



**Figure S1. Assessing the variability of metatranscriptomic read counts.** One aim of this metatranscriptomic study was to provide information on the variability in gene expression in freshwater that could be used to guide further metatranscriptomic experiments. We calculated the coefficient of variance (CoV) for each gene both within replicates and across samples (A). CoV was lower within replicates than across replicates, indicating that variation across replicates is not technical. High levels of variability were observed in genes from all three lakes. In panel B, three example eigenvectors (representative trends) of genes are shown, including trends for 1) on one day, off the next, 2) up and down across the time series, and 3) observed in only one timepoint.