**Project Goal**

The objective of this project is to compare six different fluorometers and assess their precision and accuracy as compared to extracted chlorophyll and phycocyanin.

**Project Description**

Fluorometers are a key tool for measuring algal pigments in water and are often used to analyze water samples for the presence of cyanobacteria and assess for Harmful Algal Blooms (HABs). There are many fluorometer options available for this, yet little is known about how they compare.

In this project, six fluorometers will be compared: Turner Trilogy (in vivo module), bbe AlgaeTorch, Turner, FluoroSense, AmiScience FluoroQuick, Turner CyanoFluor, and bbe Phycoprobe. The gold standard for fluorometry based methods of estimating chlorophyll and phycocyanin is extraction and this will be run on a Turner Trilogy using the chla-na (chlorophyll) and orange (phycocyanin) modules. The bbe AlgaeTorch and the Turner FluoroSense are field instruments that measure in vivo chlorophyll and phycocyanin. The Amiscience FluoroQuik and the Turner CyanoFluor are comparable units that quickly and easily measure chlorophyll, phycocyanin and the ratio between them from a small amount of fresh, whole water. The bbe Phycoprobe can be used as a field instrument or in the lab in “workstation mode”. We will be using it in its workstation mode. It quantifies chlorophyll, multiple classes of algae and unbound phycocyanin.

*Sample collection:*

Samples will be collected from a few ponds known to have high levels of cyanobacteria. Three, two liter surface samples will be collected from the edge of each pond. If any surface scum is present, it will be cleared before samples are taken to remove the possibility of differences occurring due to scum being in the samples in differing amounts. At the same time, measurements will be taken with the field instruments, the AlgaeTorch and the FluoroSense. A sonde will be used to collect temperature, dissolved oxygen, conductivity, pH and salinity.

*Sample preparation and analysis:*

Within a day after collection, each of the field samples will be analyzed in triplicate on the FluoroQuik, the Phycoprobe, and the CyanoFluor; samples will also be filtered onto 0.7 µm pre-ashed glass fiber filters, frozen, and stored at -20°C for later extraction and determination on the Trilogy. A filtered sample will be used as a blank on the CyanoFluor to correct for dissolved organic materials interference. Gluteraldehyde will be added to 125mL aliquots of each sample and will be stored in amber glass bottles for creation of permanent mounts. Permanent mounts will be used for cell counts to verify the results of the fluorometers and to look at community dominance. A 10mL aliquot will also be stored in glass scintillation vials at -20°Cfor potential microcystin analysis.

Next, each sample will be frozen for at least 4 hours and then thawed to be analyzed in the same way on each instrument. From these results a comparison between running fresh and frozen samples on all the instruments can be made. In addition, the samples will go through two more freeze/thaw cycles before being run on the FluoroQuik once again. This data will be used to look for increased consistency in analysis and normalization of samples across and within waterbodies after three freeze/thaw cycles.

In order to gain a deeper understanding of the Phycoprobe, a few other variables will be compared. Gluteraldehyde will be added to some samples to determine whether they can be preserved before being run. A time series of fluorescence measurements will be taken to examine degradation due to light exposure. Lastly, any variability due to the sample being stirred while measurements are taken will be observed.