Application Note: Cyanobacteria Detection in Water Using *In Vivo* Fluorometry

Overview

Cyanobacteria, a.k.a. blue-green algae, are common forms of photosynthetic bacteria present in most freshwater and marine systems. The monitoring of cyanobacteria is of growing interest in a number of research and monitoring fields and of particular interest is the monitoring of cyanobacteria as a public health risk. As the rates of eutrophication accelerate due to human impacts on aquatic ecosystems,algal blooms are becoming a more common problem. In the case of cyanobacterial blooms, some species can produce toxins generally referred to as cyanotoxins that can cause health risks to humans and animals. The real-time monitoring of cyanobacteria through fluorometry can serve as an early warning system for potentially hazardous conditions. In addition to potential toxin production, cyanobacteria blooms can also result in water with an unpleasant appearance, and in the case of drinking water, an unpleasant taste and odor. These problems adversely affect water quality and diminish the water's recreational utility. Also of concern are high cell concentrations causing an increase in filter run times in drinking water plants. Thus, monitoring the cyanobacteria population and distribution in lakes, reservoirs and coastal areas is extremely important for resource protection, public health and safety, and overall economics.

Turner Designs has produced a line of solid-state fluorescence instruments that can be used to detect the in vivo fluorescence (IVF) of cyanobacterial pigments in natural waters. This technology represents a new practical and robust tool for researchers and water resource managers to improve monitoring systems and improve water quality, in order to prevent the occurrence of potentially hazardous conditions.

Fluorescent Pigments

Turner Designs fluorescence instrumentation has set the standard for the monitoring of chlorophyll *a* (the primary photosynthetic pigment) levels in water. Chlorophyll *a* detection supplies data on the total algal biomass (all photosynthetic organisms contain the chlorophyll *a* pigment). However, different types of phytoplankton and cyanobacteria have unique sets of accessory pigments that serve a variety of roles for the organism. These accessory pigments are often unique to a class of algae or cyanobacteria and can be used to identify a specific group. Cyanobacteria contain accessory pigments from the phycobiliprotein family. The primary phycobilin pigments are phycocyanin (PC) and phycoerythrin (PE) that happen to have strong fluorescent signatures that do not interfere with the fluorescence of the chlorophylls (See Appendix A, Figures 1-3). This allows for the *in vivo* detection of cyanobacteria without interference from other groups of algae. PC is the predominant phycobilin in freshwater environments while PE is the predominant pigment in marine environments. When purchasing an instrument a decision must be made as to which phycobilin pigment the instrument will be configured for.

Methods of Cyanobacteria Detection

In Vivo Fluorescence: A simple technique for locating and measuring algae has been in use by oceanographers and limnologists for over 30 years (Lorenzen, C.J., 1966). It is called "in-vivo fluorometry (IVF)", and is based on the direct measurement of the fluorescence of the chlorophyll in the living algal cells. The same methodology is used to detect the phycobilin pigments of cyanobacteria in water. The benefits of IVF include ease, speed and the ability to collect large quantities of data. There is no special sample handling or processing required, making IVF ideal for profiling, moored and on-line instrument systems for real-time data collection.

IVF is the easiest method for collecting large quantities of data but there are variables associated with IVF that result in errors and interference. The fluorescence for a given cell concentration is affected by a number of factors including; the amount of light the cell was exposed to prior to the measurement and variation amongst different species, physiological states and environmental conditions. For the most accurate data, IVF data is correlated to quantitative data that can be collected by taking occasional samples to be analyzed for pigment concentration by a technique that is not affected by the conditions of the live sample. Unlike the chlorophylls that have relatively easy and well-established extraction methods (Arar, E. J. and Collins, G. B., 1992; Strickland, J.D.H., and Parsons, T.R., 1968; Wright, S. W., et. al., 1991), phycocyanin and phycoerythrin are water soluble pigments which makes extractive methods more challenging. The most common quantitative detection method is high performance liquid chromatography (HPLC) (Wright, S. W., et. al., 1991). Other methods for quantitation include cell counting and identification and detection of specific cyanobacterial toxins. For detailed information on IVF methods please visit the Turner Designs website for information and reference lists.

Despite these factors, IVF is an excellent monitoring tool for researchers and technicians, as it permits biomass data to be recorded continuously in the field or on-line. It not only replaces the equivalent of thousands of individual measurements, but it permits more accurate mapping. For example, phytoplankton typically form thin layers or patches in the water column. Unless an enormous number of discrete samples are taken at close intervals it is

likely that individual samples will miss a strata or patch of algae or cyanobacteria. However, the chances of locating phytoplankton populations using a fluorometer are greatly improved due to high speed and continuous sampling of natural water. This can be done in real-time with the use of a submersible (vertical or horizontal profiling), or an on-line instrument (water is continually pumped through the sensor).

Cell Counting Method: One method of tracking algae growth is to take samples for microscopic counting and identification. There are several well-known drawbacks to this approach. First, it is expensive in that it requires many samples to be analyzed to follow trends and a highly-trained person. Thus, many water companies send the samples to a water lab, which requires valuable time. By the time the results come back, they are frequently just an "after-the-fact" confirmation of a problem, which has already produced clogged filters or a taste and odor problem. Finally, such examination may not even show the problem. Because very thin horizontal layering is common, samples taken without guidance will likely completely miss the "hot" zones.

HPLC: The most accurate means of quanitating the concentration of algal pigments is High Performance Liquid Chromatography (HPLC) (Wright, S.W., et. al., 1991; Jeffrey, S.W., et. al., 1997). HPLC also is the most involved and expensive system that requires highly trained technicians. IVF methods are commonly used with periodic correlation to quantitative extraction methods that include fluorometric, spectrophotometric or HPLC methods.

IVF Sensor Performance

The performance of Turner Designs IVF Cyanobacteria sensors (See Appendix A, Figure 12) has been extensively tested to identify the performance specifications. The data presented here was performed using the SCUFA® submersible fluorometer instrument platform, the CYCLOPS-7® submersible fluorometer and the CyanoWatch® on-line fluorometer. The same optical filters and LED light sources are used on all four of our 'solid-state' instrument platforms comprised of the SCUFA® submersible fluorometer, Aquafluor handheld fluorometer, CYCLOPS-7® submersible fluorometer and the CyanoWatch® on-line fluorometer. The optical specifications for phycocyanin and phycoerythrin sensors are depicted in Appendix A, Figures 1-3.

Unlike IVF of chlorophyll a, the IVF of cyanobacteria is typically correlated to cell counts rather than the concentration of extracted pigment due to the complications in extracting phycobilin pigments. IVF can also be correlated to other meaningful measures that the cyanobacteria effects, such as filter run times, occurrence of taste and odor in drinking water or the presence of cyanotoxins. For example, if the occurrence of a taste and odor event corresponds to an IVF reading of 100RFU (relative fluorescence units), you can then use this information to establish warning triggers for future events. Using the on-line CyanoWatch® system, a user could set an alarm to trigger if the IVF value remained above 75RFU for 20 minutes or more. This could then provide early warning for the potential onset of a taste and odor event.

Testing of the phycocyanin optical kit took place at a third-party laboratory. Monocultures of three freshwater cyanobacteria species (*Cylindrospermopsis raciborskii, Aphanizomenon flos-aquae, Microcystis aeruginosa*) (See Appendix A, Figures 4-8) and one culture of a green algae (*Chlorella* sp) as a control were used to test the sensor (See Appendix A, Figures 9). As is the case with IVF of chlorophyll *a*, different species of cyanobacteria have slightly different **fluorescence : cell concentration** relationships. For example, cell size, cell packaging, and accessory pigments effect the amount of fluorescence per cell. In nature, these 'species effects' are averaged out to a large degree and tests using monocultures represent a worst-case scenario in terms of variation in the **fluorescence : cell concentration** relationship.

During the trial *C. raciborskii* displayed the best detection level while *A. flos-aquae* was the next most optically sensitive and *M. aeruginosa* displayed the lowest optical sensitivity. The detection limit of the sensors is approximately 500 cells/mL. However, optimization of the sensor has been accomplished since the data was collected with plans to improve the detection limit to approximately 250 cells/mL. Throughout both trials the control green alga, *Chlorella* sp., remained constant with a background fluorescence reading not exceeding 10 units.

A separate trial was performed that investigated the interference between natural lake water and humic acids encountered in the natural water column (See Appendix A, Figure 10). Humic acid was used as the natural interference at dilutions of 0-20 mg/L. Humic acid was introduced in two concentrations at low and high algal cell densities to evaluate background interference and instrument detection response. All trial species evaluated at high cell densities with the addition of humic acid exhibited an inverse response on fluorescence readings bringing fluorescence readings down 2-4 units. In contrast, additions of low cell densities with humic acid increased fluorescence readings by 5-7 units, indicating that humic interference while detectable is statistically insignificant.

Performance of the phycoerythrin optical kit was conducted on the CYCLOPS-7® submersible fluorometer instrument platform. Laboratory tests were run using two purified and commercially available phycoerythrin pigments, B-phycoerythrin and R-phycoerythrin. A combination of the various phycoerythrin pigments would be present in natural systems and dilutions of both were tested using the CYCLOPS-7® (See Appendix A, Figure 11). Excellent signal:noise and linearity values were achieved. Field testing of the phycoerythrin systems is underway and results will be posted to the Turner Designs website as soon as possible.

Applications

Cyanobacteria has been found to be a numerically abundant faction of the phytoplankton community. Their roles in primary production, community structure, and spatial and temporal distribution are of interest for numerous scientific studies as well as natural water monitoring. Since chlorophyll fluorescence cannot be used to accurately determine cyanobacterial presence, analyzing phycobilin concentrations is essential for detecting, quantifying, and monitoring cyanobacterial levels.

- Early Warning of Harmful Algal Blooms
- Improve The Quality Of Water Supplied
- Taste & Odor
- Reservoir Management
- Increase Filter-Run Time
- Reduce Algaecide Required and Optimize Algaecide Application
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Early Warning of Harmful Algal Blooms

Interest in cyanobacteria occurrence and toxin production has been growing rapidly in recent years. Consumer awareness and concern is growing too. Utilities must have reliable data on cyanobacteria and possible toxins from their source water supplies to address these concerns. Taking precautionary measures to avoid potential health risks by cyanotoxins from a Harmful Algal Bloom is a responsibility of all agencies or organizations with the mandate to monitor and protect water resources or recreational waters. IVF of cyanobacterial pigments represents an important new technology that should not be overlooked. IVF will become a key parameter that will improve monitoring systems to provide data on cyanobacteria biomass.

Many species of cyanobacteria produce toxins generally referred to as cyanotoxins. In a cyanobacteria bloom, these toxins can cause health risks to humans and animals and the real-time monitoring of cyanobacteria can serve as an early warning system for potentially hazardous conditions. Drinking water sources, recreational lakes, ponds and coastal areas are all susceptible to the impacts of cyanobacterial blooms.

The US EPA has listed cyanobacteria to their Water Contaminant Candidate List (http://www.epa.gov/safewater/ccl/cclfs.html) and currently list cyanobacteria as an unregulated water contaminant. The EPA Unregulated Contaminant Monitoring Rule (UCMR, http://www.epa.gov/safewater/ucmr.html) may be revised in the future to include a twelve (12) month monitoring program for many drinking water treatment utilities for cyanotoxins and/or cyanobacteria. The “Drinking Water Contaminant Candidate List 2:, Notice” that was published in the Federal Register on April 2, 2004, pages 17406 – 17415, included “Cyanobacteria (blue-green algae), other freshwater algae, and their toxins” in the microbiological contaminant candidate list. Utilities should prepare well in advance for future monitoring requirements such as this by monitoring now to develop an occurrence database and assessment.

Improve Water Treatment Efficiency

Fluorometric methods enable you to correct potential problems before they become problems in the form of customer complaints or non-compliance with regulations. The added benefits include saving cost and amount of algaecides, reducing costs of activated carbon (where applicable), and less frequent regeneration of filters.

The ability to plan ahead is a primary requirement of an efficient operation. Even a few days notice of the development of a bloom permits corrective action to be taken to prevent clogged filters and adverse effects on the quality of water delivered to customers. Knowledge of predictable seasonal or annual trends is valuable in deciding on management strategies.

Typically water resource managers use IVF to monitor for chlorophyll and cyanobacteria in drinking water directly at the water source. However, water monitoring just prior to the treatment process holds many economic advantages. Immediate pre-treatment monitoring enables the facility operator to optimize the amount of treatment chemical added and therefore minimizes the downtime and expense of plugged filters.

Taste & Odor

In addition to the dangers of cyanotoxins, the water resource industry has an additional interest in cyanobacteria because of their production of two compounds, geosmin and MIB (2-methylisoborneol) which cause taste and odor problems in drinking water. On-line fluorescence sensors can replace or reduce the need for manual cell identification and counting procedures and can be used to trigger tests to evaluate taste and odor, cell identifications or the presence of specific toxins in a water supply. Current monitoring techniques (cell counting, turbidity, periodic sampling for laboratory analysis, etc...) do not give a useful picture of what is going on.

Reservoir Management

In reservoir management, however, strict quantitative information is seldom necessary. Frequently, what is required is the location of algae or warning of a bloom so that adequate treatment may be applied. In such cases, watching for increasing trends or peak fluorometer readings will provide the necessary information. For more quantitative information, calibrate seasonally to correct for local conditions by an extraction method, or by sending occasional samples to a commercial water lab.

Increase Filter-Run Time

In many cases, the first indication of an algae problem is plugging of the filter. In-vivo fluorometry of chlorophyll a and cyanobacteria permits monitoring of algae growth so that corrective action may be taken before the algae becomes a problem. In one reservoir studied by Rich, P.H., 1984, a fluorometer was used to continuously monitor the intake water. This proved to be a very effective way of following trends and provided early warning of excessive algae.

Corrective action to prevent unacceptably short filter run-times need not always involve treatment with algaecide. As discussed in the next section, a thorough knowledge of the system may permit a much less expensive alternative.

Reduce Algaecide Required and Optimize Algaecide Application

The in-vivo fluorescence method was used to monitor four Connecticut reservoirs weekly during the summer and fall (Rich, P.H., 1984) and proved valuable in identifying, up to three weeks in advance, the onset of growth conditions that would eventually require algaecide treatment. In one instance, treatment was avoided altogether simply by changing the depth of the intake. This required the knowledge that the algae was layered and that the intake was at the level of the layer. Since only small changes are required, even systems that are not designed for it can easily be rigged with siphons to vary intake depth.

In-vivo fluorescence provides a means of monitoring the effectiveness of algaecide treatment. Given a stable stratified condition, experiments can be run in a remote section of the reservoir to determine the optimum quantity and means of application. This can markedly reduce the annual cost of algaecide.

Failure to recognize that the algae is layered deep below the surface may result in the use of insufficient algaecide at the surface. A killing concentration does not reach the layer, and the treatment is wasted. However, if it is known that the algae is concentrated in a thin layer a specific distance below the surface (8 meters for example), then surface application is probably not the best course. Why add excessive algaecide to produce a killing concentration in the enormous volume of water above the "target" layer?

Conclusion

Cyanobacteria monitoring with IVF sensors represents an important development in water monitoring technology. Routine monitoring labor can be reduced significantly by replacing the need for regular cell counting and cell identification with IVF. In addition, the ability to monitor in real-time ,and the accuracy and reliability of fluorescence sensors, will significantly improve the efficiency of the monitoring system. Dramatic savings in treatment chemical use, filter run times and an overall improvement in water quality are very attainable results through the implementation of fluorescence sensors. Finally, real-time monitoring of cyanobacteria can provide a valuable early-warning system to potentially hazardous conditions. Increasing fluorescence signals or the attainment of a threshold concentration can be used to trigger more specific and expensive testing that are able to identify specific species or toxins of concern.

Turner Designs is committed to developing more affordable, easy-to-use and accurate fluorescence sensors for environmental applications. In addition, we will continually work with partners and customers to make as much real-world data available to the public on the effectiveness and accuracy of our sensors for the applications described above. Please do not hesitate to contact us or visit our website for the most recent field data and user information for all of our cyanobacteria sensors.

APPENDIX A – Figures & Graph

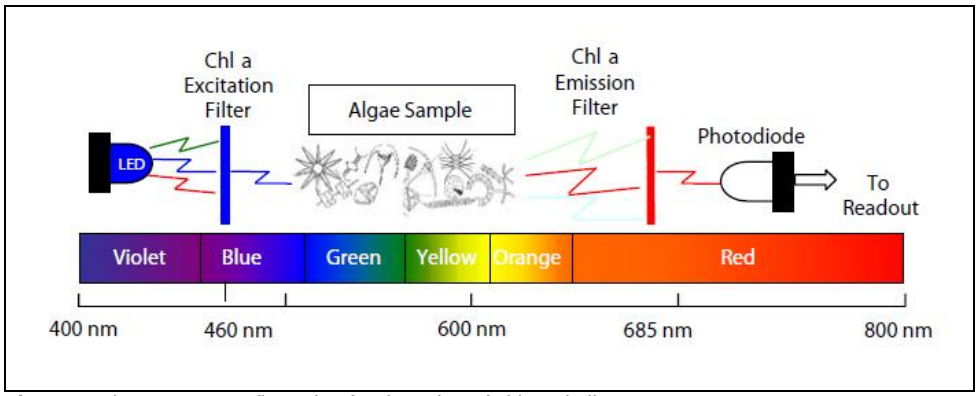

Figure 1: Fluorometer configuration for detection of chlorophyll *I*

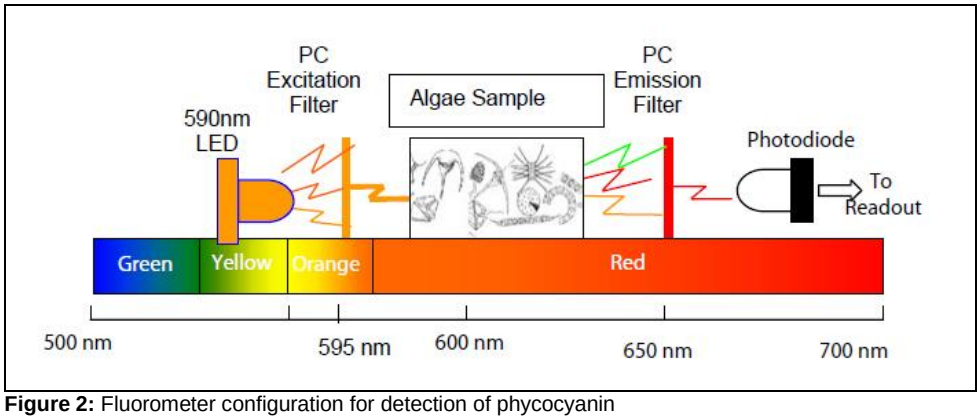

Figure 2: Fluorometer configuration for detection of phycocyanin

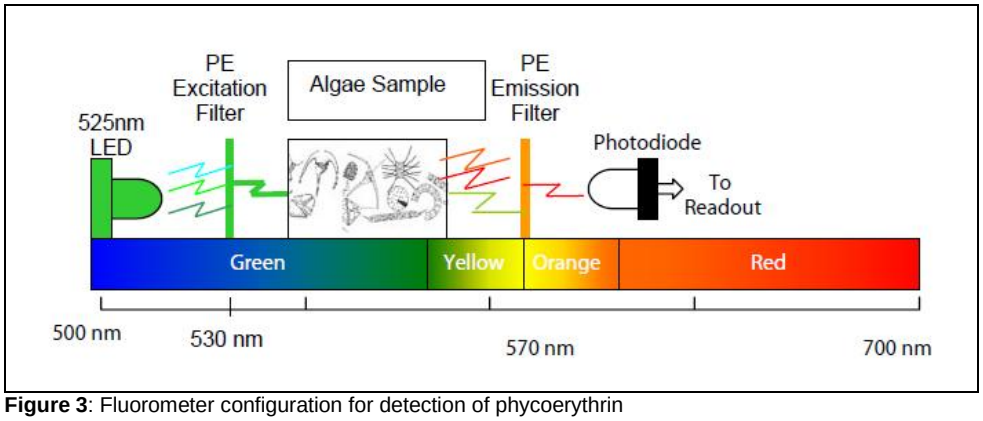

Figure 3: Fluorometer configuration for detection of phycoerythrin

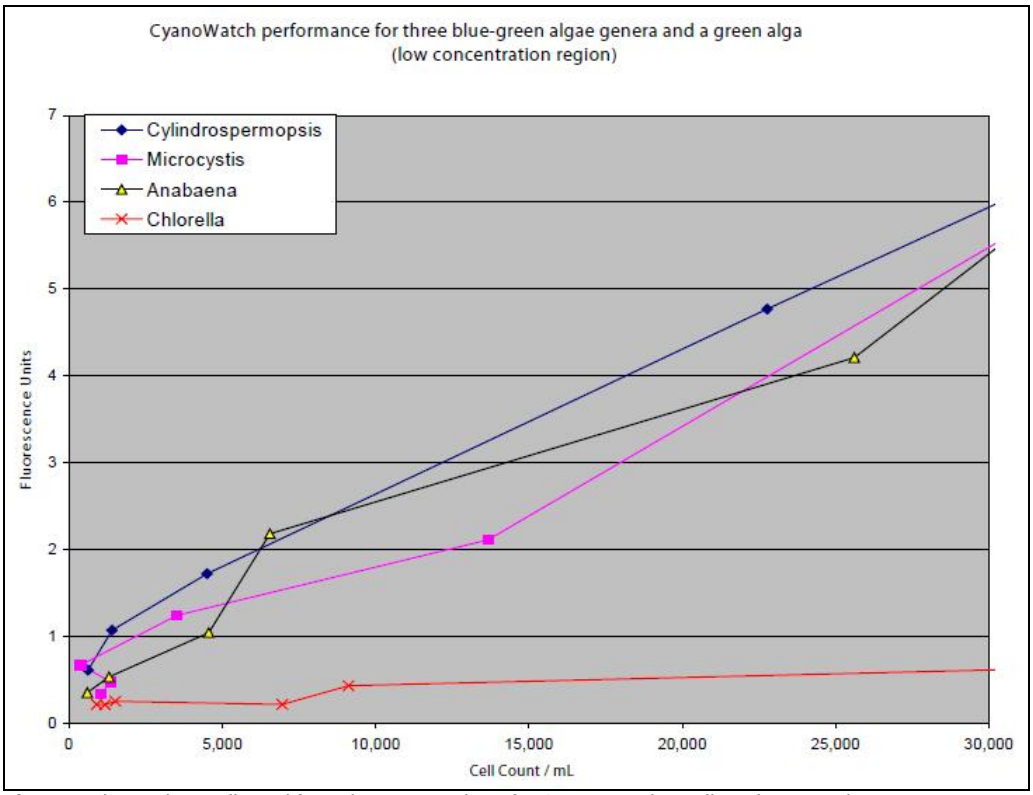

Figure 4 shows data collected from the Beta testing of a CyanoWatch on-line phycocyanin instrument. Three cultures of phycocyanin containing cyanobacteria were tested and one species of green algae as a control. The data indicates that the instrument detected all three cyanobacteria cultures at low concentrations while the green alga was not detected.

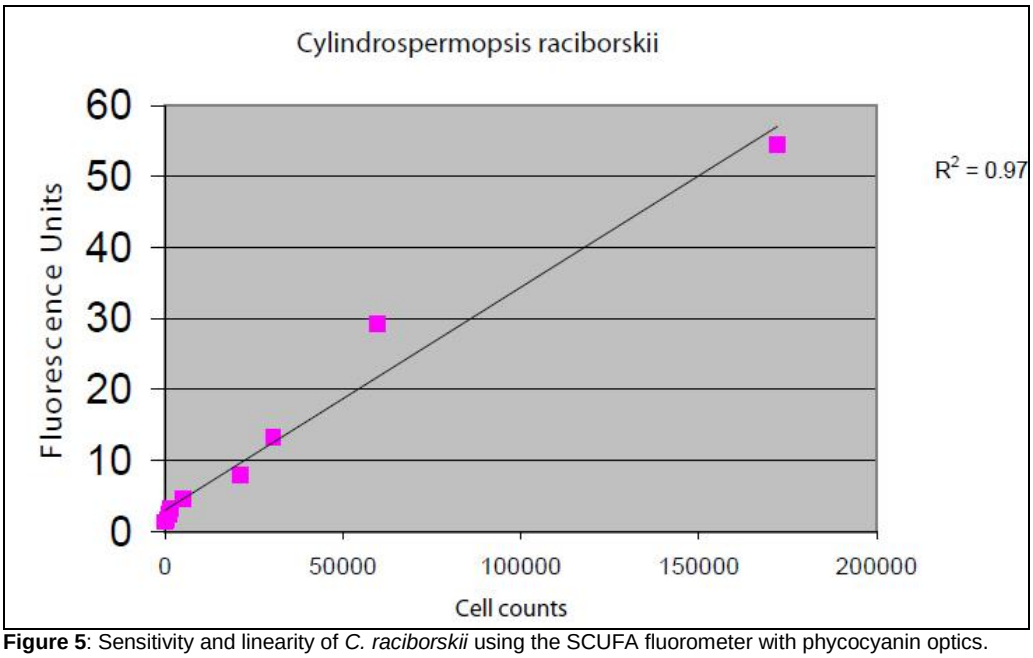

Figure 5: Sensitivity and linearity of *C. raciborskii* using the SCUFA fluorometer with phycocyanin optics.

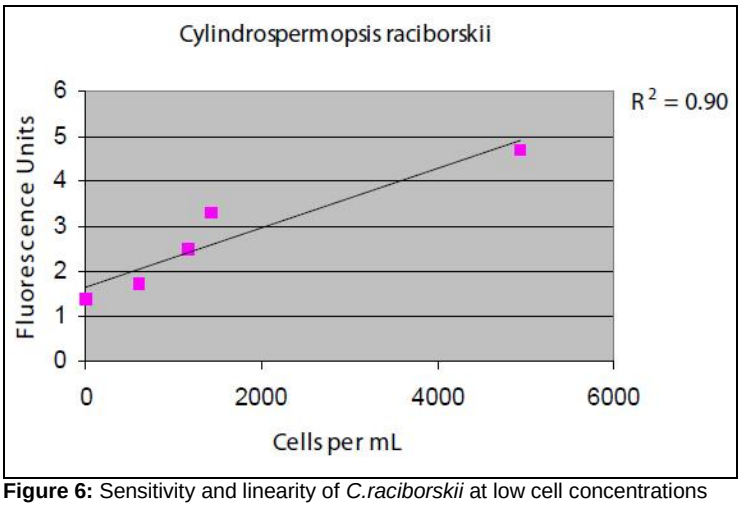

Figure 6: Sensitivity and linearity of *C.raciborskii* at low cell concentrations using the SCUFA fluorometer with phycocyanin optics.

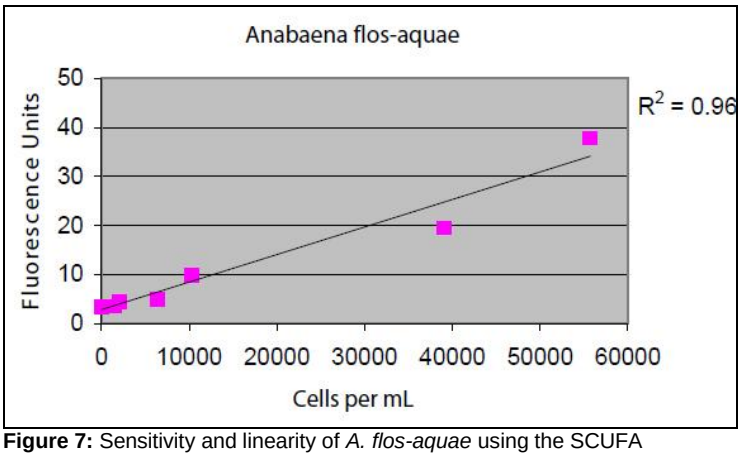

Figure 7: Sensitivity and linearity of *A. flos-aquae* using the SCUFA fluorometer with phycocyanin optics.

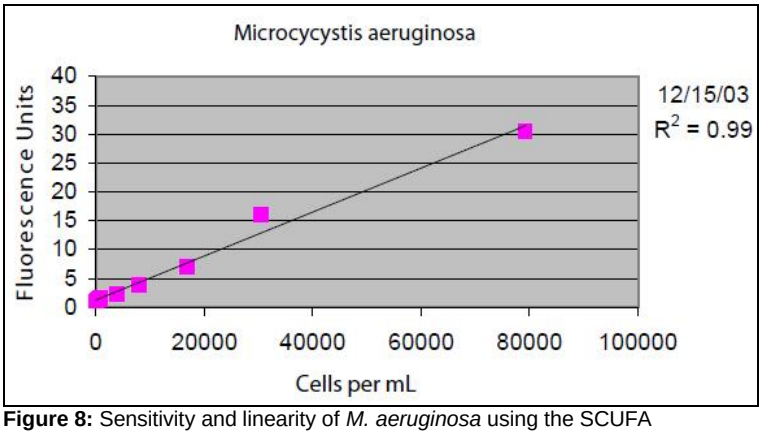

Figure 8: Sensitivity and linearity of *M. aeruginosa* using the SCUFA fluorometer with phycocyanin optics.

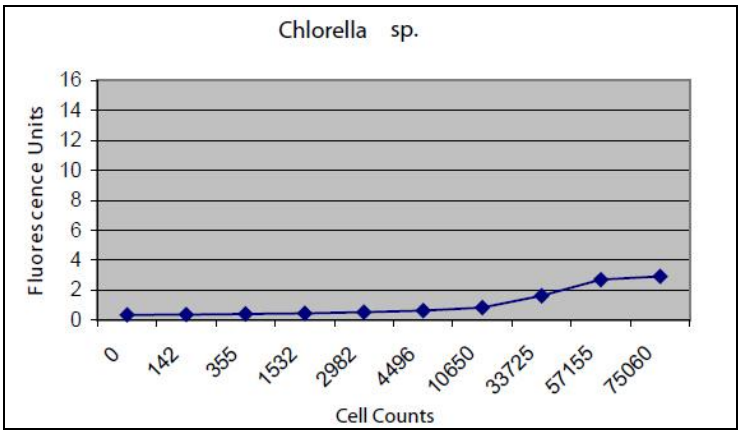

Figure 9: *Chlorella* sp.(a green alga containing no phycobilin pigments) sensitivity of the SCUFA· fluorometer with phycocyanin optics. The graph indicates that algal groups not containing phycocyanin do not interfere with the fluorescence readings.

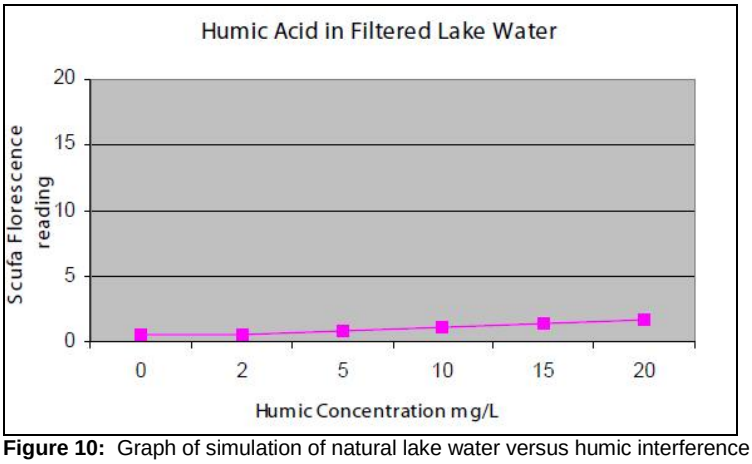

Figure 10: Graph of simulation of natural lake water versus humic interference in optical detection of the SCUFA fluorometer with phycocyanin optics. Even at high humic concentrations there was not a significant fluorescence signal indicating that the optics are not susceptible to humic interference.

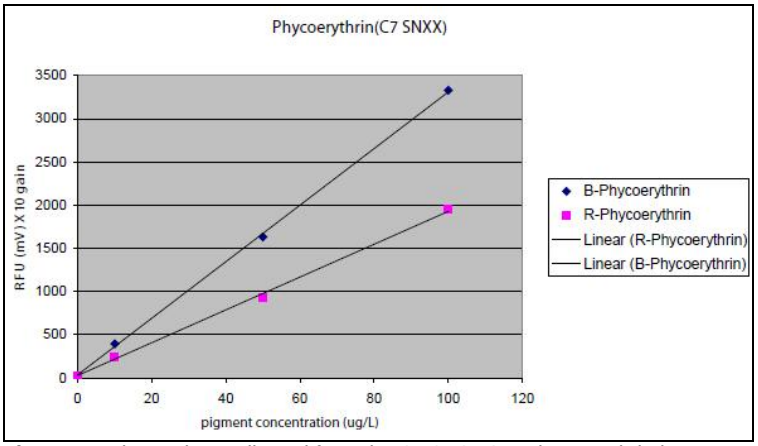

Figure 11: shows data collected from the CYCLOPS-7 phycoerythrin instrument tested with dilutions of purified B-phycoerythrin and R-phycoerythrin pigments.

Fluorometer Selection Guide by Instrument Type/Application

Fluorometer Selection Guide

Instrument Type / Application	Discrete Water Sampling			Continuous Water Sampling		
				Flowthrough	Submersible	
	Laboratory	Handheld	Field	On-line	Standalone	Integration
						
	TD-700	Aquafluor	10-AU	AlgaeWatch/ CyanoWatch	SCUFA	Cyclops-7
Chlorophyll	x	x	x	x	x	x
Rhodamine WT	x	x	x	Custom	x	x
Fluorescein	x	x	x	Custom	Custom	x
Oil	x		x			
Ammonium	x	x	x			
Phycoerythrin[1]	x		x	x	x	x
Phycocyanin[1]	x	x	x	x	x	x
CDOM	x	x	x		Custom	
Brightners	x	x	x		Custom	x
Turbidity		x			x	

Figure 12: Turner Designs Instrument Line with available applications and sample systems.

References

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