lake, which had a specific conductance between 90 and 95 μS . The specific conductance of the lake water only control containers (0% wetland water) had an average specific conductance of 94 μS for all three trials of this study. Each wetland water treatment replicate had an increasing specific conductance based upon the percent wetland water added. Average *in situ* water chemistry for each treatment is shown on Table 4.5 and all *in situ* water chemistry values for each replicate are listed in Appendix A.

Table 4.5. Average *in situ* water chemistry for 2-day trials

	0%	3%	5%	8%	10%	12%
pH	8.62	8.45	8.70	8.60	8.51	8.48
Conductivity (μS)	94	102	106	113	117	123
DO (mg)	8.9	8.4	8.9	8.5	8.5	8.1

Over the length of each 2-day trial, conductivity was conservative from start to finish. Dissolved oxygen was roughly equal for all treatments and control replicates in each trial, most likely due to the mixing that occurred when water was pumped and poured into each container at the beginning of each trial. Almost every treatment and control replicate gained 1-2 milligrams of dissolved oxygen over the 2-day trial, most likely due to phytoplankton photosynthesis. Control and treatment replicates also increased in pH over the 2-day trial. Almost every treatment and control replicate had an initial pH between 8-9 (the 5% treatment from trial three was the only exception with a pH of 9.17), and every treatment and control replicate ended with a pH between 8.91-

9.15. It indicates that all of the replicates showed a slight increase in pH over each 2-day trial, except for the 5% treatment during trial 3 which decreased by 0.12 pH units. The pH increases were also attributed to phytoplankton photosynthesis.

Table 4.6. *In situ* water chemistry for 8-day trial

	pH		Conductivity (μ S)		Dissolved Oxygen (mg)	
	0%	10%	0%	10%	0%	10%
6/21	8.41	8.1	94	117	9.05	8.55
6/23	8.91	8.87	97	121	9.25	9.84
6/24	9.3	9.17	98	122	10.14	10.09
6/26	9.48	9.35	100	124	12.91	12.81
6/27	9.89	9.76	110	130	15.2	12.53
6/29	10.0	9.73	114	133	15.54	12.89

The *in situ* chemistry measurements of the 8-day trials showed similar changes in pH and dissolved oxygen to the 2-day trials from June 21 to June 23. The conductivity also changed over the first two days of the 8-day trial, but the relatively small changes compared to the magnitude of the measured values did not indicate a difference between these changes and the conductivity values in the 2-day trials. However, after the third day of the trial, all *in situ* values increased at a faster rate, with each container showing large increases in pH, conductivity, and dissolved oxygen from day 1 to day 8 (Table 4.6).

Nutrients

The source water for the treatment and control replicates of this study showed consistent results for nitrate/nitrite (NO_2/NO_3), ammonia/ammonium (NH_4), total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP) across all

dates sampled (Table 4.7). Agency Lake had the lowest nutrient levels. Putnams Point had similarly low levels of most nutrients, except for TN, which was higher than Agency Lake and may have been due to nitrogen fixation from the high density of *A. flos-aquae* rafts. There were few/no *A. flos-aquae* rafts at the Agency Lake collection site. The Wood River Wetland had much higher levels for all measured nutrients in this study. The high nutrient levels directly influenced the nutrient levels in the wetland water treatment replicates, leading to increased nutrient levels with each increase in wetland water percent.

Table 4.7. Nutrients data for Agency Lake, Putnams Point, and Wood River Wetland collected on the morning of the beginning of each trial. All units mg/L

	Putnams Point			Wood River Wetland			Agency Lake		
	6/21	6/24	6/27	6/21	6/24	6/27	6/21	6/24	6/27
NO₂/NO₃	0.16	0.09	0.13	0.96	0.9	0.82	0.03	0.06	0.03
NH₄	0.06	0.08	0.06	0.3	0.42	0.23	0.06	0.05	0.03
TN	2.83	2.61	2.95	4.46	5.22	4.13	0.62	0.12	0.75
SRP	0.02	0.01	0.02	1.39	1.34	1.22	0.09	0.09	0.09
TP	0.5	0.53	0.4	1.53	1.92	2.03	0.18	0.31	0.38

Due to the fact that the source water nutrient levels were consistent across all sample dates, the initial nutrient levels for each treatment and control for each trial were also very consistent. The mean nutrient levels for each treatment and control are shown on Table 4.8 and nutrient levels of each replicate are shown on Appendix B. As expected the nutrient levels increased as wetland water increased, with the highest nutrient levels having occurred in the 12% wetland water treatment. The only exception to this trend was NH₄, which increased from the control to the 8% treatment, then leveled off at 0.07 mg/L in the 10% and 12% treatments.

Table 4.8. Nutrient means for 2-day trial treatment replicates. All units mg/L.

	0%	3%	5%	8%	10%	12%
NO₂/NO₃	0.15	0.18	0.2	0.22	0.24	0.26
NH₄	0.04	0.05	0.06	0.08	0.07	0.07
TN	0.78	0.91	0.99	1.11	1.2	1.28
SRP	0.07	0.12	0.13	0.17	0.2	0.22
TP	0.22	0.26	0.30	0.34	0.38	0.47

The 8-day trial containers had similar initial nutrient levels to the 0% and 10% means in the initial 2-day trials. Levels of NH₄, TN, and SRP were almost identical between the 2-day means and the 8-day measurements for NH₄, TN, and SRP. TP levels, while similar to the 2-day trial means, were lower in the initial 8-day trials. However, the initial 8-day TP measurements were almost identical to the initial measurements for TP in the first 2-day trial initiated on June 21, the same day as the 8-day trials. This indicates that the nutrients at the beginning of each 2-day trial and 8-day trial were consistent and showed that nutrient variation between the trials was not a source of variability. Nutrient levels for the 8-day trials are listed on Table 4.9.

Table 4.9. Nutrient data for 8-day trial. All units mg/L.

	NH₄		TN		SRP		TP	
	<u>0%</u>	<u>10%</u>	<u>0%</u>	<u>10%</u>	<u>0%</u>	<u>10%</u>	<u>0%</u>	<u>10%</u>
6/21	0.05	0.07	0.9	1.3	0.08	0.18	0.19	0.29
6/23	0.03	0.05	0.94	1.04	0.06	0.17	0.16	0.26
6/24	0.05	0.05	0.77	0.87	0.04	0.16	0.22	0.32
6/26	0.06	0.05	0.79	0.45	0.02	0.12	0.13	0.23
6/27	0.05	0.05	0.86	0.18	0.02	0.08	0.22	0.3
6/29	0.06	0.09	0.86	0.02	0.01	0.02	0.19	0.23

Sodium, potassium, and calcium cations

The concentrations of sodium, potassium, and calcium in the source water for the treatments and control were consistent across all three days of collection for this study. Table 4.10 shows the mean concentration and variance at each collection site for all three ion types and Appendix C shows the measured values for each collection day. Variances for each ion type at each site were very low, indicating consistent concentrations during the length of the study. The concentration differences between each collection sites was similar to the trend for nutrients: the Wood River Wetland had much higher concentrations of all three ions, while Putnams Point and Agency Lake concentrations were much lower and very similar to each other.

Table 4.10. Mean and variance of sodium, potassium, and calcium concentrations measured from samples taken on each day that water was collected from Putnams Point, Wood River Wetland, and Agency Lake for trials 1-3 of this study. Units of mean values are mg/L.

	Putnams Point		Wood River Wetland		Agency Lake	
	Mean	Variance	Mean	Variance	Mean	Variance
Sodium	9.31	0.03	43.05	0.87	7.57	0.02
Potassium	1.98	0.01	7.8	0.05	2.24	0.01
Calcium	6.65	0.01	18.89	0.07	6.57	0.05

The concentration of sodium, potassium, and calcium ions in the Wood River Wetland water was the main determinant for the differences between the treatments and the control for these cations. Due to the fact that water from the Wood River Wetland had a much higher concentration of all three cation types, the amount of wetland water added to each treatment led to the differences in the cation concentration in each

treatment. The mean concentration of sodium, potassium, and calcium measured for each treatment across all three trials are shown in Table 4.11 (all values listed in Appendix C) and indicates that cation concentration increased as wetland water increased. Differences between treatments for cation concentration across all trials was very small, with low variance between replicates for all treatments and the control (variance not shown here). Cation concentration remained conservative throughout each 2-day trial with very small differences between the initial and final concentration measurements.

Table 4.11. Mean sodium, potassium, and calcium concentrations for each 2-day wetland water treatment and control container. All units mg/L.

	0%	3%	5%	8%	10%	12%
Sodium	7.69	8.59	9.41	10.38	10.89	11.50
Potassium	2.19	2.31	2.42	2.59	2.64	2.81
Calcium	6.56	6.77	7.12	7.43	7.65	8.04

The 8-day trial echoed the same trend for sodium, potassium, and calcium shown in the 2-day trials. The differences in the initial concentration of all three ion types was determined by added wetland water. The 0% container had an initial concentration of 7.68, 2.19, and 6.46 mg/L respectively for sodium, potassium, and calcium. These values are almost identical to the 0% treatment averages in the 2-day trials. The 10% container had an initial concentration of 10.79, 2.65, and 7.47 mg/L respectively for sodium, potassium, and calcium. These values were very similar to the mean values for the 10% treatment in the 2-day trial and the initial values for the 10% treatment in trial one of the 2-day trials. Over the first two days of the 8-day trial, these values changed very little. After day 4, they increased slowly each day to reach values of 9.23, 2.12, 7.03 and 12.85,

2.63, 8.45 mg/L respectively for sodium, potassium and calcium. This showed that concentrations of sodium and calcium increased in both containers while potassium concentrations decreased in both containers over the length of the 8-day trial.

Principle Component Analysis

The principle component analysis (PCA) for this study was used to compare the treatment and control replicates based upon all of the *in situ*, nutrient, and ion measurements and analyses. The biplot for this analysis is shown on Figure 4.9. The highest magnitude loadings for PC1 include conductivity, SRP, sodium, and potassium. The highest magnitude loadings for PC2 are temperature, NH_4 , SRP, TP, and calcium.

Figure 4.9. Biplot of the 2-day trial principle component analysis. Treatment analysis is based upon temperature, pH, conductivity, DO (mg), NO_2/NO_3 , NH_4 , TN, SRP, TP, sodium, potassium, and calcium. Replicate codes indicate trial number and wetland percent (ex 1.12% represents the 12% treatment of trial 1).

The biplot of the PCA shows that the treatments in trial 1 and 2 were separated distinctly across PC1 from positive to negative. Each of the separate treatments occurs at a different loading point across PC1, from right-to-left on Figure 4.9. Trial one treatments occur in order starting with 1.0% in the lower right and ending with 1.15% in the middle left. Trial two treatments also start in the lower right with 2.0% and end in the middle left with 2.12%. For trial one, parameters most associated with the variability of the low-level treatments are SRP and NH_4 and the parameters are most associated with the high level treatments are potassium, sodium, and conductivity. For trial two, parameters most associated with the variability of the low-level treatments are TP and TN and the parameters are most associated with the high level treatments are potassium, sodium, and nitrate/nitrite. Taken altogether, the PCA of trials one and two indicate that the high-level treatments were most influenced by ions and conductivity while the low-level treatments were most influenced by nutrients.

The treatments of trial three were not clearly separated by either PC1 or PC2 alone. The low-level treatments and the control (3.0%, 3.3%, and 3.5%) were clearly separated by the positive loadings of PC1, but they were not separated in order and 3.0% and 3.3% are not closely associated with any measured or analyzed parameter. The high-level treatments (3.8%, 3.10% and 3.12%) were distinctly separated by PC2, but also not in order. These high-level treatments were more closely associated with temperature than any other measured or analyzed parameter. Due to the fact that trial three had the highest average temperature for all treatments from the sunny and warm weather conditions, it is logical that temperature would be a key parameter that influenced the trial three treatments.

Discussion

The main finding of the larger scale trials was that the 10% treatment was the most effective at inducing turgor pressure and reducing surface raft accumulation. This main finding supports the first hypothesis for this chapter about the 2-day trials and the second hypothesis about the 8-day trial. However, it is important to note that the effect of the 10% wetland water treatment was less pronounced in the large-scale, 2-day trials than in the small-scale study. Many of the buoyancy analyses comparisons were statistically different ($p < 0.05$) in the small-scale study but none were different enough in the large-scale study to be statistically different at $p < 0.05$. It is also important to note that the expectations of an increasing effect of wetland water below 10% and a decreasing effect above 10% were not supported by this study. While rafts in the 8% treatment level showed results similar to the rafts in the 10% treatment level, many of the other treatment levels showed results more similar to the rafts in the control or showed similar results that were between the values of the controls and the 10% treatment. Thus, hypotheses two and three were not supported by the data collected in this study.

I attribute two main factors to the difference between the small-scale trials and the larger scale trials: the longer duration of the larger scale trials (compared to the small-scale trials) and the difference in light intensity between trials one, two, and three. The longer time scale most likely introduced factors that led to the less pronounced effect of the 10% wetland treatments in the large-scale trials. Major factors that influence buoyancy in *A. flos-aquae*, that were minimized in the small-scale trials due to controlled conditions and the short time length, were ballast accumulation, light attenuation, and

nutrients. Fluctuations in all three of these can trigger gas vesicle formation and influence buoyancy over timescales longer than two hours.

Ballast accumulates over many hours of time for *A. flos-aquae* (Kromkamp et al. 1986), and can lead to buoyancy changes that take place over the length of a day in natural conditions (Konopka et al. 1978). The short time-scale of the small-scale trials minimized the effect of ballast accumulation, but this is a natural process that occurs in all buoyant cyanobacteria taxa over hours and days of time (Konopka and Schnur 1980) so it definitely occurred in the cells of the *A. flos-aquae* rafts in the larger, longer scale trials. However, its influence on variations in buoyancy between the *A. flos-aquae* rafts in the large-scale treatments is unclear. Light attenuation by wetland water should have led to lower productivity in the 10% treatments than the 0% control, resulting in less ballast accumulation and a higher percentage of surface rafts (Lean, 1998). Due to the fact that the 10% wetland water treatments had fewer surface rafts than the controls, the results of this study indicate that ballast influences and light attenuation on buoyancy were minimal here. If ballast accumulation and light attenuation had been bigger factors, the higher wetland water treatments would have had a greater number of rafts at the surface after the dark-adapted period or the low wetland water treatments and controls would have had fewer surface rafts and a deeper average suspension depth for non-surface rafts.

External concentration of phosphorus over multiple days may also have influenced the results of this study. Konopka et al. (1987) showed that increases in the external concentration of phosphate triggered gas vesicle formation and increased buoyancy. Since this process takes 12-24 hours to influence buoyancy, it may not have

been a factor in the small-scale trials, but a factor in the large-scale trials. As wetland water was added, phosphorus levels increased accordingly. Based upon the results of Konopka et al. (1987), Konopka and Schnur (1980), and Konopka et al. (1978), *A. flos-aquae* rafts should have gained buoyancy over the two days and eight days of the large-scale trials in the wetland water treatments due high levels of phosphorus triggering gas vesicle formation. Thus, increasing external phosphorus concentration should have led to a greater number of surface rafts and a shallower average suspended raft depth over days of time. Despite higher phosphorus levels, wetland water addition in the 10% treatment led to a decrease in surface accumulation and a decrease in the depth of suspended rafts.

While the length of the trials, ballast accumulation, light attenuation, and phosphorus concentration did not alter the overall result of the 10% treatment being the most effective, they probably contributed to the less pronounced effect on *A. flos-aquae* buoyancy in the large-scale trials compared to the small-scale trials. Simple correlation plots between each *in situ* water chemistry parameter and each measured water chemistry parameter did not reveal any clear relationships with buoyancy measurements (data not shown here). However, PCA provided some clarity to these relationships by separating the treatments of trials one and two across PC1, grouping high-level treatments and low-level treatments, and showing that variability in trial three was influenced more by temperature than the other two trials. As noted in the results section, the conditions during trial three were much warmer than trials one and two. This led to the water temperature in each treatment of trial three to be much higher. It also provided a different light environment in each container. The PCA grouped the high-level treatments of trial three together along an axis with a high influence from the positive

loading of temperature. The high-level treatments of trials one and two were grouped along an axis with a strong influence from the positive loadings of sodium, potassium, and conductivity. This indicates that different factors were driving the results of trials one and two than were driving the results of trial three.

By comparing the buoyancy loss curves for trials one and two with the curves from trial three, the influence of ions and temperature become more clearly defined. In Figure 4.4 and Figure 4.5, the buoyancy loss curves of trials one and two show that many of treatments have curves that occur at lower applied pressures than the control, especially over the last half of the curves. This indicates that the treatments in these trials outperformed the controls by causing a higher turgor pressure in the cells of the *A. flos-aquae* rafts, especially for the surface rafts. The trial three curves in Figures 4.4 and 4.5 show a different trend. The *A. flos-aquae* rafts in the control group of trial three outperformed almost all of the treatments, causing a higher turgor pressure in the rafts and leading to buoyancy loss curves similar to the 10% treatment. While the 10% treatment was still effective in trial three, this indicates that high temperatures may have reduced the potency of the high-level wetland water treatments at increasing the cellular turgor pressure in *A. flos-aquae* rafts. At lower temperatures, the PCA and buoyancy loss curves indicate that potassium and sodium were prominent influences for increasing cellular turgor pressure in *A. flos-aquae* rafts in the high-level wetland water treatments.

The relationship between buoyancy control and temperature is a key finding for the large-scale trials of this study. Cyanobacteria blooms are associated with higher temperature worldwide (Paerl and Otten, 2016), so a diminished effect of the treatments due to higher temperature in this study could drastically change the application of this

study to bloom reduction. However, temperature in this study was directly related to higher light intensity. As the weather changed from cloudy to sunny, the near-lake containers increased in temperature due to being exposed to much higher light intensity. So it is unclear from this data if the difference in turgor pressure and buoyancy from trial three and trials one and two are related to light intensity or temperature changes. In the small-scale trials, an increase in light intensity led to an increase in turgor pressure, which could explain the increase in turgor pressure of the rafts in the control between trial one and trial three. Similar changes in turgor pressure with increased light intensity were also seen in small-scale trials by Walsby et al. (1971) and Kromkamp et al. (1986), but these experiments did not isolate temperature from light intensity. Other studies on cyanobacteria dominance by Zevenboom and Mur (1980) and Tilman et al. (1982) also focus on light as a factor that can limit cyanobacteria at high intensities, but do not discuss specific temperature based limits or advantages. The main driver for cyanobacteria dominance at higher temperatures is considered to be community succession (Paerl and Otten, 2016) and taxa specific temperature preferences (Dokulil and Teubner, 2000). Physiological advantages for buoyancy at higher temperature has yet to be clarified in peer reviewed publications, making this an excellent question to be answered in future research. For the influence of temperature on turgor pressure and surface accumulation, the buoyancy loss method described in this dissertation would be a viable way to come up with an answer to this question in small-scale, laboratory trials. Preconditioning *A. flos-aquae* rafts at different temperatures before measuring water position and turgor pressure would reveal the direct effects of temperature differences on buoyancy control.

Conclusion

The purpose of the large-scale trials was to compare the buoyancy measurements used in the small-scale treatments with the same measurements taken from large-scale treatments performed over a longer time period and under non-controlled conditions. The overall results here showed the 10% wetland water treatment to be the most effective for inducing cellular turgor pressure and disrupting buoyancy. This was the same overall result obtained from the small-scale treatments, so the large scale-treatments in this study were considered a success. The large-scale treatments also indicated the importance of potassium and sodium in inducing turgor pressure and disrupting buoyancy in *A. flos-aquae* rafts. This also parallels the results of the small-scale treatments which showed that additions of potassium and sodium induced cellular turgor pressure in *A. flos-aquae* rafts, reduced surface raft percentage, and increased the suspension depth of non-surface rafts. The large-scale trials also indicated that high temperatures from increased light intensity may reduce the potency of the wetland water treatments. Since temperature was controlled in the small-scale trials, this has no comparable result from the small-scale trials. However, reduction of the potency of the 10% wetland water treatment by high temperature is an important observation for the application of wetland water as a tool to obviate cyanobacteria surface accumulation.

Chapter 5. Study Conclusions

The purpose of this research was to investigate the effect of wetland water and ion additions on turgor pressure and buoyancy control of *A. flos-aquae* rafts from UKL. The main goal of this research was to determine an effective dose of wetland water that could reduce surface blooms or accumulation in UKL. The determination of the most effective dose was based upon the percent wetland water treatment that induced the highest cellular turgor pressure, lowest surface accumulation, and lowest depth of suspension for non-surface *A. flos-aquae* rafts. These factors were studied in small-scale, controlled, lab trials and in large-scale, lake-side trials. A secondary goal was to investigate the influence of sodium, potassium, and calcium cations on cellular turgor pressure and buoyancy of *A. flos-aquae* rafts in small-scale, controlled, lab trials and through analysis of the large-scale wetland trials. To achieve these goals, I adapted the buoyancy analysis method of Walsby to be able to simultaneously assess buoyancy state and turgor pressure with surface position or suspension depth.

The buoyancy loss method

The buoyancy loss method developed in this dissertation that uses direct observations in a pressure chamber is a more useful and robust method than the spectrophotometric pressure collapse curve method developed by Walsby (1971). Many of Walsby's experiments included one replicate or two replicates per treatment, giving the results very little statistical power. For the buoyancy loss method, I relied on a minimum of 6 replicates, and in some of the small-scale wetland experiments I included 24 replicates. While not all of the results of these experiments using the buoyancy loss method had statistical significance, the experiments used to validate this method using

light and duration were significant. Based upon these results, this is a valid method and would be useful tool for small-scale, laboratory experiments to test the influence of individual factors on buoyancy control by macroscopic cyanobacteria taxa.

Small-scale experiments

The small-scale experiments in this dissertation were effective at isolating a single factor in order to determine its effects on turgor pressure and surface accumulation. By isolating the single factors, I was able to determine that the target dose for the larger scale wetland trials was the 10% wetland water treatment and that each individual ion (potassium, sodium, and calcium) had an effect on turgor pressure and surface accumulation. I was also able to identify that the least effective dose of wetland water was the 100% treatment. The small-scale experiments helped me to conclude these findings quickly and with adequate replication. The main conclusion I have to make about the small-scale trials themselves is that they provided an invaluable way to isolate factors and determine their effect on buoyancy for larger scale trials. Small-scale experiments using this method would be useful for answering some new questions created by this research about the specificity of the 10% wetland water target, the threshold for the effective dose of external ion concentrations, and the influence of temperature not related to light intensity.

Large-scale trials

The large-scale trials were exposed to considerable differences in air temperature, water temperature, and exposure to direct sunlight over the 10 days of the 2-day trials due to natural fluctuations in the weather. The PCA and buoyancy loss results indicated that when temperature was cooler and the containers were mixed by wind, potassium and

sodium concentrations explained more of the variability in buoyancy. Under these conditions, the 10% treatment was clearly the most effective treatment at inducing turgor pressure and reducing surface raft accumulation. When temperatures were higher and wind was not present to mix the containers, the 10% treatment had the lowest percentage of surface rafts and induced the highest turgor pressure, but it was not as effective as when temperature was lower and wind was higher. PCA and buoyancy analyses also indicated that nutrient concentrations were consistent throughout the study and did not play a major role in *A. flos-aquae* buoyancy in the high-level treatments. However, nutrients played more important role in the 3% and 5% wetland water treatments. This supports the idea that adding nutrients above saturation diminishes their effect on buoyancy.

The 10% wetland water treatment

The 10% wetland water treatment was the most effective treatment in this study, in both the small-scale and larger scale trials, but the mechanism of its effectiveness is still unclear. This experiment was designed to test the effectiveness of wetland water over two different-length time periods, which should have changed the overall result. In the short-term trials, the high conductivity of the wetland water should have led to an overall decrease in turgor pressure and the increase of potassium and sodium in the wetland water should have led to an increase in turgor pressure (Allison and Waslby, 1981; Sherer et al., 1988). These two competing process seemed to reach a zenith in the 10% treatment, with the 100% treatment most affected by the high conductivity and the control most affected by the lower availability of sodium and potassium for ion exchange. Also involved here is the exposure of *A. flos-aquae* rafts in the controls to higher levels

of light intensity than wetland treatments due to light attenuation, which should increase turgor pressure (Kromkamp et al., 1986). All of these short-term processes led the conditions in the 10% treatment to result in the highest turgor pressure for cells in *A. flos-aquae* rafts.

In the 2-day trials, all of the short-term processes still influenced cellular turgor pressure, with the addition of longer-term processes that trigger vesicle formation. The higher phosphate levels and reduced light intensity due to AHS should have triggered gas vesicle formation and should have led to lower cellular turgor pressure and a higher percentage of surface rafts (Konopka et al., 1987; Oliver, 1994; Lean, 1998). These added influences should have led to a more effective wetland water treatment at 12% and 15%. However, the data did not show that here. Rather, it showed the most effective 2-day treatments to be 8% and 10% wetland water. However, these longer term process that trigger vesicle formation were likely the cause for the less pronounced effect of the 10% wetland water treatment in the larger-scale trials

Based upon the competing short-term and longer-term influences here, there is not a simple explanation for the mechanism that led to the success of the 10% wetland water treatment in this study. The best answer that can be provided here is that all of the competing influences combined to make this the most effective treatment. However, future research about this result should focus first on the light environment. The 10% treatment provides some light attenuation, but not very much. In computer models of cyanobacteria dominance based upon light, low and medium levels of light intensity led to the most competitive environment for buoyant cyanobacteria and non-buoyant algae (Huisman et al., 1999; Klausmeier and Litchman, 2001). This might indicate that the

10% wetland water treatment creates a light environment that is the least favorable for *A. flos-aquae* buoyancy in UKL. To better understand the dynamics of light and buoyancy for *A. flos-aquae* rafts, short-term experiments that reduce the light environment (i.e. shading some replicates with screens of different mesh size) and longer term experiments in the lake that measure phytoplankton taxa dynamics with wetland water additions would enlighten this mechanism.

Application to future studies

This research created many new questions about thresholds of effective doses, the cellular mechanisms involved to lead to each effective dose, and the physical influences in natural systems on buoyancy control. Applicable topics for future research would be to investigate the threshold of effect for the ions used in this study, the influence of temperature changes on *A. flos-aquae* buoyancy without an increase in light intensity, and the influence of shading on *A. flos-aquae* buoyancy without a direct change in light intensity. Questions for studies in systems other than UKL should include the effectiveness of wetland water on *A. flos-aquae* in other lakes, and on other buoyant cyanobacteria taxa like *Dolichospermum*, *Microcystis*, and *Gloeotrichia*. A next step for this research in UKL is to investigate the most effective doses used in this study on a larger scale in and over a longer period of time. This should either include large limnocorrals with differing treatment levels of wetland water, or a large-scale introduction of wetland water to a part of the lake in order to measure the long-term effects. A larger scale limnocorral study should include season long measurements of water chemistry and plankton dynamics as well as an investigation of the best time of year to introduce wetland water to achieve the best outcome.

Application to lake management

The underlying goal of this work was to reduce the surface accumulation of buoyant CyanoHABs in order to diminish their physical dominance at the top of the water column in a lake. Wetland water and ion additions used in this study did not completely eliminate surface rafts, but the number of rafts at the surface was reduced and the depth of the remaining suspended rafts was expanded over a larger depth creating less surface density. Computer models of vertical algal movement and distribution show that concentrated surface layers of buoyant cyanobacteria lead to appreciable shading (Klausmeier and Litchman 2001). *A. flos-aquae* has a very low critical light intensity, so shading from concentrated layers of surface rafts creates conditions that are ideal for growth, allowing *A. flos-aquae* to out-compete other phytoplankton species to become the dominant taxa (Huisman et al. 1999). When the surface accumulation depth of buoyant cyanobacteria is reduced or spread out over a deeper amount of space in computer simulations, the photic zone is expanded allowing for better competition for light between phytoplankton taxa (Klausmeier and Litchman 2001). Higher incident light that allows for better competition between species reduces the advantage of low light growth by some buoyant cyanobacteria and often leads to their displacement as the dominant taxa by green algae or other buoyant cyanobacteria (Huisman et al. 1999). Thus, methods to reduce surface accumulations of cyanobacteria could lead to higher algal species richness and reductions of the negative impacts from CyanoHABs.

The practical use of this strategy would be to apply wetland water to a lake in order to obviate CyanoHABs surface blooms. This could be on a lake-wide scale or the introduction of wetland water to a small bay or shoreline area that traditionally suffers

from cyanobacteria blooms. However, the practical use of this strategy would require a lot of wetland water to achieve a 10% treatment to a lake. For example, UKL has a volume of $\sim 1,00,000 \text{ m}^3$, which would require at least $100,000 \text{ m}^3$ for treatment. This represents an impractical amount of wetland water to be added to the lake from a wetland on a consistent basis. For Agency Lake, with a volume of $34,000 \text{ m}^3$ this represents a more practical volume of at least $3,400 \text{ m}^3$ for an addition to reach 10% by volume. While the wood river wetland, with an area of 11 km^2 , has enough standing water to supply that volume, it does not receive enough input to supply that volume on a consistent basis. Since Agency Lake is a small lake, and the Wood River Wetland is a relatively large wetland, this represents a large practical hurdle for application of this technique. This makes a larger scale trial of this technique a crucial next step in order to understand the time and longer term influence of wetland additions.

The use of this addition as a management strategy should be as a preventative measure, before a bloom occurs, and not as an active treatment method for the reduction of a surface bloom after it forms. This strategy would most likely not completely prevent CyanoHABs in lakes with consistently heavy blooms, but could reduce their effects or delay their onset. For lakes such as UKL, with consistently heavy blooms of *A. flos-aquae*, surface reduction would allow for greater phytoplankton diversity, which could reduce the unhealthy water quality effects associated with thick surface blooms that can lead to lethally low oxygen events and native fish die-offs. A delay in bloom formation could also reduce or prevent the onset of microcystin producing blooms of *Microcystis aeruginosa*, which form after early and mid-season blooms of *A. flos-aquae* elevate nitrogen levels in the system (Eldridge et al. 2017). Large scale manipulations have been

shown to lead to a delay in the onset of an *A. flos-aquae* blooms in other systems, delaying or preventing late season *Microcystis* blooms (McDonald and Lehman 2013), so a large-scale wetland water addition could produce a comparable, desirable outcome in UKL.

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Appendix A. 2-day trial *In situ* water chemistry.

Trial	Day	Bin	Condition	Date	Temp	pH	Conductivity (μ siemens)	DO (%)	DO (mg)
1	0	10	control.lake	06.21.16	22.15	8.00	91	108.8	8.20
1	0	11	control.wet	06.21.16	18.67	7.36	332	82.2	6.41
1	0	8	0%	06.21.16	22.04	8.19	94	113.2	8.54
1	0	3	3%	06.21.16	22.24	8.25	102	114.8	8.67
1	0	4	5%	06.21.16	21.99	8.21	106	113.0	8.53
1	0	5	8%	06.21.16	22.01	8.14	112	115.2	8.68
1	1	9	10%	06.21.16	22.05	8.10	117	113.9	8.55
1	0	6	12%	06.21.16	21.78	8.07	120	112.9	8.55
1	0	7	15%	06.21.16	21.67	7.98	127	108.1	8.24
1	1	10	control.lake	06.22.16	23.11	8.19	93	104.9	7.65
1	1	11	control.wet	06.22.16	18.18	7.16	333	20.8	1.64
1	1	8	0%	06.22.16	23.02	8.69	96	117.6	8.64
1	1	3	3%	06.22.16	23.14	8.74	103	122.1	8.69
1	1	4	5%	06.22.16	22.83	8.66	107	120.3	8.86
1	1	5	8%	06.22.16	22.53	8.60	114	121.3	8.99
1	1	9	10%	06.22.16	22.51	8.48	119	116.3	8.59
1	1	6	12%	06.22.16	22.89	8.56	122	116.3	8.54
1	1	7	15%	06.22.16	22.60	8.47	129	113.2	8.52
1	2	10	control.lake	06.23.16	20.99	8.19	95	103.4	7.90
1	2	11	control.wet	06.23.16	17.45	7.58	342	62.4	5.06
1	2	8	0%	06.23.16	20.55	8.94	97	129.6	9.97
1	2	3	3%	06.23.16	20.79	8.97	105	131.3	10.02
1	2	4	5%	06.23.16	20.36	8.90	109	124.7	9.64
1	2	5	8%	06.23.16	20.20	8.89	116	127.2	9.87
1	2	9	10%	06.23.16	20.68	8.87	121	127.9	9.84
1	2	6	12%	06.23.16	20.56	8.93	123	138.1	10.62
1	2	7	15%	06.23.16	19.82	8.80	131	123.7	9.66
2	0	3	control.lake	06.24.16	20.75	8.53	94	101.2	7.79
2	0	2	control.wet	06.24.16	17.93	7.68	335	69.1	5.62
2	0	10	0%	06.24.16	20.59	8.83	96	106.1	8.19
2	0	8	3%	06.24.16	20.54	8.71	103	104.4	8.07
2	0	6	5%	06.24.16	20.50	8.72	108	103.8	8.04
2	0	7	8%	06.24.16	20.31	8.81	114	106.4	8.28
2	0	4	10%	06.24.16	20.20	8.44	119	103.0	8.02
2	0	5	12%	06.24.16	20.11	8.40	125	99.0	7.71
2	1	3	control.lake	06.25.16	17.45	8.40	96	98.9	8.12
2	1	2	control.wet	06.25.16	16.26	7.91	340	67.2	5.67
2	1	10	0%	06.25.16	17.61	8.99	112	106.4	9.19
2	1	8	3%	06.25.16	17.56	8.95	104	112.4	9.16
2	1	6	5%	06.25.16	17.74	8.90	110	112.2	9.11
2	1	7	8%	06.25.16	16.91	8.98	116	115.8	9.65
2	1	4	10%	06.25.16	16.64	8.90	120	111.9	9.38
2	1	5	12%	06.25.16	17.58	8.91	126	114.2	9.40

Appendix A *continued*. 2-day *in situ* water chemistry

Trial	Day	Bin	Condition	Date	Temp	pH	Conductivity (μ siemens)	DO (%)	DO (mg)
2	2	3	control.lake	06.26.16	25.23	8.14	95	107.8	7.60
2	2	2	control.wet	06.26.16	21.10	7.79	336	69.4	5.30
2	2	10	0%	06.26.16	25.60	9.07	97	152.0	10.65
2	2	8	3%	06.26.16	25.12	9.02	103	146.5	10.39
2	2	6	5%	06.26.16	25.05	9.10	109	142.8	10.08
2	2	7	8%	06.26.16	24.21	9.15	116	135.4	9.76
2	2	4	10%	06.26.16	23.46	8.90	120	123.4	9.06
2	2	5	12%	06.26.16	23.77	9.01	125	140.4	10.12
3	0	8	control.lake	06.27.16	26.17	8.72	91	114.8	7.97
3	0	4	control.wet	06.27.16	20.49	8.46	332	66.8	5.17
3	0	2	0%	06.27.16	25.10	8.85	93	145.4	10.06
3	0	6	3%	06.27.16	25.08	8.40	100	118.4	8.38
3	0	10	5%	06.27.16	25.55	9.17	105	147.5	10.26
3	0	3	8%	06.27.16	24.95	8.85	112	119.9	8.52
3	0	7	10%	06.27.16	24.98	8.91	116	115.3	8.82
3	0	5	12%	06.27.16	25.02	8.99	123	115.0	8.16
3	1	8	control.lake	06.28.16	24.27	8.23	91	107.8	7.74
3	1	4	control.wet	06.28.16	19.99	7.61	332	70.1	5.41
3	1	2	0%	06.28.16	24.91	8.99	93	125.4	8.90
3	1	6	3%	06.28.16	24.84	9.03	100	134.5	9.55
3	1	10	5%	06.28.16	24.71	9.07	106	138.6	9.86
3	1	3	8%	06.28.16	24.03	9.02	111	140.1	10.11
3	1	5	10%	06.28.16	23.76	8.94	115	133.9	9.72
3	1	7	12%	06.28.16	23.67	8.99	123	142.8	10.37
3	2	8	control.lake	06.29.16	25.09	8.25	91	102.3	7.22
3	2	4	control.wet	06.29.16	21.32	7.62	332	61.4	4.64
3	2	2	0%	06.29.16	25.97	8.99	93	123.9	8.60
3	2	6	3%	06.29.16	26.12	9.05	101	143.6	9.95
3	2	10	5%	06.29.16	26.05	9.05	106	146.4	10.15
3	2	3	8%	06.29.16	25.31	9.05	112	147.5	10.36
3	2	5	10%	06.29.16	24.84	9.00	116	141.3	10.02
3	2	7	12%	06.29.16	25.03	9.03	124	148.3	10.48

Appendix B. 2-day trial nutrient concentrations.

Trial	Bin	Condition	Date	NO2/NO3	NH₄	TN	SRP	TP	TN/TP
1	NA	putnams	6.21.16	0.1605	0.0629	2.8339	0.0174	0.4981	14.2995
1	NA	agency.lake	6.21.16	0.0270	0.0572	0.6227	0.0931	0.1800	3.4591
1	NA	wood.wetland	6.21.16	0.9569	0.2961	4.4585	1.3920	1.5303	2.9136
1	8	0%	6.21.16	0.0437	0.0440	0.8991	0.0749	0.1755	4.8754
1	3	3%	6.21.16	0.0716	0.0606	1.0142	0.1140	0.2186	3.0964
1	4	5%	6.21.16	0.0902	0.0589	1.0909	0.1282	0.2368	3.5451
1	5	8%	6.21.16	0.1181	0.0637	1.2060	0.1743	0.2617	2.7242
1	2	10%	6.21.16	0.1367	0.0743	1.2827	0.1991	0.3132	4.4815
1	6	12%	6.21.16	0.1553	0.0734	1.3594	0.2157	0.0439	22.3200
1	7	15%	6.21.16	0.1832	0.0706	1.4745	0.2441	0.3631	3.3060
1	1	0%	6.21.16	NA	0.0509	0.8991	0.0753	0.1876	3.8729
1	9	10%	6.21.16	NA	0.0752	1.2827	0.1806	0.2920	3.6629
1	10	control.lake	6.21.16	NA	0.0480	0.3375	0.0860	0.1483	2.2767
1	11	control.wet	6.21.16	NA	0.2775	3.8718	1.2922	1.6301	2.3752
1	8	0%	6.23.16	NA	0.0394	0.9133	0.0501	0.1377	3.4745
1	3	3%	6.23.16	NA	0.0440	1.0805	0.0856	0.1823	3.7329
1	4	5%	6.23.16	NA	0.0506	1.1017	0.1080	0.2065	3.3259
1	5	8%	6.23.16	NA	0.0586	1.0289	0.1447	0.2307	4.4596
1	2	10%	6.23.16	NA	0.0486	1.0887	0.1589	0.1589	5.9089
1	6	12%	6.23.16	NA	0.0672	1.2605	0.1751	0.2814	3.7687
1	7	15%	6.23.16	NA	0.0674	1.3348	0.2196	0.3328	2.5083
1	1	0%	6.23.16	NA	0.0340	0.9433	0.0607	0.1611	3.3722
1	9	10%	6.23.16	NA	0.0492	1.0369	0.1715	0.2625	1.1691
1	19	control.lake	6.23.16	NA	0.0397	0.3357	0.0789	0.1619	2.0741
1	11	control.wet	6.23.16	NA	0.3092	3.3394	1.3316	1.5023	2.2229
2	NA	putnams	6.24.16	0.0897	0.0780	2.6139	0.0051	0.5306	11.7120
2	NA	agency.lake	6.24.16	0.0569	0.0457	0.1218	0.0911	0.3109	0.3919
2	NA	wood.wetland	6.24.16	0.8991	0.4207	5.2166	1.3442	1.9274	2.7066
2	10	0%	6.24.16	0.3765	0.0432	0.4333	0.0726	0.1710	3.8012
2	8	3%	6.24.16	0.4018	0.0532	0.5862	0.1325	0.2194	4.4146
2	6	5%	6.24.16	0.4186	0.0457	0.6881	0.1285	0.2640	4.3350
2	7	8%	6.24.16	0.4439	0.0600	0.8409	0.1684	0.3101	2.5900
2	4	10%	6.24.16	0.4607	0.0614	0.9428	0.1849	0.4172	6.0791
2	5	12%	6.24.16	0.4776	0.0692	1.0447	0.2177	0.3661	2.3666
2	1	0%	6.24.16	NA	0.0460	0.7662	0.0382	0.2188	3.5024
2	9	10%	6.24.16	NA	0.0520	0.8664	0.1589	0.3154	2.7468
2	3	control.lake	6.24.16	NA	0.0483	0.1296	0.0883	0.2808	1.0672
2	2	control.wet	6.24.16	NA	0.3355	5.4801	1.3214	1.7281	3.7499
2	10	0%	6.26.16	NA	0.0449	0.5135	0.0489	0.1316	3.9017
2	8	3%	6.26.16	NA	0.0466	0.5993	0.0891	0.1702	2.9961
2	6	5%	6.26.16	NA	0.0432	0.6854	0.0990	0.1899	3.3465
2	7	8%	6.26.16	NA	0.0472	0.8309	0.1250	0.2330	0.5617
2	4	10%	6.26.16	NA	0.0477	0.9315	0.1601	0.2852	0.6013
2	5	12%	6.26.16	NA	0.0489	1.0121	0.1893	0.2988	0.7098
2	1	0%	6.26.16	NA	0.0597	0.7861	0.0193	0.1316	5.9726
2	9	10%	6.26.16	NA	0.0537	0.4468	0.1175	0.2300	1.9428
2	3	control.lake	6.26.16	NA	0.0317	0.1240	0.0808	0.1710	1.8953
2	2	control.wet	6.26.16	NA	0.3861	5.2536	1.4452	1.6596	31.6558

Appendix B *continued*. 2-day trial nutrient concentrations.

Trial	Bin	Condition	Date	NO ₂ /NO ₃	NH ₄	TN	SRP	TP	TN/TP
3	NA	putnams	6.27.16	0.1333	0.0583	2.9513	0.0185	0.3968	7.4374
3	NA	agency.lake	6.27.16	0.0333	0.0312	0.7518	0.0891	0.3835	1.9602
3	NA	wood.wetland	6.27.16	0.8217	0.2329	4.1336	1.2192	2.0328	2.0335
3	2	0%	6.27.16	0.0458	0.0406	1.0267	0.0753	0.3260	1.1436
3	6	3%	6.27.16	0.0695	0.0497	1.1282	0.1211	0.3472	1.1723
3	10	5%	6.27.16	0.0852	0.0669	1.1958	0.1435	0.4039	1.2156
3	3	8%	6.27.16	0.1089	0.1097	1.2973	0.1774	0.4632	2.8004
3	5	10%	6.27.16	0.1246	0.0660	1.3649	0.2070	0.4553	2.9980
3	7	12%	6.27.16	0.1404	0.0812	1.4325	0.2259	0.5430	2.6384
3	1	0%	6.27.16	NA	0.0466	0.8554	0.0197	0.2205	3.4252
3	9	10%	6.27.16	NA	0.0552	0.1827	0.0840	0.3003	0.0601
3	8	control.lake	6.27.16	NA	0.0417	0.7151	0.0903	0.3047	0.5746
3	4	control.wet	6.27.16	NA	0.3118	4.1621	1.4113	1.7529	0.8341
3	2	0%	6.29.16	NA	0.0443	1.0917	0.0410	0.2489	3.5827
3	6	3%	6.29.16	NA	0.0454	1.1504	0.0938	0.2852	3.3322
3	10	5%	6.29.16	NA	0.0532	1.0529	0.1246	0.3251	3.0235
3	3	8%	6.29.16	NA	0.0586	1.1437	0.1514	0.3561	0.6844
3	5	10%	6.29.16	NA	0.0634	1.2082	0.1727	0.3862	1.5074
3	7	12%	6.29.16	NA	0.0669	1.2830	0.2358	0.4712	0.2298
3	1	0%	6.29.16	NA	0.0614	0.8616	0.0126	0.1922	3.4419
3	9	10%	6.29.16	NA	0.0892	0.0181	0.0213	0.2267	0.0796
3	8	control.lake	6.29.16	NA	0.0409	0.7507	0.0903	0.2710	0.5561
3	4	control.wet	6.29.16	NA	0.3778	3.8303	1.3273	1.8229	0.5941

* All units in mg/L

Appendix C. 2-day trial ion concentrations.

Trial	Bin	Condition	Date	Sodium	Potassium	Calcium
1	NA	putnams	6.21.16	9.1152	1.9451	NA
1	NA	agency.lake	6.21.16	7.5081	2.2561	6.3627
1	NA	wood.wetland	6.21.16	42.0059	8.0419	18.9217
1	8	0%	6.21.16	7.6156	2.1856	6.4406
1	3	3%	6.21.16	8.6935	2.3710	6.7537
1	4	5%	6.21.16	9.2812	2.4087	6.7272
1	5	8%	6.21.16	10.2281	2.5855	7.2498
1	2	10%	6.21.16	10.6687	2.5882	7.2279
1	6	12%	6.21.16	11.2493	2.7295	7.6527
1	7	15%	6.21.16	12.2715	2.9078	7.9266
1	1	0%	6.21.16	7.6751	2.1898	6.4558
1	9	10%	6.21.16	10.7869	2.6480	7.4689
1	10	control.lake	6.21.16	7.3582	2.1770	6.3340
1	11	control.wet	6.21.16	40.2106	7.4695	17.9598
1	8	0%	6.23.16	8.1915	2.2816	6.7703
1	3	3%	6.23.16	9.0153	2.3721	6.9620
1	4	5%	6.23.16	9.4969	2.4389	7.0792
1	5	8%	6.23.16	10.9333	NA	7.5102
1	2	10%	6.23.16	11.3717	2.7130	7.3962
1	6	12%	6.23.16	12.1320	2.8573	8.0205
1	7	15%	6.23.16	13.2424	3.0447	8.4851
1	1	0%	6.23.16	7.9471	2.2292	6.4830
1	9	10%	6.23.16	11.6777	2.8130	8.0327
1	19	control.lake	6.23.16	7.9597	2.3431	6.7686
1	11	control.wet	6.23.16	44.1892	8.4360	20.0120
2	NA	putnams	6.24.16	9.4041	2.1055	6.6467
2	NA	agency.lake	6.24.16	7.7090	2.3192	6.7908
2	NA	wood.wetland	6.24.16	43.2971	7.7743	19.1396
2	10	0%	6.24.16	7.8690	2.2808	6.7302
2	8	3%	6.24.16	8.5467	2.3908	6.7668
2	6	5%	6.24.16	9.6609	2.5656	7.3832
2	7	8%	6.24.16	10.5922	2.6942	7.6513
2	4	10%	6.24.16	11.2182	2.7497	7.9016
2	5	12%	6.24.16	12.0359	2.8901	8.1303
2	1	0%	6.24.16	8.3197	2.3223	6.9662
2	9	10%	6.24.16	11.7462	2.7610	7.9121
2	3	control.lake	6.24.16	7.7196	2.3586	6.8438
2	2	control.wet	6.24.16	43.0080	7.8846	19.1441
2	10	0%	6.26.16	7.9873	2.2802	6.7609
2	8	3%	6.26.16	9.1322	2.4658	7.1887
2	6	5%	6.26.16	10.2181	2.6756	7.7912
2	7	8%	6.26.16	11.1355	2.7432	7.8555
2	4	10%	6.26.16	12.1776	2.9853	8.4396
2	5	12%	6.26.16	12.8888	3.0850	8.6390
2	1	0%	6.26.16	9.1203	2.4153	7.2970
2	9	10%	6.26.16	11.9769	2.7492	8.1545
2	3	control.lake	6.26.16	8.4049	2.5247	7.2998
2	2	control.wet	6.26.16	47.5037	8.8259	20.7448

Appendix C *continued*. 2-day trial ion concentrations

Trial	Bin	Condition	Date	Sodium	Potassium	Calcium
3	NA	putnams	6.27.16	9.4057	1.8962	6.7731
3	NA	agency.lake	6.27.16	7.4829	2.1571	6.5694
3	NA	wood.wetland	6.27.16	43.8197	7.5856	18.6197
3	2	0%	6.27.16	7.5991	2.1118	6.5046
3	6	3%	6.27.16	8.5332	2.1776	6.8043
3	10	5%	6.27.16	9.2940	2.2791	7.2347
3	3	8%	6.27.16	10.3425	2.4996	7.4012
3	5	10%	6.27.16	10.7881	2.5752	7.8347
3	7	12%	6.27.16	11.2204	2.8264	8.3420
3	1	0%	6.27.16	9.0123	2.1341	6.9822
3	9	10%	6.27.16	12.0453	2.7322	8.2493
3	8	control.lake	6.27.16	7.3804	2.0514	1.8347
3	4	control.wet	6.27.16	42.1676	7.6955	18.1902
3	2	0%	6.29.16	7.8992	2.0982	6.8347
3	6	3%	6.29.16	8.9767	2.2641	7.0347
3	10	5%	6.29.16	9.7063	2.3712	7.3460
3	3	8%	6.29.16	10.5939	2.4786	7.5021
3	5	10%	6.29.16	11.2766	2.5932	7.8480
3	7	12%	6.29.16	12.4222	2.7502	8.0975
3	1	0%	6.29.16	9.2313	2.1187	7.0266
3	9	10%	6.29.16	12.8534	2.6330	8.4502
3	8	control.lake	6.29.16	7.6692	2.1496	6.6749
3	4	control.wet	6.29.16	44.7266	8.0404	19.9321

* All units in mg/L

Appendix D. GK-12 Fellowship Report

Assessing inquiry-based science curriculum using qualitative assessment

Introduction

Traditional science teaching includes many in class activities that are picked by the teacher and focus on surveying concepts found in standard textbooks (Yager and Akcay 2008). The focus is on learning and memorizing information that is already known, has been chosen by the instructor from the textbook, and involving activities that happen only in the classroom (Yager and Akcay 2008, Marx et al. 2004). Assessment of this traditional science teaching involves written quizzes and exam that are also completed in the classroom.

Science standards are based upon traditional science teaching and assessment, but the American Association for the Advancement of Science (1993) and the National Research Council suggests that there be less of a focus on learning textbook based facts and more of an focus on exploring through inquiry (Marx et al. 2004). Based upon this recommendation, there has been a shift in the emphasis for middle and high school science education from traditional teaching and assessment to inquiry-based projects and labs that promote the process of answering questions and using the scientific method (Yager and Akcay 2008, Marx et al. 2004). Over that time, students who learn science through inquiry-based activities and labs learn basic science concepts just as well as students who learn through traditional science teaching (Yager and Akcay 2008). The

advantages of using inquiry-based science instruction are that students are better able to apply the concepts they learn, learn and use scientific concepts in their daily lives, and have an overall more positive attitude toward science (Yager and Akcay 2008, Wolf and Fraser 2008, Brookhart and Walsh 2006, Gibson and Chase 2002). While all of these are important for understanding and learning science, a positive attitude toward science in early science learning is a key long-term success in science (Wolf and Fraser 2008, Brookhart and Walsh 2006, Gibson and Chase 2002).

Inquiry based learning is specifically effective in urban school districts with a history of traditional science instruction (Marx et al. 2004). This has shown to lead to an increased interest in science from students who view science as impractical and non-essential (Yates and Akcay 2008). The key to the success of inquiry-based learning in urban area is the adaptation of inquiry activities to fit the school environment and student interests (Fogleman and Krajcik 2011, Jones and Eick 2007). From a practical standpoint, this can be the most difficult part of teaching inquiry science to middle school students, and can make or break the success of the activities. However, this hurdle can be overcome with constant re-evaluation of activities and consistent in-service training (Jones and Eick 2007). As an urban school district, PPS is a perfect fit for district-wide inquiry science curriculum due to its' wide range of economic resource issues and access to many natural areas for out of classroom observations and natural world inquiry opportunities.

My inquiry-based lessons presented here were conducted at Lentz Elementary in Portland, Oregon. Lentz Elementary is in a low-income area of the Portland, and science resources at the school mainly consist of textbooks. This made Lentz Elementary a great

candidate school to institute an inquiry-based project. Also, I conducted my lessons in a 6th grade classroom, which is the first year that students at Lentz Elementary attend science-only lessons. This means that the students had not been previously exposed to traditional science teaching. My GK-12 fellowship lasted two years. In the first year, I conducted a year-long project teaching the students how to compare streams using macroinvertebrates. The students came up with their own questions about streams and macroinvertebrates from which we collected data to try and answer their questions. However, I struggled with developing a way to measure their learning. For the second year, I developed a series of quizzes about macroinvertebrates and streams in order to measure their knowledge at each step of the way. It was through that process that I realized I learned more about their knowledge from reading their lab books and analyzing their end of the year posters than I learned from the quizzes I administered throughout the year. The information presented in this paper represents the comparison of my second year application of these two assessment methods: tradition style assessment through quizzes and qualitative assessment from reading lab books and posters.

Method

For this project, I chose to use structured inquiry, which involved providing a question for the students to answer and a procedure for collecting information. Other forms of inquiry-based learning included confirmation inquiry, guided inquiry, and open inquiry. These all differ in the amount of information and structure that is provided to the students. Confirmation inquiry provides the questions and procedure that will lead to a pre-determined answer to the question. Structured inquiry provides a question and a

procedure, which the students must use to collect data and formulate conclusions based upon the collected data. In this form of inquiry, the answer is not known or pre-determined. Guided inquiry supplies only the question to be answered and in open inquiry nothing is supplied. For open inquiry, students must determine their own questions, procedures, and analysis techniques.. As 6th graders and first year science students, I felt structured inquiry would be the most appropriate. I also chose this form of inquiry-based learning so that I could have all of the students working on the same question in order to see the range of understanding from each group on the same basic concepts.

The overall topic of this structured inquiry-based project was to use macroinvertebrates to compare streams from Portland, the Cascade mountains, and the coast range mountains. To do this, students needed to be able to organized macroinvertebrates into categories based upon physical appearance and then compare the groups to determine if they were similar or different. Therefore, the activities of this project were centered around two types of activities: organizing information into groups and comparing groups.

Organizing information into groups

The initial activity for this project was to have the students play the game SET[®]. SET[®] is a game of grouping items based upon common characteristics of number, color, pattern, and shape. Since these are the skills needed to group stream macroinvertebrates, it is the perfect introduction to identifying characteristics that are used to form groups. The students played this game in groups of 4 during three different class periods. In the fourth class period, they were given a piece of paper with many different

macroinvertebrates printed on it from the groups stonefly, mayfly, and caddisfly. They were not given names or any information, they were simply asked to cut out each macroinvertebrate picture, organize them based upon common features, and glue them into pages of their lab books based upon the groups they created. During the fifth class period, I gave a Powerpoint-based lecture about these three macroinvertebrate groups. The Powerpoint had many pictures of different example organisms from each group and a few slides with limited words. The students were required to write key characteristics of each macroinvertebrate group into their lab books. After the presentation, they were given a 10-question visual quiz. The quiz consisted of a picture of an example macroinvertebrate for which the students needed to write down the group to which it belonged (i.e. mayfly, caddisfly, or mayfly).

Comparing groups

The group comparison consisted of identifying macroinvertebrates from the three rivers and then determining similarities and differences between the macroinvertebrate groups. The three rivers were Jones Creek in the coast range, the Sandy River in the Cascade Range, and Johnson Creek in Portland. Each river was compared three different times throughout the school year. In the Fall, students visited Jones Creek, collected macroinvertebrates from the stream, and identified the number of macroinvertebrates in each group. The next two weeks in the classroom, the students identified the number of macroinvertebrates in each group from samples collected by me from the Sandy River and Johnson Creek. In the winter, I collected samples from all three rivers for the students to identify in the classroom. In the spring, students visited the Sandy River, collected macroinvertebrates from the stream, and identified the number of

macroinvertebrates in each group. The next two weeks in the classroom, the students identified the number of macroinvertebrates in each group from samples collected by me from Jones Creek and Johnson Creek. After each identification round of all three streams for each season, the students were required to write out their comparisons of each stream. At the end of the project, the students created posters to describe macroinvertebrates, the results of each seasonal identification, and an overall assessment of the stream comparisons.

Results

Organizing information into groups

There are two main ways to make sets in the game of SET[®]. The first is by getting three cards in which everything is the same. The other way is to get three cards on which every thing is different. This is very tricky for 6th graders who have not played the game before so it took 3 class periods of playing for each student to understand the game. Once they all got it, it really helped them group things based upon similarities and differences.

Grouping pictures of macroinvertebrates together based upon similar traits was an easy next task after SET[®]. The students were able to group pictures of mayflies, stoneflies, and caddisflies together without much help. Looking at their lab books, it was clear that the game of SET[®] helped them to organized organisms into groups based upon physical characteristics. Almost every student correctly grouped all of the pictures into the correct group.

In the Powerpoint presentation, the same pictures from the grouping activity were used to assign macroinvertebrate names and provide some common characteristics of each group. The students seemed to understand the short presentation well, based on

student questions and instructor group questions. For the quiz, I provided them with a list of possible answers for each question (Caddisfly, Mayfly, and Stonefly), showed them each picture individually, and they were asked write down the name of the category that applied to each picture. There were 43 students who attempted the quiz. The mean score was 6.48 out of 10, the median and mode scores were 7 out of 10. The standard deviation was 1.53 with a single low score of 3 and a single high score of 10.

Comparing groups

After each season (fall, winter, spring) the students were required to look at the data they collected for all three streams and decide if each stream was similar or different. The data consisted of numbers of caddisflies, mayflies, and stoneflies that they identified and counted in each collected water sample. Since they were asked a specific question, they often gave direct answers and did not elaborate on other observations that they made. Below are six example answers that compared two of the streams after a season. The answers were not edited for grammar, spelling, or content.

- Yes, they were different. One was really dirty and had little bugs
- My predictions were correct. I think they were correct because I do not see a lot of the same animals in both of them.
- I think these streams are similar. They each have big bugs and really similar tails and antennae.
- These streams are opposite. One had more stuff and the other had less stuff.
- No, they are not the same. Sandy River has more stoneflies and caddisflies than Johnson Creek. Johnson Creek has no stoneflies or caddisflies.
- There was more insects in Sandy River than Jones Creek

Answering specific questions was not a requirement for the end of the year posters.

Each group of four students was simply required to create a poster about the project.

They mostly consisted of a description of each group of stream macroinvertebrates, a small data table with numbers of each type found in each stream during each season, and a conclusion section. Since no specific questions were required to be answered, most groups simply described what they thought the conclusions were. Few groups answered the question(s) about stream similarity. Most simply included information about what they learned. Below are some example conclusion points.

- Clean streams have more stoneflies and caddisflies
- Rivers in Portland are more polluted than rivers in the mountains
- Stream pollution can be measured using insects that live in streams
- Colder water is better for stream insects so we need to plant trees to shade streams
- Every stream has insects that live in the dirt and under the rocks at the bottom.
You just have to look for them
- Streams in the cascades and the coast mountains are the same, Portland Rivers are dirtier.

Discussion

In each of the activities of this year-long project, I asked for answers to specific questions and also give students freedom to provide information on what they learned.

The answers I received from the specific questions did not indicate very much about what the students learned from the activity. On the macroinvertebrate quiz, I only learned if they could remember which name corresponded with the each group based upon common

characteristics. A correct answer did not tell me very much about their knowledge of the group characteristics, it just indicated that the correct name could be assigned to at least one of the characteristics. For the students who answered many of the questions incorrectly, they still had a lot of knowledge about each groups' characteristics that wasn't accounted for in their answers. The best way to assess their knowledge of each group was to look at the way they grouped each picture and then ask them questions about specific pictures that they grouped incorrectly or specific characteristics. Since inquiry learning is about the process of answering a question, assigning a name to each group does not seem as important as identifying common characteristics in order to create a group. In this specific case, the qualitative information gained from looking at the groupings and asking about common characteristics was a better method of assessment than a quiz asking for group name recall.

For the stream comparisons, the answers I received after each season were much less informative than the conclusions on the posters. After each season I asked for an answer to a specific question, which was based mainly on whether or not each stream was similar to or different from each of the other streams. The answers I received to these questions provided no extra details, simply an answer about similarity and difference. On the posters, in the conclusion sections, I provided no structure for the students to follow. They simply included the conclusions that they felt were most important to them. This proved to be a much better way to understand student knowledge about the streams than specific questions about each stream pair. The qualitative information I gained from the poster conclusion sections was a much better representation of student knowledge than the answers to my specific questions after each season.

It was clear from the many activities involved in this project that the attitudes of the students about science were much different during traditional activities in which I presented information, had them take notes, and then take a quiz. Similar to the findings of Broohart and Walsh (2006), students were self-motivated to discover new information, look up details about macroinvertebrates on the internet or in provided books, and find answers to questions when they were trying to find an answer to a question that they came up with during the process. Questions like “Do stoneflies live in polluted streams?”, “Why do stream insects live in the dirt under rocks?”, or “How do stream insects breathe?” were all student questions that motivated self-guided learning with a positive attitude. The host teacher often noted that student attitudes about science class changed on days that I was scheduled to lead activities. The most common reason given by students for this was “We do science and answer questions but on other days we just read books and do boring stuff.” These observations correlate well with the findings of Wolf and Fraser (2008) that show how a slight shift in student attitude can motivate student learning and increase student understanding.

It is important to note here that the students never completed a full exam or written test when conducting this project. This was by design, in order to be able to complete all of the activities and the end of year posters. However, many small quizzes throughout an inquiry-based project have been shown to be effective (McDaniel et al. 2013). In this project, I only had the students take one quiz, and I did not gain much clarity about their understanding of the project from the results. That may have been due to the low number of quizzes or the type of quiz I included. Future projects like this may benefit from more frequent, small quizzes in order to keep key terms in the vocabulary of

the students throughout the project and to make sure the students are applying key concepts to the different parts of the project. If I conducted this project again, I would include a series of small quizzes throughout the year in order to gain understanding of their growth of knowledge throughout the year. With just one quiz, I only gained a snapshot of their grouping knowledge. With a few more quizzes throughout the year, I might have learned more about knowledge improvement over time.

A key aspect for the success of this project were the adaptations the classroom teacher and I made throughout the year. Similar to Fogleman et al. (2011) and Jones and Eick (2007), we had to make constant adjustments to our plan based upon student progress and school resources. After every class period, we would meet, assess our progress, discuss student outcomes, and organize the next part of the activity. For example, we originally planned to play SET[®] only once at the beginning of the year but added more days based upon student understanding of the game and how much we felt it would help them categorize macroinvertebrates. Similar to the findings of Fogleman et al. (2001), I feel our constant re-evaluation was a big influence on the success of this project.

Conclusion

Learning outcomes from inquiry-based can be hard to assess using traditional assessment methods. When each student is guided by their own questions for learning, designing an exam or quiz to capture student learning for all students can be difficult or impossible. Non-traditions, qualitative assessment in this project allowed for a better understanding of student learning than traditional quizzes. I learned more from student questions, reading lab book descriptions, and reading non-scripted conclusion comments

on posters than I did from answers to quiz questions. Future science inquiry-based projects should include a series of small quizzes, but should mainly be assessed using unscripted descriptions of findings from students.