

PML

Plymouth Marine
Laboratory

Research excellence supporting a sustainable ocean

Novel Fluorescence Induction Protocols For Cyanobacteria Detection In Natural Samples

Stefan Simis

Emilie Courtecuisse¹, Peter Hunter², Kevin Oxborough³, Gavin Tilstone¹, Evangelos Spyrakos², Stefan Simis¹

¹Plymouth Marine Laboratory, United Kingdom; ²University of Stirling, Scotland, United Kingdom; ³Chelsea Technologies Ltd, United Kingdom

LabSTAF : new active fluorometer



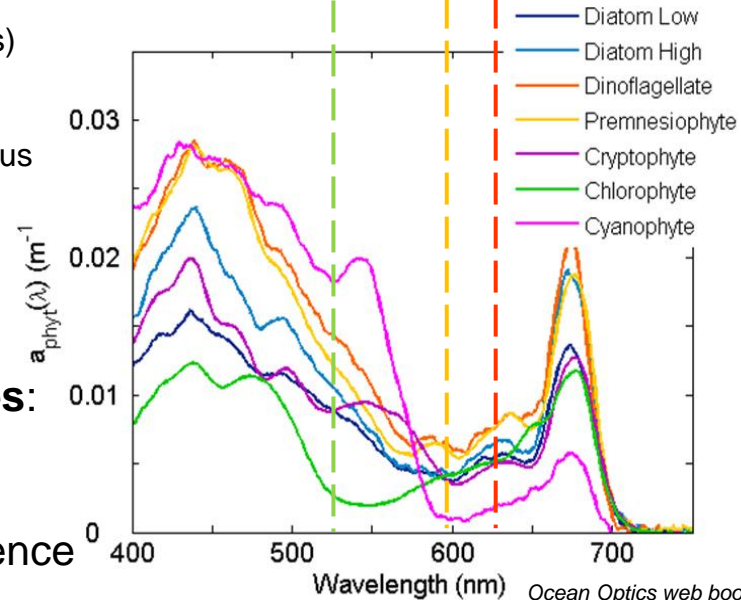
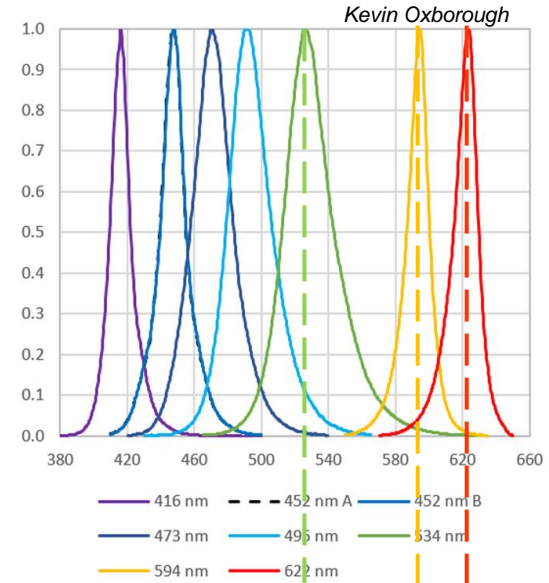
<https://chelsea.co.uk/>

Optical properties

- ▶ 2 emission wavebands: 685 and 730 nm
- ▶ 7 Excitation LEDs channels, used alone or combined
- ▶ 1 actinic light
- ▶ high sensitivity to low fluorescence signals
- ▶ ST (Single turnovers) measurements

Measurements

- ▶ Fluorescence Light Curves (FLCs)
- ▶ Excitation spectra using the excitation LEDs
- ▶ Flow-through system for continuous measurements or **punctual measurements**
- ▶ T° C measured



→ Advantages to target cyanobacteria in natural samples:

- ▶ LEDs targeting phycobilipigments (Phycocyanin and Phycoerythrin)
- ▶ Determination of physiology and productivity from fluorescence parameters
- ▶ Excitation spectra

Study site: Roadford lake

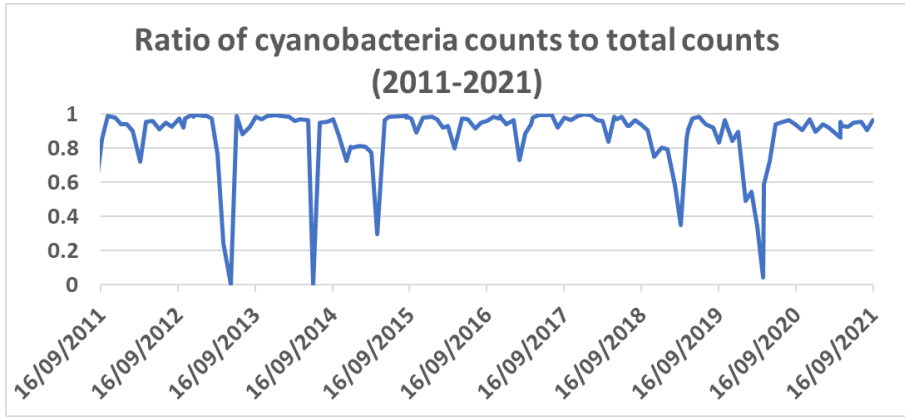
<https://commons.wikimedia.org/>



Sampling site (50.690424, -4.2300678)



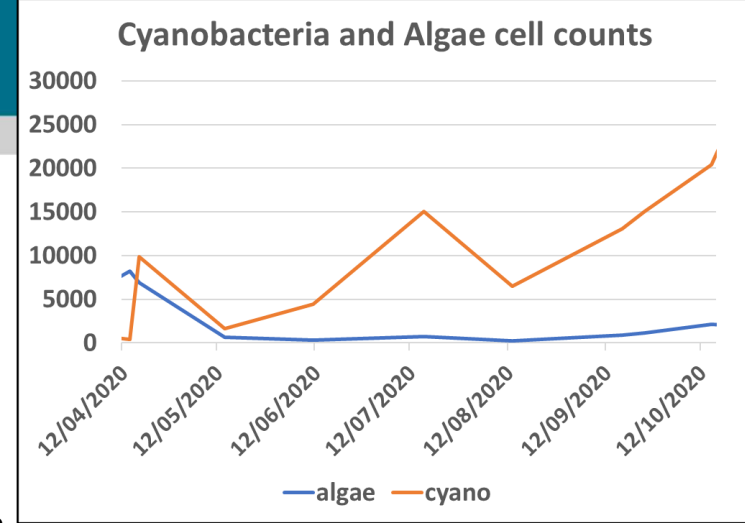
Presence of cyanobacterial blooms since at least a decade



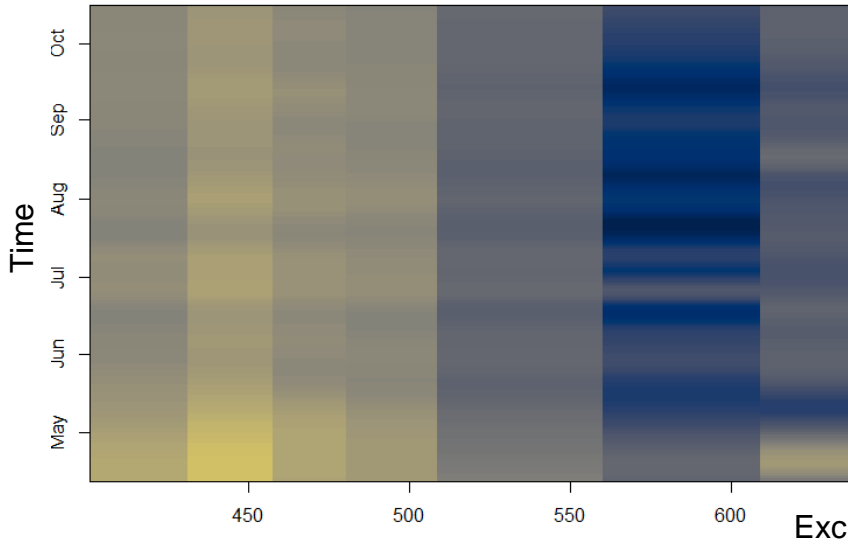
- Weekly sampling of sub-surface water between April and October 2020
- Measurements in the laboratory: fluorescence, chlorophyll a (Chla), nutrients concentration
- Data from *South West Water*: nutrients concentration/ phytoplankton counts and identification
- **LabSTAF**: Determination of 5 protocols with different LEDs combination to target different phytoplanktonic groups:
 - B**: 452 nm (*2)
 - YORB**: 452 nm(*2), 534 nm, 594 nm, 622 nm,
 - YOB**: 452 nm(*2), 534 nm, 594 nm
 - YOR**: 534nm, 594 nm. 622 nm
 - YO**: 534 nm, 594 nm

Excitation spectra: σ PSII absorption cross section of photosystem II (PSII)

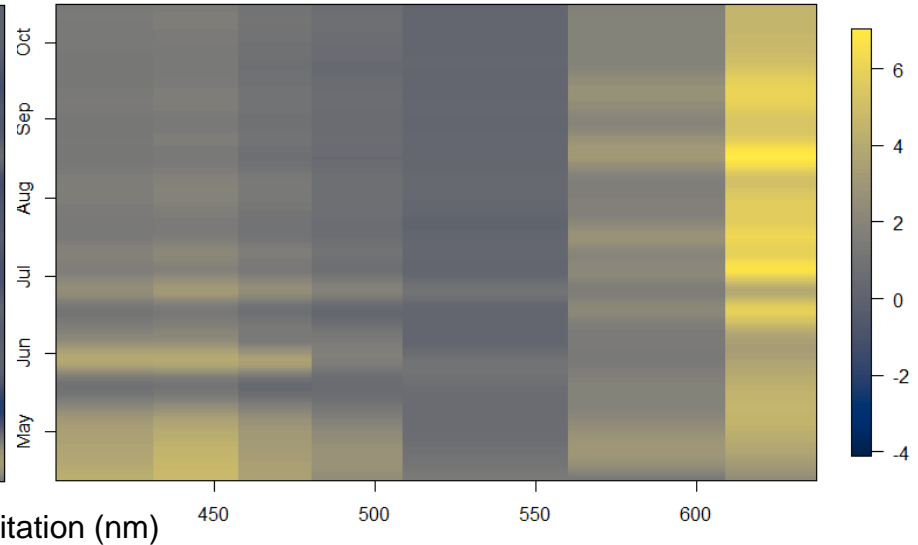
σ PSII



Protocol B



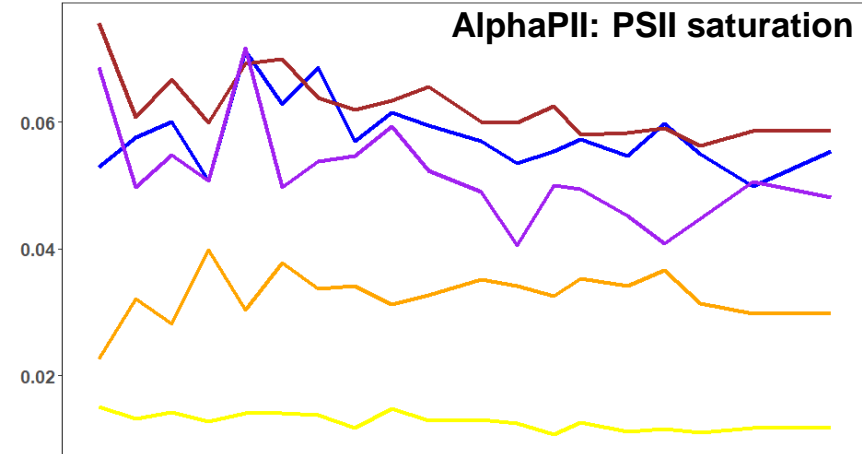
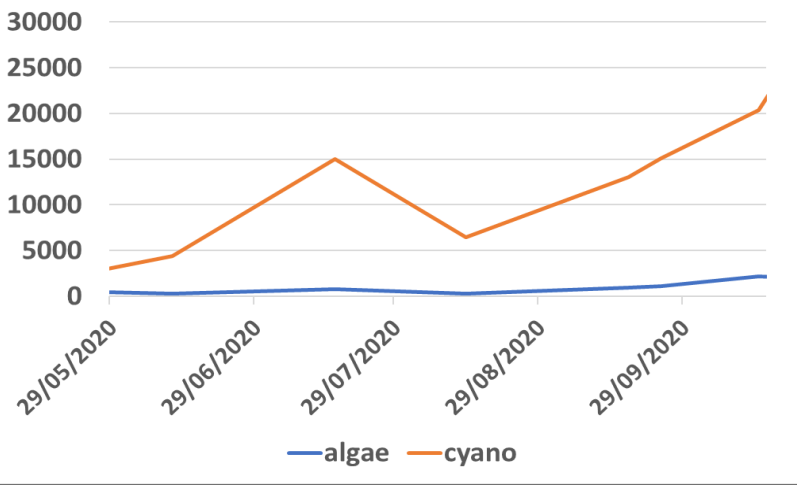
Protocol YOR



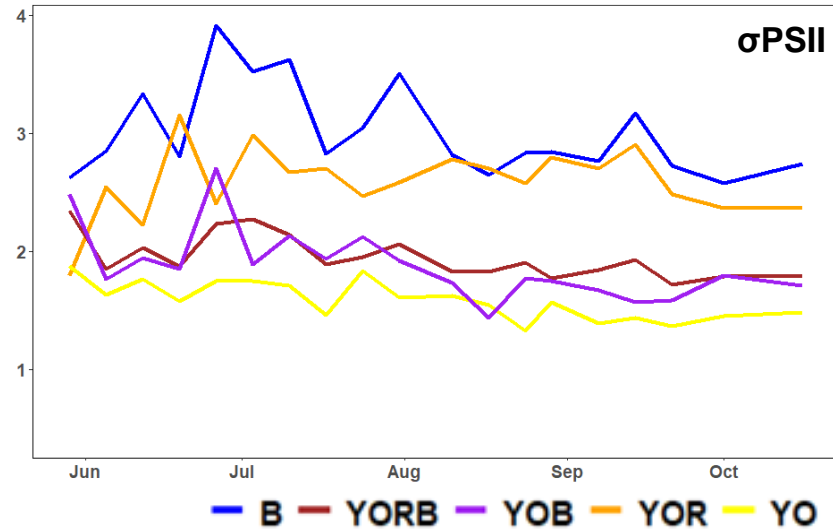
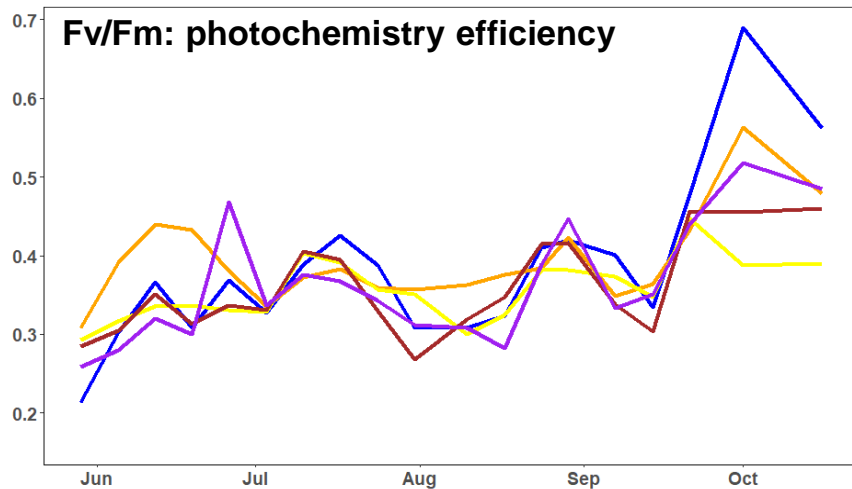
- ▶ Higher σ PSII values in shorter wavelengths at the beginning of the sampling period in YOR and B protocols
- ▶ Higher σ PSII values in longer wavelengths in the YOR protocol only towards the end of the sampling period
 - ▶ YOR and B protocol target different community responses and seems to follow the trends of algae vs cyanobacteria abundance

PSII saturation and productivity parameters

Cyanobacteria and Algae cell counts



Fv/Fm: photochemistry efficiency



- ▶ **Saturation obtained (AlphaPll between 0.05 and 0.07) with three protocols only**
- ▶ **Fv/Fm increase over the sampling period, protocols give similar results, but small differences are observed in B and YOR protocols**
- ▶ **σPSII show an increase at the beginning of the sampling period, YOR and B protocols have higher values than with other protocols**

Conclusion

- ▶ **YOR and B protocols target different communities responses** but...
 - YOR protocol is not able to fully saturate PSII
 - B protocol is also susceptible to induce a fluorescence signal by cyanobacteria with the 452 nm excitation LEDs

- ▶ **Those results are preliminary** and the fluorescence parameters should be compared to Chla concentration

- ▶ To correctly assess the presence of cyanobacteria vs. algae, **efficient Y, O and R LEDs (534, 594 and 622 nm respectively)** should be added to the LabSTAF to allow the saturation of cyanobacteria with YOR and YO protocols

- ▶ **Ratio of fluorescence recorded at 685 nm to fluorescence recorded at 730 nm** (under YOR and B protocols) should be compared to Chla concentration and phytoplankton counts

Challenge and priorities for next steps

Challenge: Accurate estimation of biomass and physiology of cyanobacteria in natural samples

Next steps to achieve the challenge:

- ▶ **in 1 year:** Measurements of fluorescence emission at 685 nm and 730 nm (*LabSTAF*), measurements with the ameliorated version of the *LabSTAF* (new excitation LEDs)- *My work until the end of the PhD*
- ▶ **in 5 years:** Continuous measurements with the *LabSTAF* during blooms periods in the field, measurements of fluorescence in oceanic and freshwater environments to test the sensitivity range of the instrument
- ▶ **in 10 years:** Determination of optical properties able to discriminate cyanobacteria in mixed communities, and consequently, modification of *LabSTAF* optical features