Technical Note: Algae Measurements Using *In Vivo* Fluorescence

Phytoplankton are microscopic, free-floating photosynthetic plants and bacteria that are commonly found in surface waters throughout the world. Aquatic scientists and water resource managers measure phytoplankton to gain a more in depth understanding on ecological dynamics, ecological health, nutrient status, and harmful algal bloom potential in aquatic systems. Submersible fluorescence sensors help to make this measurement easy, efficient, and economical by enabling real-time field estimates of phytoplankton biomass that can be directly correlated to quantitative laboratory measurements using standard methods.

Figure 1. Integrated submersible fluorescence sensor - configured on a HYDROLAB® HL7 multiparameter water quality sonde

Phytoplankton contain naturally fluorescent pigments such as chlorophyll *a*, phycocyanin, and phycoerythrin that provide a vital function by harvesting light energy needed for photosynthesis. In this process, light energy from the sun drives a reaction where carbon dioxide and water are converted into sugars. Oxygen is given off as a reaction byproduct. As phytoplankton are able to create their own chemical energy through this process, they are commonly referred to as "primary producers" within aquatic ecosystems. These primary producers form the foundation of aquatic food webs, where they are consumed by organisms higher up on the food chain such as zooplankton, invertebrates and fish.

In this technical note we will discuss important topics such as: variables that affect *in vivo* measurements, the role HYDROLAB® sondes play and best practices to enhance overall data quality.

What Variables Affect *In Vivo* Phytoplankton Measurements?

To ensure a good correlation between *in vivo* phytoplankton, fluorescence measurements and actual concentration estimates, it is important to have a general understanding of the variables that can affect their measurements. Variables mainly include environmental changes that affect the measurement area and changes within phytoplankton that affect the fluorescence output.

Key variables affecting fluorescence measurement include:

- Water Temperature
- Turbidity
- CDOM Interference
- Light History
- Phytoplankton Health
- Sensor Fouling
- Quenching

Water Temperature

If sensor deployments are made in temperature stable environments the variable of temperature should be relatively insignificant. In these cases it is recommended to calibrate at the same temperature as your samples, if possible.

In situations where there are significant environmental changes in temperature while monitoring (such as vertical profiling in stratified lakes or applications where the sensor is moved throughout the water column in systems with significant temperature variations), optional temperature compensation can be considered. Multiparameter water quality sondes, such as HYDROLAB®, come standard with a temperature sensor; this sensor automatically captures the data needed for making an optional temperature correction.

Turbidity

If turbidity is a significant and fluctuating parameter, it is advised to capture turbidity data while fluorescence readings are taken. Using data sets for *in vivo* fluorescence and turbidity, combined with correlating quantitative laboratory data for phytoplankton biomass, one can perform a statistical adjustment that can help to account for turbidity fluctuations.

CDOM Interference

If significant CDOM interferences are suspected, it is advised to use sample water that has been filtered to remove phytoplankton as a blank during the calibration process. This filtration is commonly performed using a vacuum pump and filter paper with a pore size appropriate for the phytoplankton species present. By using the sample water free of the target analyte as a blank, the signal effects of the background water can be determined and then factored into subsequent fluorescence sensor readings.

Figure 2. Lake with Algae

Interference from other Chlorophyll Variant Pigments and Pheophytins

In regards to potential interferences from other chlorophyll variant pigments and pheophytins, these can be identified and addressed through periodic extractions that go along with *in vivo* data collection. More details on this can be found within EPA Method 445.0.

Light History

The amount of light that phytoplankton are acclimated to can not only affect their growth, but also their fluorescence output. To remove or minimize the variable of light history that affects the fluorescence measurements of living phytoplankton, steps can be taken to dark adapt phytoplankton cells prior to *in vivo* measurement. Taking samples at night can completely remove this variable, and this can be easily achieved during unattended deployments as sensors can be programmed to take readings at any given time. For attended measurements taken during the day, phytoplankton can be dark adapted either by measuring samples placed in a dark container for spot sampling, or by using pump to pump sample water through a darkened flow cell with opaque tubing for profiling applications.

Phytoplankton Health & Assemblage Dynamics

In natural water bodies that contain phytoplankton, it is important to be aware that these systems are very dynamic in regards to the types of phytoplankton species present as well as the growth stage and physiological health of the individual phytoplankton cells. Different species have different typical fluorescence yields, and individual phytoplankton cells that are in different stages of growth and health can also yield different fluorescence outputs. Although these variations are not able to be controlled or corrected, it is good to be aware of them and to understand that they tend to average out in natural systems. These variables also give additional reasons to take periodic grab samples for quantitative laboratory analysis.

Biological and Particulate Fouling in Long-Term Deployments

Sensors that are set up for long-term deployments are susceptible to biological and/or particulate fouling at a rate that is dependent on the environmental conditions of the deployment site. The more eutrophic an aquatic system is, the more biological fouling is a concern due to higher productivity potential. HYDROLAB® sondes offer multiple sensor-based solutions to address fouling when they are deployed in the field. Solutions include automatic mechanical wipers that physically wipe away fouling on the measurement portion of the sensor, as well as the integration of copper accessories into the sensor area that deter the colonization of biological organisms. These examples, and other solutions, combined with well-defined field maintenance protocols can effectively keep fouling influences on sensor data collection under control.

Quenching

Concentrations where phytoplankton levels are highly visible in the water, an effect called "quenching", can occur when taking a fluorescence measurement. When concentration levels of any fluorescent analyte gets too high, outputted light from the fluorescence sensor is absorbed by the high concentration of the analyte, thereby outputting lower values than what would be anticipated to go to the detector. To address this, grab samples can be placed in containers to enable controlled dilutions. If a specific dilution percentage change of the sample equals the same fluorescence signal percentage change outputted by the sensor, it can be assessed that the sensor is reading within the linear range.

Best Practices for *In Vivo* Fluorescence Measurements of Phytoplankton

In addition to being aware of the inherent variables associated with *in vivo* phytoplankton measurement, there are additional best practices that can help to enhance overall data quality. These practices range from how to best deploy these sensors, to incorporating an understanding of environmental deployment dynamics and how certain dynamics can affect field generated data. Examples of these best practices include:

- Identifying Phytoplankton Grouping Patterns
- Using the Right Calibration Standards
- Monitoring Multiple Aquatic Systems with One Sensor
- Monitoring at Multiple Locations

Identifying Phytoplankton Grouping Patterns

A recommended practice prior to monitoring phytoplankton using *in vivo* fluorescence is to have a general understanding of the phytoplankton grouping patterns present in the aquatic system(s) to be monitored. Many laboratories offer this service if outsourcing is required. With knowledge of the species present, one can then determine which sensor is most suitable for the monitoring application and objectives. Although *in vivo* chlorophyll *a* sensors can be used for all phytoplankton monitoring applications, it would be advised to use either a phycocyanin or phycoerythrin sensor for aquatic systems that are more populated with cyanobacteria or for systems where cyanobacteria levels are specifically of interest.

Using the Right Calibration Standards

In vivo phytoplankton sensors require a two-point calibration, including a blank (de-ionized water or filtered sample water) and a non-zero standard. Unlike sensors for pH, conductivity and turbidity, there are no bottled primary calibration standards readily available for *in vivo* phytoplankton as this analyte is part of a living biological organism. A representative grab sample from the environment is the true standard for *in vivo* phytoplankton monitoring; it simply requires an additional step of *in vitro* laboratory quantification if meaningful concentration units are desired.

Figure 3. HYDROLAB® HL7 Sonde

Once this representative field grab sample is measured both *in vivo* as well as in the laboratory, one can then use a stable secondary standard to use as a relative reference for future calibration purposes. Stable secondary standards can also be used for monitoring sensor drift to make sure the sensor always reads consistently. The ideal tool to use as a secondary standard is a solid secondary standard accessory that is specific to the Turner Designs phytoplankton fluorescence sensors on HYDROLAB® sondes. This tool fits onto the top of the sensor and provides a stable and repeatable fluorescence output.

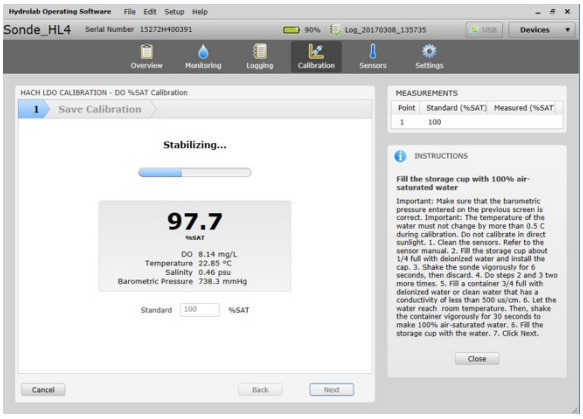

Figure 4. HYDROLAB® Operating Software calibrates sensors and checks sensor calibrations with guided and semi-automated calibration routines

It is possible to use a synthetic fluorescent tracer dye as a secondary standard, however it is important to know that synthetic dyes are not the same as a primary standard for phytoplankton pigments. Various concentrations of Rhodamine WT tracer dye are commonly used as a secondary standard, but it is important to keep in mind there are multiple sources of variability that can affect

the accuracy of using dye for this purpose. Sources of variability include concentration accuracy of the initial dye stock (powder or liquid), potential human errors in the dilution process (which can be significant), and dye fluorescence output variations due to fluctuations in temperature (an inherent variable of most synthetic fluorescent dyes, including Rhodamine WT).

Liquid chlorophyll a standards are available in the market, however it is important to note that they are typically in a solvent medium (such as acetone) which should not be in contact with the *in vivo* fluorescence sensors. These standards are intended for use in test tubes that are placed within laboratory instrumentation for extracted pigment analysis. Phycocyanin and phycoerythrin standards are also available in the market, but these standards are typically used for laboratory instrumentation as they have a very limited shelf life and need to be used within hours of procurement.

Monitoring Multiple Aquatic Systems with One Sensor

Although in some cases one sensor may only be used for monitoring one body of water, there are cases where one sensor is used for monitoring multiple water bodies. In the case of the latter, it is important to keep in mind that the properties of any two bodies of water can significantly differ from one another even if they are right next to each other geographically. If accurate quantitative values are important for monitoring efforts and one sensor is needed for monitoring more than one water body, then it is advised to determine a correlation coefficient between *in vivo* and quantitative readings for each water body being monitored. In addition, it is also a good practice to compare the background signals and data readings of the various water bodies to see if data adjustment may be necessary. This could also apply to large aquatic systems that are monitored in different areas that have significant variations in water quality parameters and/or phytoplankton assemblages.

Monitoring at Multiple Locations

Although spot sampling at one depth can provide a helpful data point at one place and one time, more data points taken over a wider spatial range can provide even more insight. For example, an extended deployment taking continuous readings every 15 minutes can capture daily and seasonal fluctuations of phytoplankton levels, while vertical and horizontal profiling of a water body can capture phytoplankton biomass that may be missed during spot sampling due to inherent patchiness in phytoplankton distribution.

Figure 5. Long-term deployment of HL7

Conclusion

In conclusion, many variables affect *in vivo* measurements. Luckily, incorporating *in vivo* phytoplankton fluorescence sensors on HYDROLAB® sondes enables the ability to gather a tremendous amount of insight on the

ecological status and health of an aquatic system, especially with continuous, long-term data sets. Combining that with some additional best practices will greatly help enhance overall data quality.

References & Resources

Standard Methods for the Examination of Water and Wastewater 19th edition (Section 10200)

EPA Method 445.0:
In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence

EPA Method 446.0:
In Vitro Determination of Chlorophylls a, b, c + c and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry

EPA Method 447.0:
Determination of Chlorophylls a and b and Identification Of Other Pigments of Interest in Marine and Freshwater Algae Using High Performance Liquid Chromatography with Visible Wavelength Detection

Turner Designs Website:
www.turnerdesigns.com

For more information on Algae Measurements Using *In Vivo* Fluorescence, contact OTT Hydromet.

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