United State Geological Survey Nutrient Treatment Study

Algal Community Composition Protocol

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**Introduction**

A nutrient enrichment study was completed by using nutrient diffusing substrate to assess the effects of macro and micronutrients on epiphytic algal community. A sister study was also completed to assess the effects on the planktonic community. The study measured the effect of 10 difference nutrient treatments on chlorophyll-a content, ash free dry mass (AFDM), microcystin concentration, and community composition to holistically understand the effects of nutrients on the community.

**Methodology**

**Field methods**

Samples were taken by a USGS employees by scraping a glass chip on a nutrient diffusion agar cup with a toothbrush and rinsing the algal community into a 20 mL glass vial with DI water. Each vial was preserved with Lugol’s iodine. These samples were taken at each of the 10 locations of the project (within Lake Michigan and Lake Erie), from each agar cup (10 at each site; one per treatment) placed for community analysis. Other agar cups were placed for chlorophyll-a, ash free dry mass and microcystin concentration analysis. This cups were submerged with a light monitor in coastal sites for 2 weeks, then collected and processed.

**Epiphytic Algal Community Assessment**

To assess the community composition of each sample, each sample is homogenized by inverting the sample vigorously for 10 seconds. 100 microliters of subsample aliquot is immediately pipetted into a clean plankton-settling chamber with 1 mL of DI water. A glass cover is placed over the chamber to ensure no contamination from the atmosphere. An Olympus IX81 inverted scope with 10x ocular lenses and 60x oil immersion objective lens (600x total magnification) is used to view the microscopic community. Using a SensiCam camera attached to the microscope, a transect of the settling chamber is viewed scanning from left to right on the diameter of the settling chamber. 400 natural units (individual cells, cyanobacteria colonies and filaments count as 1 natural unit, while eukaryotic filaments and colonies are counted as individual units) are counted from the view of the SensiCam on the computer screen.

Everything in the field of view of the SensiCam determined not to be detritus is counted and identified to the lowest possible taxonomic level (this may only be to ‘bi-raphid diatom, maybe Navicula sp., or to genus, depending on the condition of each sample). This data is immediately inputted into a spreadsheet, containing the sample identification information as the title, organisms identified with images, natural community counts, algal division and any interesting characteristics or notes for each identified taxa. Notes on the density of the cells in the sample, the time taken to process the sample, and the approximated portion of the setting chamber scrolled over are recorded. A folder of images of each organism identified and any questionable organisms will also accompany each sample. Natural units were counted by using a counter while scrolling for the common taxa, and using tally marks for the rare taxa. Once the recorded knew they were at or above 400 units, they would input all counts into the google sheet. If taxa appeared similar but slightly different they were recorded as separate taxa. Some taxa were later determined to be identical and counts for those were summed. This procedure will be duplicated for all 100 samples.

**Mesocosm Planktonic Algal Community Assessment**

The mesocosm experiment was completed for 4 of the 10 sites with water shipped by by the USGS to KSU during summer 2018. Sites included Maumee Bay, North Lake Erie, Green Bay and Ford River. Water was poured into 300 mL clear plastic bottles, nutrients were added in replication of 4, and bottles were incubated in a lighted incubator at 25 degrees Celsius for 48 hours. Bottles were inverted every 8 hours and randomly rearranged in the incubator after each inversion. After 48 hours, samples were taken for AFDM, chlorophyll-a, microcystin concentration and community composition analysis. Water was mixed with a stirring rod on medium speed while 20mLs of sample was pipetted out into 20mL bottles and preserved with Lugol’s Iodine.

For mesocosm samples, the aliquot will be 1.000 mL of sample, therefore undiluted unlike the biofilm samples. This is because the biofilm samples have been found to be more concentrated and more detritus ridden than the mesocosm samples. The same procedure was followed from there on.

**Notes on Microscopy**

The ocular number 22 divided by the objective value 60 yields a field of view that is about 300nm. The SensiCam field of view is about half of the ocular field of view.

Researcher has found that an exposure of about 15ms with a lamp level of about 30.0% seems to yield the best image. SlideBook is the application on the computer used by the microscope and camera.

Recommended to read about Kohler illumination.

**Metadata for Maumee Community Assessment**

**Data collected by:** Jordyn Stoll (jstoll7@kent.edu)

**Data collection time period:** Samples processed1-5-2018 to 3-18-2018

**Data format:** Percentage of community for each taxa. Taxa represented in each column. Treatment, total natural units counted per sample, and sample ID in columns as well. Each row represents one sample. There were 36 bottles in the experiment with 9 treatments in replicate of 4. An initial sample was taken, making 37 samples total.

**Notes:** There is an individual Google sheet for each of the Maumee samples including the natural unit counts for each taxa. Percentage of the community for each taxa was calculated by dividing the count for a taxa by the grand total of natural units counted in the sample (at least 400).

It was found that some taxa counted separately were actually identical. Counts for these taxa were added together and replicate columns were removed. Zeros were filled in for taxa that were not present in a sample. Data was then ready to be analyzed in R.