**Table 1. Comparison of Sparkling Lake, Lake Mendota, and Trout Bog.** These three lakes were chosen for comparative metatranscriptomics because of their varying trophic statuses, extensive historical data, and previous microbial sampling. Data on surface area, maximum depth, dissolved organic carbon, and development on shoreline courtesy of NTL-LTER <lter.limnology.wisc.edu>. Temperature, dissolved oxygen, pH, and conductivity were measured using a HydroLab DS5x Sonde and are averaged over all sampling depths and timepoints for each lake. Chlorophyll and phaeophytin concentrations were measured from the integrated epilimnion samples using a methanol extraction protocol and averaged over all timepoints. Secchi depth was measured at the first timepoint for each lake. Bacterial production was quantified via C14-leucine incorporation and averaged over all timepoints. Total and dissolved nitrogen and phosphorus concentrations were measured via colorometric HPLC; concentrations are within the typical ranges of these lakes. Due to thunderstorms the night of July 8th, the final 1AM timepoint in Sparkling Lake was collected on July 9th instead.

\*Dissolved organic carbon was measured by the North Temperate Lakes - Long-Term Ecological Research project and is available at <lter.limnology.wisc.edu>. The measurement closest to the date of sampling is reported here; this was July 5, 2016 for Lake Mendota, July 19, 2016 for Trout Bog Lake, and July 21, 2016

**Figure 1. Abundance vs. expression by lake.** To determine which phyla were most abundant or most expressed during our time series, we analyzed metagenomic

and metatranscriptomic read counts. All read counts are reported in transcripts per liter. The expression of clustered, nonredundant genes was aggregated by phylum and

compared to the coverage of those phyla in metagenomes and colored by kingdom(A-C). Axis are reported in proportion of reads assigned to each phylum across the time

series. Genes that could not be classi\_ed into a phylum were not included in this analysis. Proteobacteria were split into classes due to the high diversity of this phylum. No

positive relationship was observed between expression and abundance. We repeated this analysis at a \_ner resolution by investigating freshwater clades (D-F). Clades are

color-coded by phylum to provide taxonomic context.

**Figure 2. Cyclic trends in photosynthesis-related genes.** Cyclic trends with a 12 hour phase were detected in the top 20,000 most expressed genes in each lake. Here, we present an example of these cyclic trends in genes related to photosynthesis. Read counts in transcripts per liter were z-score normalized for the purpose of visualization. Each gene trend is color-coded by its time of maximal expression in the first 24 hour period. Because of missing samples in Day 2 of Trout Bog, only Day 1 is displayed.

**Figure 3. Cyclic trends by functional category and lake.** For each lake, we grouped genes by functional category based on their annotations and plotted the time of their maximal expression in the two day time series. Only genes with significant variation (greater than 20% coefficient of

variance) are plotted. Genes are color-coded by whether they have a significant cyclic pattern or not. This analysis revealed many categories contained cyclic genes in all three lakes, including anoxygenic aerobic photosynthesis (AAP), general sugar transport, reactive oxygen species defense (ROS), and carbon fixation (RuBisCO). The time of maximal expression varied by lake and category, with 1:00 as a time of maximal expression in Sparkling Lake and 9:00 in Trout Bog.

**Figure 4. Taxonomic composition of functional categories by time and lake.** We next investigated the taxonomy of functional categories and how phylogenetic groups change expression over time. The x-axis indicates the number of genes from each category assigned to each phylum, summed across both days of the time series. Proteobacteria were split by class due to the high diversity of this phylum. RNA polymerase, used as an indicator of growth, was phylogenetically diverse in all lakes, although less well-classified in Sparkling Lake, likely due to the lack of reference genomes from this site. Cyanobacteria contributed to photosynthesis in all lakes, particularly in Lake Mendota. General sugar transport was encoded primarily by Cyanobacteria and Actinobacteria in Lake Mendota, Actinobacteria and Betaproteobacteria in Trout Bog, and Actinobacteria and Armatimonadetes in Sparkling Lake.