The ability of barley straw, cypress leaves and L-lysine to inhibit cyanobacteria in Lake Hancock, a hypereutrophic lake in Florida

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Abstract Lake Hancock is a hypereutrophic lake in Central Florida dominated by nitrogen-fixing cyanobacteria. In this study, we conducted a replicated aquarium study to determine if additions of barley straw, cypress leaves or the amino acid L-lysine could reduce levels of cyanobacteria in Lake Hancock, as measured by changes in chlorophyll *a*. Additions of L-lysine brought about the quickest reduction in chlorophyll *a* compared to controls. However, the effect of L-lysine levelled off after the first week, while aquaria treated with barley straw and cypress leaves showed increased benefits over time. At the end of the three week experiment, the addition of cypress leaves resulted in an 87 percent reduction in chlorophyll *a* concentrations compared to controls. Prior assessments of water quality have found that lakes that have retained hydrologic connections to their fringing wetlands have higher levels of colored dissolved organic matter (CDOM) than lakes that have been lowered below the elevations of their historical contiguous wetlands. Furthermore, lakes with higher CDOM levels are less susceptible to the influence of nutrient supply. This preliminary study suggests that CDOM may provide lakes with compounds that may moderate the growth of cyanobacteria in lakes with healthy intact wetland fringes.

Keywords Chlorophyll *a*, cyanobacteria, hydrology, Lake Hancock, nutrients

Introduction

Water quality of the various lakes within the Peace River watershed is important not only to those lakes themselves, but also to the downstream waters of Charlotte Harbor. The combined watersheds of the Winter Haven Chain of Lakes (WHCOL), Banana Lake and Lake Hancock are just over 1,000 square kilometers in size, comprising approximately 17 percent of the Peace River watershed. Water quality within Lake Hancock is so poor, due to elevated levels of nutrients and cyanobacteria, that the Peace River Manasota Regional Water Supply Authority is able to detect impacts from lake discharges into the Peace River at its intake structure more than 130 km downstream. The poor water quality within Lake Hancock is such that it is unlikely that it can be restored (Tomasko et al. 2009), which has led to the development of the Lake Hancock Outfall Treatment Marsh. The influence of the polluted waters of Lake Hancock is so extensive that the Lake Hancock Outfall Treatment Marsh alone is expected to meet the Pollutant Load Reduction Goal for the downstream waters of Charlotte Harbor by treating

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Corresponding author: David Tomasko, dtomasko@esassoc.com discharges out of the lake prior to the water entering the upper Peace River (SWFWMD 2000).

In addition to the influence of Lake Hancock, the 24 inter-connected lakes of the WHCOL system are connected to the Peace River through the Peace Creek and Wahneta Farms drainage canals. The watershed of the WHCOL is approximate 83 square kilometers in size (FDEP 2007) and many of the lakes within the WHCOL system have been negatively impacted through historical point and non-point sources of pollution and have been listed as impaired by FDEP.

Recent studies conducted for the City of Winter Haven and Polk County have shown that water quality in local lakes is often more strongly correlated with lake levels than nutrient concentrations (Atkins 2008, Tomasko et al. 2009, Atkins 2010), which is consistent with a state-wide assessment conducted by Terrell et al. (2000). In particular, lakes that have been lowered below their historical condition via regional drainage networks were shown to be more likely to be impaired for water quality than lakes without such impacts, regardless of the degree of urbanization of their watersheds (Atkins 2010). Levels of colored dissolved organic matter (CDOM) in lakes, which correlate with the amount of forested wetlands along the lake boundary (Atkins 2008), appear to moderate the transformation of nutrients into phytoplankton (Atkins 2008, Atkins 2010), a finding consistent with the results obtained by Terrell et al. (2000).

At present, there is no clear understanding of the precise basis for the ability of wetland-associated CDOM to moderate algal populations in Florida lakes. However, research related to the link between decomposition of barley straw and nuisance algal blooms (mostly cyanobacteria) may shed some light on the processes involved. As early as the 1970s, decomposing barley straw had been used to control the growth of cyanobacteria in freshwater lakes in the UK and elsewhere (Newman and Barrett 1993). Researchers in Scotland (Barrett et al. 1996), Minnesota, USA (McComas and Stuckert 2009) and Iran (Rajabi et al. 2010) have shown that decomposing barley straw is capable of reducing the abundance of nuisance algae, particularly cyanobacteria, in lakes in different climates. However, Lembi (2002) and others have pointed out that water quality in lakes does not always respond in a favorable manner to treatment with barley straw.

In addition, researchers in Japan (Hehmann et al. 2002) and Mississippi, USA (Zimba et al, 2001) have found that the amino acid L-lysine was capable of reducing the abundance of nuisance cyanobacteria. Additions of L-lysine appeared to have no impact on the populations of more benign species of chlorophytes, leading Zimba et al. (2001) to postulate that L-lysine might have practical applications for hypereutrophic lakes dominated by nitrogen-fixing cyanobacteria.

In this study, a replicated aquarium experiment was conducted to determine if additions of barley straw and L-lysine might be able to reduce the abundance of nuisance cyanobacteria in Lake Hancock, which has been classified as the most polluted large lake in Florida (FDEP 2005). In addition to these two treatments, an experimental treatment with leaves from cypress trees (*Taxodium distichum*) was included to determine if cypress leaves might have a similar ability to reduce cyanobacteria populations as had been previously documented for barley straw.

Materials and Methods

The experimental design was set up with three replicate 39-L aquaria for each of four treatments: 1) addition of barley straw, 2) addition of cypress leaves, 3) addition of L-lysine, and 4) controls.

Recommended application rates for barley straw include areal application rates (e.g. Lembi 2002, McComas and Stuckert 2009) as well as volume-based application rates (e.g. Barrett et al. 1996). Based on previously published reports (Tomasko et al. 2009 and references within), areal and volume-based application rates were normalized for Lake Hancock with a chosen dosage rate of 40 grams of dry barley straw per cubic meter of water. Scaling dosage rates to a 39-L aquarium, 1.51 grams of dry barley straw were added in a mesh bag to each of three replicate aquaria. The barley straw was purchased from a commercial pond and landscape store. Cypress (*Taxodium distichum*) leaves were collected from a central Florida wetland. Only leaves that had fallen to the ground were used, with the leaves dried to room temperature until they reached constant weight. Cypress leaves were added at the same dosage rate as barley straw, 1.51 grams dry weight per each 39-L aquarium, and they also were placed in a mesh bag at the aquarium surface.

Hehmann et al. (2002) assessed water quality responses to L-lysine at dosage rates of 0.6 to 5 mg/L, while Zimba et al. (2001) used a dosage rate of 1 mg L-lysine/L. For this study, the highest range value was used (5 mg L-lysine/L) as it appeared that cyanobacteria levels in Lake Hancock were higher than those in the experiments carried out in prior studies. In addition to the three treatments described above, three aquaria were designated as controls, with no additions of barley straw, cypress leaves or L-lysine.

The aquarium study was conducted over a three week period from May to June of 2013. Aquaria were located outdoors on property owned by the City of Winter Haven. Aquaria were located under an awning that protected them from rainfall and also were covered with clear plastic to reduce water loss through evaporation and to keep wildlife from disturbing the aquaria. The aquaria were placed on a south-facing side of the covered site and received abundant sunlight throughout the day. Each aquarium included an aeration stone fed by an air pump to ensure circulation of the contents of the aquarium for the entire three-week duration of the experiment. Water for the experiment was collected from the surface waters of Lake Hancock. Water was transported to the aquaria for use the same day as the study was initiated.

Water chemistry was measured for all aquaria at the start of the experiment and then each week for the next three weeks. Water clarity was not quantified at the start of the experiment, but it was measured for the remaining weeks through the use of a miniature Secchi disk, which was moved horizontally away from an observer at the front of the aquarium until it was no longer visible. The disk was then moved farther away still, and then brought back toward the observer, with the distances where the disk disappeared and then reappeared recorded for each aquarium. Water quality data were collected for further analysis by Polk County's water quality laboratory. The parameters, methods and practical quantification limits are listed in Table 1.

Results

All results are shown first as raw data, then for each of the three treatments for each date and then as compared to the control aquaria. Displayed raw data are the means of the three replicate aquaria for each date. When results from treatment criteria are displayed as a percentage of the mean values of controls, the data displayed are the means of the treatment aquaria for each date compared to the mean of the control aquaria for that same sampling event.

Table 1. Water quality parameters, analytical methods used, and practical quantification limits. Method standard operating procedure refers to specific edition of EPA or Standard Method used.

Parameter	Method	Units	Practical Quantification Limit
Chlorophyll *a* (corrected)	10200 H	μg/L	3
Color	2120 B	Pt-Co	5
Total Kjeldahl nitrogen	351.2	mg/L	0.40
Nitrate plus nitrite	4500NO3-I (21ed)	mg/L	0.04
Total nitrogen	351.2&4500NO-3-I	mg/L	0.44
Ortho phosphorus	4500 P-G (21-ed)	mg/L	0.04
Total phosphorus	365.4	mg/L	0.04

Water clarity in the control aquaria varied from 5.2 cm after one week to 16.6 cm after three weeks (Figure 1). At the end of the experiment, water clarity was greatest in the aquaria with the cypress leaves, while the lowest water clarity was in the control aquaria. At the end of the experiment, aquaria with the cypress leaves treatment had the greatest improvement in water clarity, with a mean value 67 percent higher than control aquaria (Figure 2).

Concentrations of chlorophyll *a* in the control aquaria varied from 677 μg/L at the start of the experiment to 160 μg/L after three weeks (Figure 3). Concentrations of chlorophyll *a* in the control aquaria remained essentially the same for the first week of the experiment, but declined by 76 percent over the next two weeks. At the end of the experiment, chlorophyll *a* concentrations were lowest in the aquaria with the cypress leaves, while the highest chlorophyll *a* values were in the control aquaria. At the end of the experiment, aquaria with

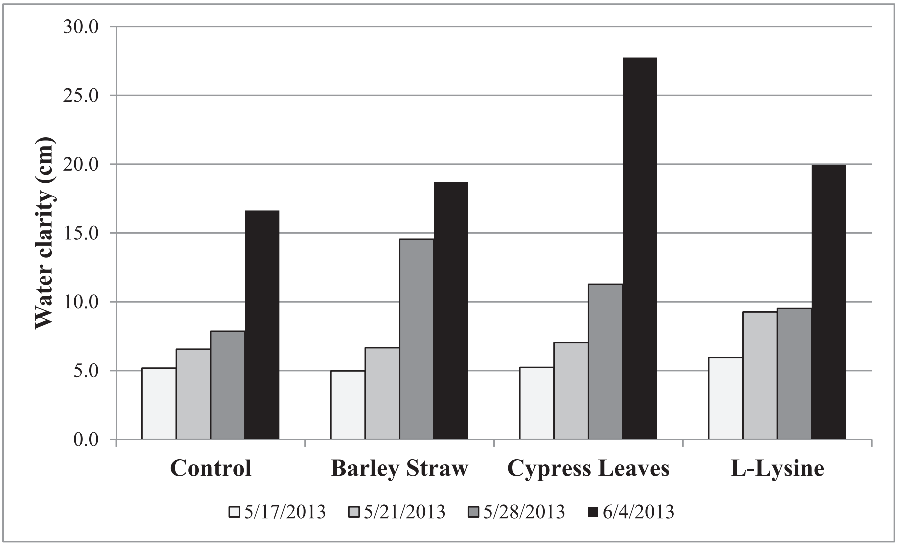

Figure 1. Water clarity (cm) in controls and aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments. Values are means of n = 3.

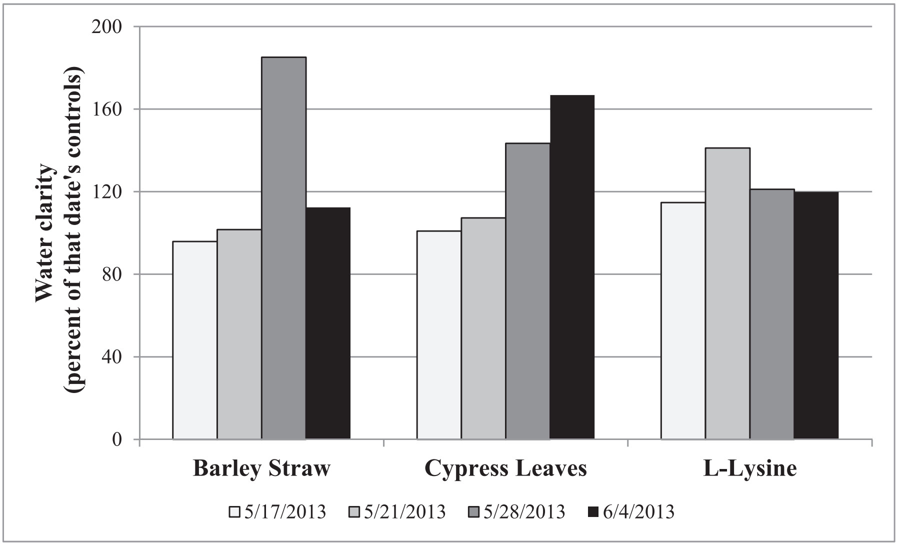

Figure 2. Water clarity (cm) in aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments as a percentage of values from controls on the same date. Values are based on comparison of means of n = 3.

the cypress leaves had the greatest reduction in concentrations in chlorophyll *a*, with a mean value more than 87 percent lower than control aquaria (Figure 4).

Concentrations of total nitrogen in all aquaria exceeded 12 mg/L at the start of the study, likely reflecting the high rates of nitrogen fixation previously

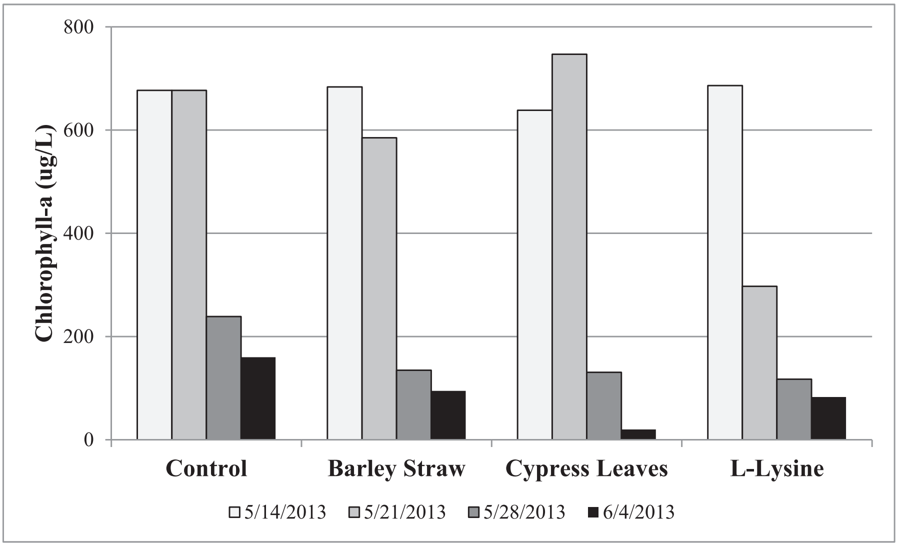

Figure 3. Chlorophyll *a* concentrations (µg Chlorophyll *a*/L) in controls and aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments. Values are means of n = 3.

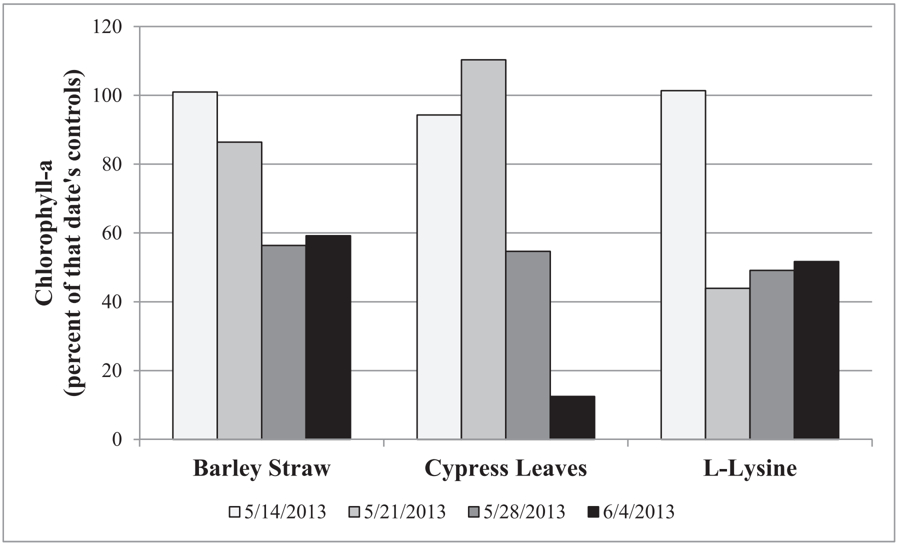

Figure 4. Chlorophyll *a* concentrations (µg Chlorophyll *a*/L) in aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments as a percentage of values from controls on the same date. Values are based on comparison of means of n = 3.

documented for Lake Hancock cyanobacteria by Tomasko et al. (2009). Concentrations of total nitrogen increased over the three weeks of the experiment, suggesting that nitrogen fixation was ongoing throughout the experiment in all four treatments (Figure 5). At the end of the experiment,

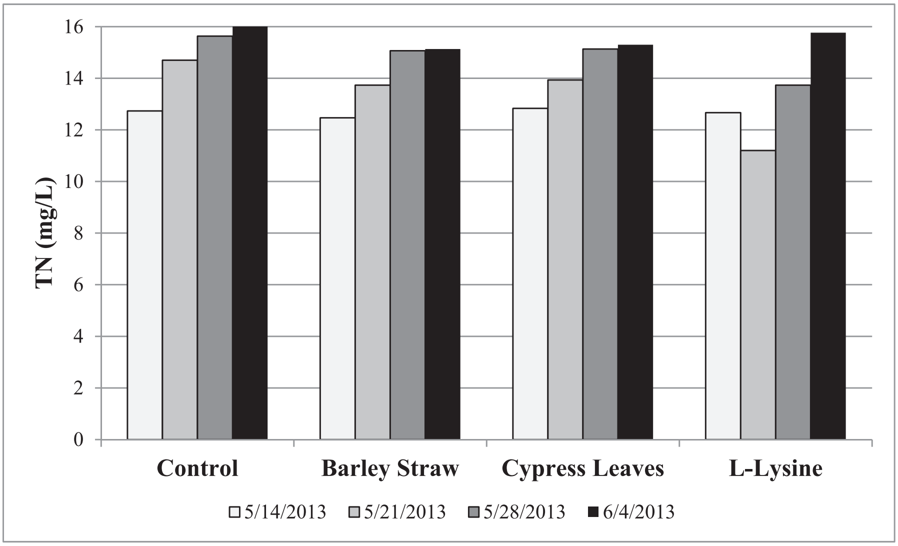

Figure 5. Total nitrogen concentrations (mg TN/L) in controls and aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments. Values are means of n = 3.

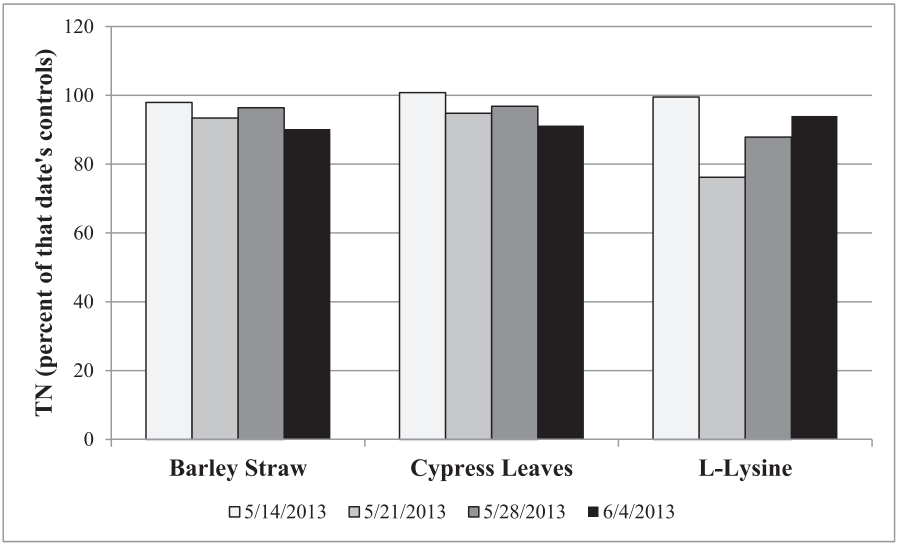

Figure 6. Total nitrogen concentrations (mg TN/L) in aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments as a percentage of values from controls on the same date. Values are based on comparison of means of n = 3.

total nitrogen concentrations were similar in all aquaria, with mean values in the three treatments within 10 percent of values from the control aquaria (Figure 6).

At the beginning of the experiment, concentrations of total phosphorus in all aquaria exceeded 0.40 mg TP/L, reflecting the very high concentrations of phosphorus that characterize Lake Hancock (FDEP 2005 and references within). In contrast to total nitrogen, concentrations of total phosphorus declined in all treatments over the three weeks of the experiment (Figure 7), suggesting that phosphorus was settling out of the water column, perhaps as dead and dying phytoplankton (as suggested by the concurrent trend of decreasing concentrations of chlorophyll *a* in the water column). At the end of the experiment, total phosphorus concentrations ranged from 15 percent lower than controls for the aquaria with cypress leaves to values 28 percent higher than controls for aquaria with L-lysine treatments (Figure 8).

Values of true color, a surrogate for CDOM, ranged between 90 and 100 platinum-cobalt units at the start of the experiment. After one week, there appeared to be an increase in color values in all aquaria. However, laboratory results became unreliable after the first week of the experiment, perhaps due to interactions between dissolved substances associated with the very high levels of phytoplankton (as indicated by chlorophyll *a*) and the techniques for quantifying levels of CDOM. Results are not shown, as the laboratory manager did not feel the technique employed was useful in such hypereutrophic conditions.

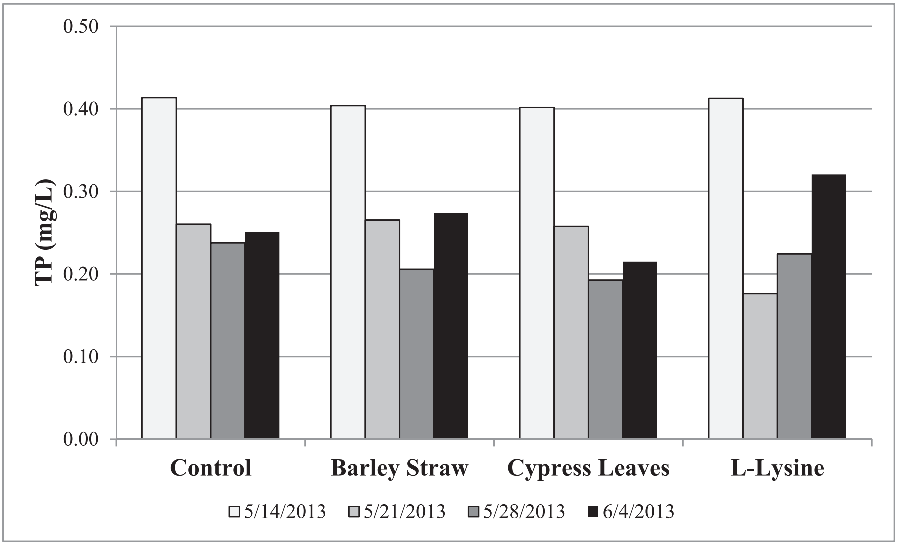

Figure 7. Total phosphorus concentrations (mg TP/L) in controls and aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments. Values are means of n = 3.

Discussion

As outlined by both FDEP and the US Environmental Protection Agency, the explicit approach to restoring water quality in Lake Hancock, the WHCOL system and elsewhere is that local, regional, state and federal resources should

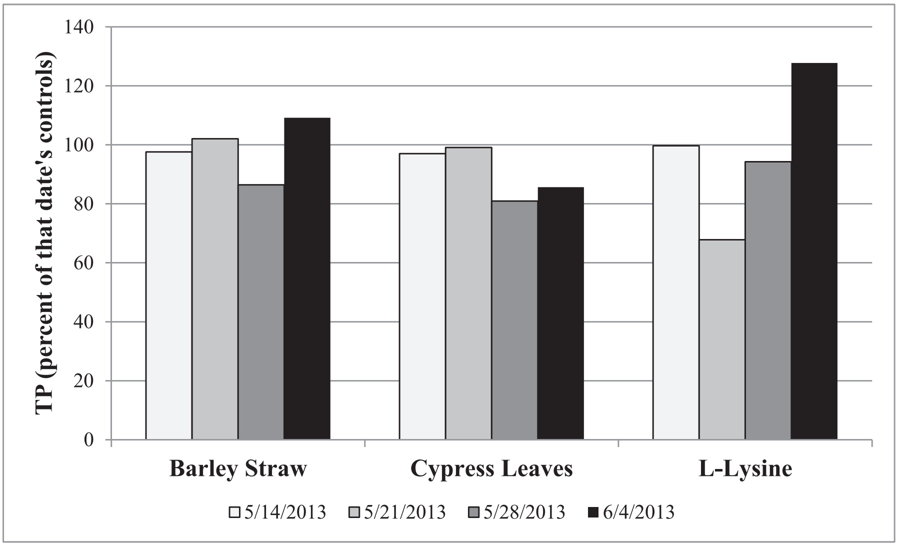

Figure 8. Total phosphorus concentrations (mg TP/L) in aquaria with Barley straw, aquaria with Cypress leaves, and aquaria with L-lysine treatments as a percentage of values from controls on the same date. Values are based on comparison of means of n = 3.

be directed at reducing the external loads of nutrients to impaired waterbodies through various regulatory and non-regulatory programs. While this approach is broadly consistent with prior successful water resource management efforts, external nutrient loads are sometimes not the primary stressor to water quality in Florida lakes (e.g. Terrell et al. 2000). Thus, acting on external nutrient loads alone has not always brought about improvements in water quality in Florida lakes (e.g. Atkins 2008, Atkins 2010).

Within the WHCOL system, Lakes Lulu, May and Shipp have all fully met their regulation-required reductions in stormwater loads of total phosphorus (TP), yet none of these three lakes showed signs of improving water clarity or decreased concentrations of chlorophyll *a* (Atkins 2008). As disappointing as the results are for Lakes Lulu, May and Shipp, they are consistent with the conclusions reached by Terrell et al. (2000) who examined water quality data from 127 lakes located throughout Florida over the period of 1967 to 1997. Over the time period examined, the overall trend in Florida lakes was that of decreasing concentrations of TP, the nutrient most commonly linked with eutrophication in freshwater systems (FDEP 2005 and 2007), and no trend in Total Nitrogen (TN). Despite the downward trend in TP and the lack of a trend in TN, there was a clear trend of increasing levels of chlorophyll *a* in those same lakes. The authors therefore concluded that altered hydrology and impacts from management efforts to control *Hydrilla verticillata* through herbicide applications were important influences on water quality.

Within the City of Winter Haven, lakes that have been lowered below their historical elevations (most often through the construction of canals and regional drainage systems) are more than four times as likely to have impaired water quality than lakes that are at their approximate historical elevations, regardless of the degree of urbanization of their watersheds (Atkins 2011).

In the Chain of Lakes Water Quality Management Plan (Atkins 2010) developed for the WHCOL system, it was determined that the lowering of lakes below their historical elevations appeared to be responsible for “disconnecting” a number of lakes from their historical wetland fringes (Atkins 2008, Atkins 2010). For example, Lake Lulu appears to be approximately 1.2 meters lower now than in its historical condition and the lake’s former swamp shoreline now lies perched above the open waters of the lake. As a result, Lake Lulu has much lower levels of CDOM than Lake Henry, where its open waters and swamp shoreline are still hydrologically connected (Atkins 2008). Lake Henry has CDOM levels more than 10 times as high as Lake Lulu and has lower concentrations of chlorophyll *a*, despite having significantly higher concentrations of both TN and TP (Atkins 2008, Atkins 2010). CDOM is mostly comprised of a mixture of tannin, lignin and humic substances (McDonald et al. 2004). Tannin is a complex molecule found in soils and decomposing vegetation, while lignin is mostly associated with woody parts of trees. Like tannin, humic substances are mostly related to the decomposition of organic matter. All three materials, tannin, lignin and humic substances, can be present through entirely natural processes (McDonald et al. 2004).

Within Florida, there appears to be a disconnect between recent lake management research and approaches to lake management encompassed within current regulatory programs. While there are locations where increased availability of nutrients is the primary stressor to a lake's water quality, the importance of stressors such as hydrologic alterations and the potentially detrimental impacts of eradication efforts focused on *Hydrilla verticillata* are usually not included in the State of Florida's regulatory approach to lake management. As the implementation of regional stormwater treatment projects for Lakes Lulu, May and Shipp did not bring about the intended benefits to water quality (Atkins 2008, Atkins 2010), a more holistic approach to water quality management appears warranted for many lakes. The reestablishment of historical hydrologic connections between lakes and their adjacent wetland fringes should be given at least as much attention as is currently focused on external nutrient loads.

While not specifically related to the topic of wetland influences, the use of barley straw for algal control may be related to wetland influences and the role of CDOM in moderating the transformation of nutrients into phytoplankton biomass. The barley straw treatment promoted by some researchers could be related to algistatic compounds released during aerobic decomposition of barley, which has an elevated concentration of lignin (Newman and Barrett 1993). While the exact mechanisms are unknown, the decomposition of lignin-rich compounds under oxygenated conditions has been postulated as bringing about the release of secondary metabolites that may interfere with the growth of cyanobacteria (Barrett et al. 1996).

Based on the results of this aquarium study, it appears that cypress leaves are capable of producing the same or better benefits as have been noted with the use of barley straw. After three weeks, water clarity in aquaria with cypress leaf additions was 67 percent greater than in controls. The increase in water clarity is likely due to the 87 percent reduction in concentrations of chlorophyll *a* compared to controls. In aquaria with barley straw and cypress leaves, the reduction in chlorophyll *a* concentrations was much greater than the decline in phosphorus concentrations, suggesting that algal growth was being reduced through mechanisms other than nutrient availability alone. Also, as the aquaria were continuously circulated and located in an outdoor setting, it is unlikely that phytoplankton reductions were due to reduced water clarity in aquaria with the added barley straw or cypress leaves.

The finding that cypress leaves have a similar, and actually greater, inhibitory effect on cyanobacteria than was found with barley straw is encouraging and consistent with studies that have concluded that disconnecting lakes from their historical wetland fringes might have contributed to increased phytoplankton populations due to the loss of this moderating influence (e.g. Terrell et al. 2000, Atkins 2008, Atkins 2010). For those lakes with lowered lake levels that could potentially be elevated again to their historical elevations (e.g. Cypress Lake in Osceola County), hydrologic restoration projects might be able to also bring about restoration of water quality through increased wetland influences on the water column. For those lakes that are lower than they were historically, but which are now surrounded by development, deployment of floating bales of barley straw or cypress leaves at appropriate application rates and with suitable techniques could be a way of bringing the lost influence of previously connected wetland fringes back to lakes that have been altered in such a manner.

The addition of L-lysine does not appear to be a good candidate in terms of long-term management techniques for dealing with hypereutrophic lakes. Those aquaria with the L-lysine treatment had the quickest response in terms of water clarity and concentrations of chlorophyll *a*, but after three weeks those aquaria lagged behind the benefits seen in the aquaria with either barley straw or cypress leaves. Also, while the barley straw and cypress leaves techniques are consistent with a larger narrative of lost wetland influences, there does not yet appear to be a nature-based paradigm that would warrant the use of widespread application of amino acids to hypereutrophic Florida Lakes.

Finally, these results should be viewed as preliminary in nature and in need of further assessments to determine their applicability outside of an aquarium setting. In particular, it would be useful to follow up this study with an outdoor and in-lake mesocosm study similar to the techniques used to study the benefits to Lake Hancock of sediment removal (e.g. Tomasko et al. 2009). If a larger-scale and in-lake mesocosm study results in similar findings, an experimental approach on a whole-lake level would be a logical next step, with system responses monitored through the use of a Before and After, Control and Impact experimental design.

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