Fluorometer Chlorophyll a Correction

Manatee County Natural Resources Department; Environmental Protection Division

Standard Operating Procedures

5/28/2024

1. **Purpose of project**

Eutrophication of bodies of water is a worldwide issue, and locally poses a threat to the many important aquatic ecosystems in and around Manatee County. The Manatee County Natural Resources department has two programs, Regional Ambient Monitoring Program (RAMP) and Surface Water Ambient Monitoring Program (SWAMP), that assess water quality in 80 sites off the coast of Tampa and Sarasota Bay, as well as in freshwater systems. The Natural Resources Department's Environmental Protection Division (EPD), among many other purposes, uses the RAMP and SWAMP to better understand the causes of eutrophication and what can be done to reduce it. To investigate eutrophication, chlorophyll a analysis must be carried out to determine the concentration of phytoplankton in the water. The current EPA approved method of chlorophyll a concentration determination requires samples from the field to be brought back to a laboratory, where analysis is time and labor intensive. With eutrophication causing many issues for the environment and the systems that require clean water in our society, the necessity for a more efficient method of chlorophyll a analysis is strong in order to investigate causes of and efforts to reduce eutrophication. Fluorometric field readings pose an opportunity to be that efficient method, as they can provide a chlorophyll a concentration measurement as fast as factors like salinity and temperature. Although field readings may result in data variation too strong to be verified and validated data, with proper analysis, a correction factor can be created to allow for field measurements that match with laboratory analyzed measurements. Once a method that eliminates the need for laboratory analysis is approved, staff in the EPD can focus on gathering more data that can prove both the effectiveness of certain efforts to reduce eutrophication, and the causes of it.

2. **Quality Assurance**

3. **Equipment and Supplies**
    I. General
        a. County vehicle
        b. GPS/Cell phone
        c. Stations map
        d. Field data sheet and pens
    II. In-situ data collection
        a. Eureka Manta2 multiprobe
        b. County laptop with Manta2 control software
        c. Manta2 underwater cable
        d. Bluetooth connection device
        e. Storage/Calibration cup
        f. Testing cup
    III. Water sample collection
        a. Water collection device (bucket, dipping rod sampler, VanDorn Bottle)

b. 500 mL amber Nalgene sample C bottles for chlorophyll (pre-labeled for each station)
c. Insulated cooler and ice
d. Vacuum pump with glass fiber filter
e. Acetone solution
f. Laboratory Fluorometer
g. Vortexer
h. Centrifuge
i. Vials

4. Timeline of Goals

I. Collect data set from field measurements and collection that match with the lab analyzed chlorophyll a concentration, investigate any trends, compare field to lab measurements.
II. Conduct field collection and measurement aimed at determining the correct depth for accurate measurements, analyze data and set depth requirements.
III. Determine range of algae levels that you are confident in field measurement accuracy.
IV. Conduct any remaining investigations of confounding factors if found in previous data trends.
V. Employ depth requirements and range of confidence to create data set from field measurements and collection that match with the lab analyzed chlorophyll a concentration.
VI. Investigate the relationship between field and lab measurements, determine correction formula.
VII. Implement new field data collection method to increase data collection.

5. Correction Procedure

Follow the steps and instructions below to correct fluorometer accuracy and develop a method of efficient data collection. The steps below match the timeline of goals outlined above. Find the instructions for in-situ data collection, sample collection, chlorophyll a analysis, and data formatting below in “Common Procedures”.

I. Uncorrected Data Collection
    i. Conduct in-situ data collection and sample collection at site
    ii. Conduct chlorophyll a analysis in laboratory
    iii. Conduct data formatting
    iv. Using a scatterplot, investigate the relationship between water parameters/measurement statistics and the correction factor (this set of data will not show much due to variability from inaccuracy and improper methods of data collection, is important to show how variability is different between uncorrected and corrected methods of data collection)
II. Depth Analysis

Because the fluorometer determines the concentration of chlorophyll a based off of the light that is hitting its sensors, light coming from the sun can affect the measurement. To collect data that is free of interference from, the depth at which sunlight is no longer a factor must be determined. This depth may be different for environments with different conditions, for example, in my testing, different salinity/conductivity environments had different depth requirements. This is because of a couple of reasons. One is that freshwater is a lot more clear than brackish or salt water, so sunlight travels further in the water causing freshwater depth requirements to be at depths closer to the bottom. However, salt water, because of the higher conductivity, is not as clear, and so sunlight dissipates quicker. Along with this, certain salt water bottoms can be lightly colored which can affect the measurements. This makes closer to the surface the proper depth requirement for salt water. Brackish water, because of its mix between the two, shows a depth between the two depths of salt and fresh water.

i. Conduct water sample collection at site where data collection will be taken from (suggested to take twice the normal amount of samples, during my testing I took 6 samples per depth analysis)
ii. Conduct rounds of in-situ data collection at separate depths until reach bottom/depth limit, keep differences between depths equal(My tests were conducting from depths 0.15-0.75 meters with intervals of 0.15 meters
iii. Conduct data formatting
iv. Create a column that is called “Error” and set it equal to the absolute value of the difference between the field determined chlorophyll a concentration and the lab determined concentration.
v. Create a scatterplot with X set as “Depth” and Y as “Error”, determine the depth at which error is the least and the range of error is the least, this is the depth at which you will record measurements
vi. Repeat depth analysis at locations that represent different conditions, I analyzed the correct depth at locations of freshwater, brackish water, and salt water

III. Range of Confidence

One of the trends I began seeing when going through the data was that the higher the chlorophyll a concentration, the more error between the field and lab determined concentrations. Through analyzing data it became clear that when a bloom was occurring, the fluorometer was under estimating the concentration and had much more variability than normal, not only in the field determined concentration but also the lab determined concentration. Because of this, A range must be set of the chlorophyll a concentrations that the fluorometer can accurately read. To formulate this range, I took historic records of chlorophyll a in the waterways I was testing and determined a 95% confidence interval at 37.5 ug/L to be my upper limit. This is a confounding factor for this project but may not be for yours, make sure to investigate your data to find any confounding factors that are relevant to your project. The data at your disposal may be different and you will most likely have to formulate your own method to determining your own range.

IV. Remaining Confounding Factors

As mentioned before, it is important to go through your data as you are collecting it and investigate any relationships that may end up being confounding factors you have to come up with requirements for. This may involve developing your own method to investigate these confounding factors.

V. Corrected Method Data Collection
    i. Conduct in-situ data collection and sample collection at site
        i. Use depth requirements set previously
        ii. Only include observations within range of confidence
        iii. Follow any other requirements formed
    ii. Conduct chlorophyll a analysis in laboratory
    iii. Conduct data formatting
VI. Correction Formula
    i. Create a scatter plot with X set as the field determined chlorophyll concentration and Y set as the lab determined concentration
    ii. Set a linear trendline and determine the R squared value
        i. If R squared value is not adequate, go back and investigate data further and develop more requirements
    iii. Use trendline as correction formula, put field determined chlorophyll concentration in as X and Y will equal the corrected value
VII. Implement new field data collection method to increase data collection.
    i. Employ measurement techniques/requirements and apply correction formula to data to employ this method and accurately monitor chlorophyll levels and subsequent algae levels

6. Common Procedures

I. In-situ data collection
    1. Calibrate Manta2 multiprobe, including fluorometer pink post-it standardization
        a. See SOP for fluorometer standardization
    2. Depart for SWAMP stations
    3. Connect Manta2 cable to Bluetooth device and pair Bluetooth device to county laptop and open Manta2 control software
    4. Remove storage/calibration cup and place testing cup on the end of the Manta2 multiprobe
    5. Drop Manta2 multiprobe into water, holding onto the cable, confirm data readings are stable, then click “Capture One Line of Data to PC with Annotation” and note the station number and the depth measured
    6. Pull Manta2 multiprobe out of the water, place storage/calibration cup with water on the end of the probe.
    7. Write down any necessary notes, pack up and repeat at next station
    8. Once all stations have been tested, save snapshot files to computer for statistical analysis
II. Water sample collection

1) Prepare 500 mL sample bottles with appropriate labels for each station
2) Prepare insulated cooler with ice and place bottles inside and load into county vehicle
3) Depart for SWAMP stations
4) Gather bottle labeled for specific station, along with water collection device upon arrival
5) Rinse sample bottles 3 times with water being collected
6) Pour collected water sample into 500 mL sample bottle, seal sample bottle
7) Place samples in insulated cooler with ice to preserve them while other SWAMP stations are visited for testing
8) Repeat steps 4-7 for each station being visited, then transport samples back to laboratory

III. Chlorophyll a analysis

1) Once back in laboratory, lay out samples, let come to room temperature
2) Filter sample(125 mL) through a glass fiber filter using a vacuum pump
3) Store resulting filter in acetone solution(90%, 8 mL), vortex sample to dissolve filter into acetone, place in fridge for 2 hours minimum (can go to 24 hours max) (or frozen at or below -20 degrees Celsius for 20 days)
4) After 2 hours, vortex sample again, then centrifuge sample to settle any suspended solids
5) Pour sample into cuvette and place in fluorometer to get chlorophyll a concentration measurement and record results

IV. Data Formatting

1) Import data from field measurements, keep any parameter recorded for investigation of trends
2) Create a column that notes the station/location where the measurement was made, as well as a column for the lab determined chlorophyll a concentration
3) Duplicate each observation/row for how many samples were analyzed, make a column that notes the replicate number for each observation
4) Enter lab determined chlorophyll a concentrations, enter the different sample measurements in the different replicates rows
5) Create a column called “Correction Factor” and make that cell be equal to the field determined chlorophyll a concentration divided by the lab determined concentration