Making Sense of TAL Sensors

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Stephanie A. Smith, Ph.D.
Outdoor Water Quality Product Segment Manager
Pigments & Sensors
**Why Pigments?**

**Why algae love them**
- Harvest light for photosynthesis
- Can regulate pigment levels
Why Pigments?

Why algae love them
• Harvest light for photosynthesis
• Can regulate pigment levels

Why we love them
• Fluorescent molecules that we can detect *in situ*

Excitation
• Absorbs light energy of a specific wavelength

Emission
• Releases light of a longer wavelength, but lower energy
Why Pigments?

Why algae love them
• Harvest light for photosynthesis
• Can regulate pigment levels

Why we love them
• Fluorescent molecules that we can detect *in situ*

Why you should love them, too
• Early detection of HABs

Monitor
Manage
Which Pigments?

Chlorophyll
• *All* algae

Phycocyanin
• Blue-green algae native to freshwater

Phycoerythrin
• Blue-green algae native to marine water
Pigments Absorb Different Wavelengths...

Chlorophyll

Phycocyanin

UV

IR

Chlorophyll Light Absorbance

Phycocyanin Light Absorbance
And Emit Different Wavelengths...

Chlorophyll a in methanol
...And Emit Different Wavelengths

Phycocyanin in water
YSI’s Algae Monitoring Platforms

6-Series
Single-channel Sensors

Continuous Monitoring

ProDSS
Spot Sampling

TAL
Dual-channel Sensors
6-Series Sensors

6131 Phycocyanin

6025 Chlorophyll
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chlorophyll</th>
<th>BGA-PC</th>
<th>BGA-PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex $\lambda$</td>
<td>470 nm (unfilt.)</td>
<td>590 ± 20 nm</td>
<td>525 ± 20 nm</td>
</tr>
<tr>
<td>Em $\lambda$ (meas.)</td>
<td>&gt;630 nm</td>
<td>640 ± 40 nm</td>
<td>590 ± 20 nm</td>
</tr>
<tr>
<td>Range</td>
<td>0-400 µg/L Chl a 0-100 RFU</td>
<td>0-280,000 cells/mL 0-100 RFU</td>
<td>0-200,000 cells/mL 0-100 RFU</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.1 µg/L Chl a 0.1 RFU</td>
<td>1 cell/mL 0.1 RFU</td>
<td>1 cell/mL 0.1 RFU</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>0.1 µg/L Chl a</td>
<td>~220 cells/mL</td>
<td>~450 cells/mL</td>
</tr>
</tbody>
</table>
EXO and ProDSS Total Algae Sensors

TAL-PC (599102-01) and TAL-PE (599103-01)

TAL-PC (626210) and TAL-PE (626211)
Fluorescence Resonance Energy Transfer (FRET)

Solid—excitation
Dotted—emission

EXO and ProDSS Sensors
### Parameter Phycocyanin Chlorophyll Phycoerythrin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phycocyanin</th>
<th>Chlorophyll</th>
<th>Phycoerythrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex λ</td>
<td>590 ± 15 nm</td>
<td>470 ± 15 nm</td>
<td>525 ± 15 nm</td>
</tr>
<tr>
<td>Em λ (meas.)</td>
<td>685 ± 20 nm</td>
<td>685 ± 20 nm</td>
<td>685 ± 20 nm</td>
</tr>
<tr>
<td>Range</td>
<td>0 to 100 RFU; 0 to 100 μg/L PC</td>
<td>0 to 100 RFU; 0 to 400 μg/L Chl</td>
<td>0 to 100 RFU; 0 to 280 μg/L PE</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.01 RFU; 0.01 μg/L PC</td>
<td>0.01 RFU; 0.01 μg/L Chl</td>
<td>0.01 RFU; 0.01 μg/L PE</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>0.01 μg/L PC</td>
<td>0.01 μg/L Chl</td>
<td>0.01 μg/L PE</td>
</tr>
</tbody>
</table>
### EXO/ProDSS vs. 6-series Phycocyanin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6-Series BGA-PC</th>
<th>EXO TAL-PC</th>
<th>So?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex LED</td>
<td>Single</td>
<td>Dual</td>
<td>Only need one sensor</td>
</tr>
<tr>
<td>Em λ (meas.)</td>
<td>640 ± 40 nm</td>
<td>685 ± 20 nm</td>
<td>Less interference</td>
</tr>
<tr>
<td>Range</td>
<td>0-280,000 cells/mL 0 to 100 RFU</td>
<td>0 to 100 μg/L: 0 to 100 RFU</td>
<td>Build cfu/mL in KorEXO</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.1 RFU; 0.1 μg/L Chl</td>
<td>0.01 RFU; 0.01 μg/L PC</td>
<td>10X better</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>0.1 RFU</td>
<td>0.01 RFU</td>
<td>10X better</td>
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Real-World Monitoring
Real-World Monitoring

- Turbidity
- IFE
- Temperature
- Sensor-to-sensor variability
- The algae don’t care about you
Turbidity

Light emitted from sensor

Suspended particles

Light from excited PC reaching detector

Light from excited PC deflected by particles

RFU

Turbidity
Inner Filter Effect (IFE)

- Light emitted from sensor
- Absorbent molecules
- Light from excited Chl absorbed by "quenchers"
- Light from excited Chl reaching detector
- Light from quencher goes undetected

RFU vs. Chlorophyll plot:
- No quenchers
- With quenchers
• **There is an inverse relationship between temperature and fluorescence**

• This example: profiling from 0-12m depth

• The question: is this showing a change in algae population, or a change in fluorescence due to temp?

• Thank you, Jamie Carr of MA DCR!
Calibration is Critical

**DRIFT HAPPENS**

- **A:** slope is same
  - What’s the temp?
- **B:** slope changed, especially affects higher readings
- **C:** slope changes, especially affects lower readings

![Graph showing drift A, B, and C with RFU on the y-axis and [RhoWT] on the x-axis.](image-url)
So, what is a “One point cal?”

A one-point calibration is a *re-zeroing* of the sensor.

- **A**: slope is same
  - What’s the temp?
- **B**: slope changed, especially affects higher readings
- **C**: slope changes, especially affects lower readings
So, what is a “One point cal?”

A one-point calibration is a *re-zeroing* of the sensor

- A was improved at the low end
- B didn’t improve at all
- C was fixed completely
Instead, do a 2-point cal

If you want your sensors to all align, do a 2-point cal, and do them together

- \( A = B = C \)

- Very important to address sensor to sensor variability
In spite of these challenges...

- Turbidity
- IFE
- Temperature
- Sensor-to-sensor variability
- The algae don’t care about you

With proper calibration and maintenance, the sensors deliver the highly-reliable early-warning signals of an HAB
Case Study: Lake Erie Monitoring

- 3M+ people drink Lake Erie Water
- *Microcystis aeruginosa*
- 2014 Toledo water crisis
- Network of monitoring buoys with EXO
- Thanks to Ed Verhamme of Limnotech for the data to follow!
Case Study: Lake Erie Monitoring

Parameters:
- Temperature
- pH & ORP
- Conductivity
- Turbidity
- Dissolved Oxygen
- Chlorophyll
- Phycocyanin
Normalize your fleet!

- Co-calibration sets the same baseline for all the sensors
- Cal checks during visits/maintenance
• Raw water intake (not finished water!)
• EXO2 TAL-PC
• Microcystin by ELISA
• Lake Erie has fairly consistent blooms of *M. aerugionosa*
• Value is in observing year over year *trends*...
• *Learn your system*
• TAL-PC alone doesn’t define *treatability*
Why this program is so successful...

- Standardized fleet of sensors
- Proper Maintenance
- Proper Calibration
- Expectations: monitor for changes from a baseline
Sensor Construction

- **Dual-channel sensors are fundamentally different from single-channel sensors**
- **More sensitive, highly specific**
- **All fluorescence-based sensors drift**
Making Sense of Algae Sensors

Monitoring in the Real World

• Normalize your fleet: same equipment, same calibration
• Monitor for changes from a baseline in your system, and look for sustained changes
• Awareness of interferences