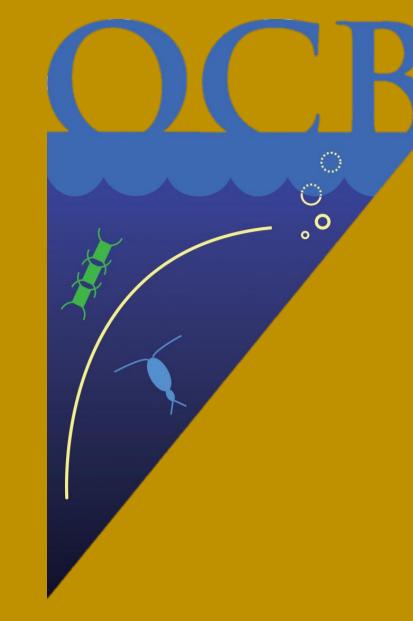
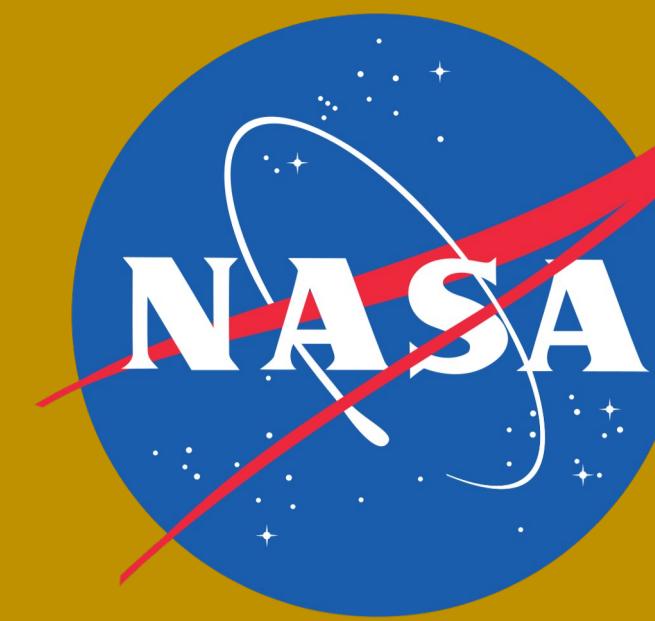
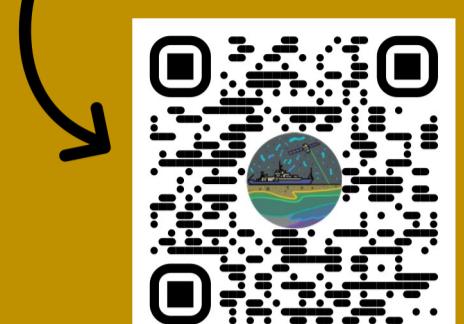


# Diel $b_{bp}$ Cycles in Cultured Phytoplankton



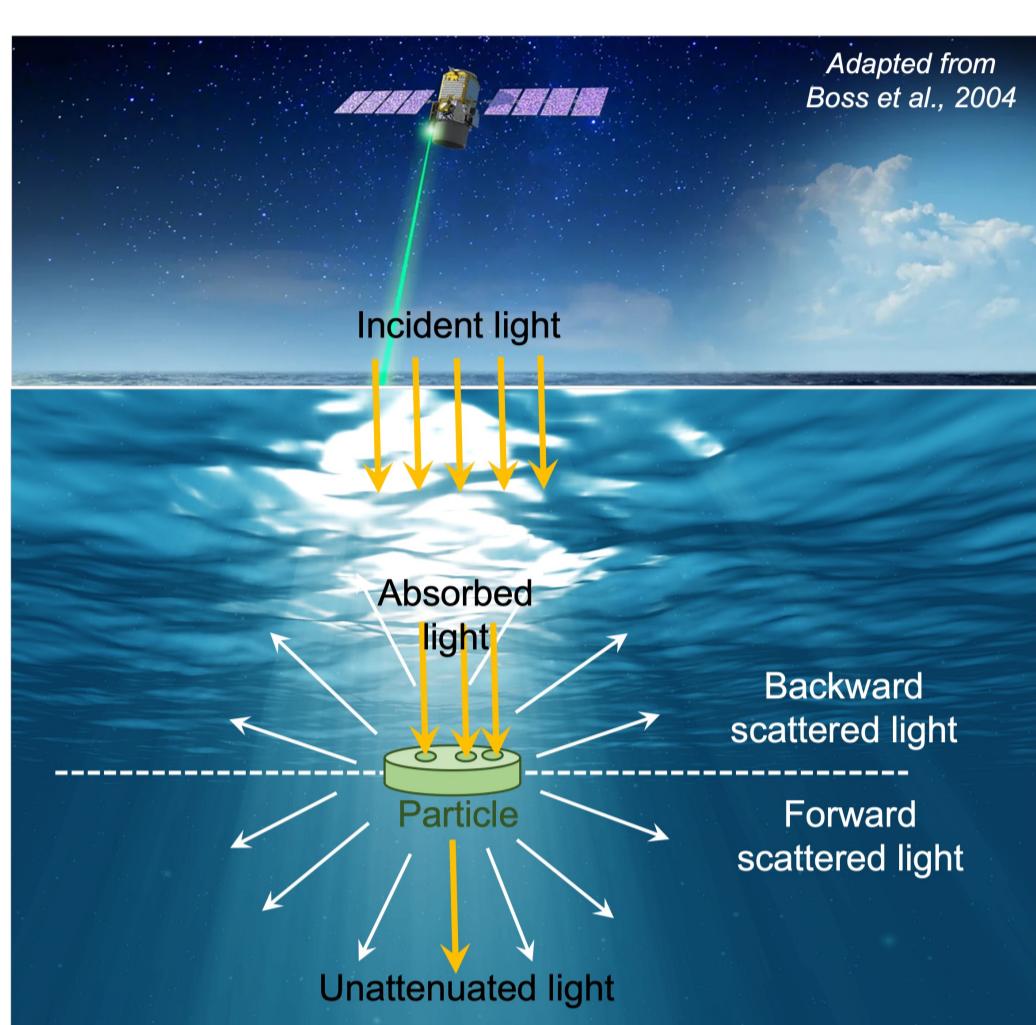
\*Homepage & Contact

Nicholas Baetge\*, Kim Halsey, Jason Graff, Brian Ver Wey, Toby Westberry, Amanda Appel, Guillaume Bourdin, Charlotte Begouen Demeaux, Emmanuel Boss, & Michael Behrenfeld



## Background & Questions

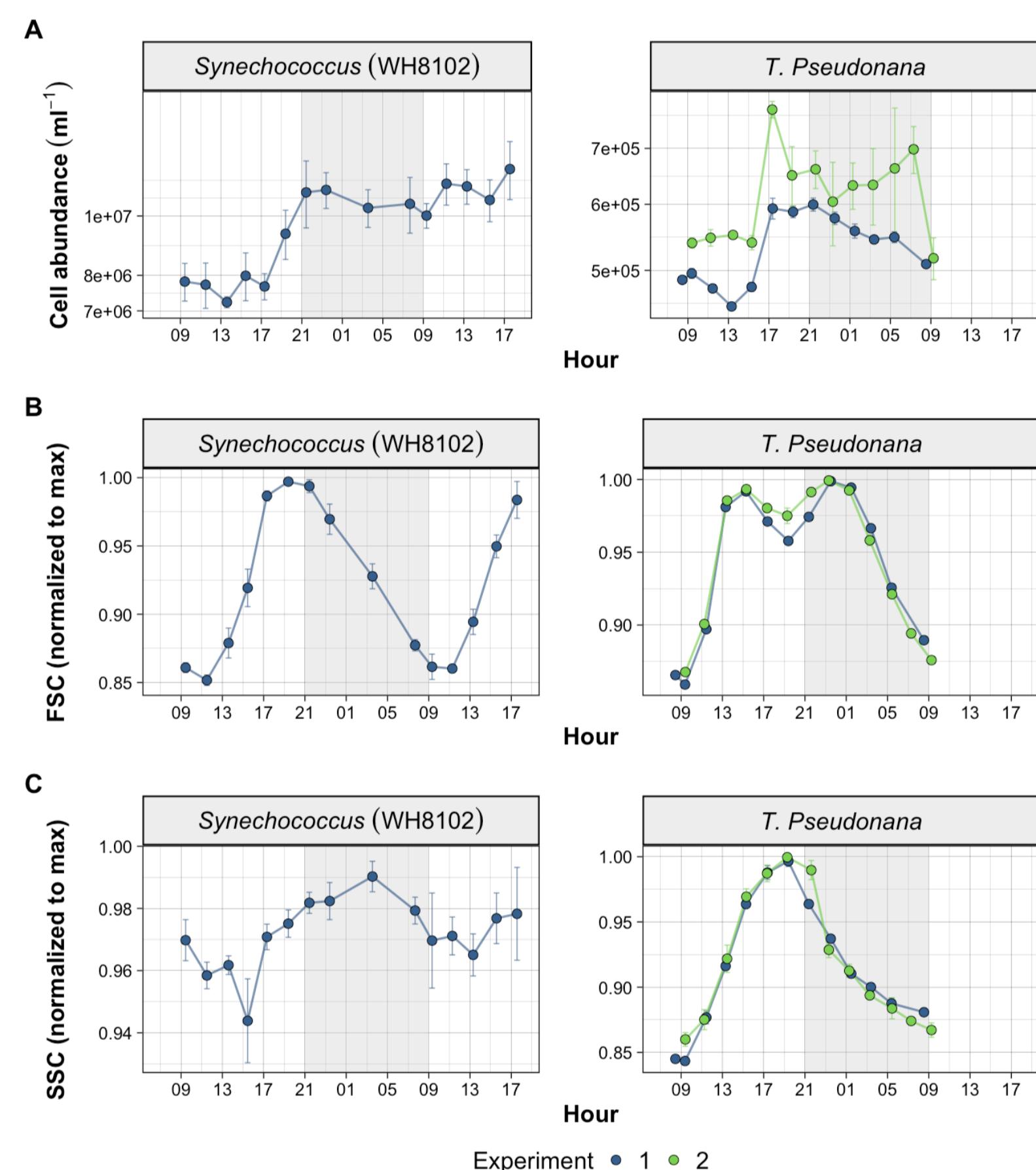
Photons scattered by particles in the backwards direction ( $b_{bp}$ ) have the potential to leave the ocean surface and be detected by remote sensing.  $b_{bp}$  is a proxy for particle abundance but is also dependent on particle refractive index. Global  $b_{bp}$  has been reported to vary over diel timescales and has been shown to increase in laboratory cultures from sunrise to sunset.



- Q1: What is the contribution of phytoplankton to  $b_{bp}$  variability over a complete diel cycle?
- Q2: How does this contribution vary across species?

**Figure 1.** Attenuation of light in the surface ocean.

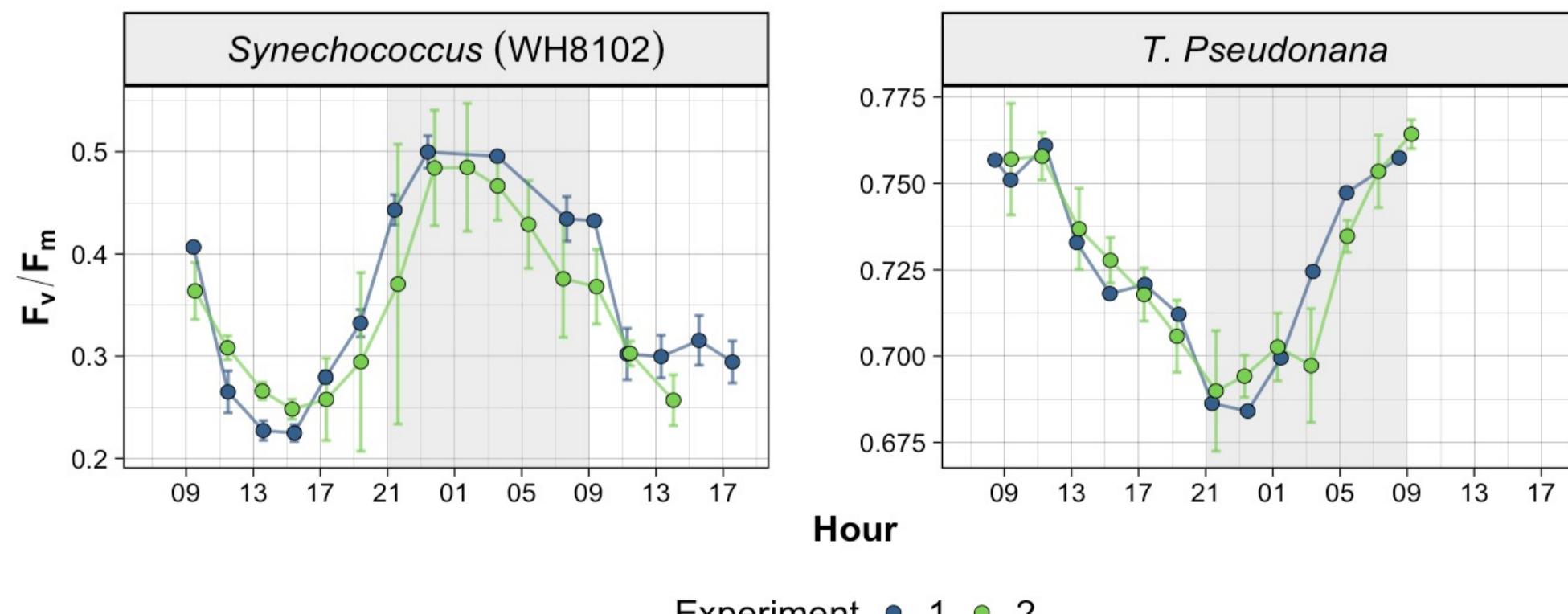
## Synchronized Cell Division



**Figure 3.** Cell abundance (A), forward scatter (FSC; B) and side scatter (SSC; C) per cell. Unshaded and shaded areas denote day/night (dawn = 900, noon = 1500, dusk = 2100). Error bars denote standard deviation from mean of biological replicates for each experiment.

- Both phytoplankton displayed synchronized cell division, with SYN division ( $\mu = 0.43 \text{ d}^{-1}$ ) occurring over shorter span than TP ( $\mu = 1.1 \text{ d}^{-1}$ ). Note: TP cultures were diluted to starting concentrations when  $\mu > 0$  (TP division between 1700 and 0900).
- FSC (cell size proxy) increased during the day and decreased during night-time division and respiration.
- SSC (proxy for cell complexity and granularity) showed similar diel cycle for TP but out of phase with division for SYN.

## Photosynthetic Efficiency



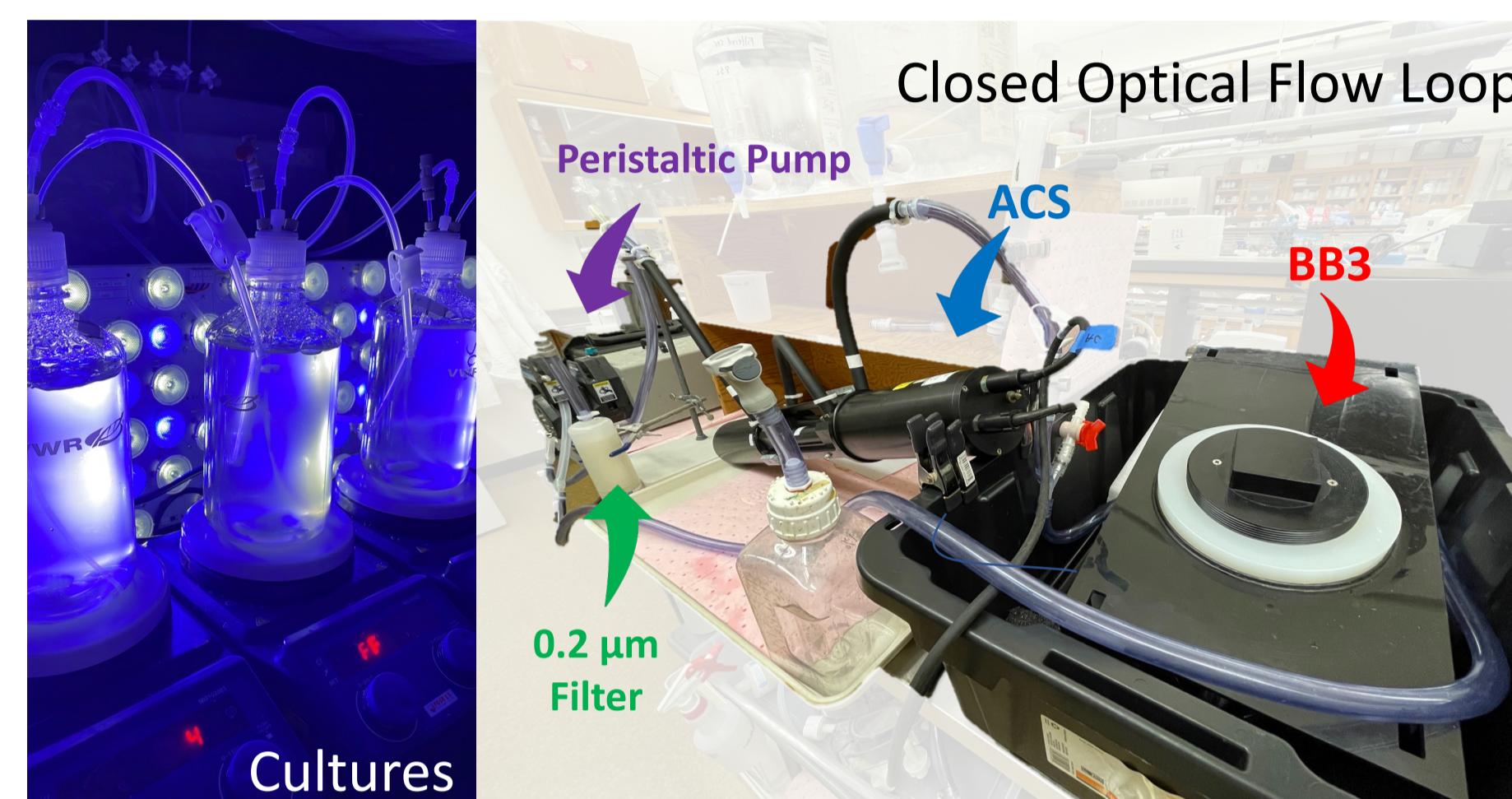
**Figure 6.** Variable to maximum fluorescence ( $F_v/F_m$ ) of photosystem II, an indicator of photosynthetic efficiency, varies in response to available light for both phytoplankton. Error bars denote the standard deviation of the mean of biological replicates for each experiment.

This work was funded by NASA award # 80NSSC21K0414. We thank Joan Hudson for her help with SEM and the Giovannoni Lab for use of the flow cytometer. This work has benefited from discussions with the Halsey Lab and Ali Chase.

## Hypotheses & Approach

H1: Diel variations of  $b_{bp}$  will reflect increases in phytoplankton size and organic carbon content during the day and decreases during the night due to division and respiration.

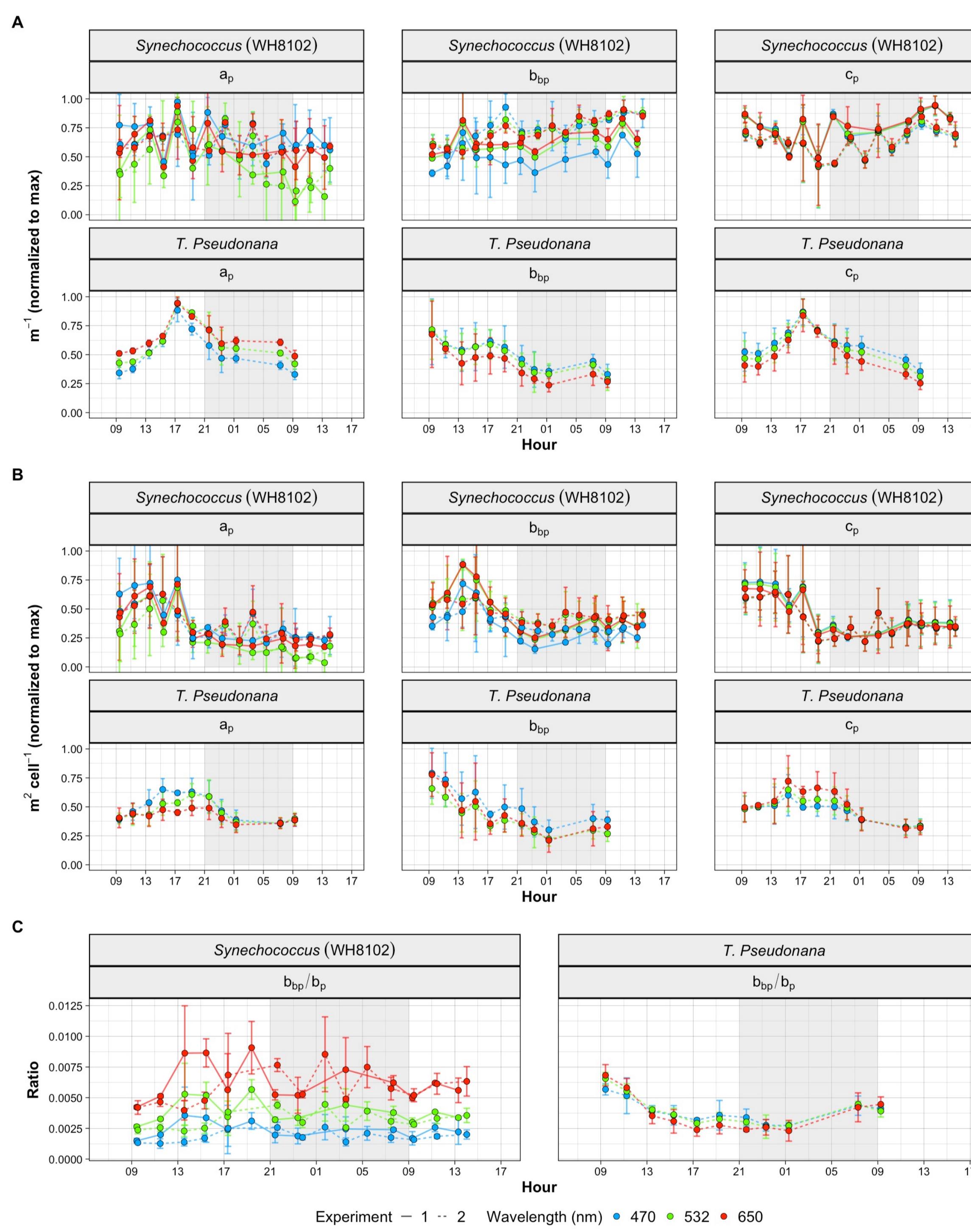
H2: The magnitude and timing of diel changes in  $b_{bp}$  will be heavily influenced by the degree of cell cycle synchronization, which can be greater for some species relative to others.



- Complete diel cycles characterized for *Thalassiosira pseudonana* (TP) and *Synechococcus* (WH8102; SYN), grown in nutrient-replete conditions at 18°C under 12:12 h sinusoidal light: dark period (maximum intensity of 400  $\mu\text{E m}^{-2} \text{s}^{-1}$ ).
- Particulate absorption ( $a_p$ ), attenuation ( $c_p$ ), and  $b_{bp}$  measurements collected using a WET Labs ACS and ECO-BB3 every 2 hours for at least 24 hours.
- Ancillary samples for cell enumeration and scattering via flow cytometry; photophysiology via fast-repetition rate fluorometry (FRRF); cell organic carbon and nitrogen; cell macromolecular composition; SEM.

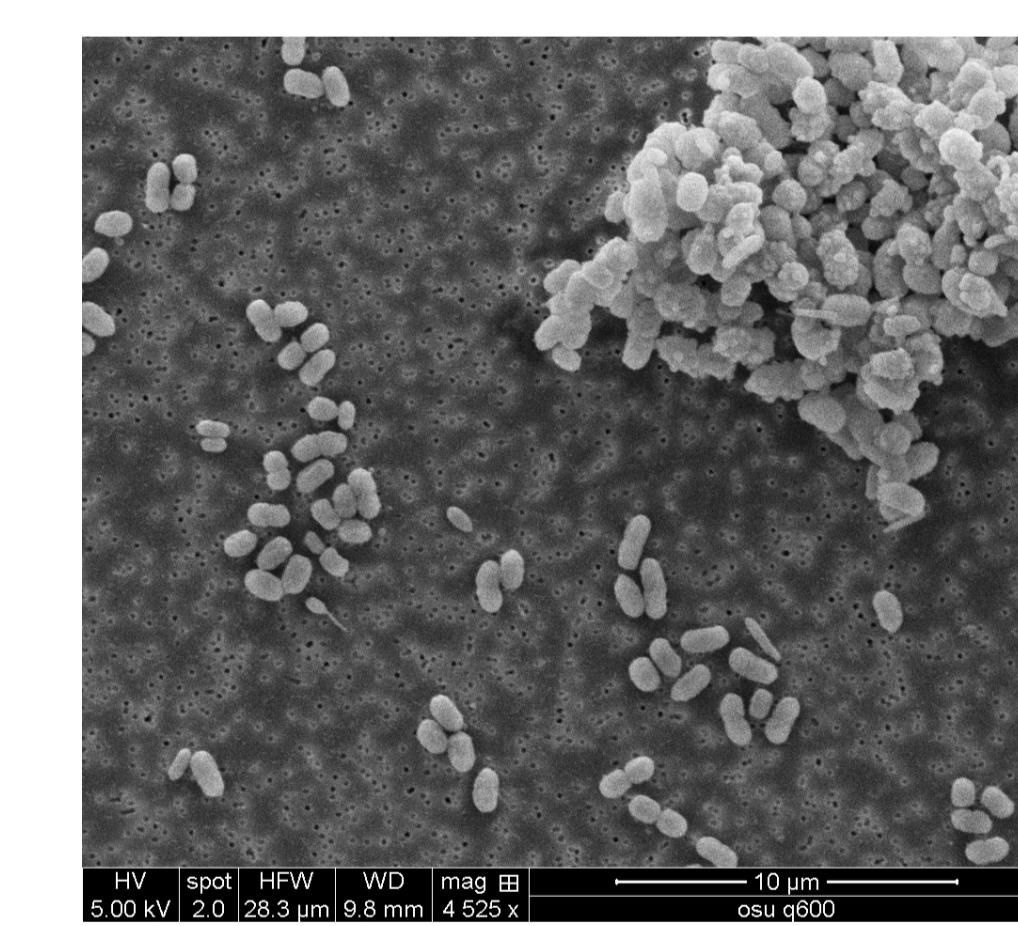
**Figure 2.** Culture conditions (left) and sampling set up of optical instruments (right).

## Diel Cycles in Optical Properties



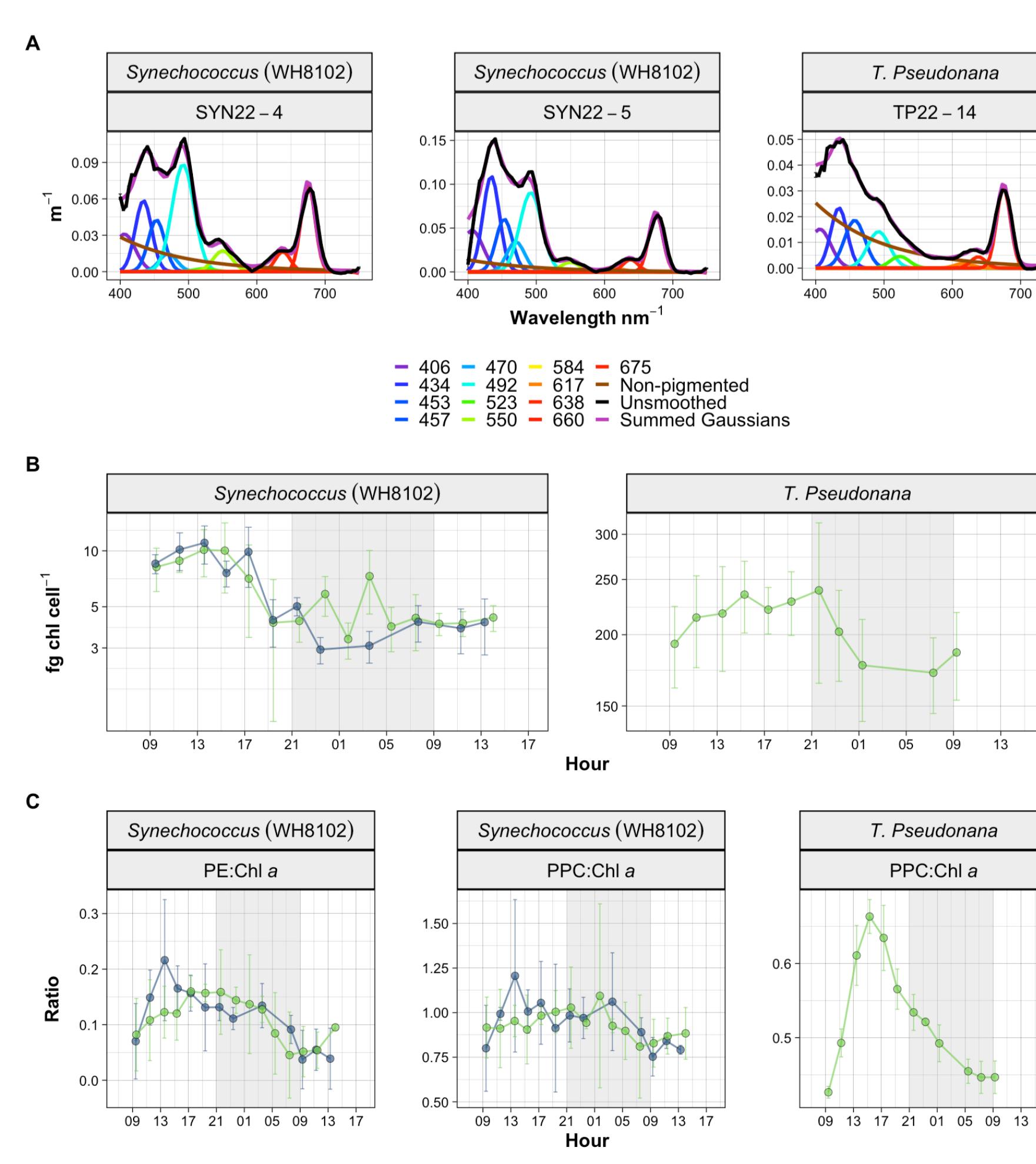
**Figure 4.** Bulk (A) and cell concentration-normalized (B) properties of  $a_p$ ,  $b_{bp}$ , and  $c_p$ . (C) Backscattering efficiency ( $b_{bp}/b_p$ ). Error bars denote the standard deviation from mean of biological replicates for each experiment.

- Bulk optical properties showed diel variability for only TP, while normalized properties show similar patterns between species.
- Coefficients are maximal prior cell division during the day and decrease following division, reaching minimums during the night.
- Changes in  $a_p$  and  $c_p$  are more pronounced for SYN while changes in  $b_{bp}$  are comparable between the two phytoplankton.
- $b_{bp}/b_p$  varies over the diel cycle for TP but not for SYN.
- Little to no spectral dependence for any of the properties for either phytoplankton, except for  $b_{bp}/b_p$  for SYN.



**Figure 5.** Scanning Electron Microscopy (SEM) image of *Synechococcus* from 0500.

## $a_p$ Spectral Decomposition & Pigment Ratios



**Figure 7.** Examples of Gaussian decomposition of absorption spectra (A). Cell-normalized chlorophyll concentrations estimated from absorption line height at 676 nm. (B) Ratios of Gaussians for phycoerythrin (PE, 550 nm), photoprotective carotenoids (PPC, 492 nm), and chlorophyll a (Chl a, 675 nm). Error bars denote the standard deviation of the mean of biological replicates for each experiment.

- Cell-normalized chlorophyll follows pattern of cell division, with a 5 – 6 h lag for TP.
- Diel patterns in pigment ratios follow changes in light intensity and are dampened for SYN relative to TP.

## Conclusions

- Diel variations in  $b_{bp}$  generally reflect changes in the cell cycle and photophysiology. However, the timing of  $b_{bp}$  peaks and valleys can be out of phase with cell abundance, photosynthetic efficiency, and pigment ratios.
- The pronounced diel changes in phytoplankton  $b_{bp}$  suggests that time of day is an important consideration for interpreting satellite  $b_{bp}$  retrievals.