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ASSESSMENT OF N_2-FIXATION IN LAKES JESUP AND MONROE, FLORIDA

Assessment of N_2-fixation in Lakes Jesup and Monroe, Florida.

FINAL REPORT to:

St. John's River Water Management District
Contract # SK42812

Sherry Brandt-Williams, Ph.D.
Water Resources Department
St. Johns River Water Management District
4049 Reid St.
Palatka, FL 32177

Submitted by:

Leonard J. Scinto[1], Serge Thomas[2], William Anderson[1,3], Makoto Ikenaga[4], and Chris Sinigalliano[5].

[1]Southeast Environmental Research Center, [3]Department of Earth Sciences
Florida International University, Miami, FL 33199. 305-348-1965. fax: 305-348-4096
scintol@fiu.edu.

[2]Florida Gulf Coast University, Fort Meyers, FL 33965.
[4]Department of Bioscience and Biotechnology, Ritsumeikan University, Shiga, Japan.
[5]National Oceanic and Atmospheric Administration, and Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami FL.

Administrative Contact:
Robert Gutierrez, Director
Office of Sponsored Research Administration
Florida International University
11200 S.W. 8th Street, MARC 430
Miami, FL 33199
305-348-2494 (ph); 305-348-4117 (fax)

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EXECUTIVE SUMMARY

Lake Jesup (surface area ≈ 4510 ha, volume ≈ 6.25 x 10^7 m^3) and Lake Monroe (sa ≈ 4000 ha, volume ≈ 7.10 x 10^7 m^3) are two lakes located in the Middle St. Johns River basin in central Florida. Both of these lakes have been verified by the Florida Department of Environmental Protection (FDEP) as impaired water bodies due to excess nutrients (nitrogen, phosphorus). The St. John's River (SJR) connects to the northern end of Lake Jesup which has a water residence time estimated to be on the order of about 80 days. Contrastingly, Lake Monroe is an open lake more like an enlargement of the SJR with a very short hydrologic residence time of approximately 10 days and is thus free-moving. FDEP has established a Total Maximum Daily Load (TMDL) for only total phosphorus (TP) in Lake Jesup's watershed load. Although FDEP established a nitrogen TMDL, no effort has been made to enforce reductions through a Basin Management Action Plan (BMAP) because there was evidence of nitrogen fixation. Both FDEP and St. Johns River Water Management District (SJRWMD) hypothesized that reduction in TP below levels leading to dominance by nitrogen fixating species would concomitantly reduce TN concentrations.

The SJRWMD identified a need to obtain estimates of annual within-lake N_2-fixation to improve their ability to adequately manage the Lake Monroe and Jesup watersheds. Our objectives in this study were to improve our understanding of the physicochemical and biological influences on cyanobacterial N_2-fixation in Lakes Jesup and Monroe to assist in the refinement of management scenarios. Our specific objectives included estimation of the annual within-lake TN loading resulting from nitrogen fixation, determination of seasonal, daily, and diel variations in N_2-fixation, correlate N_2-fixation with other important in-lake variables, and relate N_2 -fixation to molecular characterizations of the organisms and the level of gene expression responsible for N_2-fixation.

We conducted field measurements, water collections, incubations, and analysis at six sites on each of the two lakes approximately every seven weeks for total of eight sampling events throughout an annual cycle starting in February 2007 and ending in January 2008 to assess seasonal variation. Additionally, incubations to determine variation between days were conducted for one week periods during the dry and wet seasons. Diel variation was determined from multiple incubations within a 24 h period, also during the dry and wet seasons. Water physicochemistry was determined from whole water column profiles and chemical analysis of samples. The N_2-fixation was determined by acetylene reduction (AR) which was calibrated using ^{15}N incorporation. A molecular assay to determine *nif*H gene expression was conducted to correlate/quantify N_2-fixation rates. Productivity measures (light/dark bottles) were conducted along with our N_2-fixation incubations. The photosynthetic activity of the major algal groups (*Bacillariophyceae, Chlorophyceae, Cyanophyceae*) were assessed by pulse amplitude modulation (PAM) measurements. Finally, molecular techniques using DNA and RNA analysis were conducted to identify the algal communities responsible for N_2-fixation.

Lakes Jesup and Monroe are nutrient rich systems with Lake Jesup being continuously hypereutrophic and Lake Monroe being eutrophic to hypereutrophic during this study

(2007). The rate of N_2-fixaton was obtained by comparing the C_2H_2 reduction assay and the $^{15}N_2$ isotopic approach and resulted in a C_2H_2/N_2 ratio of 6.37:1. N_2-fixation varied throughout the annual cycle and was generally greater in Lake Jesup compared to Lake Monroe. Where Lake Jesup showed some level of N_2-fixation at all sampling events, Lake Monroe showed that there were periods of high fixation alternating with periods of low or non-detectable fixation. Although N-fixation rates calculated as mass per volume per time (e.g. mg L^{-1} d^{-1}) were generally higher in Lake Jesup than Lake Monroe, the larger volume of Lake Monroe compensates for the differences in rates and therefore leads to greater TN fixation. The average estimated mass contribution of TN during this 334 d study was 128.6 Mg TN for Lake Jesup and 187.7 Mg TN for Lake Monroe which equates to N-fixation rates of 0.0088 mg TN L^{-1} d^{-1} in Lake Jesup and 0.0077 mg TN L^{-1} d^{-1} in Lake Monroe. The mean TN concentration in Lake Jesup during this study was 1.38 mg L^{-1} and for Lake Monroe 1.02 mg L^{-1} which means that if these lakes were closed systems N-fixation rates could have created the mean water TN concentrations in 156 d in Lake Jesup and 132 d in Lake Monroe. However, our estimates are not related to the actual flux of N into the basins as this would require measuring the total of all fluxes into and out of these systems.

Both lakes were clearly dominated by phytoplankton vs. rooted macrophytes (Anderson et al., 2004), and specifically Cyanophyacea were an important group in both systems. Temperature was a driving factor in the primary production and the subsequent N_2 fixation, and because water temperature is a function of solar heating, irradiance is indirectly controlling the primary production and N_2-fixation in these lakes. The pH was also an important factor in both lakes and was often correlated (or indirectly correlated for Lake Jesup, since no direct correlation was found) between algal metabolism and N_2-fixation. Because of the high concentrations in both TP or TN, it is unlikely that the TN:TP ratio influenced N_2-fixation as might have been expected.

Determination of cyanobacterial dominance by both the PAM and molecular work (DNA/RNA) showed that seasonal variations in the major algal groups were observed in both lakes. Lake Jesup was largely dominated by Cyanophyceae throughout the year (PAM) and this community was likely composed largely of *Cylindrospermopsis* (RNA). Algal group dominance throughout this study was more variable and complex for Lake Monroe. Lake Monroe was dominated by Cyanophyceae during the late dry to early wet season and in December 2007 (PAM). However, relative dominance varied with Bacillariophyceae and Cyanophyceae being co-dominant generally during the winter months (PAM determination which is proportional to biovolume).

The approach used to measure the enriched samples also provides natural abundance $\delta^{15}N$ values from the "untreated" particulate organic matter (POM). Isotopic values less than 1 ‰ and near 0‰, typically reflect an atmospheric N source via fixation of N_2, versus NO_3^- utilization (Anderson et al, 2004). All data in the time-series from both Lakes were <5‰, except for one value from Lake Monroe (September of 2007), which had an extremely enriched value of 9.3‰, which indicated an N source not originating from fixation. $\delta^{15}N$ values near this level of relative enrichment are typically associated with denitrification (Teranes and Bernasconi, 2000), and/or dissolved inorganic N (DIN)

coming into the watershed from pasture lands (manure) (Kendall 1998). However, these data did not fall within the typical range of $\delta^{15}N$ values for denitrification when Lake Monroe's DIN values were considered.

Both Lake Monroe and Jesup had phytoplankton populations that fix atmospheric N_2 within the water column. This contribution of N is significant, but can be further evaluated with improved mixing and hydrodynamic models of both lakes as understanding changes in residence times during wet and dry seasons would greatly improve estimates of N loading to the basins. These data provide a baseline understanding of the N-fixation within these shallow subtropical lakes, but further work will enhance the understanding of N-flux in this region.

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ACKNOWLEDGEMENTS

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We would like to thank the St. John's River Water Management District (SJRWMD) for financial support and especially Dr. Sherry Brandt-Williams (SJRWMD) for her scientific, administrative and moral support.

1.0 INTRODUCTION

Lake Jesup is a 4317 ha lake in the Middle St. Johns River basin in central Florida. The St. Johns River Water Management District's Governing Board in 2002 designated this area as a priority basin for restoration of water quality and fish and wildlife habitats as part of Florida's Surface Water Improvement and Management (SWIM) program. Lake Jesup's watershed is highly urbanized (61% is developed) and several tributaries deliver untreated stormwater from urban Orlando into the lake. The St. John's River connects to the northern end of Lake Jesup. Water residence time is estimated to be on the order of about 80 days which is short compared to most lakes (having residence time of 8 – 10 yrs). The position of the SJR relative to Lake Jesup causes periods of reversible flow with Lake Jesup acting as a source or a sink for water (and associated nutrients) at varying times. Lake Jesup thus often behaves in a manner similar to that of a reservoir, storing water from the SJR during the dry season and providing water to the SJR during the wet season. A recent evaluation of monitoring data for the period 1995 – 2003 shows an overall net outflow of water to the SJR from Lake Jesup but not for all years (SFWMD, 2004). FDEP has established a Total Maximum Daily Load (TMDL) for only total phosphorus (TP) in Lake Jesup's watershed load. Although FDEP established a nitrogen TMDL, no effort has been made to enforce reductions through a Basin Management Action Plan (BMAP) because there was evidence of nitrogen fixation. Both FDEP and St. Johns River Water Management District (SJRWMD) hypothesized that reduction in TP below levels leading to dominance by nitrogen fixating species would concomitantly reduce TN concentrations. Estimated average TN concentration in the outflow water was greater than inflow (2.669 vs. 1.745 mg L^{-1}) during 1995 – 2003 (SFWMD, 2004). According to SJRWMD, the Florida Department of Environmental Protection recently proposed TMDLs for both nitrogen and phosphorus for Lake Jesup. The SJRWMD is developing pollution load reduction goals (PLRGs) for nitrogen and phosphorus that will assist FDEP in later refinements to their TMDLs. SJRWMD has defined a preliminary target in-lake concentration for nitrogen that would restore the lake to state water quality standards of 1.2 mg TN L^{-1}. Plant coverage in Lake Jesup as studied by FDEP Bureau of Invasive Plant Management (BIPM) was estimated at 298 ha of emergents, 63 ha of floating macrophytes, and < 2 ha of submerged aquatic vegetation summing to a small fraction (8.4 – 11.0% depending on estimated surface area) of the open water surface area (SFWMD, 2004).

Lake Monroe is a 4000 hectare lake in Seminole and Volusia Counties FL. Lake Monroe is an open lake more like an enlargement of the SJR. Lake Monroe is a flow through system with a very short hydrologic residence time of approximately 10 days and is thus free-moving. Lake Monroe was on the 2004 verified list as an impaired water body by FDEP due to excess nutrients (nitrogen, phosphorus) and for low dissolved oxygen. In a recent study of sediment accumulation in Lake Monroe, Anderson et. al. (2004) found this lake contained only a minor flocculent detrital organic layer on the sediment surface and had an average water depth of about 215 cm. Sediment analysis shows that this lake has accreted relatively low density organic rich soft sediments in the last 100 years.

The SJRWMD has identified a need to obtain estimates of annual within-lake N_2-fixation to improve their ability to adequately manage the Lake Monroe and Jesup watersheds. Numerous factors affect N_2-fixation rates including: temperature, light, other available N species (NH_4^+, NO_3^-, and organic nitrogen – ON, urea for example), available P, DO, pH, and the size and composition of the N_2-fixing phytoplanktonic communities. These factors can change seasonally, diurnally, vertically within the water column, and spatially depending on lake morphology, structure, and hydrology.

Much of peninsular Florida has a subtropical climate with seasons more determined by wet/dry cycles than by large fluctuations in temperature. Generally, the early rainy season brings a flush of nutrients from the watershed while by the end of the wet season lakes tend to be diluted and there is relatively lower importation of nutrients from the surrounding watershed. The biological fixation of N_2 is energetically expensive, utilizing energy and photoreductants produced by photosynthesis. Recently Pearl et al. (2003) found greatest N_2-fixation rates at 14:00 (2:00 p.m.), a point in the diurnal cycle where photosynthesis is most active and cells have accumulated photosynthates (e.g. glucose) from the morning's light. However, oxygen prevents N_2-fixation and therefore can reduce this ability in cells unless the algae have heterocysts (although there are physiological adaptations by which non-heterocystic algae fix N_2 in oxidized environments).

1.1 Objectives.

Our objective was to improve our understanding of the physicochemical and biological influences on cyanobacterial N_2-fixation in Lakes Jesup and Monroe with the intention that these results may be extrapolated to other lakes in the SJR system and thus allow refinement of management scenarios. The overall goal of the SJRWMD is to differentiate between nitrogen entering Lakes Jesup and Monroe from watershed/tributary loading and nitrogen in the water column due to nitrogen fixation thus contributing to a more accurate assessment of nitrogen loading from the watershed through an improved nitrogen balance around each lake.

Specific objectives include:

1) Estimation of the annual TN loading from nitrogen fixation.
2) Determine the temporal variability in nitrogen fixation and correlate with other important in-lake variables e.g. physicochemistry including nutrients and phytoplankton communities.
3) Evaluate daily fluctuations for two non-consecutive weeks, one in the middle of the wet season and one in the middle of the dry season, to determine ambient range.
4) Determine if nitrogen dynamics have any similarities between the two lakes and other similar lakes studies.
5) Use the accumulated information to build a bank of data relating specific levels of N_2 -fixation to a particular genus with an understanding of the factors that influence N_2-fiaxtion rates and the variability in these rates.

2.0 METHODS

2.1 Site Selection

Lakes Jesup and Monroe have similar surface areas (approximately 4510 ha and 4000 ha, respectively) but because of differences in depths (LJ = 2.4 m max, LM = 4.8 m max) and volume, Lake Jesup contains less open water and has more shallow and wetland areas. Morphologically, Lake Monroe has a more uniform round shape which contrasts with the elongated and meandering shoreline of Lake Jesup. Project logistics did not provide for intensive, detailed spatial sampling in these lakes. However, we employed a stratified sampling technique to insure that all areas (based on depth) of each lake were represented. In our stratified sampling we adapted the sampling effort to a per area or volume of lake of similar bathymetry (depth intervals). We did not determine bathymetric depths by field sampling prior to our selection of site locations. Rather the relative depths based on those available from Mapsource Garmin Bluechart Americas v 7.5 were used. Although lake surface area and depth will vary temporally depending on water stage we used these published values to at least partially assure that our sites were representative of overall lake water conditions and depths. Several previous studies have been conducted on each of these lakes. We selected sampling locations from among those identified in previous studies as long as they fit our stratified sampling scheme. Six sampling stations were identified in each lake based on the planar projection of the various depth strata of a similar bathymetric interval (Fig. 2.1.1 and 2.1.2).

Histograms of the percentage of total area represented by depth increments showed that when using half-meter increments > 95% of Lake Jesup is less than 1.5 m deep (Fig. 2.1.3) while Lake Monroe contained generally five depth increments (omitting a small deep spot) with depths of 2.0 to 2.5 m accounting for almost 40% of the lake surface area (Fig. 2.1.4). Lake Jesup has greater spatial variability and a more convoluted shape than Lake Monroe. Six sampling locations in each lake were identified (via GPS coordinates; Table 2.1.1) that were used for the collection of all water samples. In Lake Jesup we established 2 locations in each 0.5 m depth increment (omitting > 1.5 m depths or 4% of the lake area). In Lake Monroe one location in each depth increment was selected in each of the four increments from 0 to 2.0 m and because the 2.0 to 2.5 m increment accounts for a relatively large surface area we sampled two locations for this depth. Using these criterion sampling sites were located on each lake (Figs. 2.1.1 and 2.1.2). Site designations follow notations used in previous studies; Tomasko (2006) for Lake Jesup and Anderson et al. (2004) for Lake Monroe.

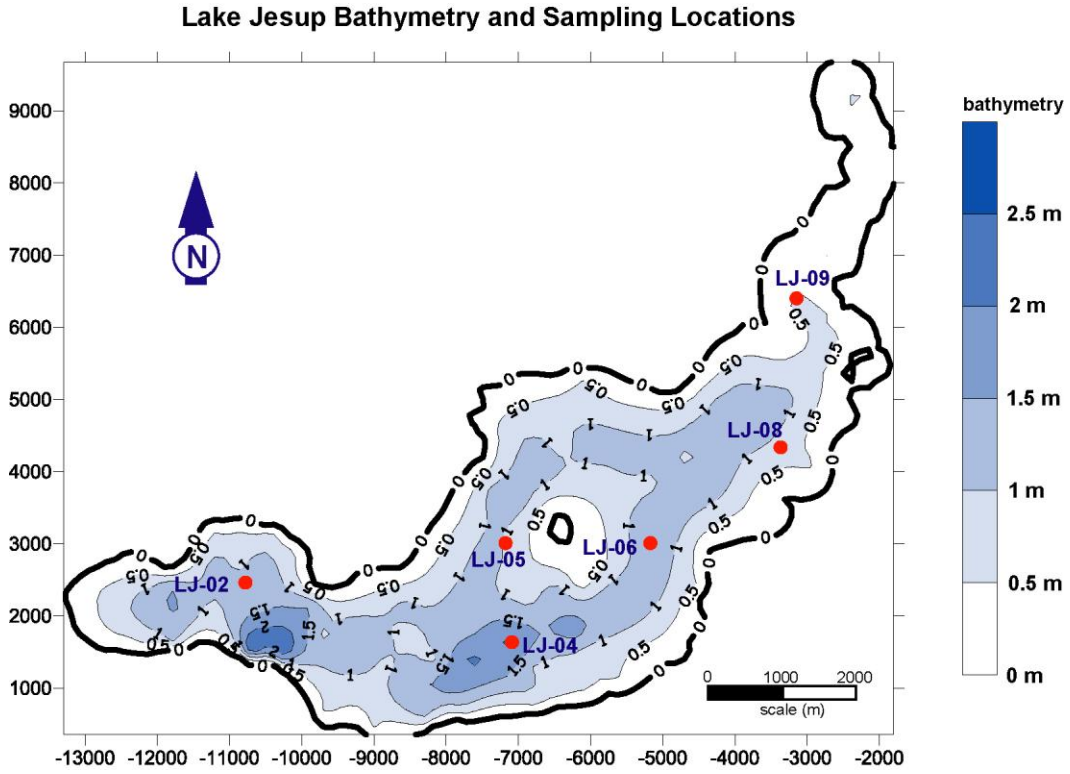

Figure 2.1.1. Lake Jesup bathymetry (depth measurements issued from Mapsource Garmin Bluechart Americas v 7.5 and data points using Surfer 8, square cells, kriging method and linear model).

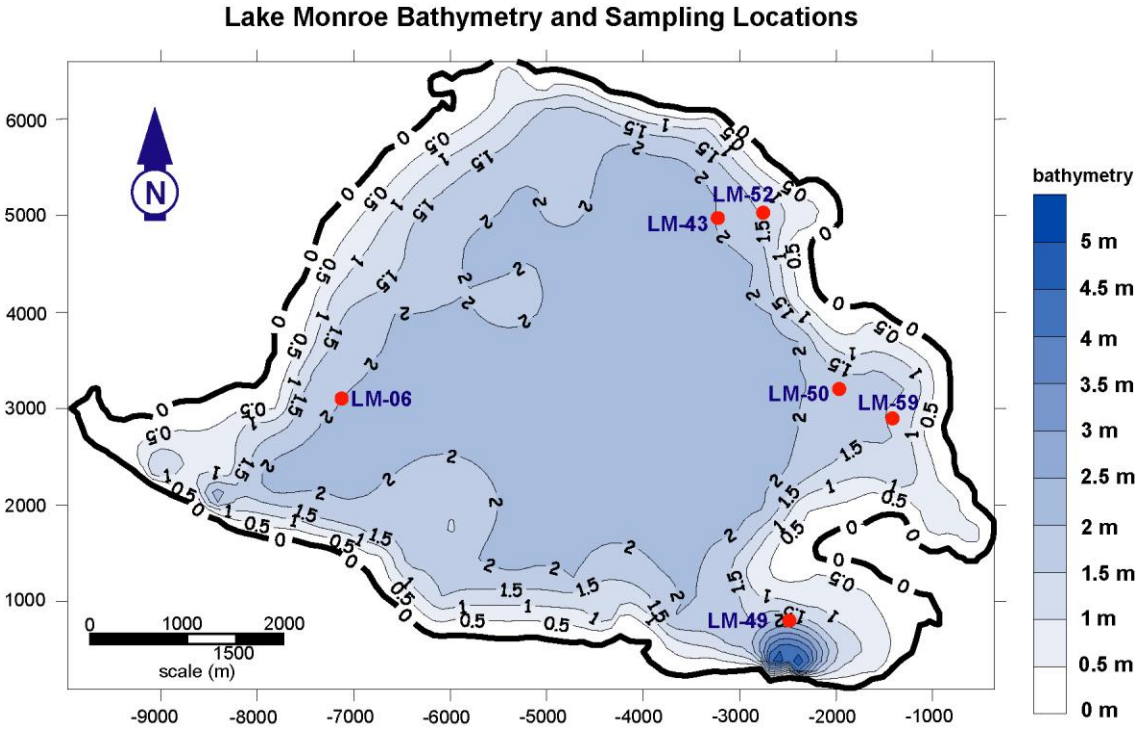

Figure 2.1.2. Lake Monroe bathymetry (same citation information as above).

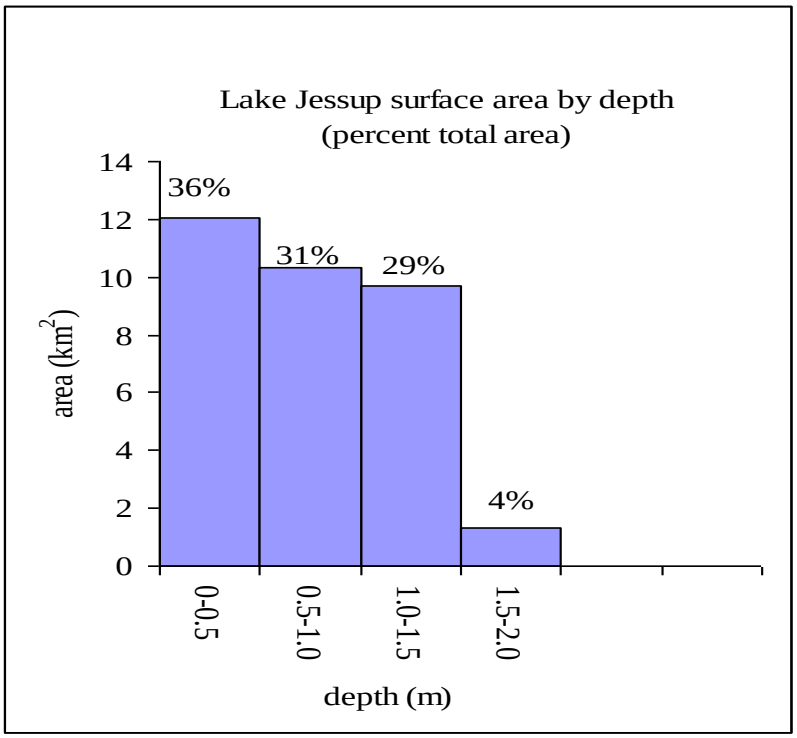

Figure. 2.1.3. Percentage of total Lake Jesup surface area within several depth increments.

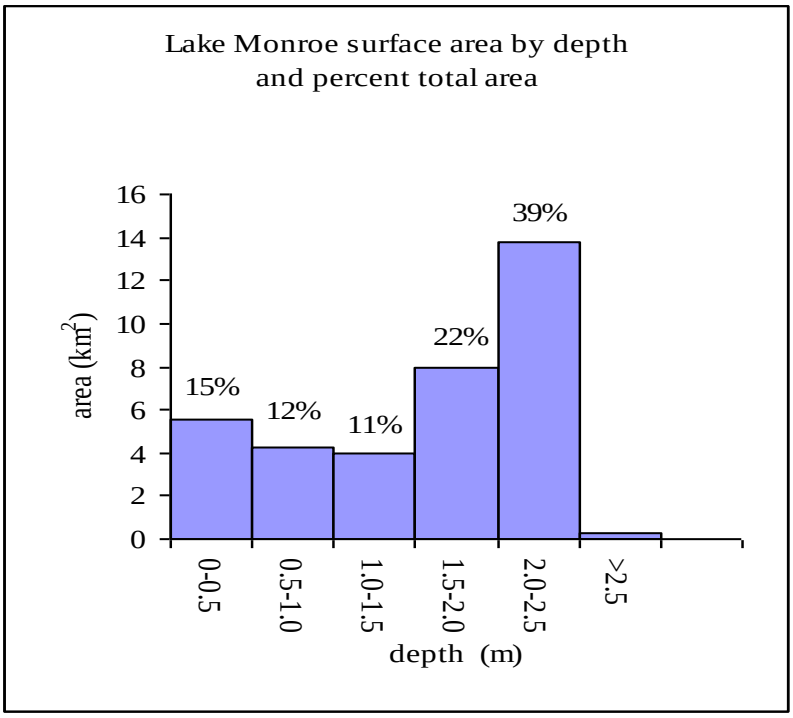

Figure 2.1.4. Percentage of total Lake Monroe surface area within several depth increments.

Table 2.1.1. Locations of sampling stations in Lake Jesup and Lake Monroe.

Site ID	UTM X Coordinates	UTM Y Coordinates
Lake Jesup		
LJ-02	474173	3176665
LJ-04	477888	3174841
LJ-05	477783	3177642
LJ-06	479790	3177177
LJ-08	481609	3178528
LJ-09	481830	3180589
Lake Monroe		
LM-06	471059	3189874
LM-43	475019	3191742
LM-49	475769	3187555
LM-50	476614	3189954
LM-52	475453	3191802
LM-59	477129	3189676

2.2. Temporal Sampling Schedule.

Seasonal variation in subtropical, peninsular Florida is determined largely by variations in the wet/dry cycle with warm wet summers and drier, slightly cooler winters. Variations in rainfall also affect watershed processes including runoff and dilution events. Although peninsular Florida does not experience the wide swings in temperature that more temperate climates experience, there is seasonal variation in temperature and day-length. The biological fixation of N_2 is done by photoautotrophs whose activity is partially regulated by photosynthesis and therefore should vary on daily and seasonal cycles. To determine seasonal variation we conducted water collections, incubations, and analysis on each lake approximately every 7 weeks for total of 8 sampling events throughout an annual cycle starting in February 2007 and ending in January 2008 (Table 2.2.1). We conducted the incubations for approximately 4 hour periods with the incubation interval generally bracketing the period around 1400 (2:00 p.m.). To assess variation between days in a weekly cycle, a series of daily assessments was conducted for a period of one week once in the middle of the dry season (April 2007) and once in the middle of the wet season (September 2007). During each of these events we sampled each lake for 6 of the 7 days in a weekly cycle. Additionally, on one day during the week long assessment we conducted our field protocols (including sampling and incubations, etc.) at either two (daytime as above and night, April 2007) or three times (morning, midday, and night, September 2007) to determine diel variation. On the days a lake was sampled for diel variation the other lake was not visited as time and logistics would not allow this. This resulted in the weekly sampling occurring on 6 of the 7 days for each lake. The nighttime incubations generally bracketed the hours around 23:00 (11:00 p.m.). The same six stations were used for all samplings allowing for the assessment of daily, weekly, and seasonal variability.

Table 2.2.1 Dates of sampling events on Lakes Jesup and Monroe.

Event	Date
1	February 28, 2007
2 and dry season week-long study	April 20 – 26, 2007
3	June 7, 2007
4	July 23, 2007
5 and wet season week-long study	September 10 – 16, 2007
6	October 30, 2007
7	December 18, 2007
8	January 28, 2008

2.3. Water and Site Physicochemical Sampling.

Water was collected with a 1.8 m clear acrylic tube (diameter = 7 cm) so that the whole water column (defined as the surface water down to approximately 10 cm above the sediment or floc layer if present) was sampled. Tubes of water were drawn repeatedly until a 10 L dark, insulated carboy was filled to overflowing. Carboys were immediately capped to minimize exposure to, and mixing with the atmosphere. Water was thoroughly mixed prior to each subsampling.

A YSI 600QS-08 probe was used to profile dissolved oxygen (DO), temperature, pH and electrical conductivity (EC) at 0.5 m intervals from the lake surface to the bottom. Probe measures yield an idea of the level of stratification in the water column. The photosynthetic photon flux density (PPFD) profile was assessed with a LICOR LI-1400 DataLogger connected to a LI-192 Quantum flat sensor. PPFD measurements were taken at 25 cm intervals.

From each carboy, an unfiltered and filtered water sample was collected and stored on ice until returned to the laboratory. Filtered water was vacuum filtered through a 0.45 μm Gelman Sciences membrane filter following return to the field station. Unfiltered water samples were analyzed for total N (TKN: ASTM D5176), P (TP; EPA 365.1), and organic C (TOC; EPA 415.1). Filtered water samples were collected for analysis of soluble nitrate, nitrite (NO_3, NO_2; EPA 353.2), ammonia (NH_3; EPA 350.1), reactive P (SRP; EPA 365.1).

Filters were collected for Chlorophyll a analysis (SM10200 H) vacuum filtering aliquots of known volume onto 25mm Whatman GF/F glass fiber (0.7um pore size) glass fiber filters (Whatman GF/F). Filters were folded into microcentrifuge tubes and stored frozen in the dark until analysis. Chlorophyll a in the algae retained on the filter was extracted for 48h with 90% cold (-20ºC) acetonic extraction. The acetonic Chl a concentration in the extract was determined on a Gilford FLUORO IV spectrophotometer (excitation 435nm, emission 667nm).

All water quality chemical analysis was conducted by the NELAC Certified (Certification # E76930; Non-potable water general chemistry) Water Quality Laboratory of the Southeast Environmental Research Center.

2.4. $^{15}N_2$-fixation and Calibration of and Acetylene Reduction Method (AR).

Tracing $^{15}N_2$ incorporation is the only direct measure of actual N fixation. When nitrogenase activity based on AR is extrapolated to actual N_2 fixation a conversion factor is used. Generally, a conversion factor of 3 – 4 moles C_2H_4 produced is equal to 1 mole of N_2 fixed. However, conversion factors can range 0.5 – 13 necessitating experimental determination for LM and LJ (Seitzinger and Garber, 1987; Montoya et al. 1996). Calibration of the AR method for N_2-fixation was conducted by using simultaneously

incubated triplicate ^{15}N vessels containing water from sites LM50 and LJ08 following the methods of Montoya et al. 1996. This water was used to overflow triplicate 250ml Pyrex bottles which were sealed with Teflon-faced septum caps. An aliquot of 0.5 ml of $^{15}N_2$ (98 atom %, Cambridge Isotope Labs) gas was injected via a gas-tight syringe with the same volume of water being removed to equalize pressure in the bottle. Sample bottles were gently mixed and placed back in the lake (at a single location) for 3-6 hour incubations. Particulate organic matter (POM) was filtered (pre-ashed GFF filters) from three aliquots of the bulk water sample and from each of the three bottles at the end of the incubation. The six filters were dried to a constant weight and were analyzed on the Elemental Analyzer- Isotope Ratio Mass Spectrometer (EA-IRMS) system at the SERC Stable Isotope Laboratory (SIL). Isotope abundances were used to measure fixation of N_2 by comparing the isotopic composition change of the POM after the addition of the $^{15}N_2$ tracer gas, over a specified incubation period, to the bulk water samples that did not undergo incubation. This straight-forward approach produces viable results because modern IRMS systems are very sensitive for this type of isotopic measurement and require small sample volumes. The initial ^{15}N at% of the tracer ranged between 14 and 17 at%, depending on amount introduced into the known lake water sample (Hamme and Emerson, 2004; Montoya et al., 1996). Results of the ^{15}N incubations were compared to their AR counterparts (same lake location) after normalization for incubation time, biomass, and volume. A total of 96 ^{15}N samples were analyzed (1 location per lake, 2 lakes, 3 t_0 replicates and 3 t_f replicates, and 8 events) during the seasonal studies.

2.5. N_2-fixation by the Acetylene Reduction Method (AR).

Assays of N_2-fixation (nitrogenase activity) using the acetylene reduction (AR) technique were conducted. This technique is relatively straightforward and uses the catalysis of the reduction of acetylene (C_2H_2) to ethylene (C_2H_4). This established method is possible because the nitrogenase system is highly versatile and reduces many low molecular weight substrates such as N_2, C_2H_2, N_2O, etc. (Weaver and Danso, 1994). However there are several caveats to using a surrogate (C_2H_2) to quantify the amount of N_2 fixed, mostly as a result of greater water solubility of C_2H_2 than N_2. Therefore using the AR method requires calibration if N_2-fixation is going to be quantified in terms of moles N_2 fixed per unit volume (or biomass or surface area etc.) and not just as a relative assay. Calibration of the AR method for N_2-fixation will be conducted by using simultaneously incubated triplicate ^{15}N vessels containing water from one of the six sampled locations in each lake (section 2.4 above).

Acetylene reduction measurements were carried out in triplicate for all sample sites and for field blanks using distilled, deionized water. Upon return to the field station, subsamples of 500 mL lake water were added to1 L transparent rectangular cell culture flasks. Flasks were capped with rubber stoppers to make airtight then 50 cm^3 of headspace was withdrawn from the air present in the bottle, and 50 cm^3 of acetylene was injected into the bottle. Acetylene was generated in the field using CaC_2 rocks, trapped in slack Tedler bags, and allowed to cool to ambient temperature before injection.

Bottles were transported to the lake for incubation immediately following injection. Routine daily incubations were conducted for approximately 4 hours bracketing 14:00 and were carried out dockside at the water surface in either Lake Jesup or Lake Monroe. Upon completion of AR incubations, a 10 cm^3 gaseous subsample of the headspace was transferred to purged headspace vials for return to the laboratory for analysis of C_2H_2 and C_2H_4 on an Flame Ionization Detector (FID) equipped Hewlett-Packard 5890 Gas Chromatograph (GC) using a Porapak Type R column (2 m). Purged GC headspace vials were prepared in the laboratory before sampling by repeatedly vacuum evacuating air from the vials and purging with N_2. Ten cm^3 of headspace was removed from the purged vials immediately before the addition of 10 cm^3 gaseous subsamples thus maintaining atmospheric equilibrium. Ethylene standards were created in the field at the time of sample collection by injecting blank purged vials with 25 µL and 250 µL additions of either 99.5 mL L^{-1} or 1000 µL L^{-1}, respectively, of Standard C_2H_4 concentration gas (Scott Specialty Gases, Troy, MI). Field blanks were analyzed along with incubation samples to insure a lack of C_2H_4 conversion during transport. The GC was calibrated using known volumes of a commercial standard (e.g. 100 µL L^{-1} C_2H_4 in He, Scott Specialty gases, Troy, MI). These methods were used during the 8 seasonal samplings, 2 week long daily samplings, and the diurnal studies. In total, this method was carried out for approximately 1100 water samples over the course of the project.

2.6. Photosynthesis and Productivity.

There can be a strong link between photosynthesis and N_2-fixation. Therefore we simultaneously measured productivity (light/dark bottles) along with our N_2-fixation incubations. In the light/dark bottles (oxygen method) the change in the dissolved oxygen (DO) concentration of the water was recorded under light (= net photosynthesis, NPP) and dark (respiration, R) BOD bottles. The difference in DO between the light and dark bottle yields gross primary production (GPP).

To determine phytoplankton metabolism, net primary production (NPP) and respiration were assessed using the change of dissolved oxygen method (DO) in duplicate 250mL light and dark BOD bottles. GPP was then subtracted from the respiration and NPP. Bottles were incubated in situ in one of the lakes on floating racks for 2-4 h depending on the irradiance intensity and percentage of DO supersaturation level in the bottle. Changes in DO were measured using an YSI 5905 probe with an YSI 58 meter. Metabolic rates were expressed in mg O_2 L^{-1} h^{-1}.

The major algal groups (*Bacillariophyceae, Chlorophyceae, Cyanophyceae*) were assessed by pulse amplitude modulation measurements using a PHYTOPAM (www.walz.com). This machine is a quadrachromatic fluorometer exciting the pigment pool at the photosystem II antenna at 645 nm (phycocyanine in *Cyanophyceae*), 470 and 665 nm (chlorophyll b in *Chlorophyceae*) and 520nm (chlorophyll c and carotenoids in *Bacillariophyceae*). More information about the Pulse Amplitude Method can be found in Hall et al., 2007. The reference spectra used to calibrate the PHYTOPAM were

generated with pure algal cultures isolated from either Lake Jesup or Monroe and grown in different culture media. The averaged reference spectrum was then used to represent each algal group and used for the deconvolution of the fluorescence signal. The rate of photosynthesis or electron transfer rate (ETR) was expressed in arbitrary units and determined at different irradiances (from 16 to 2064 μmol quanta.m-^{2}.s^{-1}) to generate a Photosynthesis-Irradiance curve (PI curve). The PI curve parameters for each algal group (ETR_{max}, Ik and the initial slope "α") were determined by non-linear regression using the PHYTOWIN software (www.walz.com).

2.7. N_2-fixing Gene Expression

Cells from the total microbial community of replicate known volumes of lake water samples were harvested under vacuum filtration onto sterile Whatman cellulose nitrate filters (0.4um pore size) within approximately 4 hours of sample collection, preserved and stored frozen until later nucleic acid extraction and analysis. Separate replicate filters were collected for RNA and DNA extraction. In the case of DNA samples, filters were rolled cell side in, placed in microfuge tubes with 400uL of buffer AP1 (Qiagen) and immediately frozen and stored on dry ice until return to the lab, then stored frozen at -80°C until further extraction and analysis. In the case of RNA samples, filters were rolled cell side in, placed in microfuge tubes with 1 mL of RNAprotect solution (Qiagen), and incubated in the RNAprotect solution for 30 min at room temperature. Excess RNAprotect solution was then gently removed with a sterile pipette, and the preserved filter was immediately frozen and stored on dry ice until return to the lab, then stored frozen at -80°C until further extraction and analysis.

DNA was extracted by thawing filters preserved in the AP1 buffer, transferring the filter and its associated AP1 buffer to a bead beat tube (lysing matrx "E", MPBiomedical/Qbiogene), and bead beating in a Fastprep instrument (MPBiomedical/Qbiogene) at a speed of 6.5 for 30 seconds. The lysate was transferred to a sterile microfuge tube, the DNA was extracted from the AP1 buffer lysate with the DNeasy Plant MiniKit (Qiagen) as per manufacturer's instructions. RNA was extracted by thawing filters and tranfering them to a bead beat tube (lysing matrix "E", MPBiomedical/Qbiogene), adding 500uL of buffer RLT (Qiagen RNeasy Plant minikit), and bead beating in a Fastprep instrument (MPBiomedical/Qbiogene) at a speed of 6.5 for 30seconds. The lysate (450uL) was transferred to a sterile microfuge tube, the RNA was extracted from the lysate with the RNeasy Plant miniKit (Qiagen) according to the manufacturer's instructions, using the lysing buffer RLT and the protocol for plant cells.

DNA samples were analyzed for presence of species specific *nifH* from *Cylindrospermopsis raciborskii* by qPCR, using a chromo4 real time qPCR thermocycler (MJResearch/BioRad), and the QuantiTect Probe Mastermix (Qiagen) using Taqman-type 5'exonuclease chemistry. Target concentration standards were prepared by cloning target amplicons from *C. raciborskii* using the TOPO TA cloning kit (Invitrogen), and using dilutions of these plasmid standards to determine copy # of target in environmental

extracts by qPCR with the same primer/probe sets. For *Cylindrospermposis raciborskii* qPCR, the dual-labeled internal 5' exonuclease probe was: 5' - 6FAM-CGA CCA GCA CAR CCT ACR CC-BHQ1- 3', the forward primer was: 5-TAA RGC TCA AAC TAC CGT AT-3', and the reverse primer was: 5-GTA GGA AAC GAA GTC TAA AT-3'. Reaction cocktail consisted of 0.5uL of target sample template, 0.125uL of each forward and reverse primer (from 100uM stock), 0.1uL of probe (from 100uM stock), 12.5uL of 2X mastermix (Qiagen QuantiTect Probe Mastermix Kit), and 11.65uL water for a final reaction volume of 25uL. Thermocycling parameters were initial denaturation at 95°C for 15 min then 45 cycles of 95°C for 15 sec and 60°C for 30 sec (followed by fluorescent plate read). For *C. raciborskii nifH* expression, RNA was analyzed with the same thermocycling conditions and the same primer/probes sets by qRT-PCR using the QuantiTect RT-PCR mastermix Kit (Qiagen), incorporating the manufacturer's recommended reverse transcription step at the beginning of the cycling parameters (i.e. 50°C for 30min before initial denaturation step). DNA samples were analyzed for general cyanobacterial *nifH* using by real-time SybrGreen dye incorporation using a chromo4 thermocycler and QuantiTect SyberGreen mastermix PCR Kit. Target concentration standards were prepared by cloning target amplicons from C. raciborskii using the TOPO TA cloning kit (Invitrogen), and using dilutions of these plasmid standards to determine copy # of target in environmental extracts by qPCR with the same primer/probe sets. Primer sets used were UNIFF forward primer for nifH (Ueada et al., 1995): 5'-GCIWTYTAYGGIAARGGIGG-3' and the nifdn reverse primer (McReynolds & Zehr, 1989): 5'-ADNGCCATCATYTCNCC-5'. Reaction cocktail consisted of 0.5uL of each primer (10uM stock), 0.5uL template, 12.5uL of 2X mastermix (Qiagen QuantiTect SYBR Mastermix PCR kit) and water to final volume of 25uL. Cycling conditions were initial denaturation at 95°C for 15 min, followed by 45 cycles of: 95°C for 15 sec, 50°C for 30 sec, and 72°C for 15 sec (followed by fluorescent plate read). For general *nifH* expression, RNA was analyzed with same cycling conditions and same primer sets by qRT-PCR using the QuantiTect SYBR mastermix RT-PCR kit, with the manufacturer's recommended initial reverse transcription step.

2.8. Identification of Algal Communities Responsible for N-fixation and Production

Sample collection. Microorganisms in lake water were filtered from water samples using 47 mm diameter filters with a pore size 0.45 micrometer, within 24 hours of water collection. The filters were prepared for DNA and RNA extractions, respectively, and filters were stored at -20 °C for DNA and -80 °C for RNA until analysis.

DNA and RNA extractions. DNA and RNA extractions were carried out using a Fast DNA SPIN for Soil (Bio101) and a FastRNA Pro Soil - Direct (Bio101), respectively, based on a physical extraction method with special beads, following manufacturer's instructions. The filter was cut to be small pieces, 2 mm x 2 mm, with a sterilized scissors before extraction. Only extracted RNA was treated with DNase I (TaKaRa) according to the manufacturer's instructions to digest any potential DNA contamination. The extracted DNA and RNA were kept at -20 °C and -80 °C until amplification, respectively.

PCR and Reverse Transcription (RT) – PCR. PCR and RT-PCR amplifications of cyanobacterial 16S rDNA and 16S rRNA were conducted with primer set, CYA106f (5′-GGG ACG GGT GAC TAA CGC GTG A-3′) and CYA781r (5′-GAC TAC WGG GGT ATC TAA TCC CYT t-3′) (Nübel et al., 1997). PCR amplification conditions were as follows: 94 °C for 3 min (initial denaturation), followed by 27 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min with a final extension at 72 °C for 8 min. One Step RNA PCR Kit (TaKaRa) was used for RT-PCR amplification. The RT reaction was conducted at 50 °C for 30 min prior to amplification. The PCR amplification conditions following the RT reaction were the same as those for PCR amplification of DNA extracts. Both amplicons thus obtained were again amplified with CYA359f-GC (5′-CGC CCG CCG CGC CCC GCG CCG GTC CCG CCG CCC CCG CCCG GGG GAA TYT TCC GCA ATG GG-3′, underlined sequence denotes GC clamp) (Nübel et al. 1997) and CYA781r. Amplification conditions are as follows: 94 °C for 3 min (initial denaturation), followed by 22 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 8 min.

Denaturing Gradient Gel Electrophoresis (DGGE). Both products derived from DNA and RNA were used for DGGE, which was performed with 8 % (wt / vol) acrylamide gel containing a linear chemical gradient ranging from 20% to 60% denaturant (Muyzer et al., 1993). Aliquots of 400 ng of concentrated PCR product were electrophoresised for 14 h at 60 °C under 100 V using the DCode universal mutation detection system (Bio Rad Laboratories). The gel was photographed under UV illumination after staining with SYBR Green I.

Band extraction and sequencing. Dominant bands in DGGE patterns were selected and excised from the gels. Excised bands were amplified under the same PCR conditions used for the primer set of CYA359f-GC and CYA781r (as above) but with the number of cycles reduced to 18. DGGE was again performed to confirm the positions of the bands in the gel. PCR products with successfully isolated bands were purified with a Millipore filtration kit and sequenced directly. The cycle sequence reaction was prepared with a Big Dye Terminator (v 3.1) kit, according to manufacturer instructions, and 3.2 pmol of CYA781r primer. Sequencing samples were run using a capillary sequencer (ABI).

Cluster analysis of DGGE patterns and BLAST Sequence. Cluster and principal component analyses were performed based on the DGGE patterns. Respective DGGE bands are classified into five categories (1-5) based on their intensities, with the strongest intensity given a value of 5. All the DGGE bands were used in the calculations. Cluster analysis was performed with the Black Box program (http://aoki2.si.gunma-u.ac.jp/bb0/BlackBox0.html) using the Ward method, while a principal component analysis is performed using SPSS software (SPSS).

Confirmed sequences of the DGGE bands were aligned to the 16S rDNA sequences obtained from the DNA Data Bank of Japan (DDBJ) database (http://blast.ddbj.nig.ac.jp/top-e.html) with the BLAST search program.

3.0 RESULTS

Note: Many boxplots are presented to describe data in this report. Bars represent median values in these boxplots. The top and bottom of the box represent the 75^{th} and 25^{th} percentile, respectively. The upper and lower bars represent the 90^{th} and 10^{th} percentile, respectively. Open circles designate "outliers" and stars represent "extreme values". This explanation holds for all similar box plots this document. Boxplots were generated using SPSS v. 14.

3.1 N_2-Fixation Rates

3.1.1. Calibration of ^{15}N and acetylene reduction.

The isotopically enriched $^{15}N_2$ method was used to measure N_2 fixation in a sub-set of samples to calibrate the acetylene reduction (AR) method. Acetylene reduction was used at all sites (6 per lake) at all times. One station from each lake was pre-selected for calibration purposes, for Lake Monroe – LM 50, and for Lake Jesup – LJ 08. To calibrate, we used linear regression of N_2 fixation nmol N_2 L^{-1} h^{-1} as calculated by the ^{15}N-method of Montoya et al. (1996). The $^{15}N_2$ fixation was compared with ethylene production (nmol $C_2H_4L^{-1}$ h^{-1}) in separate incubations using the same bulk water collected at each site for each sampling (Figs. 3.1.1 and 3.1.2). The regression for Lake Jesup gave the best relationship of the two lakes (Fig. 3.1.1), while the fixation data from Lake Monroe did not have a statistically significant relationship ($P = 0.063$, when two outliers were excluded, and was worse when all data was included, not shown). Therefore, we built our calibration data set by using all the data from Lake Jesup and several points that were < 5 nmol N_2 L^{-1} hr^{-1} as determined by the ^{15}N-method. Note there are two issues with comparing data between these two methods. First, the ^{15}N-method is more sensitive, and has a lower detection limit for N_2-fixation compared to AR, however, this limit is relatively insignificant, and is typically < 0.8 nmol N_2 L^{-1} h^{-1}. Below this limit AR would indicate zero fixation. Secondly, the range of data for Lake Monroe did not have enough high fixation values to generate a statistically significant relationship, where Lake Jesup had better spread in fixation values allowing for a proper calibration. In general, Lake Monroe did not have the level of fixation as Lake Jesup, and therefore it was harder to independently calibrate this lake. The best calibration was a combination of the two lakes. With Lake Monroe providing the N-fixation values between 0 to < 5 nmol N_2 L^{-1} h^{-1}, and Lake Jesup data including all values. The resultant calibration slope of 6.37 fell within the range of previously published measurements/calibrations (Montoya et al. 1996). The approach used to measure the enriched samples also provides natural abundance $\delta^{15}N$ values from the "untreated" particulate organic matter (POM). These data are plotted in a time-series (Fig. 3.1.3). Isotopic values less than 1 ‰ and near 0‰, typically reflect an atmospheric N source via fixation of N_2, versus NO_3^- utilization (Anderson et al, 2004). All data in the time series from both Lakes were <5‰, except for one value from Lake Monroe (September of 2007; Fig. 3.1.3), which had an extremely enriched value of 9.3‰, which indicates an N source not originating from fixation. $\delta^{15}N$

values near this level of relative enrichment are typically associated with denitrification (Teranes and Bernasconi, 2000), and or dissolved inorganic N (DIN) coming into the watershed from pasture lands (manure) (Kendall 1998). However, our data did not fall within the typical range of $\delta^{15}N$ values for denitrification when Lake Monroe's DIN values were considered.

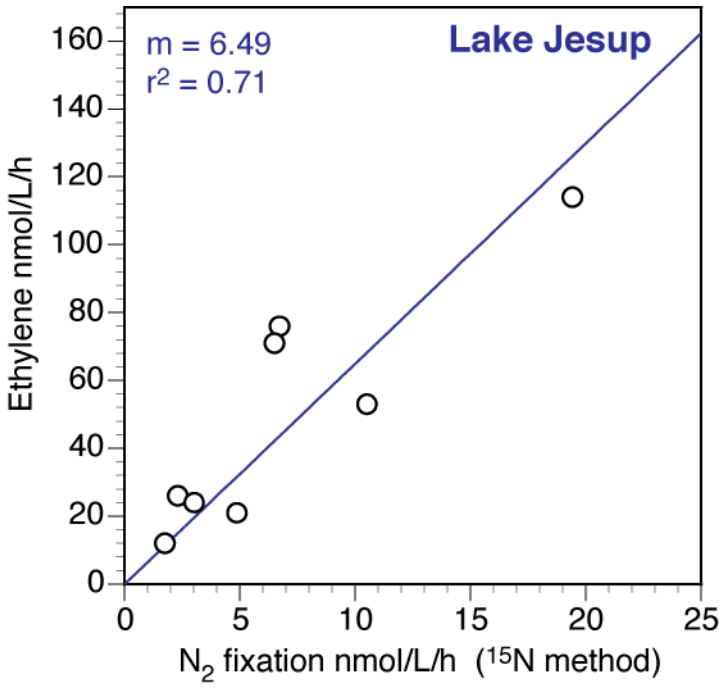

Figure 3.1.1.1. Comparison of the two methods for nitrogen fixation, ethylene produced (nmol C_2H_4 L^{-1} h^{-1}) by the Acetylene Reduction (AR) method versus $^{15}N_2$ labeled gas method of Montoya et al. (1996). Each point represents the average of three analyses for both methods, for samples collected from Lake Jesup station LJ08 (P= 0.006).

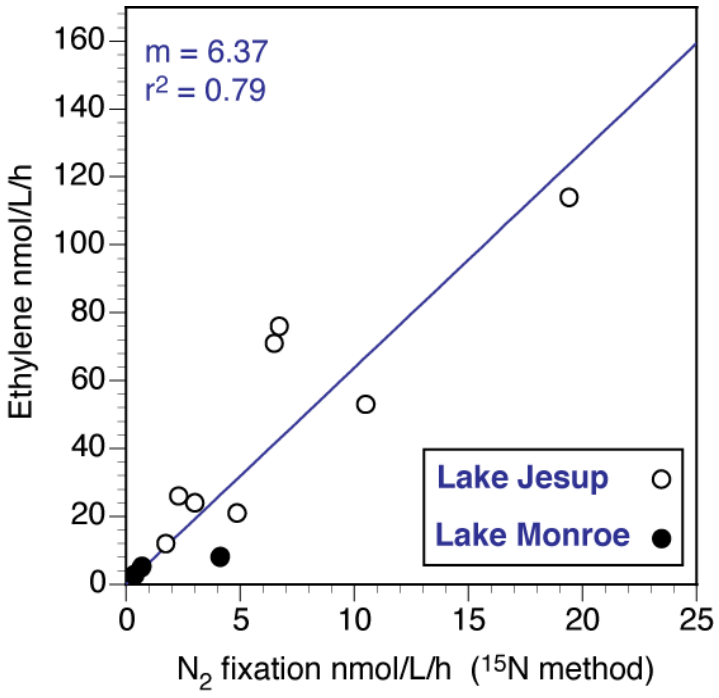

Figure 3.1.1.2. Comparison of the two methods for nitrogen fixation, ethylene produced (nmol C_2H_4 L^{-1} h^{-1}) by the Acetylene Reduction (AR) method versus $^{15}N_2$ labeled gas method of Montoya et al. (1996). Each point represents the average of three analyses for both methods for samples collected from Lake Jesup station LJ08. Data for Lake Monroe < 5 nmol N_2 L^{-1} h^{-1} are included with all of the Lake Jesup data (P = 0.0002). The ^{15}N method is more sensitive than the AR so not all data from Monroe could be directly compared. In several months Lake Monroe did not show C_2H_4 production and thus no N_2-fixation while the ^{15}N method did, but at extremely low levels.

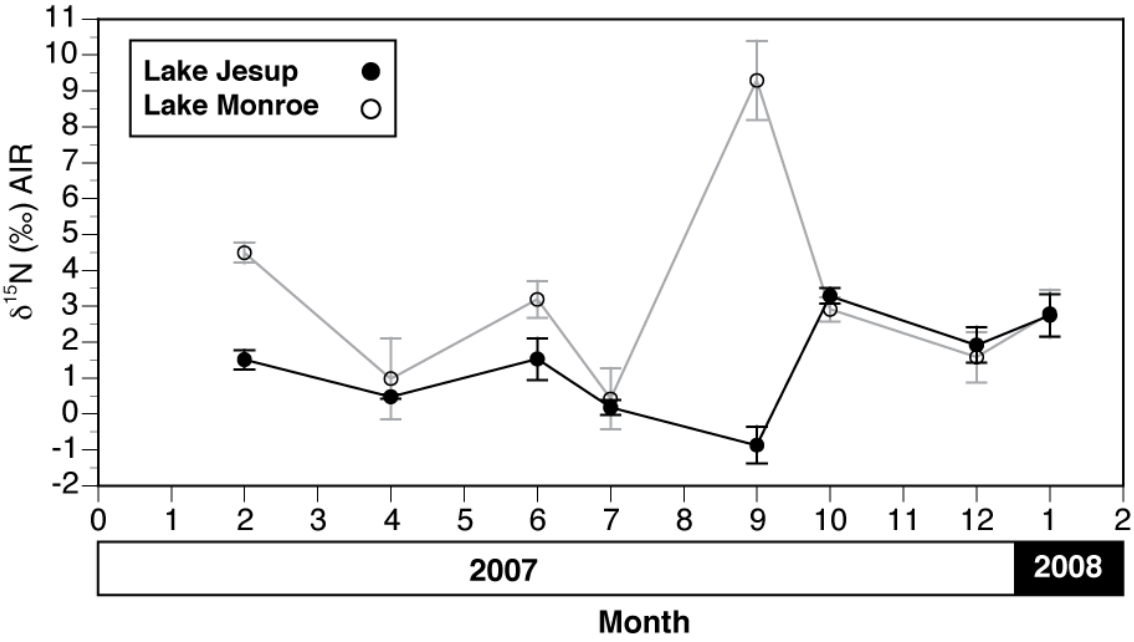

Figure 3.1.1.3. Time series of all natural abundance isotope date from particulate organic matter (POM) collected during the study. Error bars represent 1 sigma, and each data point represent three different filters from three different subsamples from Lake Monroe station LM50 and Lake Jesup station LJ08. Note, values less than 1 ‰, indicate nitrogen fixation is occurring.

3.1.2. Annual cycle of N_2-fixation.

The N_2-fixation rates varied seasonally throughout the 334 d of this study with higher rates of fixation occurring during the summer months (Fig. 3.1.2.1). Generally, Lake Jesup showed higher fixation rates compared to Lake Monroe. Lake Monroe shows little to no fixation at several sampling events (Fig. 3.1.2.1 and Table 3.1.2.1). The GC employed in our analysis is very sensitive to the determination of ethylene produced by the AR method. However, all analyses are background corrected to distilled, deionized water (DDIH2O) blanks that were incubated at the same time as lake water samples. The DDIH2O blanks generally produced acetylene reduction rates that corresponded to about 1 nmol N_2 L^{-1} h^{-1}. The within lake variation at these times showed that there was no fixation at several sites and small rates of fixation at others (Fig. 3.1.2.2). There were measurable rates of fixation at all events and all sites in Lake Jesup (Fig. 3.1.2.3). Whereas the fixation in Lake Monroe alternated between high and low during the warm events (April – September) the fixation in lake Jesup generally increased to a high in late summer (Fig. 3.1.2.1). There are signs of local variation in fixation rates which, as mentioned, appear greater in Lake Monroe (Fig. 3.1.2.2) than in Lake Jesup (Fig. 3.1.2.3).

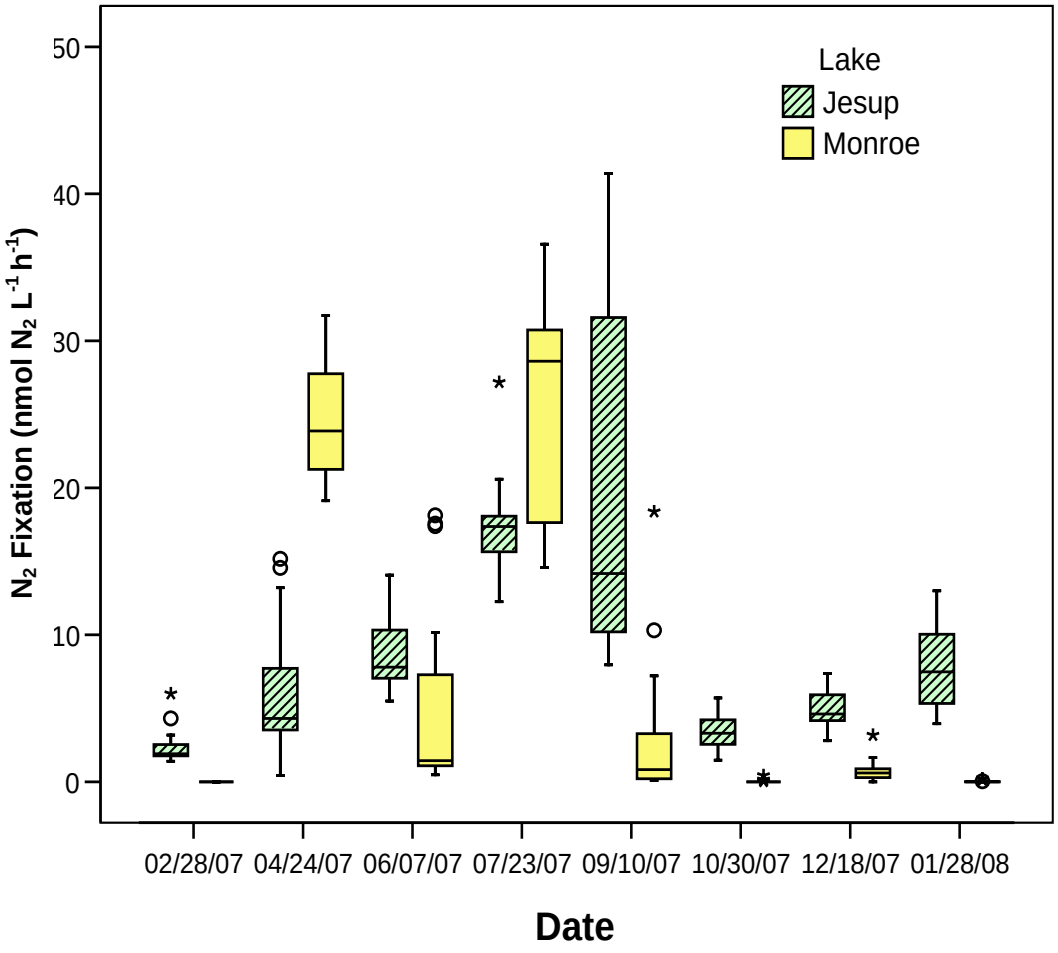

Figure 3.1.2.1. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) for all sites within Lakes Jesup and Monroe for the 8 seasonal variation sampling events conducted Feb 2007-Jan 2008. Bars represent median values. The top and bottom of the box represent the 75^{th} and 25^{th} percentile, respectively. The upper and lower bars represent the 90^{th} and 10^{th} percentile, respectively. Open circles designate "outliers" and stars represent "extreme values". This explanation holds for all similar box plots that follow in this document.

Table 3.1.2.1. Mean (± SD) N_2 fixation and N incorporation rates. ND = not detected. All reported values were blank corrected. Those shown in red are not different from zero and should be considered below detection.

Date	N_2 fixation	N incorporated	Mass N fixation
	nmol N_2 L^{-1} h^{-1}	Nmol N L^{-1} h^{-1}	ng N L^{-1} h^{-1}
Lake Jesup			
28 Feb 2007	2.44 ± 1.25	4.88 ± 2.50	68.35 ± 35.02
24 Apr 2007	6.33 ± 4.59	12.66 ± 9.18	177.33 ± 128.58
07 June 2007	8.65 ± 2.32	17.30 ± 4.64	242.32 ± 64.99
23 July 2007	17.48 ± 3.27	34.96 ± 6.54	489.68 ± 91.61
10 Sept 2007	18.97 ± 11.13	37.94 ± 22.26	531.43 ± 311.80
30 Oct 2007	3.40 ± 1.27	6.80 ± 2.54	95.25 ± 35.58
18 Dec 2007	4.85 ± 1.21	9.70 ± 2.42	135.87 ± 33.90
28 Jan 2008	7.95 ± 2.99	15.90 ± 5.98	222.71 ± 83.76
Lake Monroe			
28 Feb 2007	ND	ND	ND
24 Apr 2007	24.57 ± 4.14	49.14 ± 8.28	688.30 ± 115.98
07 June 2007	5.38 ± 6.30	10.76 ± 12.60	150.72 ± 176.49
23 July 2007	25.91 ± 7.61	51.82 ± 15.22	725.84 ± 213.19
10 Sept 2007	3.07 ± 5.00	6.14 ± 10.00	86.00 ± 140.07
30 Oct 2007	0.04 ± 0.11	0.08 ± 0.22	1.12 ± 3.08
18 Dec 2007	0.75 ± 0.74	1.50 ± 1.48	21.01 ±20.73
28 Jan 2008	0.03 ± 0.07	0.06 ± 0.14	0.84± 1.96

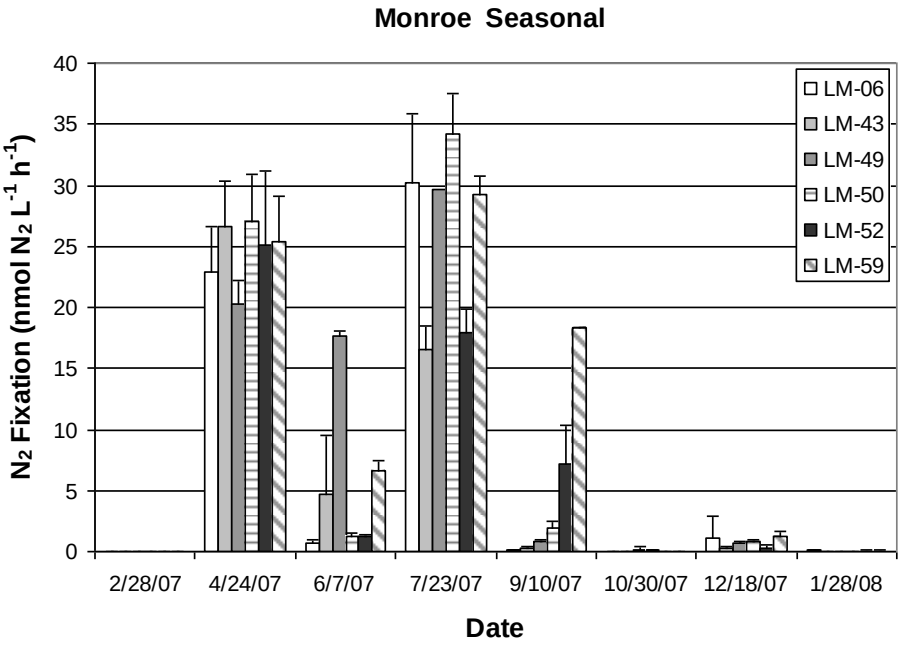

Figure 3.1.2.2. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) at each of 6 sites in Lake Monroe for 8 seasonal variation sampling events. Error bars represent standard deviation among 3 replicates per site.

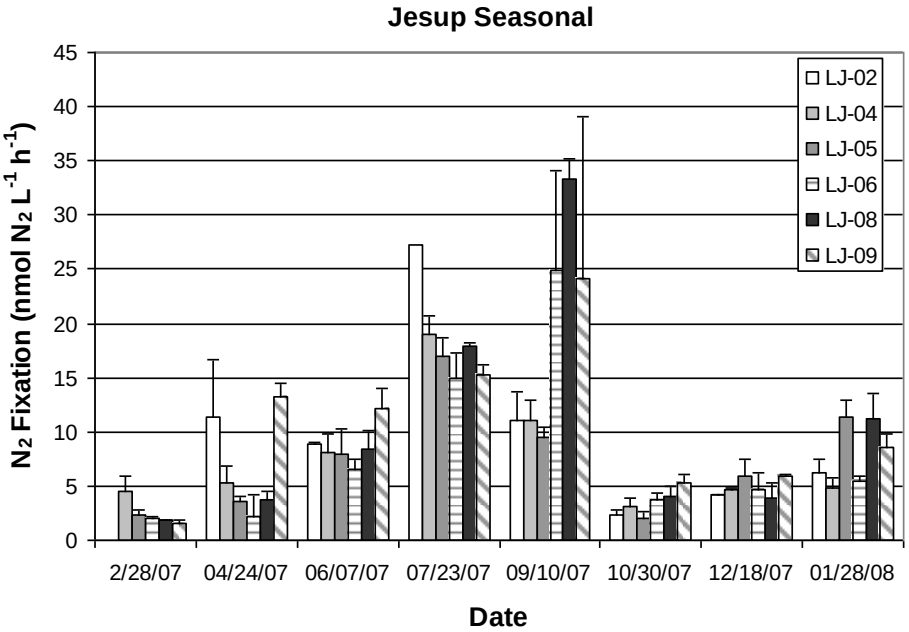

Figure. 3.1.2.3. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) at each of 6 sites in Lake Jesup for 8 seasonal variation sampling events. Error bars represent standard deviation among 3 replicates per site.

3.1.3. Diel variation in N_2-fixation.

Two different 24 hr diel samplings were conducted in both lakes during April and September 2007. These data indicate that day-time (morning and afternoon) N_2 fixation was always higher when compared to night-time measurements (Figs. 3.1.3.1 and 3.1.3.2). Generally, the day:night N_2 fixation ratios for Lake Jesup and Lake Monroe are similar, the overall average ratio for both lakes is 3.18 (Table 3.1.3.1). These ratios were used to calculate total lake fixation (section 3.1.5).

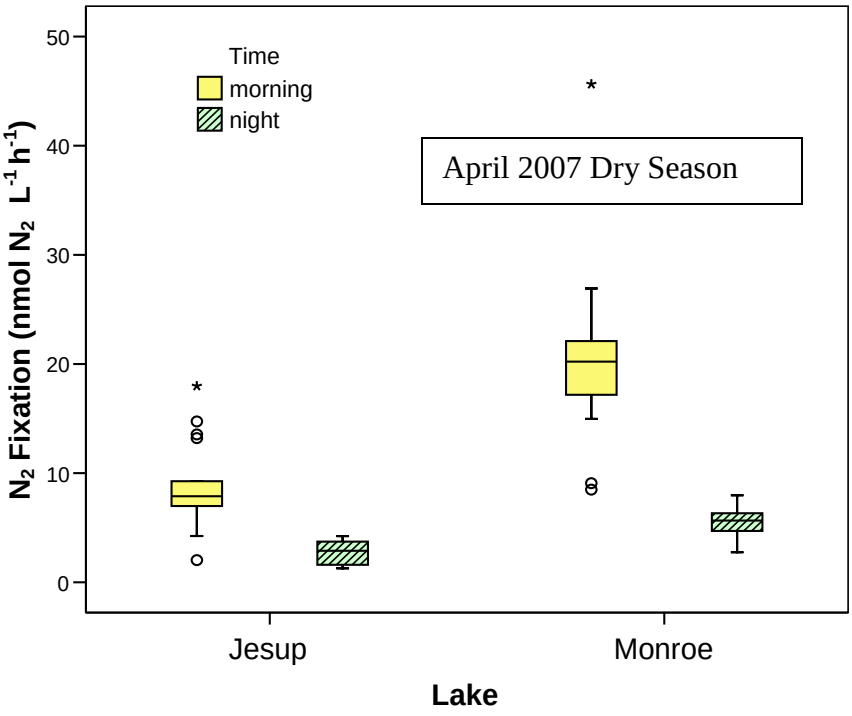

Figure 3.1.3.1. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) for Lakes Jesup and Monroe over a 24 h period with one incubation occurring in the daylight (between 1100 and 1500) and another during the night. The dry season diel events were conducted Apr 22 2007 for Lake Monroe and Apr 24-25 2007 for Lake Jesup. See Fig. 3.1.2.1 for further explanation of this boxplot.

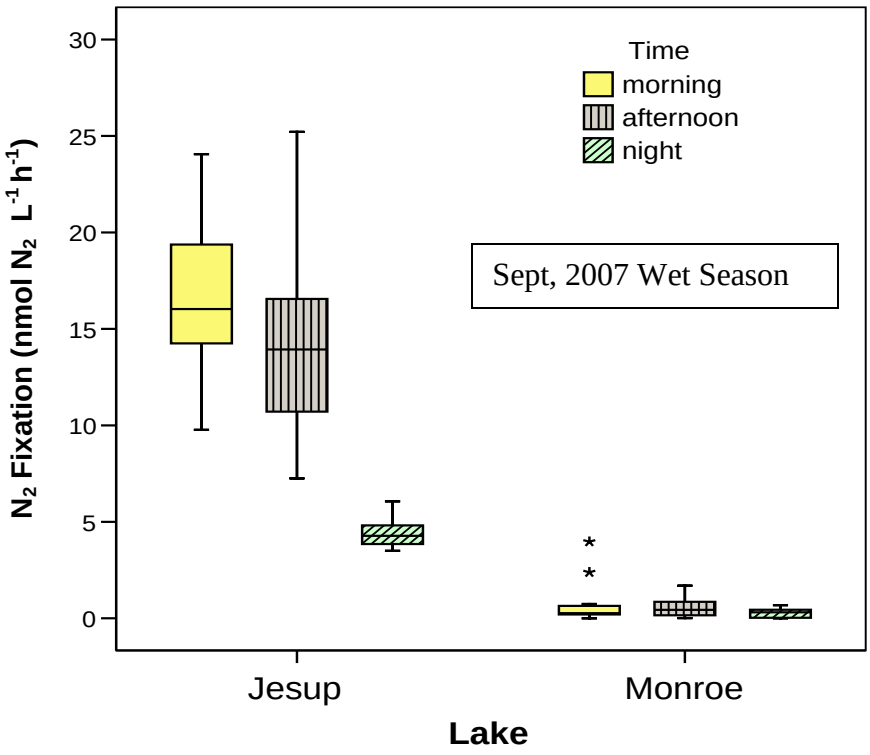

Figure 3.1.3.2. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) for Lakes Jesup and Monroe over a 24 h period with one incubation occurring in the daylight (between 1100 and 1500) and another during the night. The wet season diel events were conducted on Sep 11 2007 for Lake Jesup and Sep 14-15 2007 for Lake Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

Table 3.1.3.1 Ratios of day to night N_2 fixation rates for Lakes Jesup and Monroe. Mean values for all sites for each lake during daylight hours were divided by mean night time fixation rates to determine the ratio of day:night fixation. Two 4 h incubations (morning and night) were conducted in April 2007 while 3 (morning, afternoon, night) were conducted in September 2007 for each lake. Therefore, the averages of the morning and afternoon incubations were used to develop the day:night ratios. From these four values a comprehensive ratio was calculated.

	Lake Jesup	Lake Monroe
	nmol N_2 L^{-1} h^{-1}	nmol N_2 L^{-1} h^{-1}
April 2007		
Day	8.81	20.45
Night	2.75	5.34
Day:Night ratio	= 3.20	= 3.83
September 2007		
Morning	16.45	0.65
Afternoon	13.94	0.49
Night	4.47	0.25
Average Day:Night ratio	= 3.40	= 2.28
Comprehensive Mean (± SD)	= 3.18 ± 0.65	

3.1.4. Short-term (weekly) variation in N_2-fixation.

N_2-fixation patterns over the week long study indicated that the daily change in the lakes was variable. In fact, during the April sampling (Fig. 3.1.4.1), Lake Monroe had the greatest variation in fixation, with alternating days having larger ranges than the preceding day. These differences in daily measurements are real, and not caused by any systematic error in sampling. Additionally, the fixation rates were greater in Lake Monroe than Lake Jesup. In contrast, the September week long sampling yielded daily fixation rates that decreased over the seven days (Fig. 3.1.4.2), with Lake Jesup having the highest fixation rates of the two lakes. During September, Lake Monroe's daily N_2-fixation averages were all < 3 nmol N_2 L^{-1} h^{-1}.

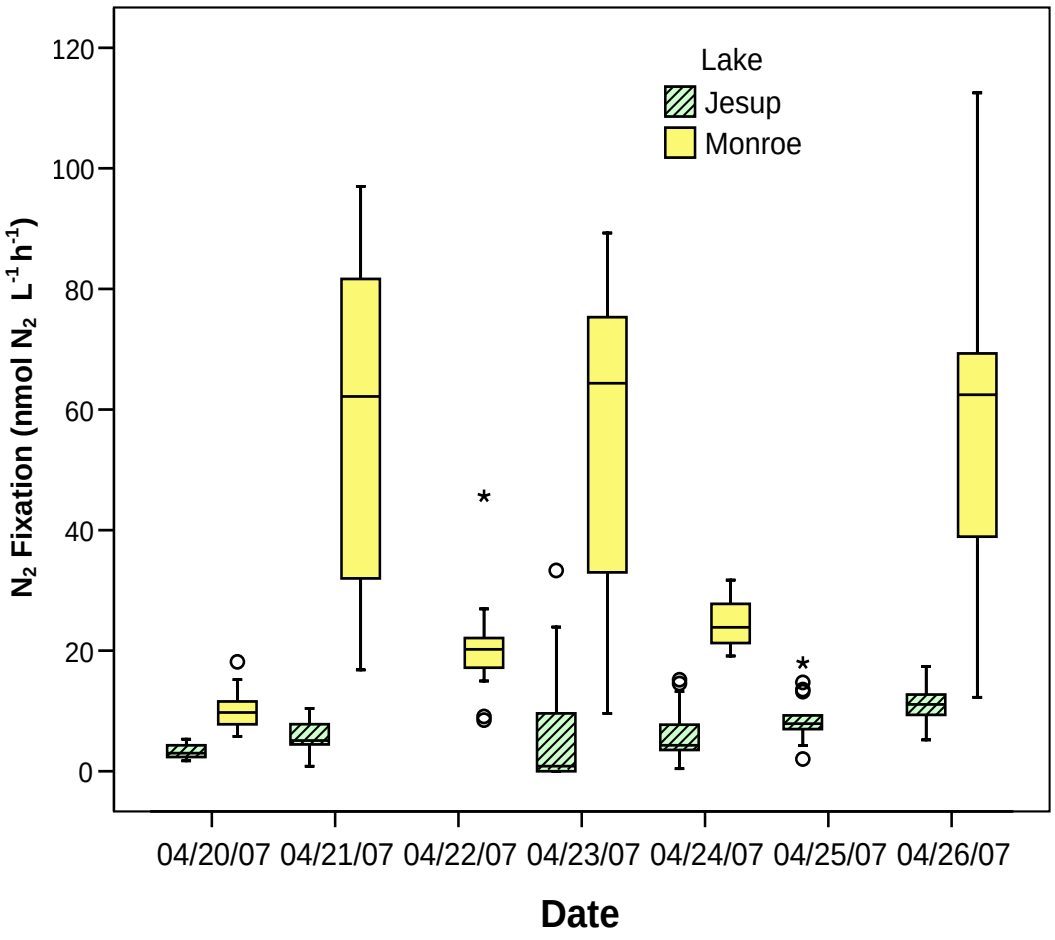

Figure 3.1.4.1. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) for all sites within Lakes Jesup and Monroe over a one-week period in Apr 2007. Only Lake Monroe was sampled on Apr 22 2007. Only Lake Jesup was sampled on Apr 25 2007. See Fig. 3.1.2.1 for further explanation of this boxplot.

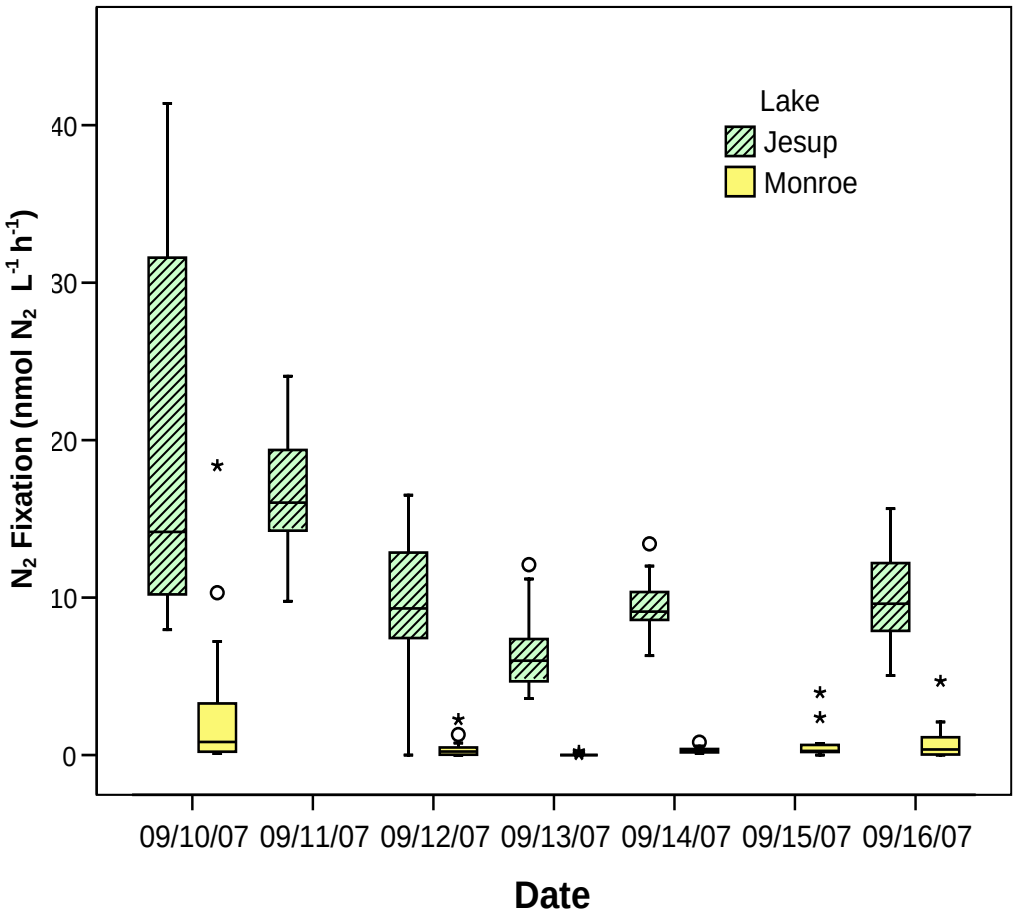

Figure 3.1.4.2. Fixation of N_2 (nmol N_2 L^{-1} h^{-1}) for all sites within Lakes Jesup and Monroe over a one-week period during Sep 2007. Only Lake Jesup was sampled on Sep 11 2007. Only Lake Monroe was sampled on Sep 15 2007. See Fig. 3.1.2.1 for further explanation of this boxplot.

3.1.5. Estimates of the role of N_2-fixation in annual N loading.

The data from our seasonal sampling events (as summarized in Fig. 3.1.2.1) and the fraction of daylight to nighttime N_2 fixation (Table 3.1.3.1) were used with approximate lake volumes to estimate the potential role N_2 fixation is contributing to Lakes Jesup and Monroe. These calculations began by making linear regressions between the average, minimum, and maximum (n = 6 sites) N_2 fixation rates (nmoles N_2 L^{-1} h^{-1}) of any two seasonal events to develop a slope that predicts the daylight N_2 fixation rate by interpolation for all days between the two sampled dates. The mean ratio of N_2 fixation during daylight (light) to nighttime (dark) hours = 3.18 (Table 3.1.3.1) was then used to calculate an estimate of nighttime hourly fixation. When plotted there was a significant linear relationship between the daylight and nighttime fixation for each lake. However, the day to night relationship is based on only 2 events (12 data pairs for each lake). We analyzed the fraction of day to night fixation for each lake to determine if the mean

values were significantly different between these lakes. The resultant mean ratios (3.30 for Lake Jesup and 3.10 for Lake Monroe) were not significantly different when analyzed by the non-parametric Mann-Whitney Test (SPSS v. 14.0) so we concluded that an overall mean ratio would be most useful. We obtained the meteorological number of daylight and nighttime hours for every day (334 days), multiplied the respective fixation rates (day or night), then summed the results to produce an estimate of the total N_2 fixed L^{-1} in that 24 hour day. We then calculated the daily volume (L) of the lakes from daily stage data provided by the SJRWMD and a characteristic curve between lake stage and cumulative volume developed for the period 1995 to 2002 by either the Florida Department of Environmental Protection for Lake Jesup (FDEP/Gao, 2005) or the SJRWMD for Lake Monroe. The resultant N_2- fixed in a given 24 h day for the entire lake was then converted to the mass (kg) of N and plotted with time (Fig. 3.1.5.1 and Fig. 3.1.5.2). Because there was variation in the mean N2 fixation rates for any given sampling event the minimum and maximum calculated contributions N by fixation were also plotted. This was done to provide a range of plausible values for possible use in mathematical models as these models often provide variable outcomes.

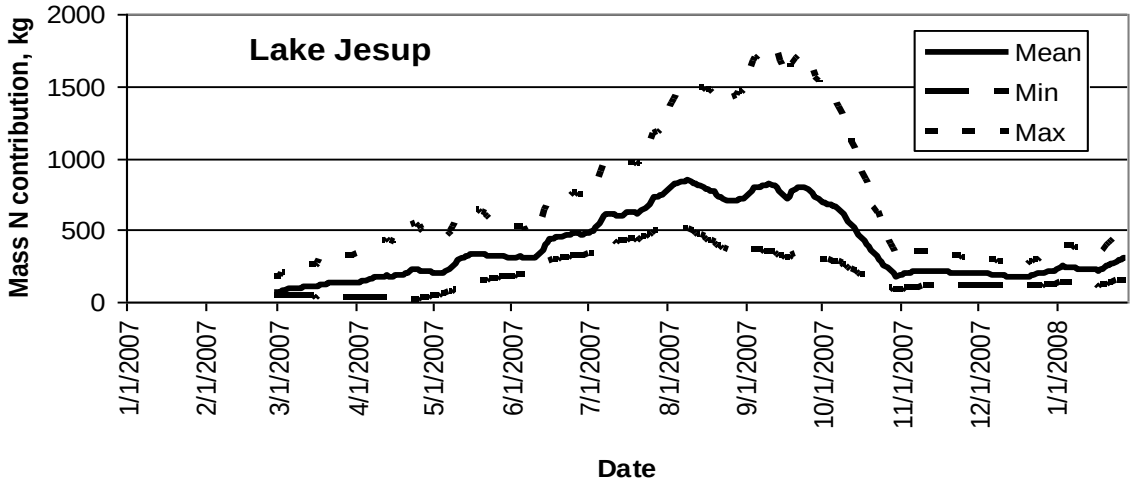

Figure 3.1.5.1. Mean, minimum, and maximum, estimates of the mass of nitrogen (N) kg added to Lake Jesup between Feb 28 2007 and Jan 28 2008.

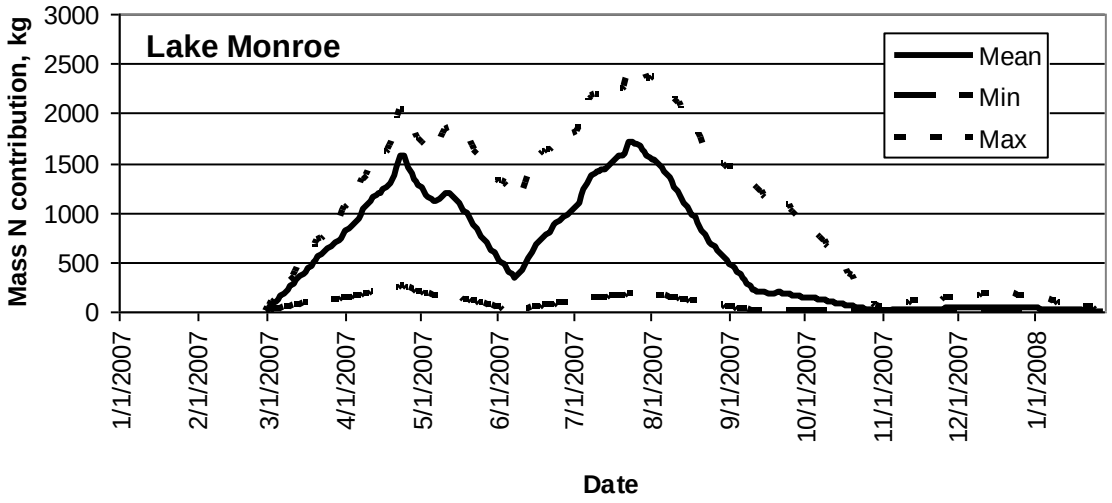

Figure 3.1.5.2. Mean, minimum, and maximum, estimates of the mass of nitrogen (N) kg added to Lake Monroe between Feb 28 2007 and Jan 28 2008.

Table 3.1.5.1. Rates of Nitrogen (mg N m^{-2} d^{-1}) contribution and total mass N (Mg N; 1 Mg = $1x10^6$g) estimated to be added to each lake during the 334 day study period.

	Mean	Range	Mean	Range
	mg N m^{-2} d^{-1}		Mg N	
Lake Jesup	8.92	4.52 – 16.67	128.6	65.2 – 240.4
Lake Monroe	13.12	1.52 – 24.20	187.7	21.7 – 346.3

3.2 Water Quality and Physicochemistry

3.2.1. Water quality and affect on N_2-fixation.

Table 3.2.1.1. Average nutrient and lake physicochemistry for 8 sampling events and 6 sites per lake conducted at 7 week intervals, February 2007- January 2008 (n = 48).

		L. Jesup		L. Monroe	
Analyte	Unit	Mean ± SD	Range	Mean ± SD	Range
Chlorophyll a	$\mu g\ L^{-1}$	230 ± 91	20 - 399	67 ± 68	2 – 255
Total Phosphorus	$\mu g\ L^{-1}$	131.1 ± 53.5	7.7 - 288.3	97.8 ± 40.7	31.0 - 229.2
Total Nitrogen	$mg\ L^{-1}$	1.38 ± 0.54	0.55 – 2.73	1.02 ± 0.41	0.4 - 1.87
Total Organic Carbon	$mg\ L^{-1}$	23.2 ± 5.6	14 - 40	21.6 ± 7.6	9.9 - 33.3
Total Inorganic Nitrogen	$mg\ L^{-1}$	0.09 ± 0.14	0.02 – 0.71	0.26 ± 0.27	0.01 - 0.93
Ammonia	$\mu g\ L^{-1}$	71.0 ± 133.6	13.8 - 672.7	95.6 ± 87.4	5.4 - 280.9
Nitrate	$\mu g\ L^{-1}$	17.2 ± 21.1	1.9 - 72.8	155.6 ± 205.9	0.0 - 844.5
Nitrite	$\mu g\ L^{-1}$	1.3 ± 1.0	0.1 – 6.3	12.0 ± 35.0	0.1 - 240.8
Total Organic Nitrogen	$mg\ L^{-1}$	1.3 ± 0.54	0.53 – 2.71	0.75 ± 0.35	0.22 - 1.76
Soluble Reactive Phosphorus	$\mu g\ L^{-1}$	8.6 ± 18.0	0.0 - 73.9	26.7 ± 38.3	0.2 - 178.4
Conductivity	$\mu S\ cm^{-1}$	1246 ± 411	559 - 2115	1483 ± 539	591 - 2439
Dissolved Oxygen	$mg\ L^{-1}$	8.4 ± 2.0	5.4 - 12.0	8.6 ± 1.8	4.5 - 11.5
pH		8.79 ± 0.35	7.87 – 9.21	8.02 ± 0.62	7.41 - 9.55
Z_{eu}	m	0.59 ± 0.11	0.45 – 0.94	1.16 ± 0.37	0.61- 2.07

Lakes are classified by the United States Environmental Protection Agency (USEPA) using the Carlson's trophic state index (TSI), based on a numeric scale of 0-100. Determination of trophic states is made based on the examination of several diverse criteria such as water TP concentration, algae abundance (as determined by Chl a concentration), and depth of light penetration (Secchi Depth) (Carlson, 1977). The TSI is one means available to examine the relationship between TP, Chl a, and transparency in a lake and the lakes' overall productivity. Lake water quality can be described as oligotrophic, mesotrophic, eutrophic, and hypereutrophic where each are assigned TSI values based on evaluation of the measures of TP, Chl a, and Secchi Depth (not determined in our study as we opted for direct measures of light penetration) according to accepted limits (Carlson and Simpson, 1996).Calculations are based on the simplified equations:

$TSI = 9.81 \ln(\text{Chl } \underline{a}) + 30.6$
$TSI = 14.42 \ln(TP) + 4.15$

Where: TSI is the Carlson trophic state index and ln the natural logarithm, Chl a is the chlorophyll a concentration in $\mu g\ L^{-1}$ and TP the total phosphorus concentration in the water column in $\mu g\ L^{-1}$. This criterion (Table 3.2.1.2) was used to characterize Lakes Jesup and Monroe at each seasonal sampling event (Table 3.2.1.3).

Table 3.2.1.2. Numerical criteria and description of the Trophic State Index (TSI) as used by the U.S. Environmental Protection Agency.

TSI	Trophic Status Description
0-40	Oligotrophic: clear water.
41-50	Mesotrophic:
51-65	Eutrophic:
66-100	Hypereutrophic: light limited productivity, dense algae and macrophytes.

According to the TSI and based on both criteria, Lake Jesup was continually hypereutrophic during the course of this study. Lake Monroe was also usually hypereutrophic although at a few seasonal samplings, generally during the cooler months this lake was eutrophic. It is doubtful that nutrient availability ever limits the productivity of either of these lakes.

Table 3.1.2.2. Trophic state index (TSI) based on either chlorophyll a (TSI_{Chla}) or total phosphorus (TSI_{TP}) for Lakes Jesup and Monroe. Values shown are derived from the means of 6 sites per lake for each sampling event. TSI values > 66 denote hypereutrophic conditions.

Date	Lake Jesup		Lake Monroe	
	$TSI_{Chl\ a}$	TSI_{TP}	$TSI_{Chl\ a}$	TSI_{TP}
28 Feb 2007	74	77	75	60
24 Apr 2007	82	76	82	75
07 June 2007	84	79	72	69
23 July 2007	84	73	75	68
10 Sept 2007	88	69	61	76
30 Oct 2007	86	68	52	69
18 Dec 2007	86	79	66	72
28 Jan 2008	82	70	57	67

3.2.2. Biological productivity and affect on N_2-fixation.

Seasonal variation in phytoplankton metabolism and productivity cycle peaked in both lakes in April, and were generally higher in Lake Jesup when compared to Lake Monroe. In Lake Jesup GPP was (mean ± SD) 1.62 ± 0.89 mg O_2 L^{-1} h^{-1} on 01/02/2008 and 02/28/07 (winter sampling events) while the rest of the time it leveled off at 2.35 ± 0.47 mg O_2 L^{-1} h^{-1} (Fig. 3.2.2.1). Lake Monroe GPP increased from 0.43 ± 0.11 mg O_2 L^{-1} h^{-1} during February 2007, to a maximum on 04/24/07 (2.15 ± 0.32 mg O_2 L^{-1} h^{-1}) then decreased steadily to reach 0.16 ± 0.11 mg O_2 L^{-1} h^{-1} on 01/28/2008. The positive NPP rate (Fig. 3.2.2.2) followed the same pattern as GPP. Annual mean GPP for Lake Jesup (2.08 ± 0.75 mg O_2 L^{-1} h^{-1}) was higher than in Lake Monroe (0.67 ± 0.67 mg O_2 L^{-1} h^{-1}) with a ratio of ~2.4 from 02/28/2007 through 08/06/07 and ~5.3 thereafter.

Respiration in Lake Jesup (0.19 ± 0.07 mg O_2 L^{-1} h^{-1}) was consistently higher than in Lake Monroe (0.09 ± 0.07 mg O_2 L^{-1} h^{-1}) (Fig. 3.2.2.3). The phytoplankton metabolism in Lakes Jesup and Monroe did not change greatly during the week long periods regardless of season (April or September 2007) ($P > 0.05$) (Figs. 3.2.2.4 GPP, 3.2.2.5 Respiration, and 3.2.2.6 NPP). The diel metabolism measurements indicate respiration in Lakes Jesup and Monroe was higher in the morning than at night ($P < 0.05$) with a ratio ~ 3.8 in April and September 2007 (Figs. 3.2.2.7 and 3.2.2.8). This ratio was lowered to ~3.1 when morning and afternoon respiration were compared in September for Lake Jesup. Respiration rates in Lake Monroe in September were close to the detection limits of light/dark BOD method. GPP and NPP were higher in the morning than in the afternoon in Lake Jesup ($P < 0.05$, ratio ~2.3) but they remain similar in Lake Monroe ($P = 0.78$).

Reference spectra calibrations for major algal groups in each lake were measured with the PHYTOPAM. A total of 12 algae (1 Cyanophycea, 6 Chlorophyceae and 5 Bacillariophyceae) were isolated from Lakes Jesup and Monroe. The different algal cultures were not identified beyond these general classifications (Fig. 3.2.2.9). The reference spectrum of each algal group was similar to the standard reference spectrum delivered with the PHYTOPAM. The Br_std refers to the standard reference spectra as initially loaded into the PHYTOPAM upon delivery from the manufacturer for Cyanophyceae, Chlorophyceae and Bacillariophyceae respectively. Seasonal variations in major algal groups were observed in both lakes. Lake Jesup was largely dominated by Cyanophyceae (> 80% total biomass) with an exclusive dominance after June 2007 (Figs. 3.2.2.10 through 3.2.2.13). Lake Monroe was dominated by Cyanophyceae (> 70% biomass) from April to July and in December 2007. The remainder of the time, Cyanophyceae dominance is lower and Bacillariophyceae and Cyanophyceae co-dominate in February and December 2007, and January 2008. Chlorophyceae were only noticeable in Lake Monroe in September 2007 (~20%). There were no variations in major algal groups on a weekly (Figs. 3.2.2.14, 3.2.2.15, and 3.2.2.16) or daily basis (Fig. 3.2.2.17) in either lake. The quantum yields (QY) of the major algal groups were similar in both lakes during the 334 day study (Fig. 3.2.2.18 and 3.2.2.19). Cyanophyceae QY was stable and similar in both lakes ($P < 0.05$) during the duration of this study; Lake Jesup (0.204 ± 0.034) or Monroe (0.202 ± 0.068). When present, Chlorophyceae and

Bacillariophyceae QYs in Lake Monroe averaged 0.828 ± 0.111 and 0.394 ± 0.209, respectively. The relative ETR_{max} parameter of the PI curves (GPP_{max}) of major algal groups showed that the Cyanophyceae ETR_{max} for Lake Jesup (41.5 ± 7.3 µmol electrons m^{-2} s^{-1}) and Lake Monroe (38.1 ± 16.2 µmol electrons m^{-2} s^{-1}) were similar (P = 0.18) and fairly constant over time (Fig. 3.2.2.20 and 3.2.2.21). Bacillariophyceae ETR_{max} for Lake Monroe was higher at 121.2 ± 88.6 µmol electrons m^{-2} s^{-1}. Chlorophyceae ETR_{max} measurements were not reliable enough to be presented here because this algal group had a minor biomass when compared to the other algal groups. Cyanophyceae I_K in Lakes Jesup (422 ± 71 µmol photons m^{-2} s^{-1}) and Monroe (462 ± 548 µmol photons m^{-2} s^{-1}.) was steady and similar over the study period (P = 0.06) (Fig. 3.2.2.22). It should be noted that the determination of Bacillariophyceae and Chlorophyceae I_K in Lake Monroe were not reliable enough to be presented here.

The ratio between the total Chlorophyll of all algal groups as determined with the PHYTOPAM and the Chl *a* as determined with the spectroflurorometer shows that 13% of the chlorophyll is other than Chl *a* (Fig. 3.2.2.23). The PHYTOPAM algal concentration also matched the total algal biovolume. Because the counts were done by two different groups, an adjustment was performed: Lowe's laboratory biovolume had to be multiplied by a constant equal to 0.6 (Fig 3.2.2.24). When comparing the plankton data from two other research groups studying Lake Jesup (R. Lowes's and WAR laboratories) and our Phytopam data, all indicate that the lake was dominated by Cyanophyceae throughout the year.

The Chla:biovolume was 7.9 µg mm^{-3} (Fig. 3.2.2.24) and was linearly correlated (P < 0.01, r^2 = 0.64, not shown). The PHYTOPAM can distinguish the different algal groups but it cannot separate N_2-fixing from the non- N_2- fixing Cyanophyceae. The algal counts exhibit a large dominance of the potentially N_2 -fixing *Cylindrospermopsis spp* encompassing 35% of the total Cyanophyceae population from January 2007 until July 2007. Thereafter, *Cylindrospermopsis spp* encompassed approximately 80% of the total Cyanophyceae population (Figure 3.2.2.25). Since Lake Jesup was dominated by Cyanophyceae, the figures can be transposed to this lakes total algal population (Fig. 3.2.2.26).

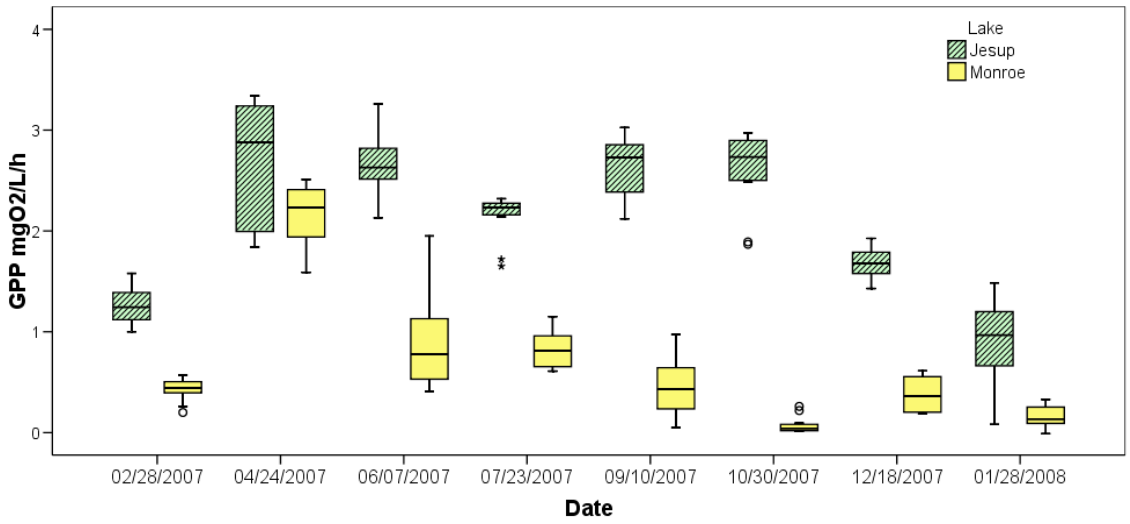

Figure 3.2.2.1: Seasonal change in GPP in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

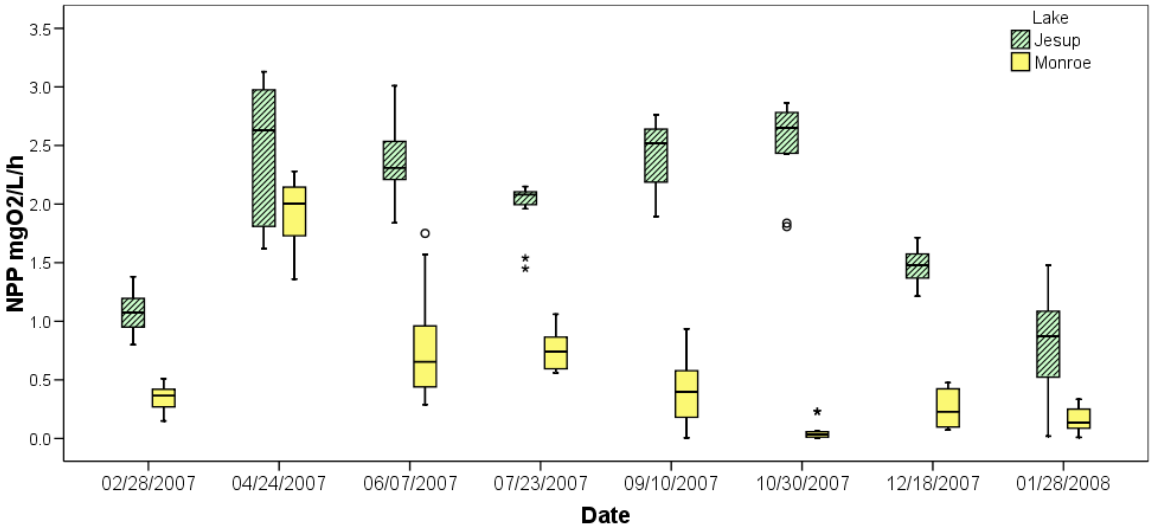

Figure 3.2.2.2: Seasonal change in NPP in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

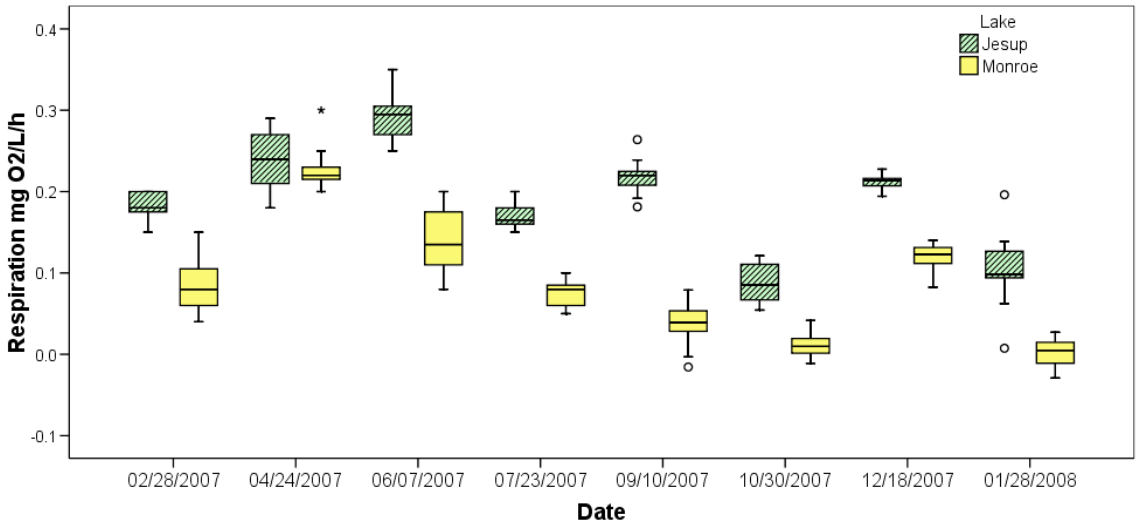

Figure 3.2.2.3: Seasonal change in respiration in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

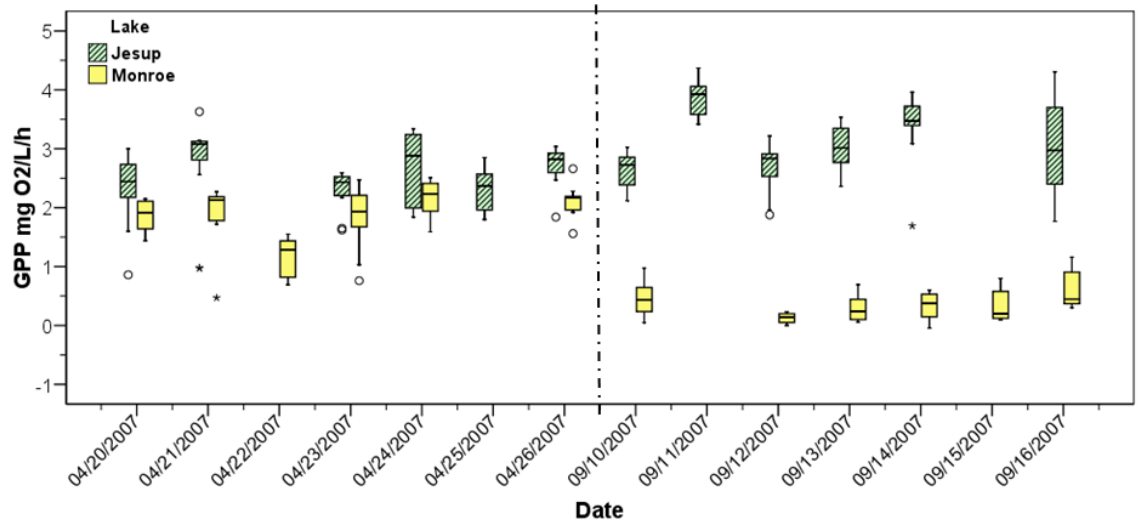

Figure 3.2.2.4: Weekly change in GPP in April and September 2007 in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

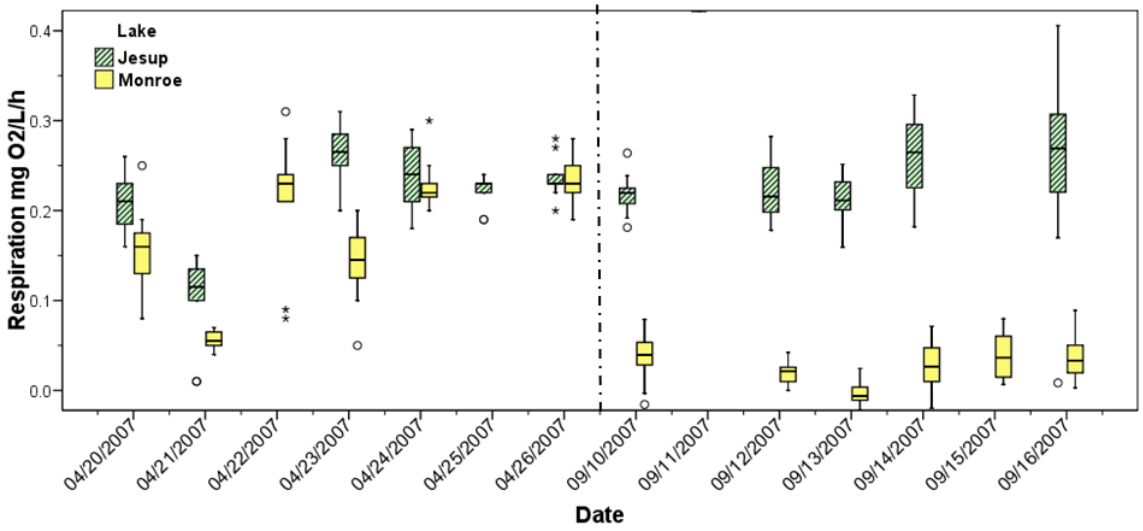

Figure 3.2.2.5: Weekly change in respiration in April and September 2007 in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

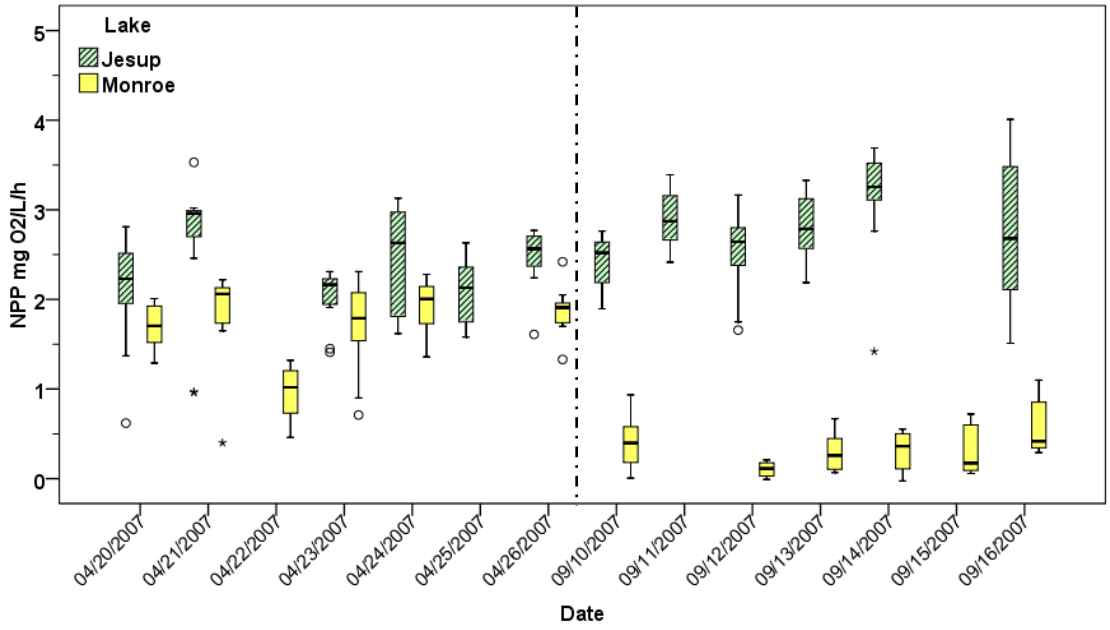

Figure 3.2.2.6: Weekly change in NPP in April and September 2007 in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

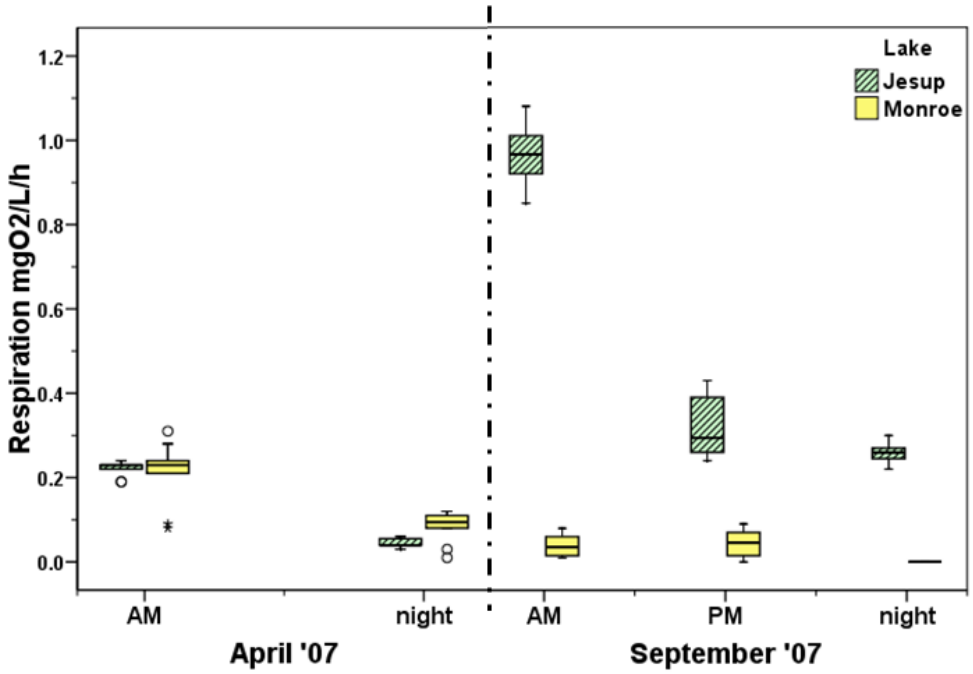

Figure 3.2.2.7: Diel changes in respiration in Lakes Jesup and Monroe in April and September 2007. See Fig. 3.1.2.1 for further explanation of this boxplot.

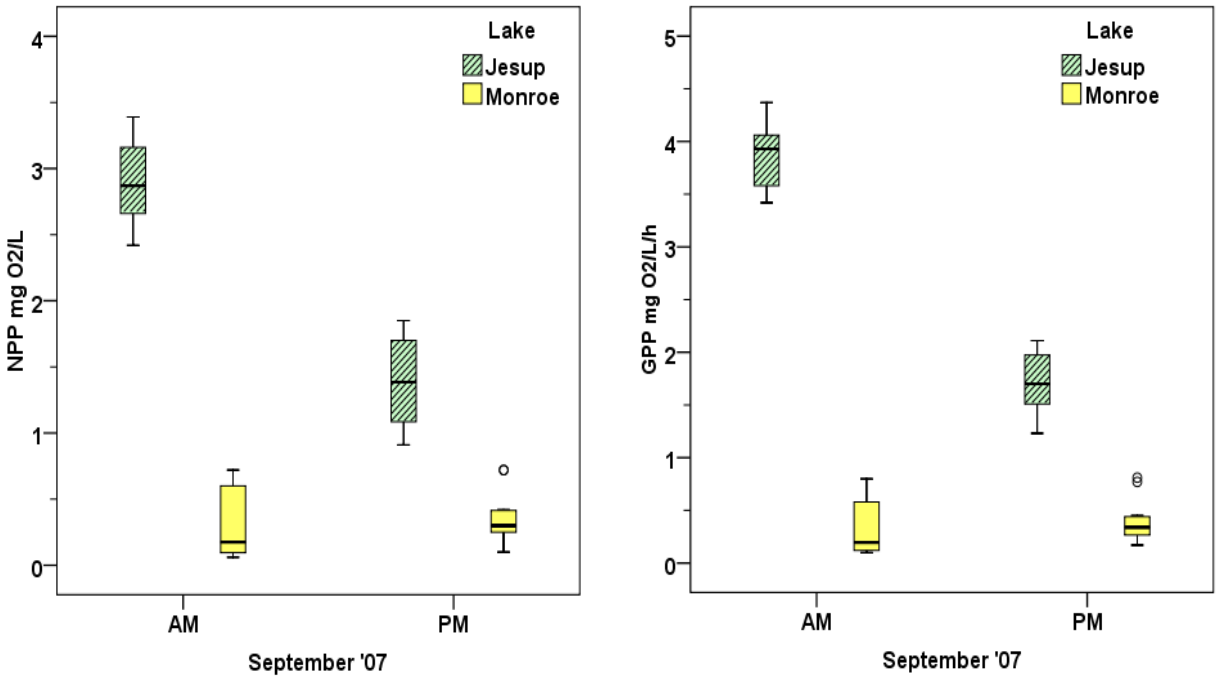

Figure 3.2.2.8: Diel changes in GPP and NPP in September 2007 in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

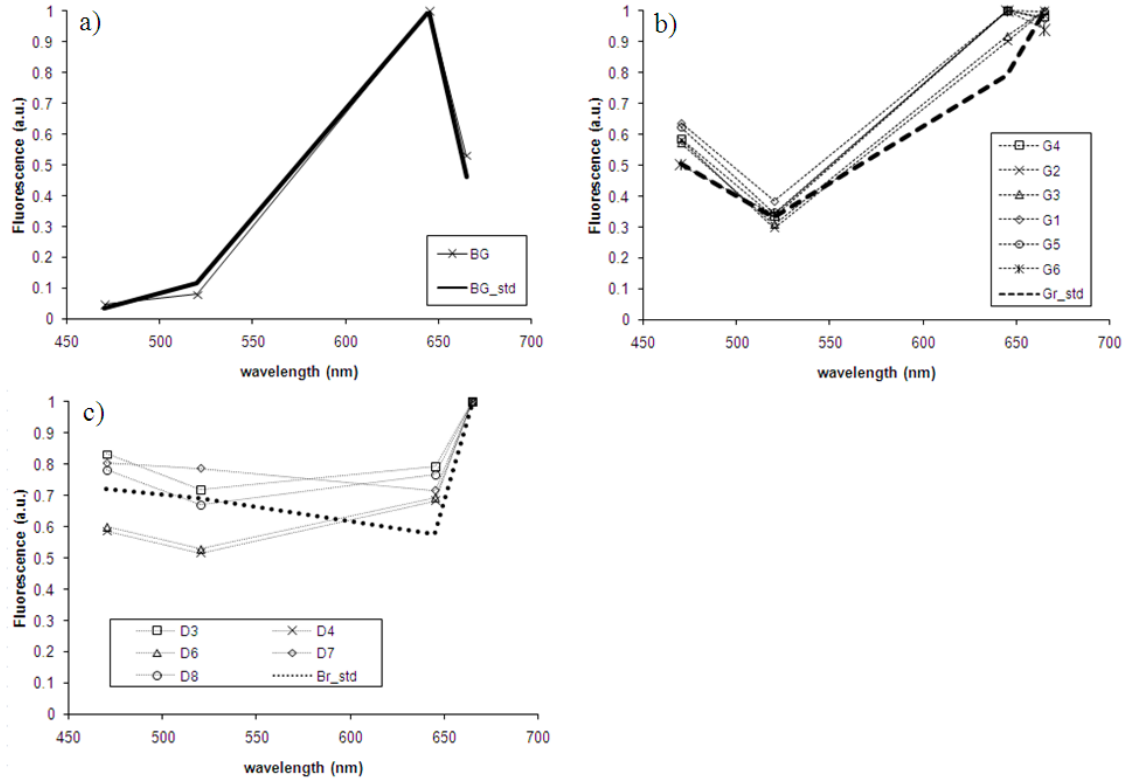

Figure 3.2.2.9: Variation in the reference spectra from Lakes Jesup and Monroe isolates for Cyanophycea (a), Chlorophyceae (b) and Bacillariophyceae (c). BG_std, Gr_std and

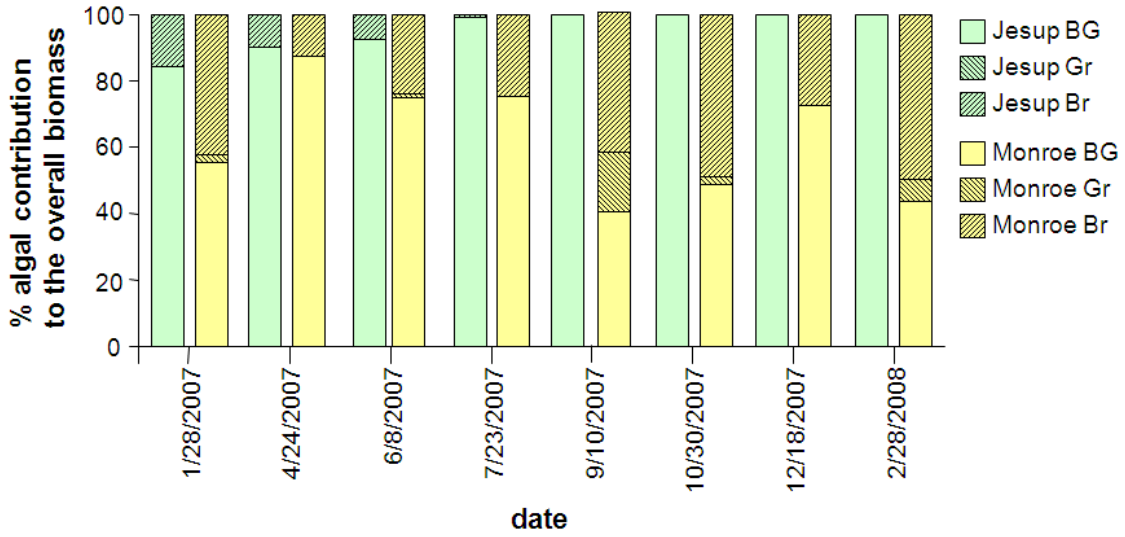

Figure 3.2.2.10: Seasonal change in percentage of Cyanophyceae (BG), Chlorophyceae (Gr) and Bacillariophyceae (Br) for Lakes Jesup (pale green) and Monroe (pale yellow) as determined with the PHYTOPAM.

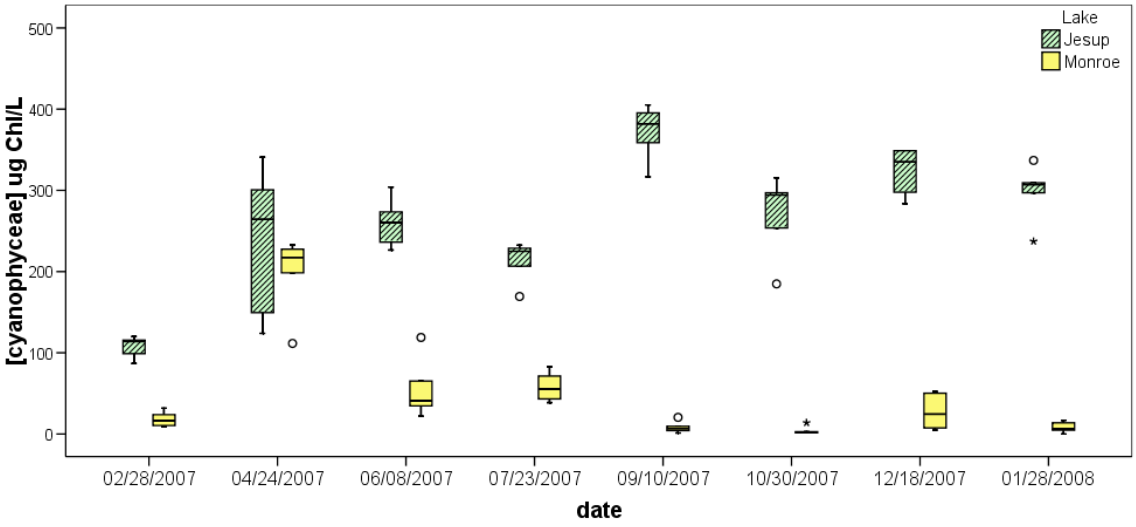

Figure 3.2.2.11: Seasonal change in concentration of Cyanophyceae in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

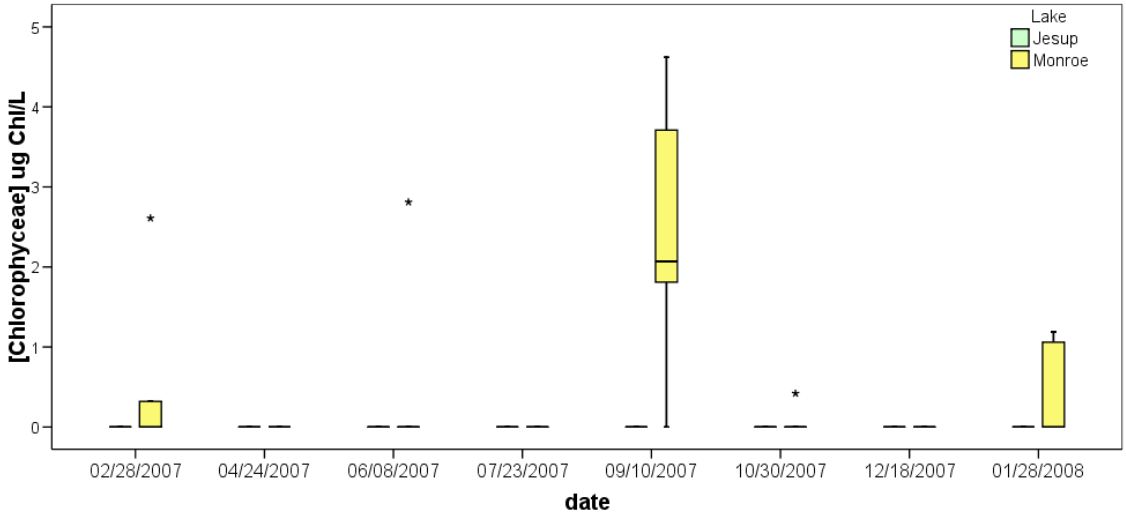

Figure 3.2.2.12: Seasonal change in concentration of Chlorophyceae in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

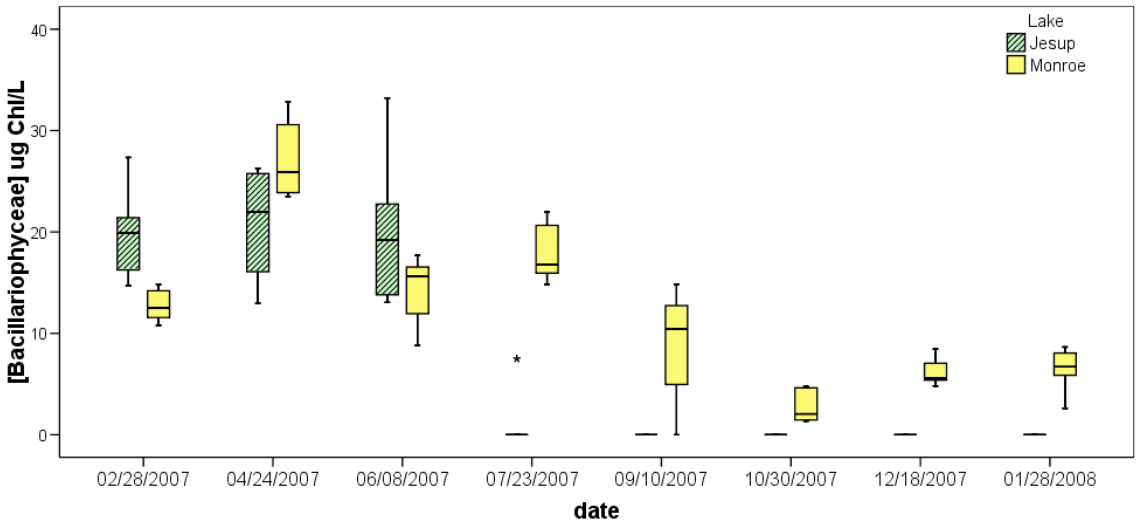

Figure 3.2.2.13: Seasonal change in concentration of Bacillariophyceae in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

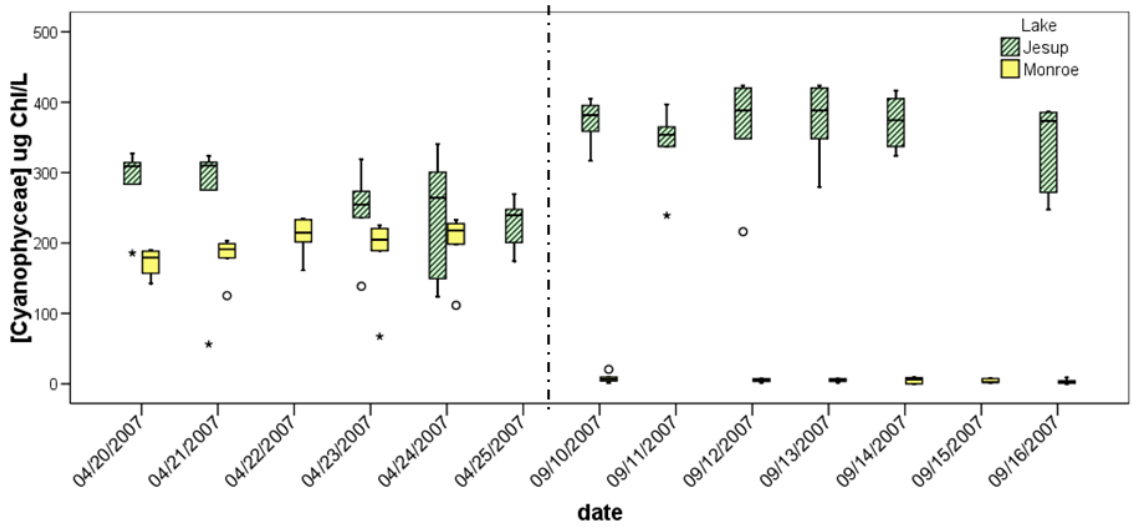

Figure 3.2.2.14: Weekly change in Cyanophyceae concentration in April and September 2007 in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

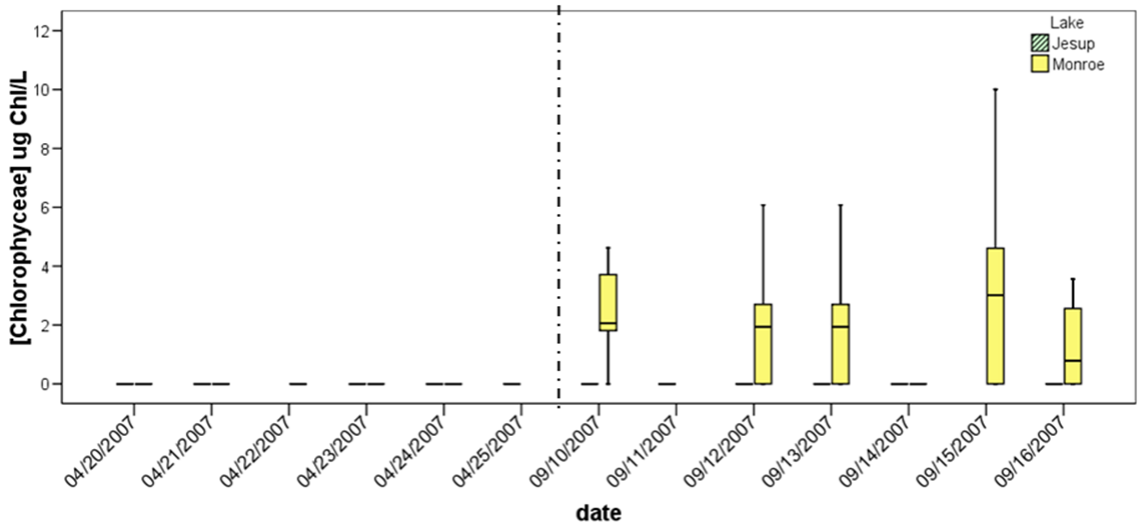

Figure 3.2.2.15: Weekly change in Chlorophyceae concentration in April and September 2007 in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

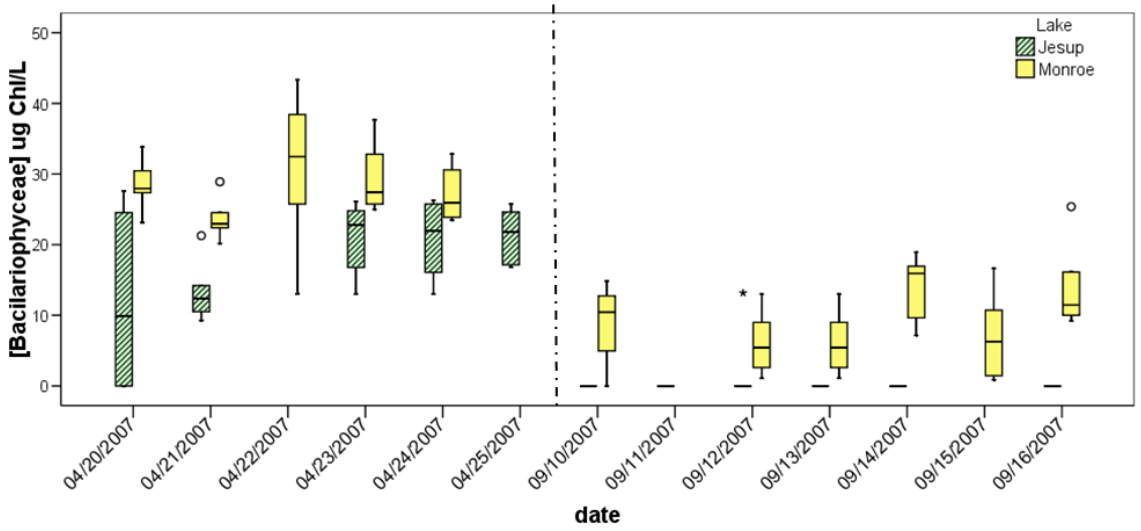

Figure 3.2.2.16: Weekly change in Bacillariophyceae concentration in April and September 2007. See Fig. 3.1.2.1 for further explanation of this boxplot.

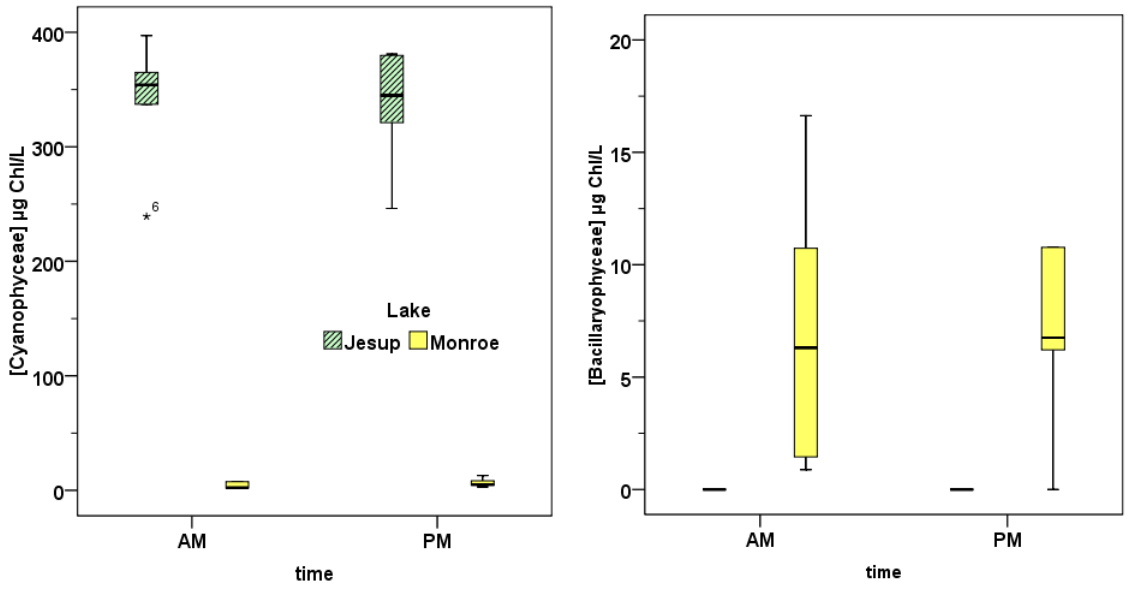

Figure 3.2.2.17: Diel change in Cyanophyceae and Bacillariophyceae concentration between the morning (AM) and the afternoon (PM) in September 2007. The night PHYTOPAM assessment was not consistently made and is thus not presented. When done, the night measurement showed similar values as during daylight hours. See Fig. 3.1.2.1 for further explanation of this boxplot.

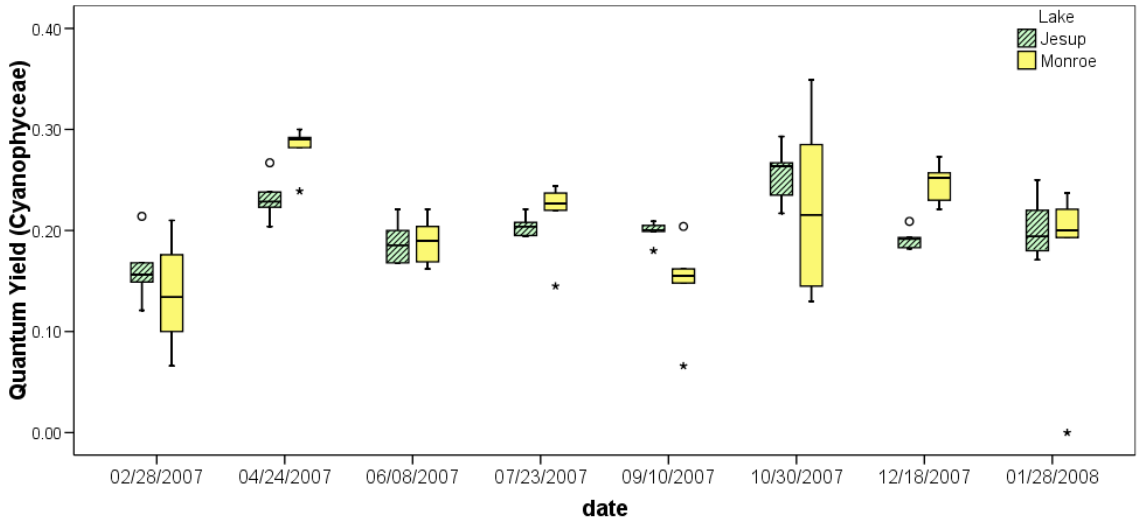

Figure 3.2.2.18: Seasonal change in Quantum yield for the Cyanophyceae in Lakes Jesup and Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

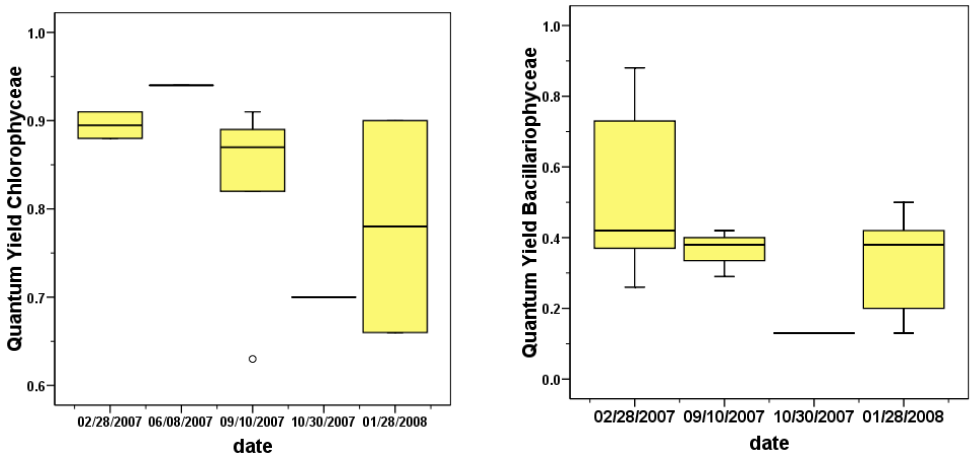

Figure 3.2.2.19: Seasonal change in Quantum yield for the Cholorophyceae and Bacillariophyceae in Lakes Monroe. See Fig. 3.1.2.1 for further explanation of this boxplot.

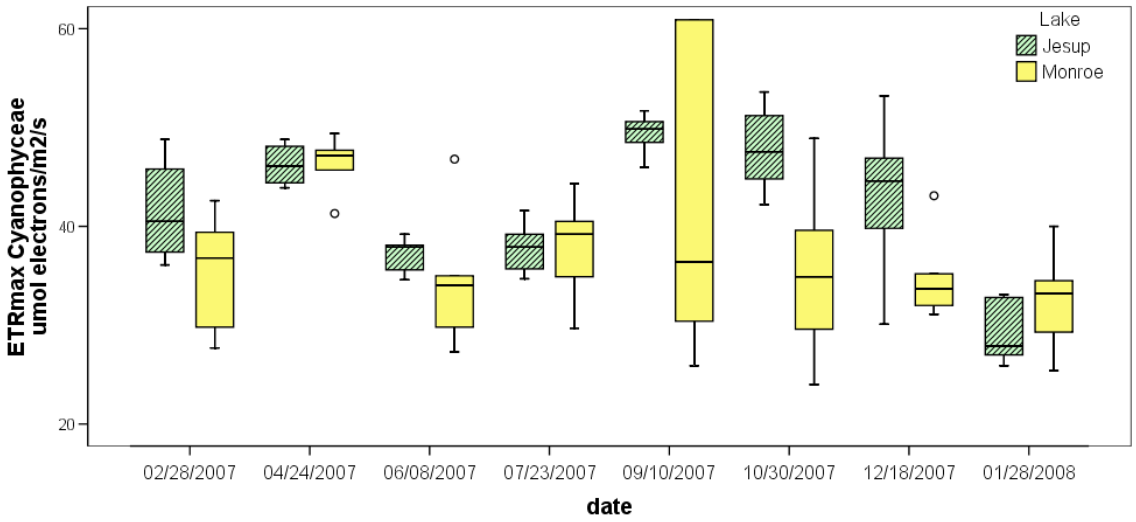

Figure 3.2.2.20: Seasonal change in relative ETR_{max} in Lakes Jesup and Monroe for the Cyanophyceae. See Fig. 3.1.2.1 for further explanation of this boxplot.

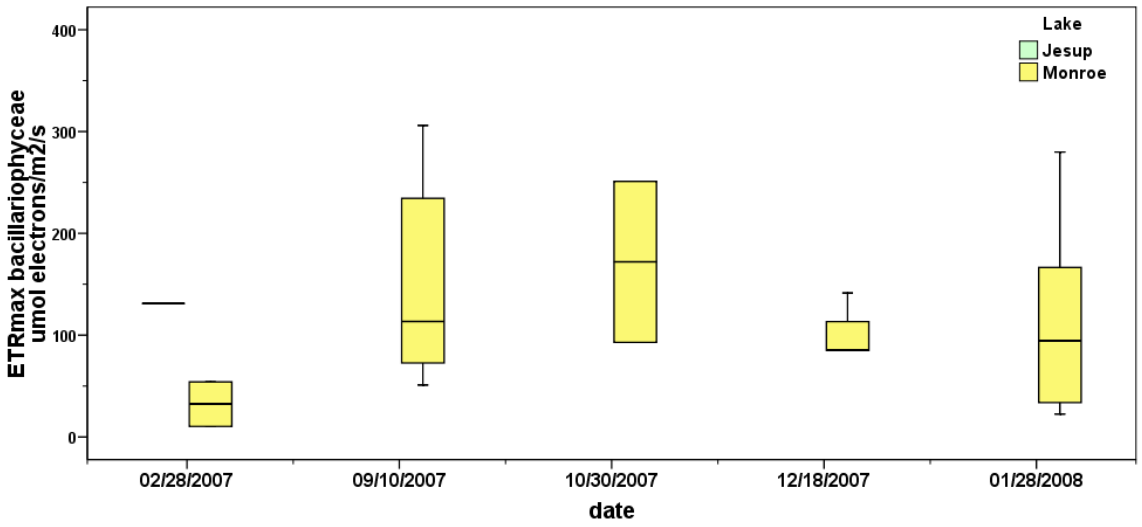

Figure 3.2.2.21: Seasonal change in relative ETR_{max} in Lake Monroe for the Bacillariophyceae. See Fig. 3.1.2.1 for further explanation of this boxplot.

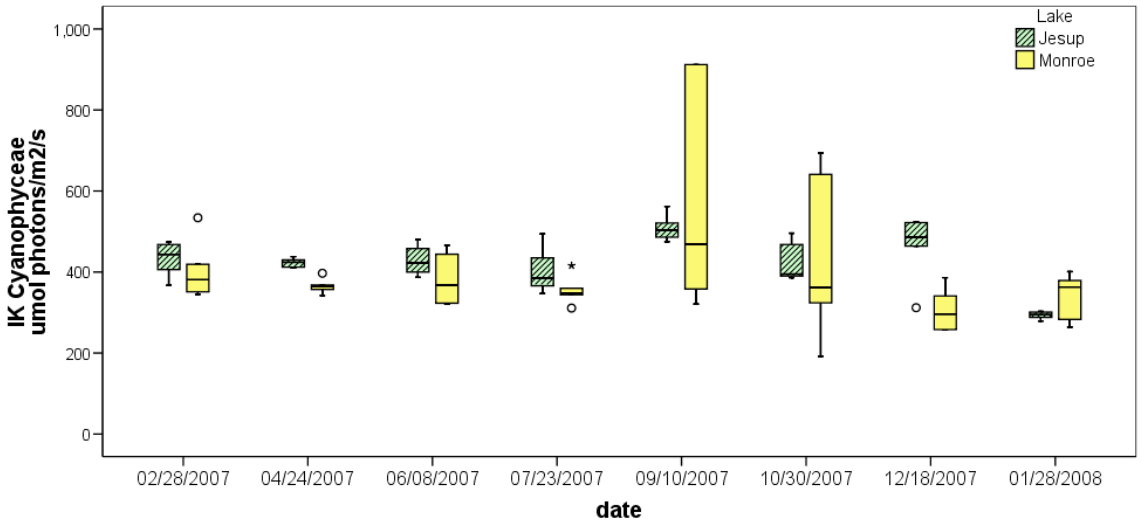

Figure 3.2.2.22: Seasonal change in I_k in Lakes Monroe and Jesup for Cyanophyceae. See Fig. 3.1.2.1 for further explanation of this boxplot.

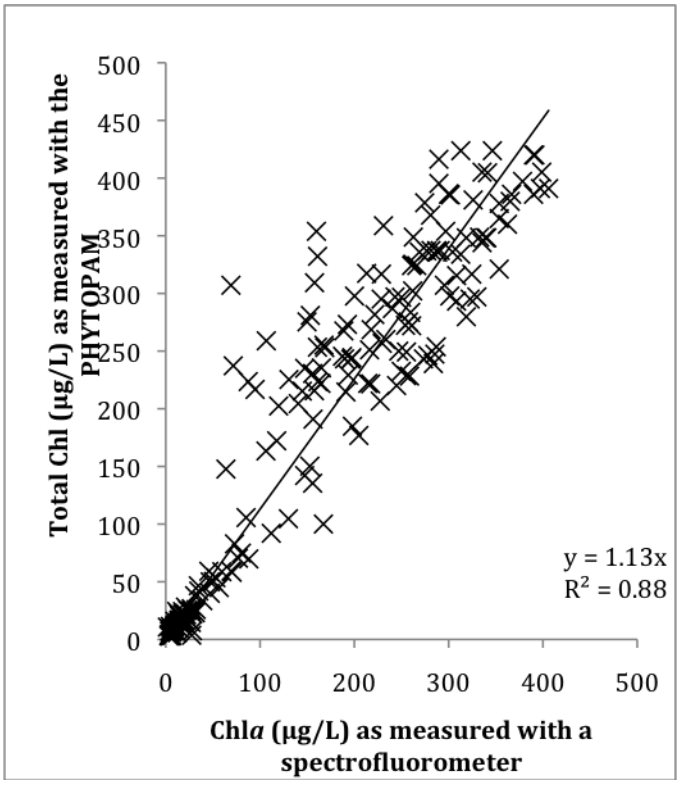

Figure 3.2.2.23: correlation between the algal biomass as measured with the spectrofluorometer and with the PHYTOPAM (all algal groups combined). Note that the PHYTOPAM measures total chlorophyll for each algal group.

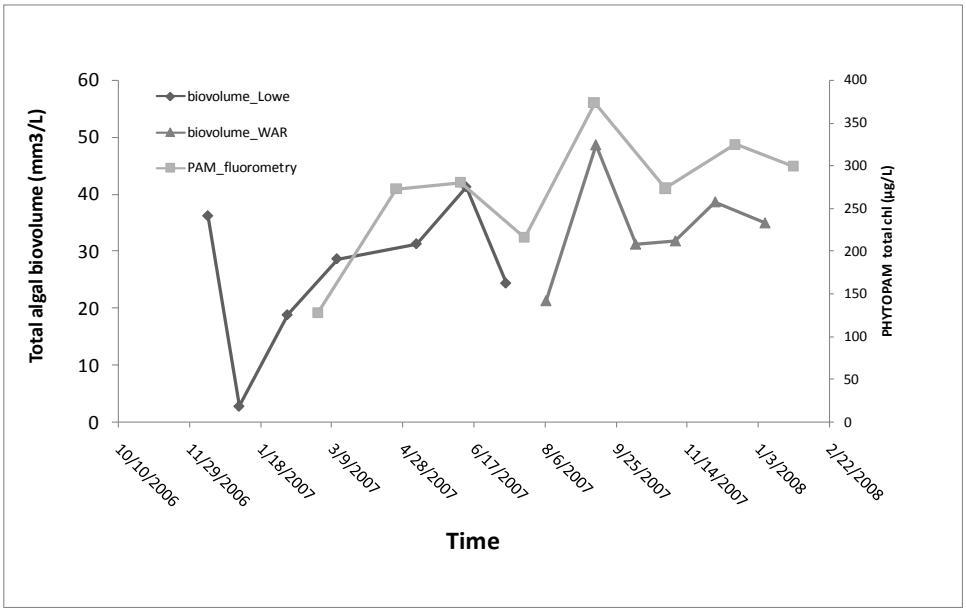

Figure 3.2.2.24: Change in the algal biomass as expressed as biovolume and determined by algal counts and the PHYTOPAM.

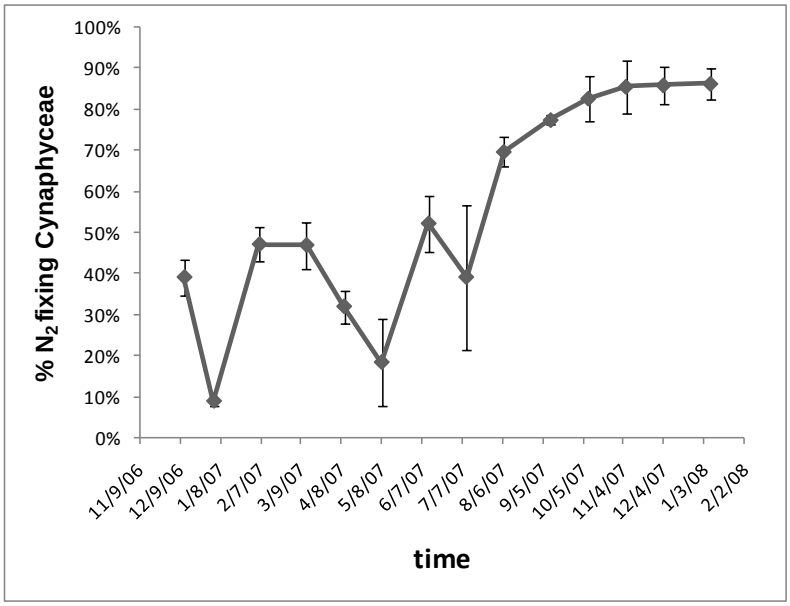

Figure 3.2.2.25. Percentage of biovolume/biovolume N_2 fixing Cynaophycea in Lake Jesup as compared to the overall Cyanophycea population

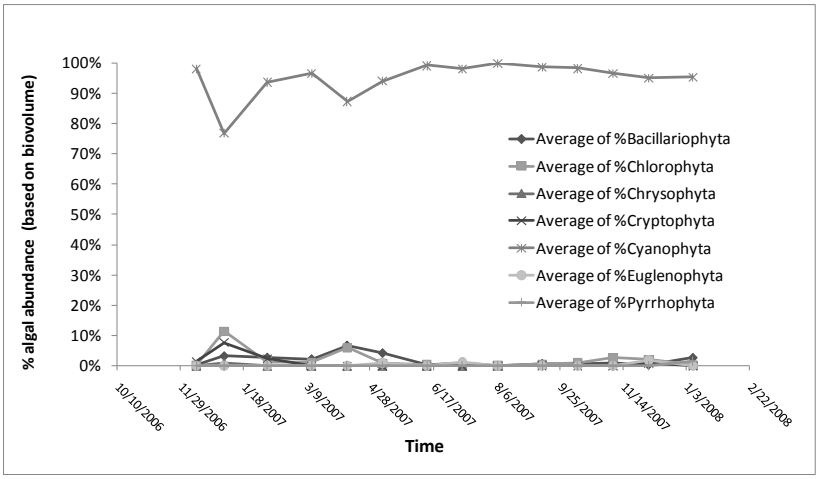

Figure 3.2.2.26: change in the contribution as of biovolume/biovolume of the different algal groups to the overall algal population in Lake Jesup. A strong Cyanophycea dominance is observed.

In our attempt to conduct a logistically feasible assay to determine seasonal variation in N_2-fixation rates we incubated all samples from all events in floating racks at the waters surface. It is well known that light level in the water column affect N_2-fixation rates (Dugdale and Dugdale, 1962). Our data also strongly support this assertion that

photosynthesis and N_2-fixation are linked. The amount of irradiance received by the algal population of Lakes Jesup and Monroe thus partially determined photosynthesis and N_2-fixation. However, the Photosynthesis-Irradiance relationship was non-linear; although linear at irradiances below the I_K threshold, photosynthesis plateaus at irradiances above I_K. During our incubations, photosynthesis (BOD) incubations and N_2 fixation were conveniently incubated subsurface on floating racks, normal for these types of studies (Montoya et al., 1996). The algal populations thus received a higher amount of light than encountered throughout the entire water column. Our study did not have the capacity to measure fixation at different depths. However, if this had been measured, our estimates of N_2-fixation potentially could be different (lower). These lakes have well mixed water columns during both the day and night. By collecting water in a "core" we sampled the entire water column in these continually mixing lakes. Therefore, the nitrogen fixing community at any time could have been distributed throughout the water column e.g. at the surface during part of the day but moving below the surface at other times. We sampled daylight fixation rates during our seasonal events and night time fixation rates (during two diels in an anuual cycle) (Fig. 3.1.3.1 and 3.1.3.2 and Table 3.1.3.1). Variations (light effects) in day/night fixation were apparent in this data which was the best estimate possible using the data collected (Figs. 3.1.5.1 & 3.1.5.2) and therefore is an assessment of how our approach during the incubations impacted our photosynthesis and N_2 fixation results.

Table 3.2.1: Comparison of the mean irradiance during the incubations and the I_K as determined with the PHYTOPAM for the Cyanophyceae of Lakes Jesup and Monroe. I_K is, with a few exceptions (denoted *), always smaller than the mean ambient irradiance.

	Mean irradiance during incubations	I_K Cyanophyceae L. Jesup	I_K Cyanophyceae L. Monroe
Date	µmol photons m^{-2} s^{-1}	µmol photons m^{-2} s^{-1}	µmol photons m^{-2} s^{-1}
02/28/07	1336	434 ± 41	402 ±71
04/24/07	977	423 ± 11	365 ±18
06/07/07	1654	428 ± 35	382 ±61
07/23/07	319	402 ± 54*	354 ± 35*
09/10/07	1213	508 ± 31	506 ± 242
10/30/07	568	422 ± 48*	429 ± 196
12/18/07	404	466 ± 79*	306 ± 51
01/28/07	469	293 ± 9.00	338 ± 61

Lakes Jesup and Monroe, being well mixed, contain algal populations that were migrating through the water column (since no major occurrences of floating Cyanophyceae blooms were noteworthy). The light regime encountered is thus likely lower than the one used during the incubations (max. light at the surface diminishing with depth). It is very likely that a large portion of the algal population never experiences saturating irradiance, except when exposed to surface waters. However, it was not possible with the data gathered in this study to assess the actual light regime for the

basins. For this to have been accomplished the above hourly lake water PPFD during the whole study period of sampling would have been needed and computed with the mean depth and the irradiance exponential decay curve in the water column to assess the average daily irradiance level received by the algal populations.

Undoubtedly, our N_2-fixation and photosynthesis rates are high estimates (because incubations took place in full available sun in the surface lake waters); but because P_{max} is generally reached (plateau) during all incubations we can make comparisons across time. Seasonal variation in N_2-fixing activity was one of the main objectives of this study and it was better to compare both parameters (photosynthesis and N_2-fixation rates) under these conditions as a uniform depth (surface) provided consistency in making seasonal assays. Furthermore, it would have been impractical to mimic the light regime encountered by the algal populations, since the incubation depth (provided a fixed location in the water column existed) would have had to have been calculated every day, depending on the amount of PPFD of the incubation day. Lastly, data from our diel study provide estimates of day-time versus night-time fixation (max. vs. min. light levels).

3.3 Molecular Analysis of N_2-Fixing Gene Expression and Cyanobacterial Identification.

3.3.1. Composition and structure of cyanobacterial communities

Sequence similarity matching was performed in DDBJ Blast. DDBJ Blast results for the 6 sequenced bands are shown in Table 3.3.1.1. Sequences for bands 1 and 2 (indicated with arrows in Fig. 3.3.1) had the highest similarity to sequences for *Cylindrospermopsis* (Table 3.3.1). These *Cylindrospermopsis* bands were dominant bands in Lake Jesup RNA patterns over the course of the entire year (Feb 2007-January 2008 Fig. 3.3.1a-e). They were also dominant bands in Monroe RNA patterns during the dry season months of February, April, and December 2007 and January 2008 (Fig. 3.3.1a,b,e). Band 2 continued to be a dominant band in L.Monroe RNA patterns for the early wet season (June and July) with Band 1 still appearing but with reduced intensity (Fig. 3.3.1b,c). While *Cylindrospermopsis* bands appeared intensely in RNA patterns for all Lake Jesup and dry season-early wet season Lake Monroe samplings, these same bands often were weak or nonexistent in the DNA DGGE patterns. *Cylindrospermopsis* bands were only intense in DNA patterns during the early-mid dry season (February and December 2007, January 2008 Fig 3.3.1a, e). This discrepancy between RNA and DNA patterns suggests that *Cylindrospermopsis* was highly active (RNA expression) even though the *Cylindrospermopsis* population constituted only a small part of the total cyanobacterial community present in the lakes (reduced DNA band intensity). Bands 1 and 2 were strong bands in the DNA patterns of Lakes Jesup and Monroe for February 2007, December 2007, and January 2008 indicating that *Cylindrospermopsis* formed a greater percentage of the community during these months (Fig. 3.3.1a-e). In April, 2007 patterns, *Cylindrospermopsis* activity (RNA DGGE band intensity) remained high while its proportion of the community declined in both lakes (weak DNA DGGE band intensity,

Fig. 3.3.1a & b). Intense DNA *Cylindrospermopsis* bands were not seen again for either lake until the following dry season (Dec, 2007 and Jan, 2008) (Fig. 3.3.1e).

Blast search results for bands 14 and 15 did not yield similar cyanobacterial sequences; rather, sequences of bands 14 and 15 had high similarity to diatom chloroplast 16S rRNA and rDNA sequences (Table 3.3.1). This was not an unexpected result as the cyanobacterial primers used in this work are capable of also amplifying plastid 16S rRNA and rDNA (Nübel et al 1997). These bands were the dominant bands for Lake Monroe DNA and RNA patterns during the later part of the rainy season (September, 2007), indicating that diatoms become dominant in Lake Monroe and had a high metabolic activity during this time period (Fig. 3.3.1c & d). These 2 bands did not appear in Lake Jesup samples. The appearance of bands 14 and 15 represented an important shift in the phytoplankton community of Lake Monroe between July and September samplings. Diatoms bands 14 and 15 were absent from Lake Monroe October DGGE patterns (Fig. 3.3.1d) suggesting a short-lived dominance of the diatoms during September and another period of transition prior to the October, 2007 event.

The remaining sequenced bands (bands 7 and 10 in Fig. 3.3.1) had the highest sequence similarity to two non-N_2 fixing cyanobacterial groups, *Oscillatoriales* and *Leptolynbya* (Table 3.3.1). The 4 most similar sequences for band 7 included the non-N_2 fixing cyanobacteria *Leptolynbya*, *Plectonema*, *Phormidium*, and the N_2 fixing cyanobacteria *Oscillatoria* (Table 3.3.1). These two bands were dominant in late dry season (April) DNA patterns for both Lake Jesup and Lake Monroe (Fig. 3.3.1 a & b). The intensity of these bands in RNA patterns was weak indicating that they formed a large proportion of the cyanobacterial population but the activity of these two cyanobacterial types was low compared with *Cylindrospermopsis* (compare intensity of bands 7 & 10 in RNA gels to bands 1 & 2 in Fig. 3.3.1 a & b). Bands 7 and 10 continued to be intense bands in Lake Jesup wet season DNA patterns, with band 7 appearing as an intense band in Lake Jesup DNA patterns for July, 2007 and the last 2 September sampling dates (Fig. 3.3.1b,d) and band 10 remaining dominant for June and July 2007 samplings (Fig. 3.3.1b,c). For bands 7 and 10, the intensities of the bands were strong in the DNA, but not the RNA, pattern. This is the converse of the appearance of *Cylindrospermopsis* bands (bands 1 and 2 in Table 3.3.1). This suggests that the cyanobacterial groups represented by bands 7 and 10 had large populations compared to other types of cyanobacteria present, but that their metabolic activity was relatively low compared to other cyanobacterial types in the lake (e.g. *Cylindrospermopsis*).

The six most dominant bands (Bands 1, 2, 7, 10, 14, and 15) out of the 36 total bands present in the DGGE gel patterns were sequenced. Further sequencing of other intense bands may indicate the presence of other N_2 fixing groups (e.g. *Anabaena*, *Microcystis*) or more non-N_2 fixing cyanobacteria. However, we focused on the most dominant groups present.

Cluster analysis revealed that the communities of Lake Jesup and Lake Monroe were relatively similar during the dry season and late dry to early wet season as determined by DGGE patterns of the DNA derived products (Fig. 3.3.2), although each lake tended to

form their own subclusters. The RNA patterns (Fig. 3.3.3) showed separation in expression between the dry and early wet seasons and the late wet season for both lakes. There were important differences between Lake Jesup and Lake Monroe for both DNA and RNA DGGE patterns during the later part of the wet season (September and October, Fig. 3.3.2-4). During this period, Lake Monroe DGGE patterns shifted to where bands 14 and 15 suddenly appeared and became dominant in September but disappeared by the October sampling (Fig. 3.3.1c,d). Lake Jesup by contrast continued to display the same intense bands (bands 1,2,7,10) seen in its February, April, June, and July DGGE patterns in its September and October patterns (Fig. 3.3.1a-d). Cluster analysis showed that while RNA and DNA patterns made up different subclusters, the differences between RNA and DNA DGGE patterns were not as great as those between patterns from different sampling dates (Fig. 3.3.4). The difference between RNA and DNA patterns was more pronounced in September patterns than April patterns (Fig. 3.3.4).

Understanding the cyanobacterial community compositions of Lakes Jesup and Monroe can aid in the understanding of seasonal or spatial variation in N_2 fixation rates. It is important to know not only when N_2 fixing types are present, but also if N_2 or non-N_2 fixing cyanobacteria are a dominant or a minor component of the community. Possible factors influencing the composition and succession of cyanobacterial communities in Lakes Jesup and Monroe include those known to be major factors in biodiversity distribution in various environments such as temperature, sunlight, and availability of nutrients (Frontier 1985, Atlas and Bartha 1997).

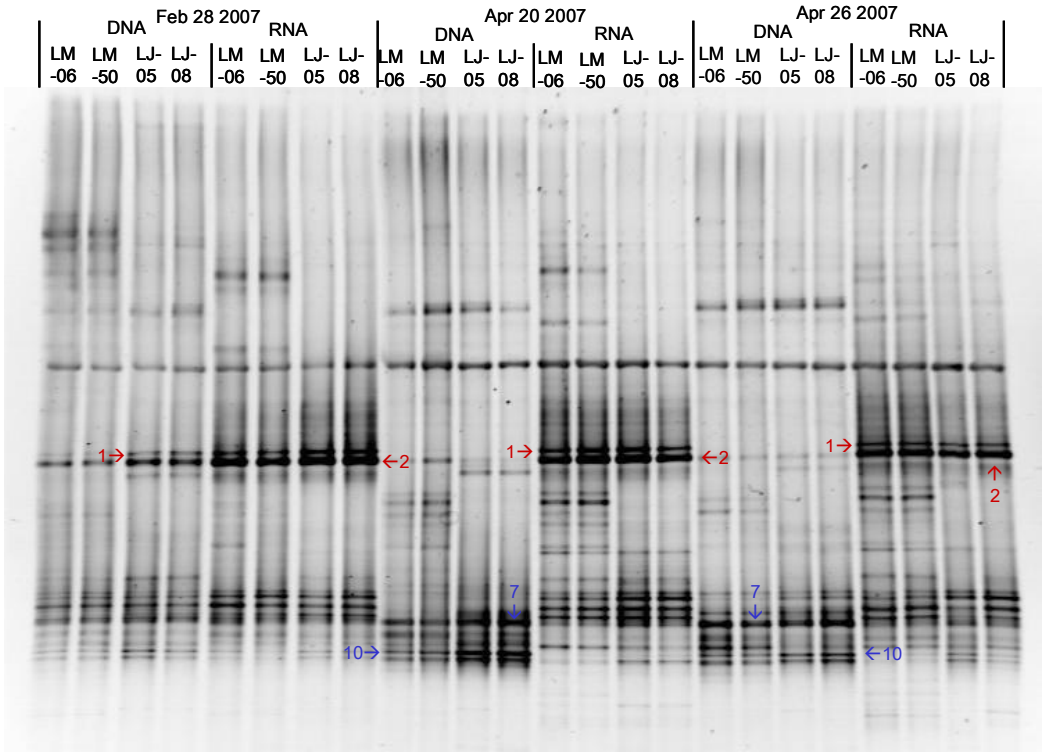

Figure 3.3.1a

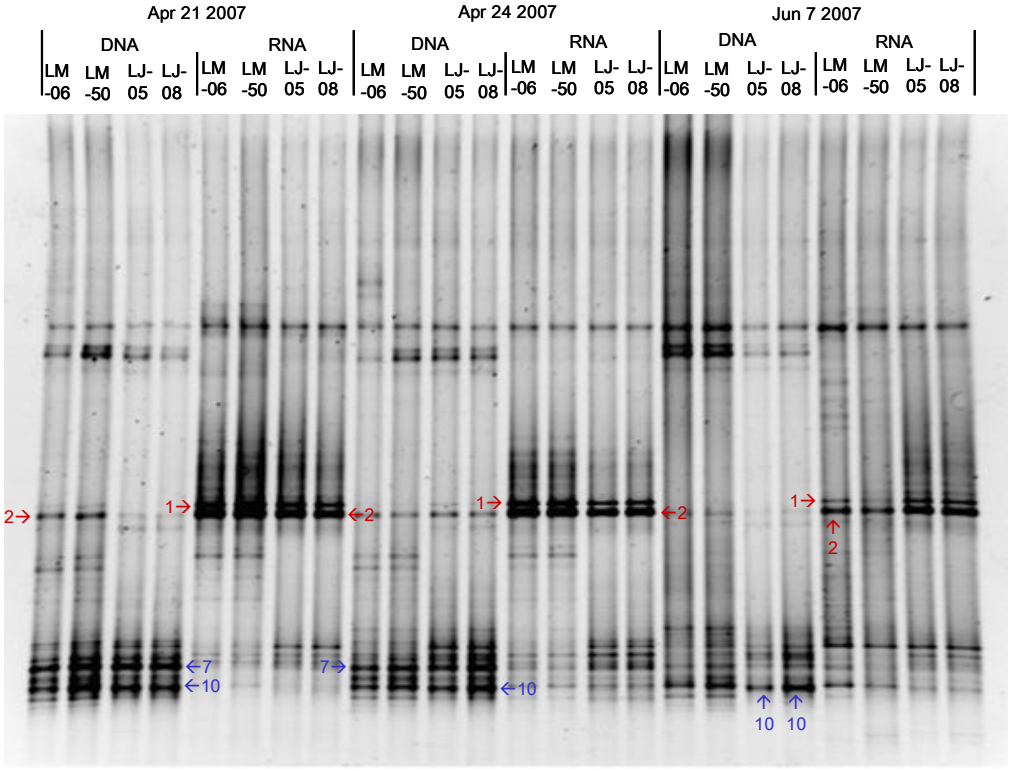

Figure 3.3.1b

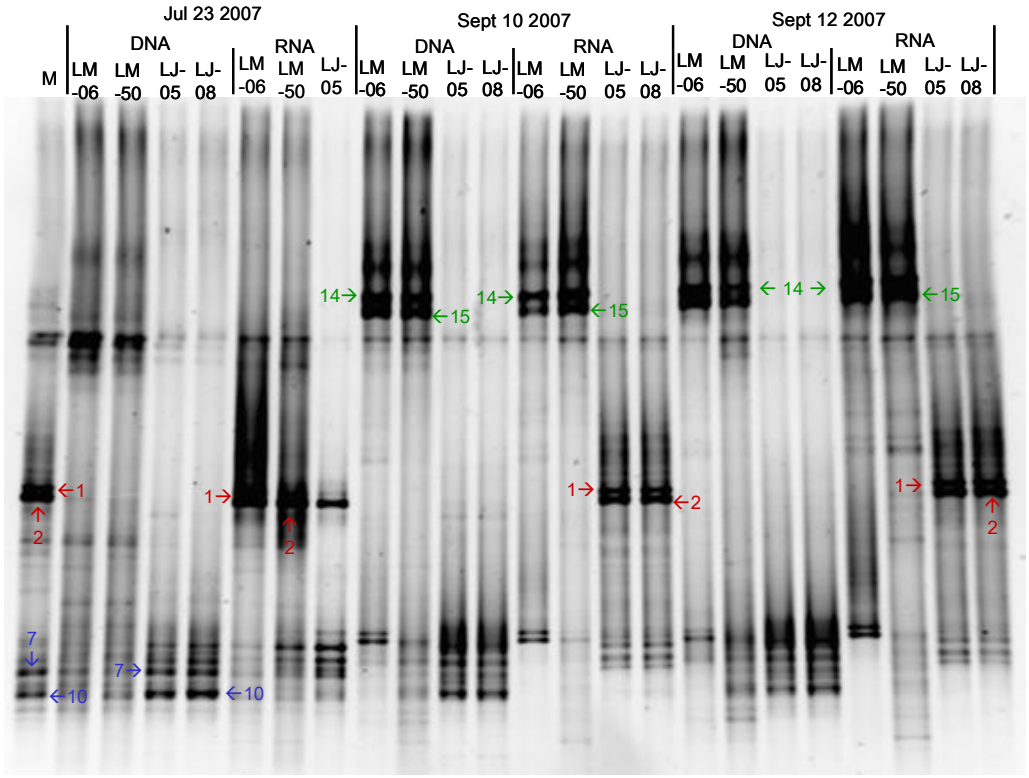

Figure 3.3.1c

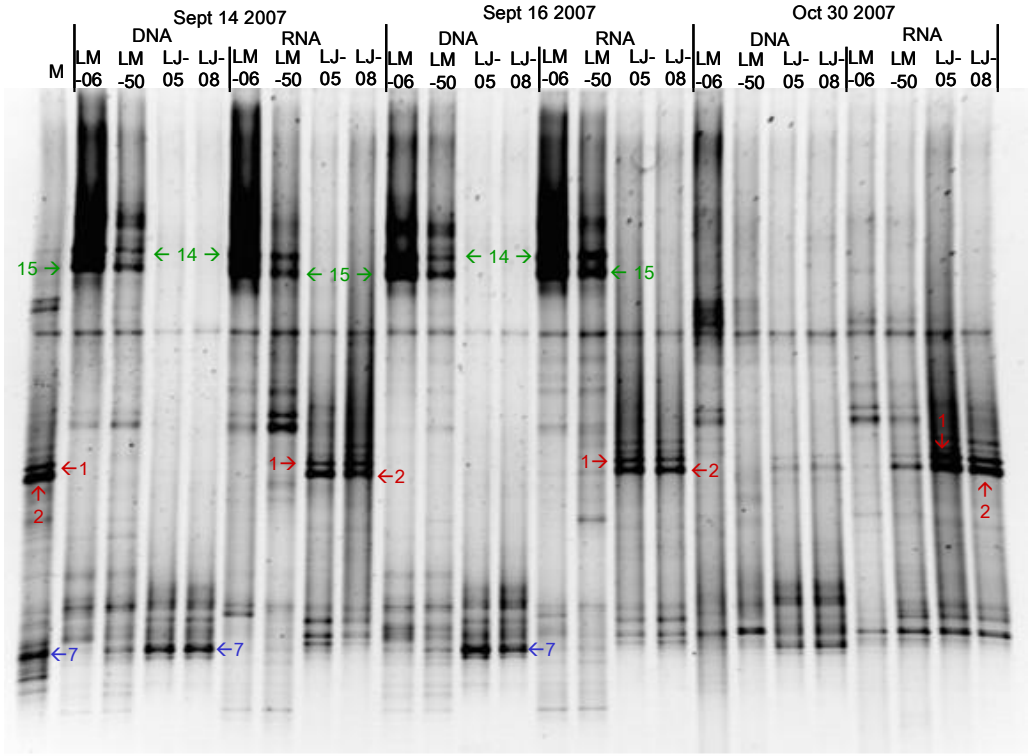

Figure 3.3.1d

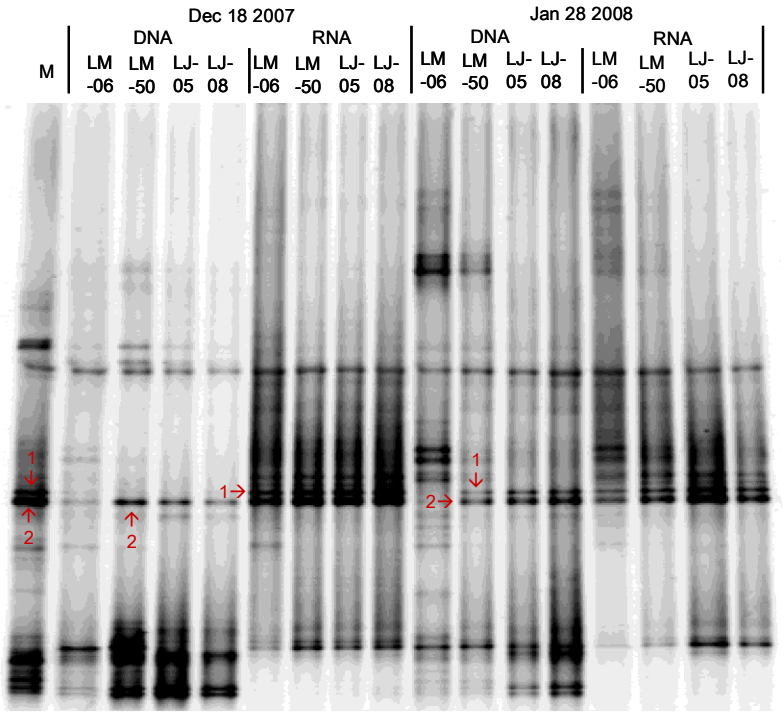

Figure 3.3.1e

Figure 3.3.1a-e DGGE patterns of nested PCR products derived from extracted 16S rDNA and 16S rRNA. Bands selected for sequencing are marked where they appear in the gels with arrows and ID numbers. A description of sequenced bands is provided in Table 3.3.1. "M" on the far left of c-e is an internal marker made from nested PCR product for LM-50 4/21/07 DNA and LM-06 4/21/07 RNA.

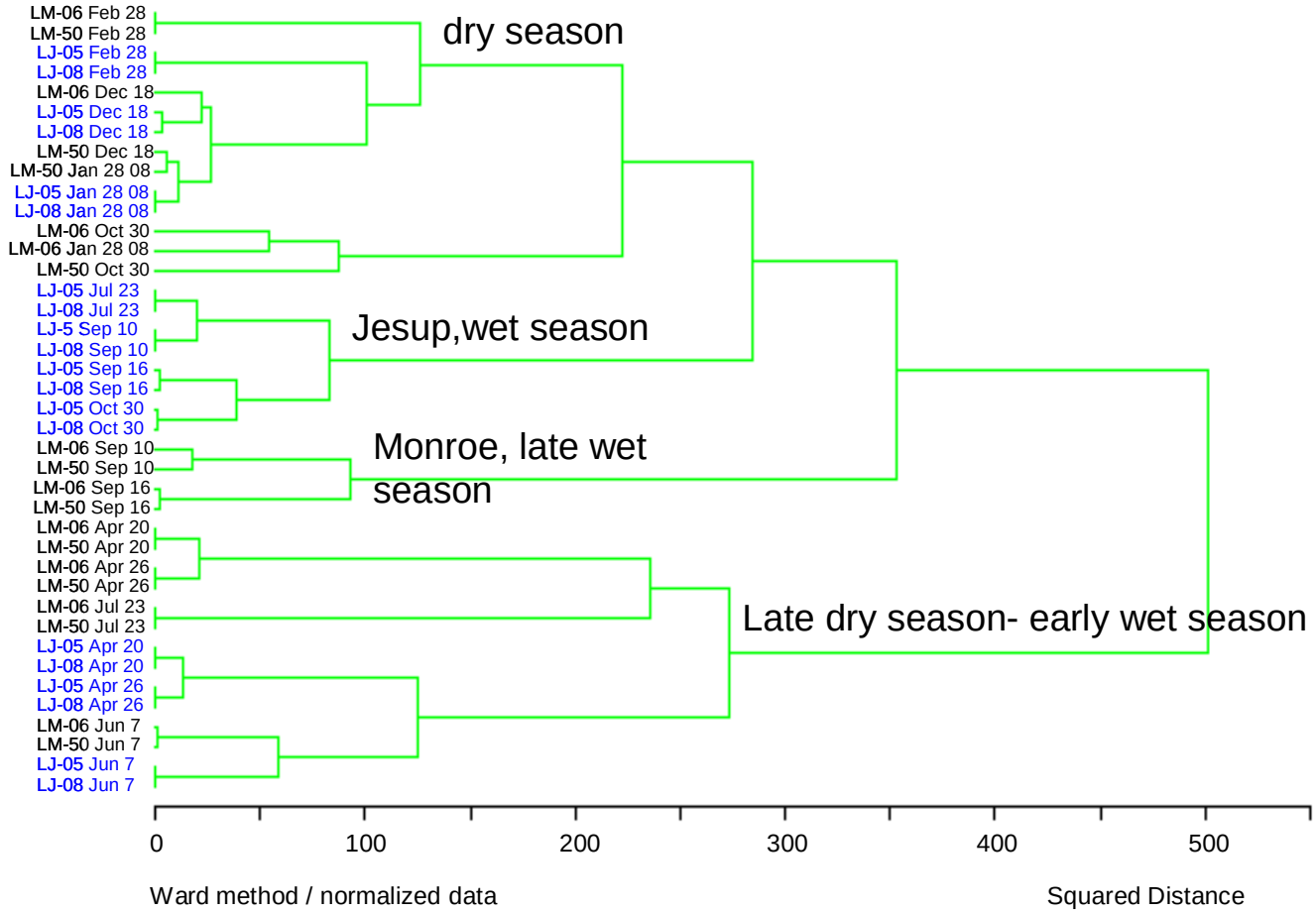

Figure 3.3.2 Cluster analysis of DGGE patterns for DNA derived products. Lake Monroe samples are shown in black text. Lake Jesup samples are shown in blue text.

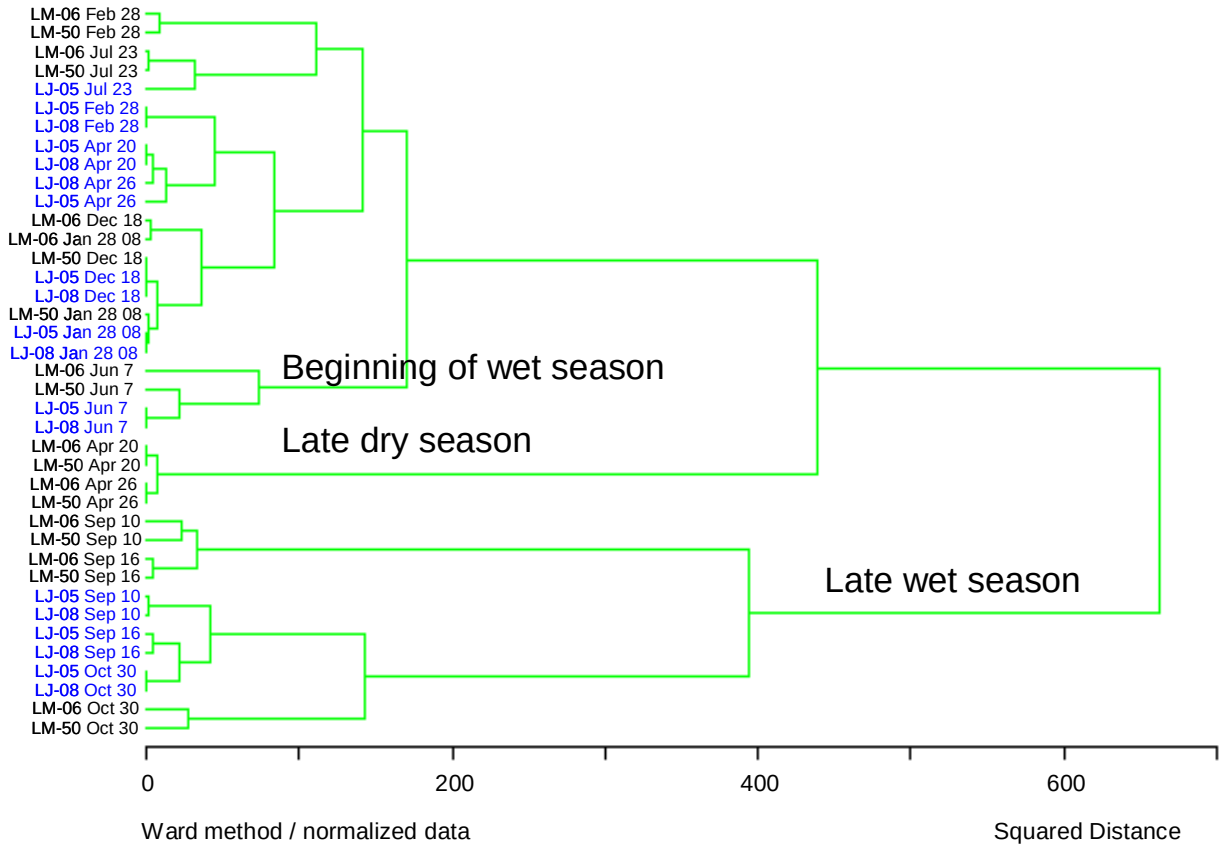

Figure 3.3.3 Cluster analysis of DGGE patterns for RNA derived products. Lake Monroe samples are shown in black text. Lake Jesup samples are shown in blue text.

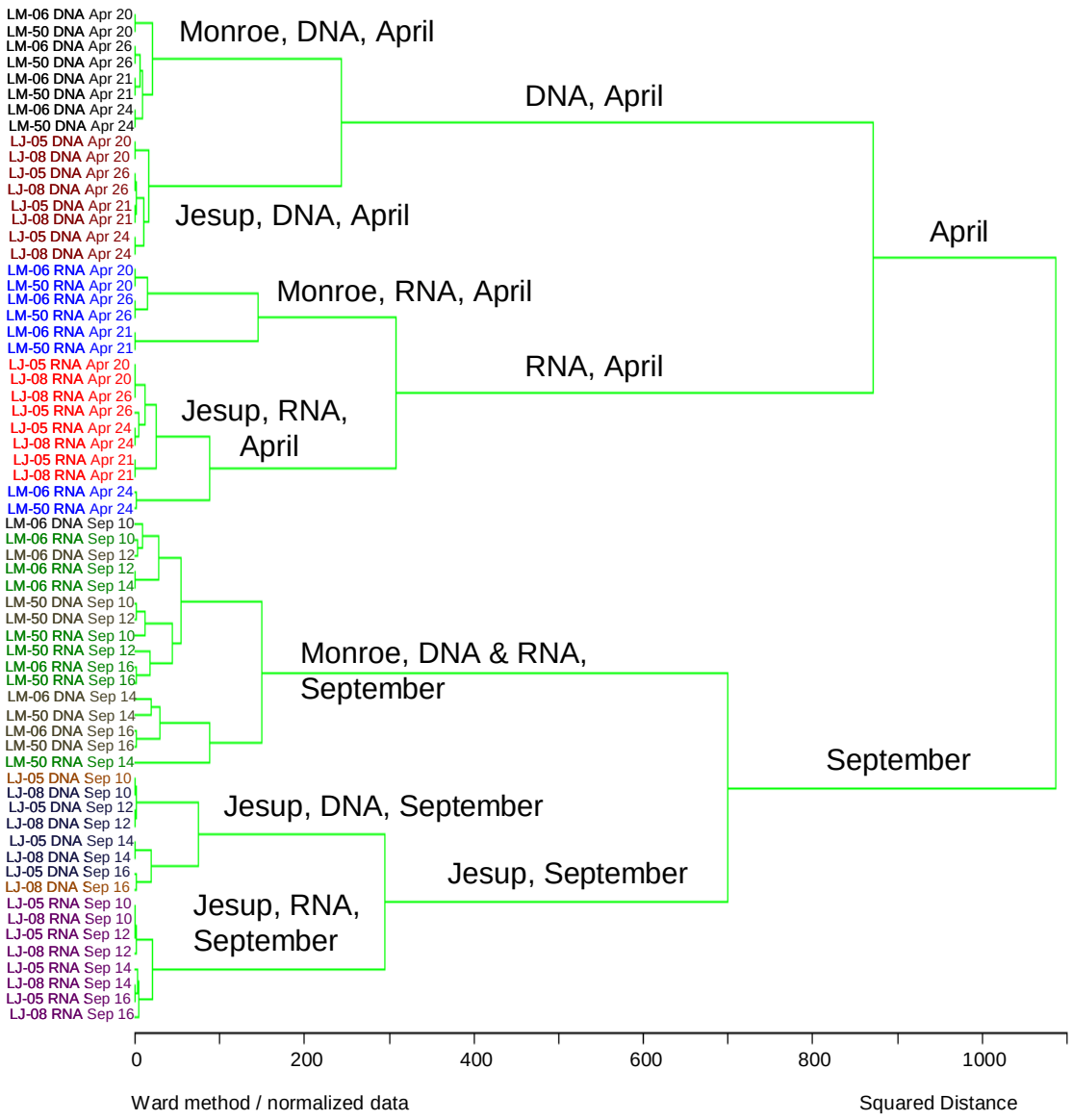

Figure 3.3.4. Cluster analysis of DGGE patterns for RNA and DNA derived products of April and September weeklong samplings. April samples were collected 4/20, 4/21, 4/24, and 4/26/2007. September samples were collected 9/10, 9/12, 9/14. 9/16/2007. Text colors are as follows: black for Lake Monroe April DNA, brown Lake Jesup April DNA, blue for Lake Monroe April RNA, red for Lake Jesup April RNA, bronze for Lake Monroe September DNA, dark blue for Lake Jesup September DNA, green for Lake Monroe September RNA, purple for Lake Jesup September RNA.

Table 3.3.1.1. Closest relatives of characteristic DGGE bands that were sequenced in duplicate from two track lanes

DGGE Band	Closest Relatives						
	Cyanobacteria and Chloroplast names	Phylogenetic Affiliations	Accession Number	%Sim-ilarity	Seq bp	Alignment	Sources
2-1	uncultured *Cylindrospermopsis* sp. clone 11	Cylindrospermopsis	EU099011	98	342	319 / 324	Nile river water
	Cylindrospermopsis raciborskii LMECYA 135	Cylindrospermopsis	EU078548	98	342	319 / 324	Portuguese freshwater
2-2	uncultured *Cylindrospermopsis* sp. clone 11	Cylindrospermopsis	EU099011	95	293	278 / 292	Nile river water
	Cylindrospermopsis raciborskii LMECYA 135	Cylindrospermopsis	EU078548	95	293	278 / 292	Portuguese freshwater
1-1	uncultured *Cylindrospermopsis* sp. clone 11	Cylindrospermopsis	EU099011	97	331	325 / 332	Nile river water
	Cylindrospermopsis raciborskii LMECYA 135	Cylindrospermopsis	EU078548	97	331	325 / 332	Portuguese freshwater
1-2	*Cylindrospermopsis raciborskii* DMKU51015	Cylindrospermopsis	AB115480	96	293	280 / 290	Reservoir
	uncultured *Cylindrospermopsis* sp. clone 11	Cylindrospermopsis	EU099011	96	293	279 / 290	Nile river water
14-1	*Thalassiosira rotula* chloroplast clone V1_138	Chloroplast	AY907300	99	340	337 / 340	Phycosphere of microalgae
	Thalassiosira eccentrica chloroplast P108	Chloroplast	AJ536458	99	340	330 / 333	N.D.
14-2	*Thalassiosira eccentrica* chloroplast P108	Chloroplast	AJ536458	97	340	270 / 277	N.D.
	Thalassiosira rotula chloroplast clone V1_138	Chloroplast	AY907300	96	291	275 / 284	Phycosphere of microalgae
15	*Thalassiosira rotula* chloroplast clone V1_138	Chloroplast	AY907300	98	335	332 / 336	Phycosphere of microalgae

Table 3.3.1.1. Continued.

DGGE Band	Closest Relatives						
	Cyanobacteria and Chloroplast names	Phylogenetic affiliations	Accession Number	%Sim-ilarity	Seq bp	Alignment	Sources
	Thalassiosira eccentrica chloroplast P108	Chloroplast	AJ536458	99	335	326 / 329	N.D.
7-1	*Spirulina laxissima* SAG 256	Oscillatoriales	DQ393278	93	286	268 / 286	N.D.
	Pseudanabaenaceae cyanobacterium DPG1-KK5	Oscillatoriales	EF654067	93	286	268 / 286	N.D.
	Phormidium sp. LMECYA 173	Oscillatoriales	EU078510	93	286	268 / 286	Portuguese freshwater
	Leptolyngbya sp. AECC1321	Oscillatoriales	EU729064	93	286	267 / 286	Lake microbial mat
7-2	*Leptolyngby*a sp. PCC 7104	Oscillatoriales	AY768404	95	337	321 / 337	N.D.
	Plectonema sp. HPC-49	Oscillatoriales	AY430152	95	337	321 / 337	Stromatolite
	Phormidium sp. LMECYA 173	Oscillatoriales	EU078510	94	337	320 / 337	Portuguese freshwater
	Oscillatoria sp. CCMEE 416	Oscillatoriales	AM398781	94	337	320 / 337	N.D.
10-1	*Leptolyngbya frigida* ANT.LH52B.3	Leptolyngbya	AY493612	91	338	307 / 337	N.D.
	Leptolyngbya frigida ANT.REIDJ.1	Leptolyngbya	AY493611	90	338	304 / 336	N.D.
10-2	*Leptolyngbya frigida* ANT.LH52B.3	Leptolyngbya	AY493612	91	340	308 / 337	N.D.
	Leptolyngbya frigida ANT.REIDJ.1	Leptolyngbya	AY493611	90	340	305 / 336	N.D.

3.3.2. *nifH* gene presence and expression.

The expression of *nifH* showed significant intersample variability both within and between lakes (Figs. 3.3.2.1 and 3.3.2.2, note that the y-axes are log scale). There was an initial increase in both cyanophyceae RNA expression and DNA concentration during the beginning of our study in both lakes (February to April 2007). There was a dramatic shift by the June sampling dates, when Lake Monroe showed a large decrease in *nifH* expression while Lake Jesup continued to show elevated levels (Fig. 3.3.2.1). By the July event the reduction in the RNA expression and DNA quantity was apparent for Lake Monroe. The levels of both RNA and DNA remained low and fairly constant in Lake Monroe for the duration of the study. Conversely, Lake Jesup showed increasing RNA activity and DNA concentrations through the late summer (September 2007) followed by a slow decrease into the winter month with the most dramatic decrease occurring between the December, 2007 and the January, 2008 events (Fig. 3.3.2.1 and 3.3.2.2).

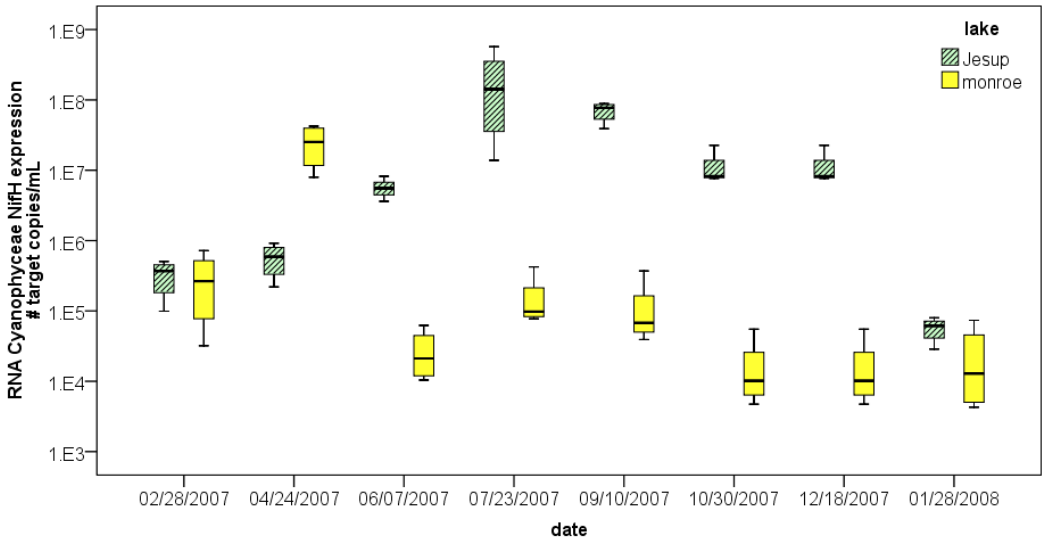

Figure. 3.3.2.1. RNA cyanobacterial *nif*H expression. The *nif*H expression is related to the presence of the gene that allows the bacteria to fix N_2.

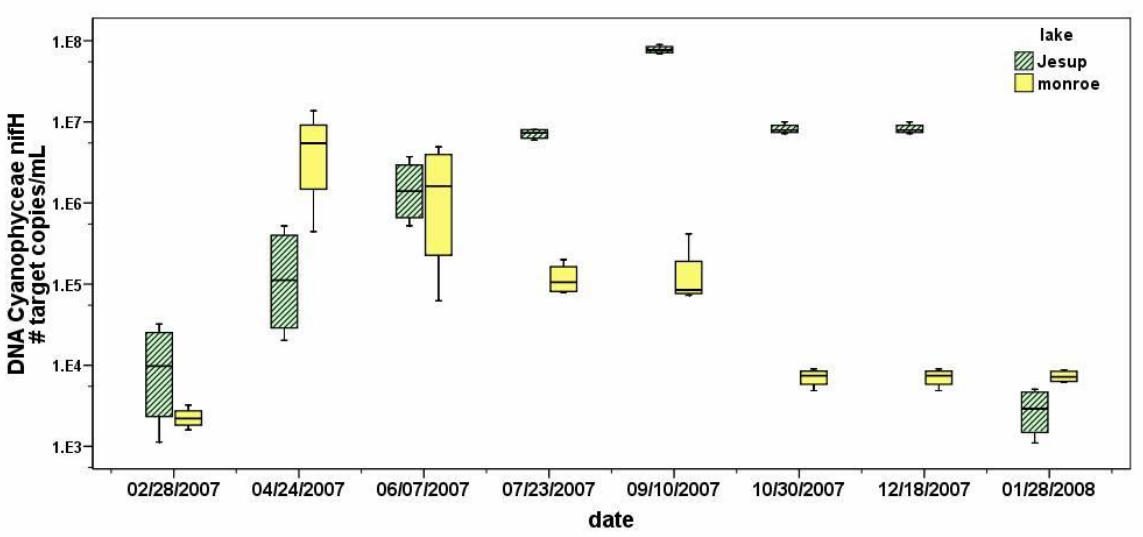

Figure. 3.3.2.2. DNA cyanobacterial *nif*H presence. The *nif*H presence is related to amount of the gene that allows the bacteria to fix N_2.

3.4 Correlations and Relationships.

3.4.1. Multivariate analysis of the data collected in Lakes Jesup and Monroe.

One principal component analysis was conducted with the non-transformed data collected for each lake and for the seasonal events only. The correlation matrix was used to develop principal components analyses (PCAs). Our data collected during the weekly events did not change significantly over the course of a week.

The genomic data as well as the yield and the PI curve parameters were not included in the PCAs. Average values for the water column parameters (temperature, DO, conductivity) were used to compute the PCAs. The euphotic zone depth (Z_{eu}) was used for the assessment of the light penetration depth in the water column. The total chlorophyll concentration for combined algal group and, separately, for each algal group was used in lieu of the chlorophyll *a* concentration. All the other variables used can be considered as averages of the water column since the measurements were made from a composite water sample (encompassing the whole water column).

TABLE 3.4.1.1: variables and PCA codes used to compute the PCAs for Lakes Jesup and Monroe.

Variables	PCA code
Bacillaryophyceae total chlorophyll	CBr
Chlorophyceae total chlorophyll	CGr
Conductivity	Cond
Cyanophytes total chlorophyll	CBl
Dissolved oxygen	DO
Euphotic zone depth	Zeu
Gross Primary Production	GPP
Nitrogen fixation	N2fix
pH	pH
Respiration	Resp.
SRP	SRP
Temperature	Temp
TN	TN
Total chlorophyll (all algae)	Ctot

In the PCA analysis for Lake Jesup redundant variables were removed which resulted in these variables retained for the final PCA: N2Fix, TN, TP, SRP, Temp, pH, Ctot and Zeu. The temperature and the DO were negatively correlated ($P < 0.05$, Fig. 3.4.1.1) as expected, while the converse was found for the temperature, the conductivity and the GPP ($P < 0.05$, Fig. 3.4.1.2). The DO and GPP were negatively correlated ($P < 0.05$, Fig. 3.4.1.1) and GPP was positively correlated with the respiration, the total chlorophyll

concentration and TOC ($P < 0.05$, Fig. 3.4.1.1). The total chlorophyll concentration of Bacillaryophyceae and Chlorophyceae were insignificant for Lake Jesup to be taken into account in the PCA. The total chlorophyll concentration of Cyanophyceae was nearly identical to the total chlorophyll concentration of all the algal populations (Fig. 3.2.23, paragraphs on algal populations). The first three components of the PCA encompassed 74.5% of the overall data variability (Fig. 3.4.1.2). The events were fairly well grouped together with the exception of event 2, which is fairly spread out. Event 1 was very different from events 3 through 8, which are pooled. N_2-fixation was positioned close to temperature on the two planes of the PCAs and the two variables were positively correlated ($P < 0.05$, $r^2 = 0.24$, Fig. 3.4.1.3). TN and TP were also pooled in both planes of the PCA, and were positively correlated ($P < 0.05$, $r^2 = 0.12$, Fig. 3.4.1.2). The euphotic zone was consistently on the opposite side of the total chlorophyll concentration, emphasizing a negative correlation ($P < 0.05$, $r^2 = 0.41$, Fig. 3.4.1.4).

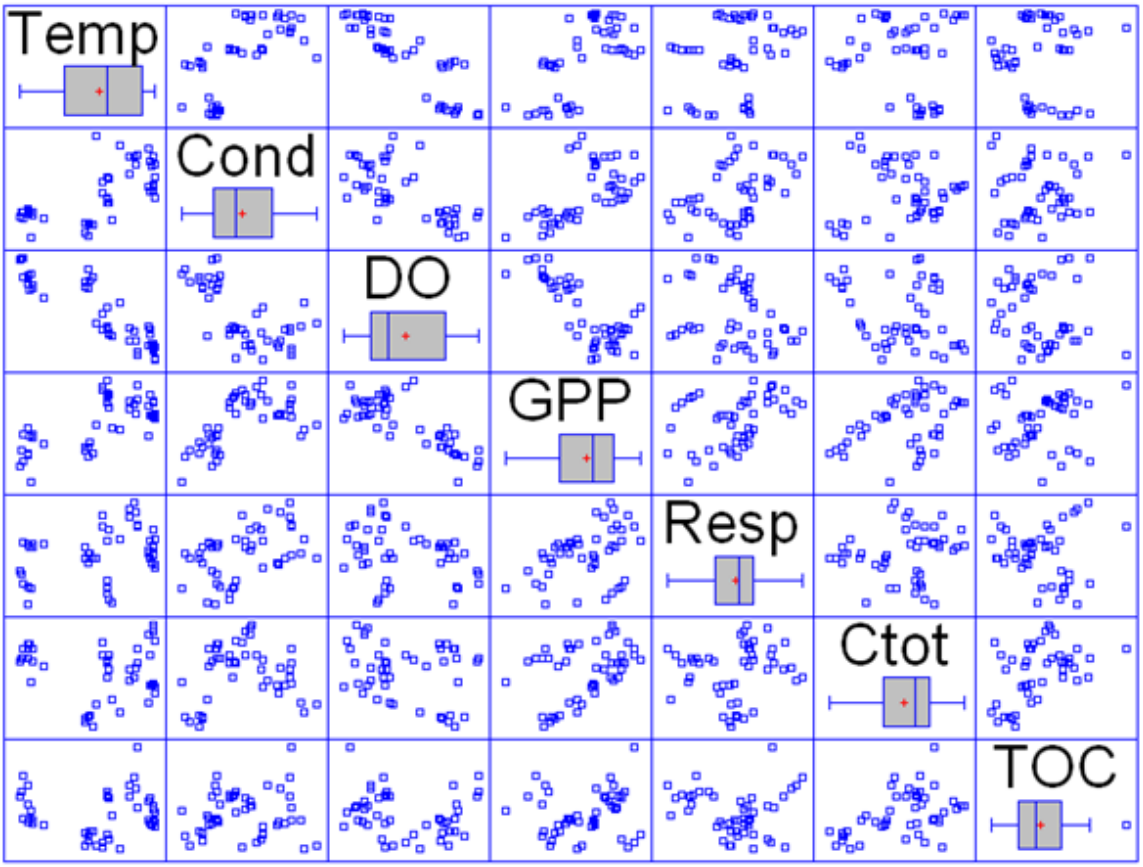

Fig. 3.4.1.1: Correlation matrix showing the redundant variables. Refer to the text above for more details.

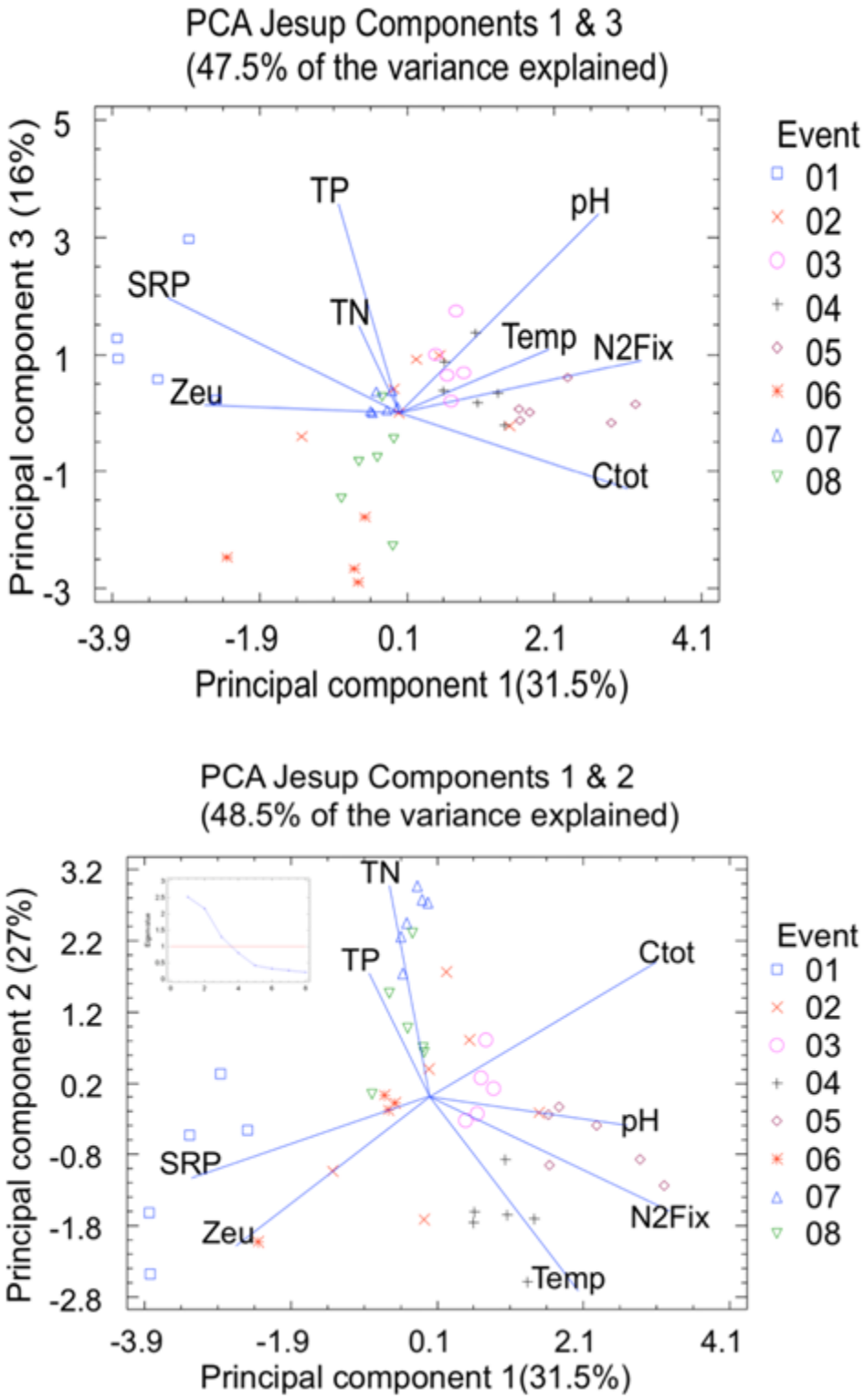

Figure 3.4.1.2 Principal components analysis (PCA) for Lake Jesup showing the main non-redundant variables and the two principal planes as defined with the PC 1 & 2 (48.5%) and PC 1 & 3 (47.5%).

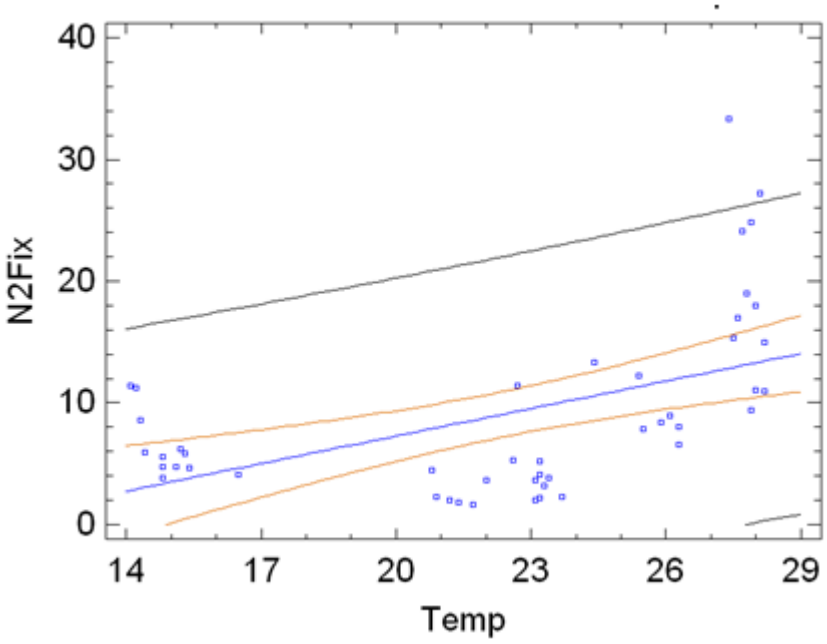

Figure 3.4.1.3 Positive correlation between the temperature (Temp) and the N_2-fixation (N2Fix) in Lake Jesup.

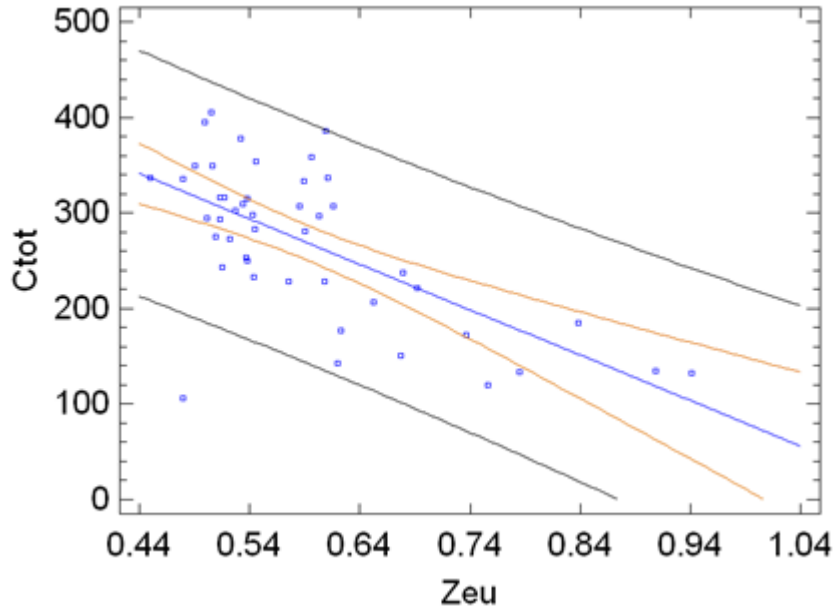

Figure 3.4.1.4. Negative correlation between the depth of the euphotic zone and the total chlorophyll concentration.

In the PCA analysis for Lake Monroe redundant variables were removed which resulted in the following variables retained for the PCA: N_2Fix, pH, DO, TN, TOC, SRP, Temp and TP. TP was then not retained for the final PCA because this variable was too close to the center of inertia (not shown). The pH was positively correlated with the total chlorophyll concentration of both the Bacillaryophyceae and the Cyanophyceae or all the algal groups combined. The pH was also positively correlated with the GPP and the respiration ($P < 0.05$). The two first principal components were retained for the PCA analysis as they accounted for 79% of the total variation. The first component encompassing 51% of the total variation was driven by the N_2-fixation and TOC (Fig. 3.4.1.5). The pH as well as the redundant variables (total chlorophyll concentration of Cyanophyceae and Bacillaryophyceae, GPP, Respiration) were all correlated with the N_2-fixation ($P < 0.05$, $r^2 = 0.66$). N_2-fixation was negatively correlated with TN and TOC. The temperature and the DO were also negatively correlated ($P < 0.05$, $r^2 = 0.51$).

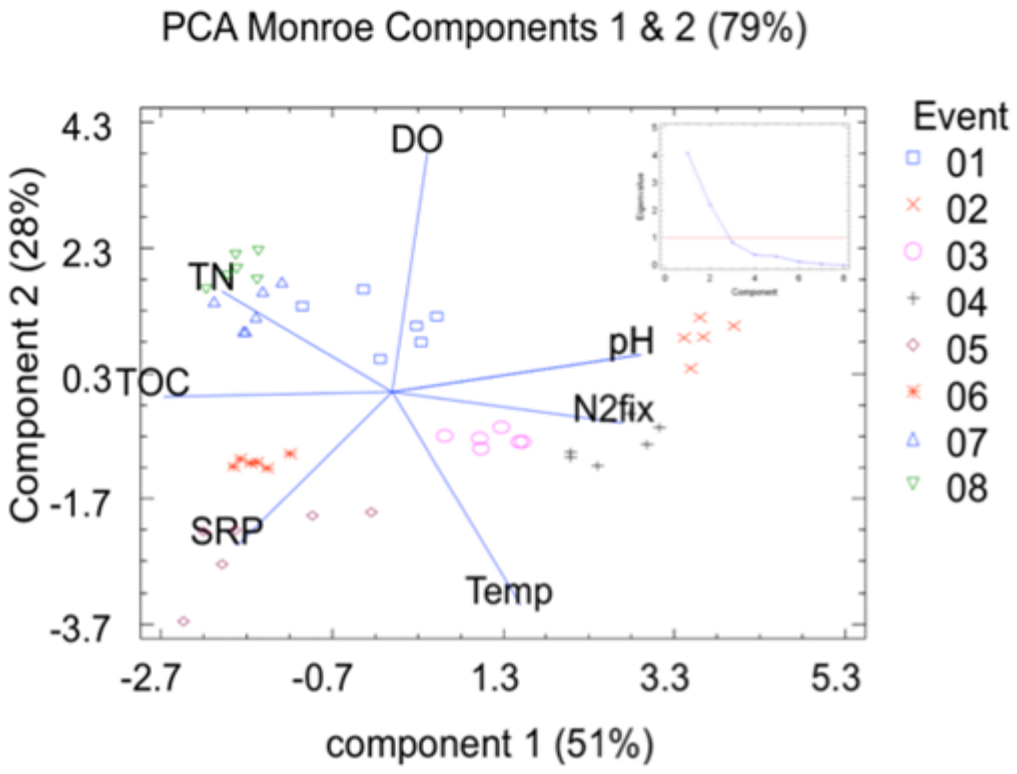

Figure 3.4.1.5. Principal components analysis (PCA) for Lake Monroe showing the main non-redundant variables and the plane defined by PC 1 & 2 (79%).

3.4.2. Correlations between *nifH* gene presence and expression and selected physicochemical parameters.

Correlation analysis (Spearmans non-parametric rho) was performed between measured values of DNA presence and RNA expression in *Cylindrospermopsis* sp. (Cyl) and general cyanobacterial (Cyano) species and selected water physicochemical properties (Table 3.4.2.1 and 3.4.2.2). Physicochemical properties included N_2-fixation rates, total N (TN) and P (TP), the molar ratios of TN to TP (TN:TP) and dissolved inorganic N (DIN) to soluble reactive P (SRP) (DIN:SRP), water temperature (T), electrical conductivity (EC), and pH. The DIN was the sum of NH_4 and NO_2 + NO_3. All measures of DNA or RNA were highly correlated with each other for both lakes. All DNA and RNA metrics were correlated to N_2-fixation except the DNA of the general cyanophytes in Lake Jesup and *Cylindrospermopsis* spp. RNA in Lake Monroe. Highly significant relationships between the Lake Monroe *Cylindrospermopsis* spp. and cyanobacterial DNA and N_2-fixation suggested that fixation was greatest when theses groups were present in large numbers even if their *nifH* expression was not prominent (lack of significance in *Cylindrospermopsis* spp.RNA and significant but weaker correlations with cyanobacterial RNA). The RNA expression in Lake Jesup was significantly correlated to N_2-fixation for both the *Cylindrospermopsis* spp. and general cyanobacteria although the correlations for that of *Cylindrospermopsis* spp. was higher, which agrees with other measures showing general cyanobacterial (section 3.2.2) and *Cylindrospermopsis* spp. (section 3.3.1) dominance. Total N was significantly inversely correlated to all DNA and RNA measures in Lake Jesup and to the general cyanobacterial DNA and RNA in Lake Monroe (but to a lesser extent). In Lake Monroe TN was not correlated to *Cylindrospermopsis* spp. DNA or RNA. This suggests that when TN is high the presence and expression of genes for N fixation are reduced in both lakes but that this effect is greater in Lake Jesup. However, gene presence and expression is not the only factor controlling N_2-fixation (see section 3.4.1 above). The TN:TP was not significantly related to genetic characteristics (DNA presence, RNA expression) in Lake Jesup but it was significantly inversely correlated for Lake Monroe suggesting there might be more of a nutrient control over phytoplanktonic populations and activity in Lake Monroe. Temperature was more significantly correlated to genetic characteristics in Lake Jesup than in Lake Monroe where there were contradictory correlations between *Cylindrospermopsis* sp. (Cyl) and general cyanobacterial (Cyano) species DNA and RNA parameters.

TABLE 3.4.2.1. Correlation analysis between *Cylindrospermopsis* sp. DNA or RNA (Cyl) or general cyanobacterial DNA and RNA (Cyano) and selected physicochemical properties of lake water samples in Lake Jesup. Correlations were conducted using Spearman's r in SPSS v. 14.0.

	Cyl DNA	*P*	Cyano DNA	*P*	Cyl RNA	*P*	Cyano RNA	*P*
Lake Jesup								
N_2 Fix	0.381	0.035	0.296	ns	0.493	0.008	0.408	0.023
TN	-0.549	0.001	-0.426	0.015	-0.554	0.002	-0.423	0.016
TP	-0.370	0.037	-0.344	ns	-0.493	0.007	-0.281	ns
TN:TP	-0.040	ns	0.034	ns	-0.047	ns	0.008	ns
DIN:SRP	0.414	0.026	0.567	0.001	0.311	ns	0.430	0.020
SRP	-0.289	0.108	-0.227	ns	-0.039	ns	-0.168	ns
T	0.745	<0.001	0.773	<0.001	0.900	<0.001	0.819	<0.001
EC	0.551	0.001	0.469	0.007	0.566	0.001	0.462	0.008
pH	0.194	ns	0.210	ns	0.267	ns	0.315	ns
DIN	-0.216	ns	-0.011	ns	0.013	ns	0.011	ns
Cyano DNA	0.892	<0.001						
Cyl RNA	0.908	<0.001						
Cyano RNA	0.870	<0.001						

TABLE 3.4.2.2. Correlation analysis between *Cylindrospermopsis* sp. DNA or RNA (Cyl) or general cyanobacterial DNA and RNA (Cyano) and selected physicochemical properties of lake water samples in Lake Monroe. Correlations were conducted using Spearman's r in SPSS v. 14.0.

	Cyl DNA	*P*	Cyano DNA	*P*	Cyl RNA	*P*	Cyano RNA	*P*
Lake Monroe								
N_2 Fix	0.781	<0.001	0.689	<0.001	0.231	ns	0.438	0.012
TN	-0.133	ns	-0.379	0.032	0.037	ns	-0.378	0.033
TP	0.602	0.004	0.310	ns	0.400	ns	0.105	ns
TN:TPm	-0.571	0.007	-0.754	<0.001	-0.488	0.047	-0.455	0.009
DIN:SRPm	-0.044	ns	-0.248	ns	-0.069	ns	-0.335	ns
SRP	-0.553	0.009	-0.190	ns	-0.278	ns	-0.164	ns
T	0.285	ns	0.591	<0.001	-0.505	0.039	0.384	0.030
EC	0.509	0.019	0.591	<0.001	-0.005	ns	0.519	0.002
pH	0.721	<0.001	0.471	0.006	0.635	0.006	0.510	0.003
DIN	-0.623	0.003	-0.410	0.020	-0.397	ns	-0.486	0.005
Cyano DNA	0.904	<0.001						
Cyl RNA	0.783	<0.001						
Cyano RNA	0.656	0.001						

4.0 DISCUSSION.

Lakes Jesup and Monroe are nutrient rich systems with Lake Jesup being continuously hypereutrophic and Lake Monroe being eutrophic to hypereutrophic during this study (2007). A basic goal of this project was to measure the rate of nitrogen fixation in both of these lakes, and these data form the foundation of this work. Therefore the rate of N_2-fixaton was obtained by comparing the C_2H_2 reduction assay and the $^{15}N_2$ isotopic approach. The calculated C_2H_2/N_2 ratio of 6.37:1 (Fig. 3.1.1.2) was based on the calibration of C_2H_2 production compared to N_2 fixed as measured by the isotopic enriched labeled $^{15}N_2$ method of Montoya et al. (1996). In theory, the ratio of moles of C_2H_2 produce to actual N_2-fixation is 3:1, which is based on the assumption that C_2H_2 production from acetylene by nitrogenase is comparable to the fixation of ammonia from N_2 (Howarth et al., 1988). The 3:1 ratio is often used when ^{15}N calibration approach is not, but published ratios show that N_2-fixation by plankton often occurs at higher ratios (Graham et al. 1980). When planktonic cyanobacteria are present in the freshwater systems, ratios >3:1 can be observed due to the blocking of C_2H_2 formation caused by nitrogenase-mediated hydrogen formations (Paerl 1982). Additionally, many species of bacteria can produce C_2H_2 without the presence of acetylene (Primrose 1979). Therefore, it is important to calibrate the acetylene reduction method with the ^{15}N method. The acetylene reduction method is more accurate than the isotopic method, but when background levels of C_2H_2 are relatively high, the ^{15}N approach is not affected by this situation. However, the best results for comparing both methods occur when similar incubation periods are used between the two different approaches (Howarth et al, 1988), and for this study, both sets of incubations were timed for the same length and occurred in the same location in the lake.

N_2-fixation varied throughout the annual cycle and was generally greater in Lake Jesup compared to Lake Monroe, except for the events of April and July 2007 (Fig. 3.1.2.1). Whereas Lake Jesup showed some level of N_2-fixation at all sampling events, Lake Monroe showed that there were periods of high fixation alternating with periods of low or non-detectable fixation. The range of variability in Lake Monroe has also been observed in other basins with longer periods of observation, e.g. greater than 1 year (Tõnno and Nõges, 2003). Thus the range of fixation was greater for Lake Monroe than Lake Jesup (Fig. 3.1.2.1). The average estimated mass contribution of TN during this 334 d study was 128.6 Mg TN for Lake Jesup and 187.7 Mg TN for Lake Monroe (Table 3.1.5.1). Although N-fixation rates calculated as mass per volume per time (e.g. mg L^{-1} d^{-1}) were generally higher in Lake Jesup than Lake Monroe, the larger volume of Lake Monroe compensates for the differences in rates and therefore leads to greater TN fixation. Dividing these amounts of TN by the number of days in this study (334 d) and the mean volume of each lake produces rates of N-fixation equal to 0.0088 mg TN L^{-1} d^{-1} in Lake Jesup and 0.0077 mg TN L^{-1} d^{-1} in Lake Monroe. The mean TN concentration in Lake Jesup during this study was 1.38 mg L^{-1} and for Lake Monroe 1.02 mg L^{-1} (Table 3.2.1.1). Dividing these concentrations by these rates showed that 0.64% of the TN existing in Lake Jesup and 0.76% of the TN present in Lake Monroe are contributed in a day. If these lakes were closed systems N-fixation at these rates could have created the mean

water TN concentrations in 156 d in Lake Jesup and 132 d in Lake Monroe. In comparison, it has been estimated that in Lake Okeechobee, N-fixation can account for 30% of the N inputs within that system (Havens et al., 2001). However, our estimates are not related to the actual flux of N into the basins. The approach of this work was to measure the rate of N-fixation in both lakes, and not the total flux of all N (DIN, TON, and DON). This point is important as the calculations of the net N-flux into these basins would require monitoring of the all inlets and outlet, and a comprehensive sediment trap network to collect all of the necessary parameters/data (which is not trivial for shallow eutrophic lakes). While this project collected all the nutrient data from these study sites, a different approach must be used to calculate the fluxes. The contribution of N-fixation to these basins is real, and should be considered when implementing water quality measures. These data provide one of the first comprehensive base-line studies of N-fixation in these basins.

Both lakes were clearly dominated by phytoplankton vs. rooted macrophytes (Anderson et al., 2004), and specifically *Cyanophyacea* were an important group in both systems. Cyanophyceae are phostosynthetic bacteria that are most successful in "extreme" environments. Since subtropical Lakes Jesup and Monroe were generally hypereutrophic on the Carlson index and classified impaired water bodies by FDEP, it is thus not surprising that a strong *Cyanophyceae* dominance was found. Additionally, other possible reasons why *Cyanophyceae* were more successful than the other algal groups was likely linked to factors that generally limit algal growth, namely pH and nutrients. Irradiance in shallow lakes should not be a limiting factor because of the relatively shallow water depths and well mixed water column. However, temperature is driving the primary production and the subsequent N_2 fixation, and because water temperature is a function of solar heating, irradiance is indirectly controlling the primary production and N_2-fixation. The increase in water temperature is also enhanced by the darkening color of the lake water column due to algal production. Temperature also limits oxygen solubility as suggested by the PCAs, which translates into a lower pH than if the water remained cold. This can possibly mitigate the negative impact of high pH on algal GPP. The pH is a strong driving factor in both lakes as it is often correlated (or indirectly correlated for Lake Jesup, since no direct correlation was found) between algal metabolism and N_2-fixation.

Many studies have documented that physiochemical parameters will affect plankton communities and N-fixation (Vinner, 1985; Tõnno and Nõges, 2003). The pH in both Lake Jesup and Lake Monroe was often above 8.3, which translates in a low availability in carbon dioxide concentration (e.g. King, 1970). The ability of Cyanophyceae to utilize bicarbonate (HCO_3^-) by using the enzyme carbonate anhydrase (CA) that catalyzes the dehydration of bicarbonates, makes them more competitive than the other algae in such a low CO_2 environment (e.g. Shapiro, 1990). However, this characteristic is not totally restricted to Cyanophyceae, since other specialized eukaryotic algae can also utilize HCO_3^- as carbon source (e.g. Talling 1976). Furthermore, the low availability of CO_2 can also increase the presence of gas-vacuolate Cyanophycea, which float on the water surface for better access to atmospheric CO_2. It is not known whether the Cynaophyceae

were actively expressing the gene coding for the CA Additionally, the correlation between lower pH and diatom dominance in Lake Monroe is very striking (Fig. 3.2.10).

Thus, it appears that temperature (and indirectly the amount of solar radiation, which translates to irradiance) and pH have a positive feedback on the overall Cyanophyceae population encompassing the N_2-fixing species. The dominance of N_2-fixing *Cylindrospermopsis* spp. in Lake Jesup confirms this assertion, while in Lake Monroe it has yet to be demonstrated since algal counts were not available. *Cylindrospermopsis raciborskii*, favors warm waters with optimal growth around 30º C and is adapted to low light environments as found in Lake Jesup (eg. Shafik et al. 2001, Chapman and Schelske 1997). *C. raciborskii* has a competitive advantage because it also has a high affinity for, and can store excess P, but, it normally loses this advantage when P, such as in these lakes, is found in large amounts. During our survey we did not find a negative correlation with *C. raciborskii* and TP as it was the case in Lake Jesup in the past (Dobberfuhl (2003). However, C. raciborskii does not necessarily depend on N_2- fixation as ammonium is its preferred N source (Briand et al. 2002) but may be prominent here as it prefers high pH environments (pH = 8.0 to 8.7; Padisák 1997) as are present in Lakes Jesup and Monroe. The two first components of the PCA do show lower N_2-fixation at the end of the study when TP and TN concentrations were high, but the rest of the time, this inverse relationship was not found. Furthermore, integrating TN:TP ratio into the PCA did not draw any noteworthy results (data not shown). TN:TP ratio seems to be triggering N_2-fixing Cyanophyceae blooms in lakes when the molar N:P ratio is lower than values ranging 16 (Howarth et al. 1988) to 67 (Smith, 1983). Lakes Jesup and Monroe TN:TP ratio were within this range during the entire study and should translate into continuous N_2-fixation. Since this was not the case and because of the high concentrations in both TP or TN, it is very likely that the TN:TP ratio does not influence N_2-fixation (Flynn 2002). It is not always possible to determine the amount of N-fixation based on hydrochemistry and phytoplankton species composition, because even if an N-fixing species is dominate, the rate of fixation will not always correlate directly (Tõnno and Nõges, 2003).

Determination of cyanobacterial dominance by both the PAM and molecular work (DNA/RNA) showed that seasonal variations in the major algal groups were observed in both lakes. Lake Jesup was largely dominated by Cyanophyceae throughout the year (PAM) and this community was likely composed largely of *Cylindrospermopsis* (RNA). Algal group dominance throughout this study was more variable and complex for Lake Monroe. Lake Monroe was dominated by Cyanophyceae during the late dry to early wet season and in December 2007. However, dominance varied with Bacillariophyceae and Cyanophyceae being co-dominant generally during the winter months. Additionally, DNA/RNA patterns showed a shift in the phytoplankton community of Lake Monroe between the July and September samplings which corresponded to an increase in the PAM-determined increase in the importance of Chlorophyceae (~20% of total population). However this shift was short-lived and not apparent by the following event. Cluster analysis revealed that the communities of Lake Jesup and Lake Monroe are relatively similar during the dry season and late dry to early wet season.

Correlations between DNA and RNA metrics and N_2-fixation showed that in Lake Monroe N_2-fixation was greatest when *Cylindrospermopsis* spp. and general cyanobacteria were present in large numbers. However, there is a discrepancy in that the RNA and DNA patterns suggest that *Cylindrospermopsis* RNA expression was active even though the *Cylindrospermopsis* population was a small part of the total Cyanophyceae community present in the lake. In Lake Jesup N_2-fixation was significantly correlated to both the *Cylindrospermopsis* spp. and general cyanobacteria RNA expression which agrees with other measures showing general Cyanophyceae (section 3.2.2) and *Cylindrospermopsis* spp. (section 3.3.1) dominance. All DNA and RNA measures in Lake Jesup and the general Cyanophyceae DNA and RNA in Lake Monroe were significantly inversely correlated to Total N. This relationship suggests that when TN is high the presence and expression of genes for N_2-fixation are reduced in both lakes. But, gene presence and expression is not the only factor controlling N_2-fixation (as PCA discussion above suggests). The TN:TP ratios were not significantly related to genetic characteristics in Lake Jesup but were significantly inversely correlated for Lake Monroe suggesting there may be more of a nutrient control over phytoplanktonic populations and activity in Lake Monroe.

Water characteristics, phytoplanktonic communities, and N_2-fixation varied with season in both lakes but this variation was less in Lake Jesup than in Lake Monroe. Lake Jesup water was relatively clear, in February 2007, and then gradually shifted to a more turbid state linked to a much higher Cyanophyceae concentration, which persisted until the end of the study. During this second phase there was an obvious change in N_2-fixation that was linked to the temperature and pH without corresponding changes in the N_2-fixing population. As shown by the PCAs (section 3.4.4), the highest concentrations of TN and TP occurred at the end of the study which corresponded to a reduction in N_2-fixation (for both lakes the N_2-fixation vector is on the opposite side of the origin for TN or TP with the event values clustered around the nutrient vector). Lake Monroe showed greater variation with peak N_2-fixation for events 2, 3, 4, which corresponded to higher pH and Temperature (or GPP, algal concentrations), and no noticeable N_2-fixation at the end of the study (January 2008) with lower temperature and pH. Variability between events in Lake Monroe may be due to its very short water residence time (10 d) compared to that of Lake Jesup (80 d). However, hydrodynamic evaluation of these water bodies was not part of this project; a longer study period will be needed to better evaluate seasonality, which would have to be longer than one year.

5.0 CONCLUSION

Both Lakes Monroe and Jesup have phytoplankton populations that fix atmospheric N_2 within the water column. This contribution of N is significant, but can be further evaluated with improved mixing and hydrodynamic models of both lakes, as understanding changes in residence times during wet and dry seasons will greatly improve estimates of N loading to the basins. When compared to other shallow eutrophic lakes, Lake Monroe and Lake Jesup display similar characteristics, where typically lower N:P ratios is correlated with increased N-fixation. Yet, it is important to realize that this

scenario does not always hold true. Even if the abundance of N-fixing cynaobacteria is high in a lake, it does not always mean the rate of N-fixation will follow suit. Basically, presence alone does not mean fixation will occur, or predict rate. This circumstance is observed in our data when a species is present, but the *nifH* is not expressed. Therefore it is always best to make direct measurements of fixation. Additionally, in order to better observe seasonal trends, a longer period of study will be required, with at least 2-3 years of observation. These data provide a baseline understanding of the N-fixation with these shallow subtropical lakes, but further work will enhance the understanding of N-flux in this region.

6.0. REFERENCES

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