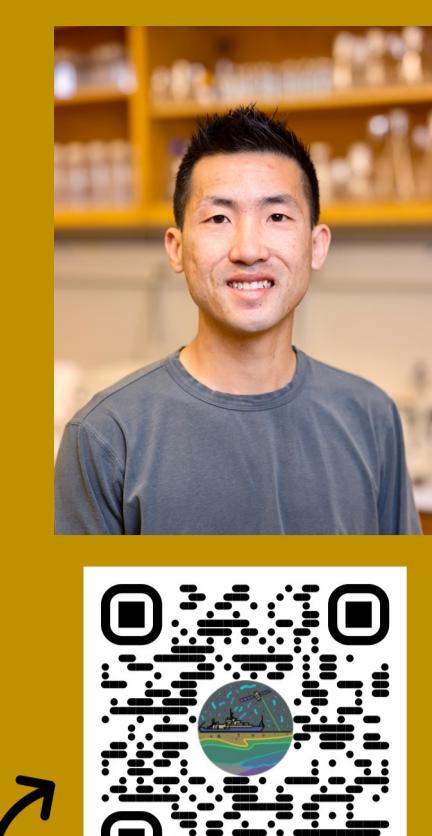
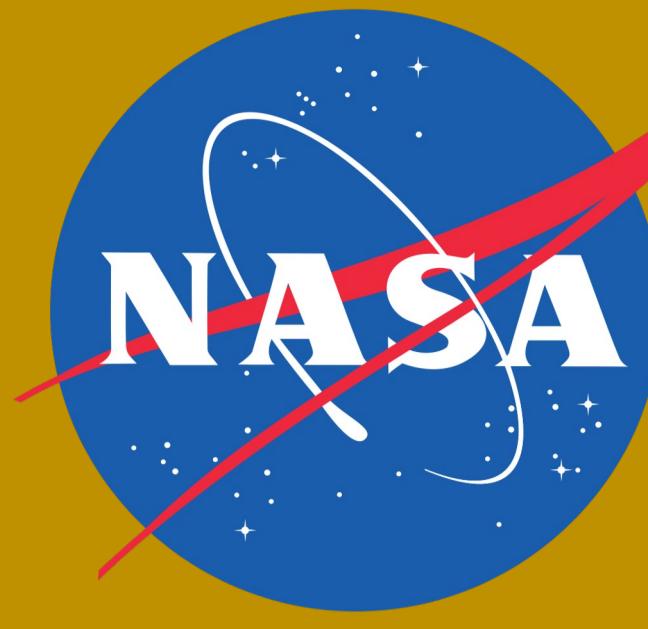


# Diel $b_{bp}$ Cycles in Cultured Phytoplankton

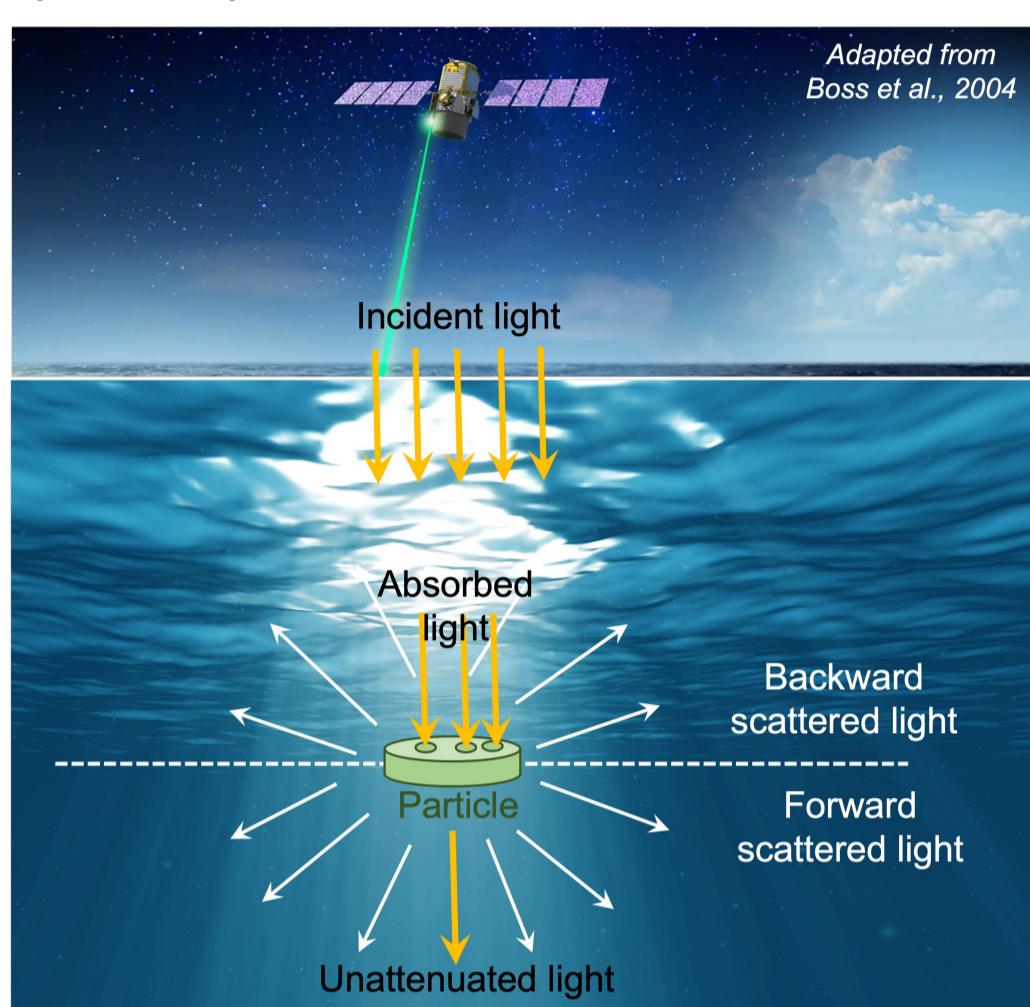
Nicholas Baetge\*, Kim Halsey, Jason Graff, Brian Ver Wey, Toby Westberry, Amanda Appel, Guillaume Bourdin, Charlotte Begouen Demeaux, Emmanuel Boss, & Michael Behrenfeld



\*Homepage & Contact

## Background & Questions

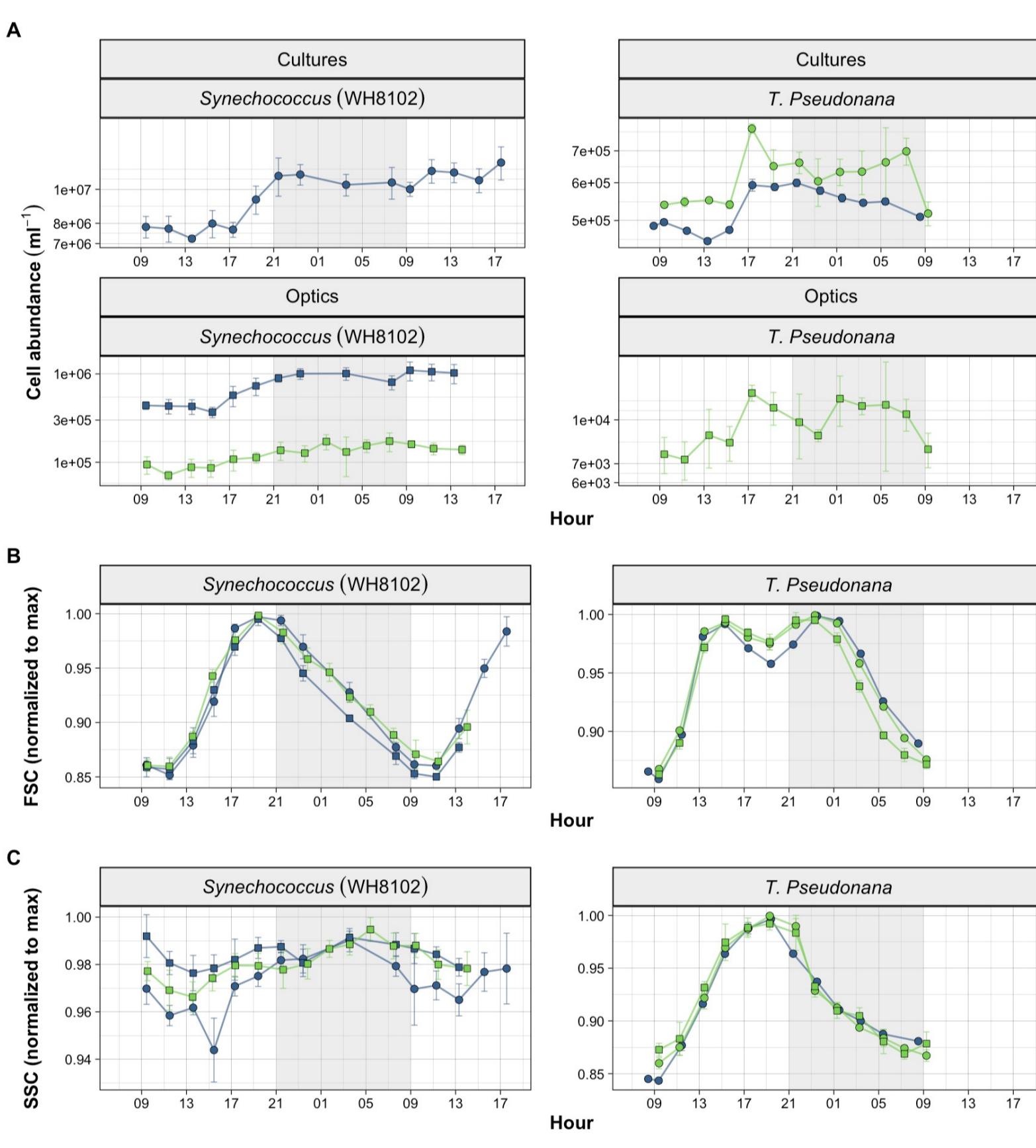
Photons scattered by particles in the backwards direction ( $b_{bp}$ ) have the potential to leave the ocean surface and be detected by remote sensing.  $b_{bp}$  is a proxy for particle abundance but is also dependent on particle refractive index. Global  $b_{bp}$  has been reported to vary over diel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

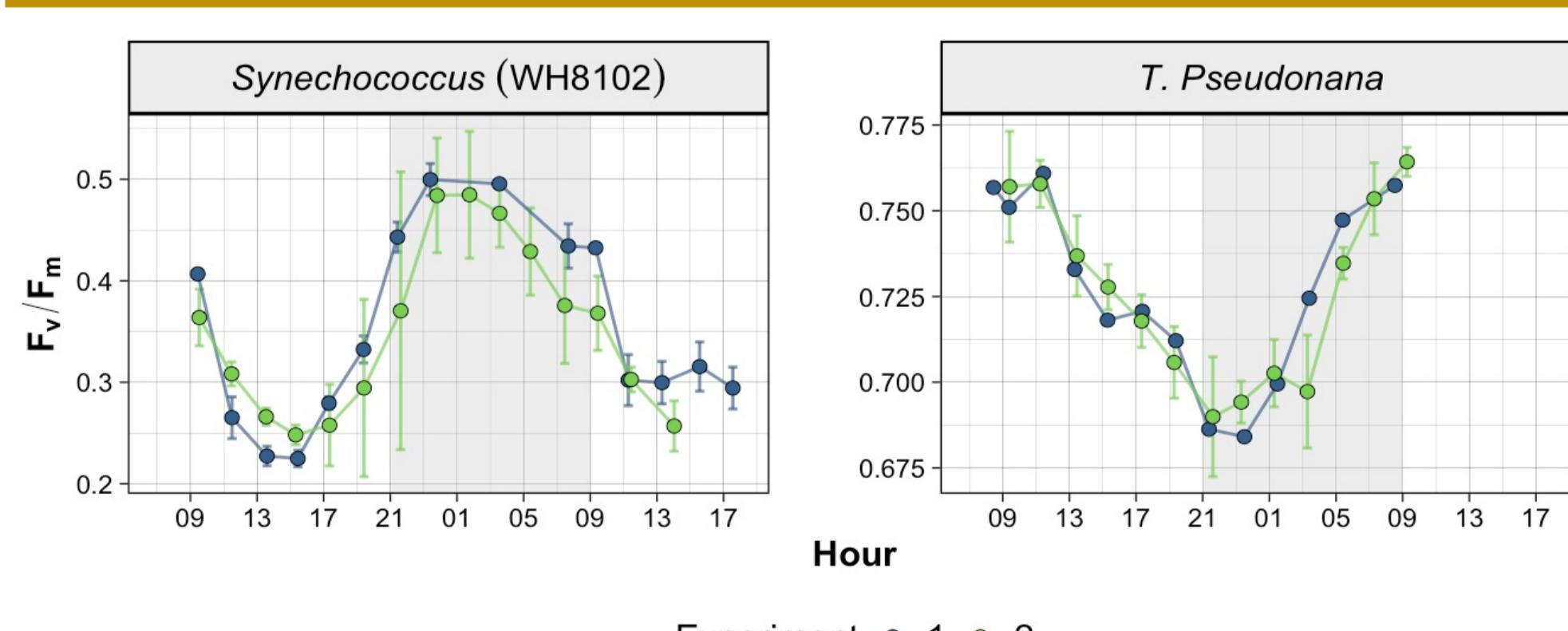
## Synchronized Cell Division



**Figure 3.** Cell abundance in the cultures and optics instruments (A), forward scatter (FSC; B) and side scatter (SSC; C) per cell. Unshaded and shaded areas denote day/night (dawn = 9:00, noon = 15:00, dusk = 21:00). Error bars denote standard deviation from mean of biological replicates for each experiment.

- Both phytoplankton species displayed synchronized cell division, with SYN division ( $\mu = 0.43 \text{ d}^{-1}$ ) occurring over a shorter time 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 (changes with size, but more sensitive to cell complexity and granularity than FSC) showed similar diel cycle for TP but out of phase with division for SYN.

## Photosynthetic Efficiency



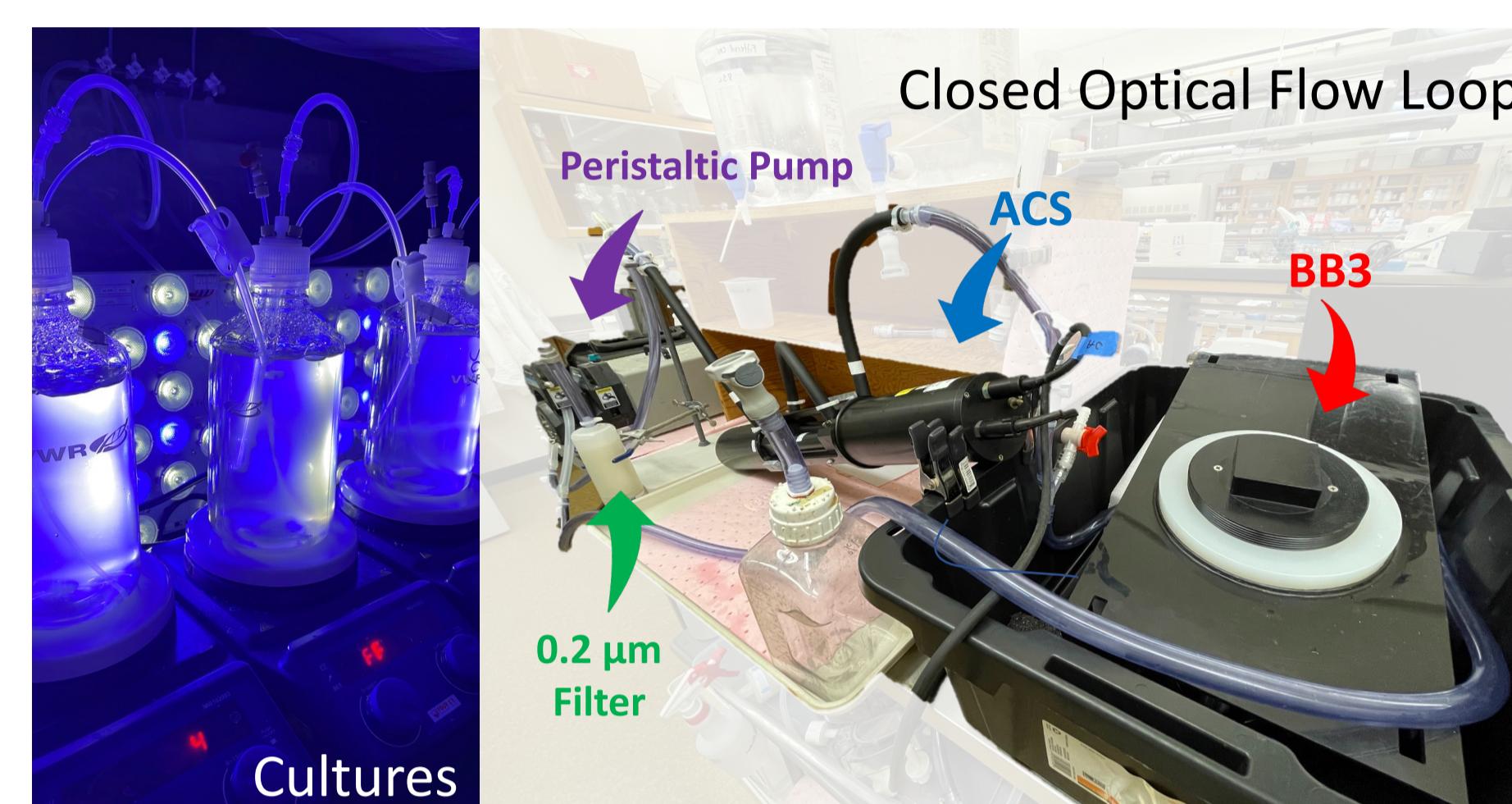
**Figure 6.** Variable to maximum fluorescence ( $F_v/F_m$ ) of changes in response to available light for both phytoplankton. Error bars denote the standard deviation of the mean of biological replicates for each experiment.

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## 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.

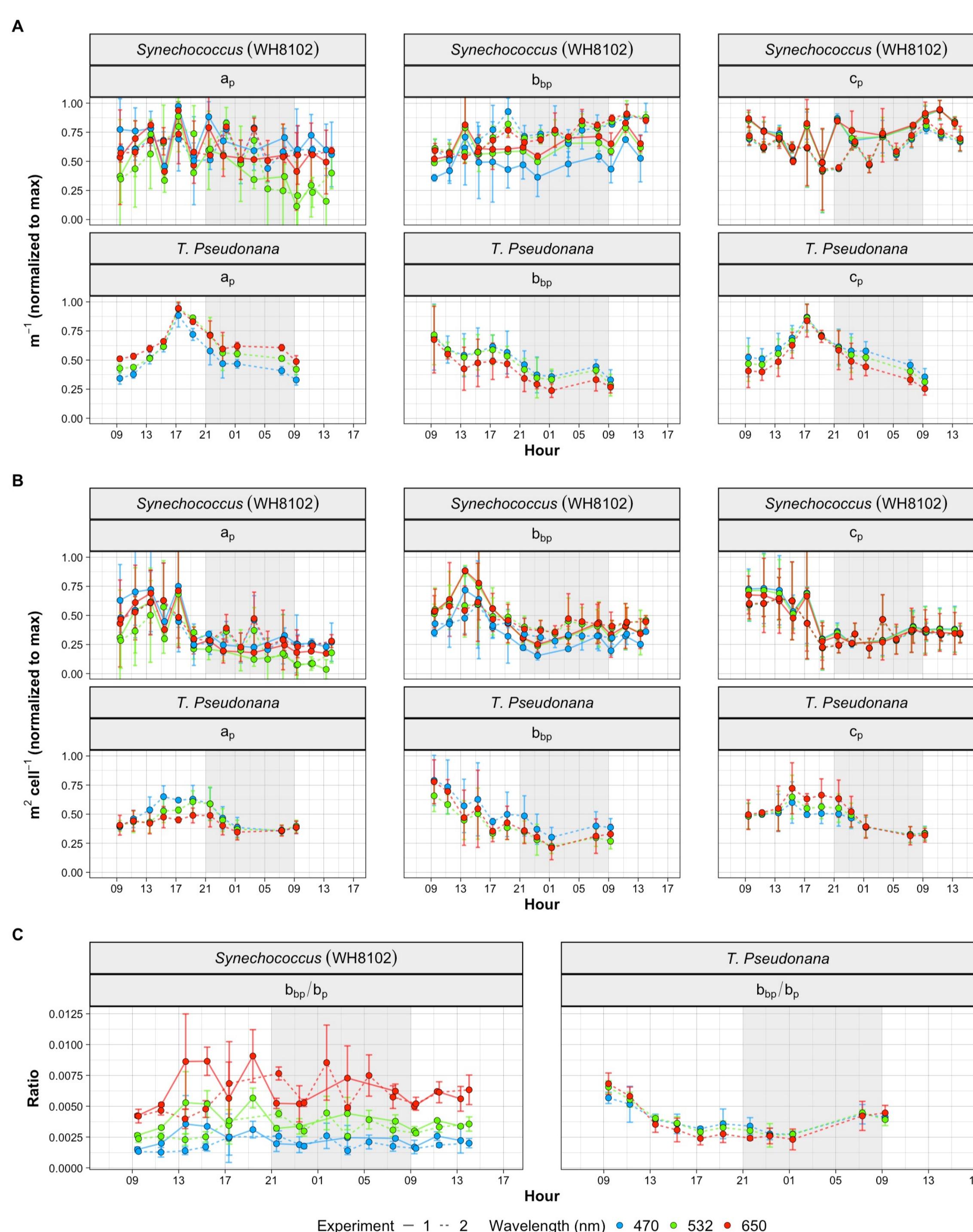
H2: The magnitude and timing of diel changes in  $b_{bp}$  will be heavily influenced by the degree of cell cycle synchronization, which can be greater for some species relative to others.



- 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.

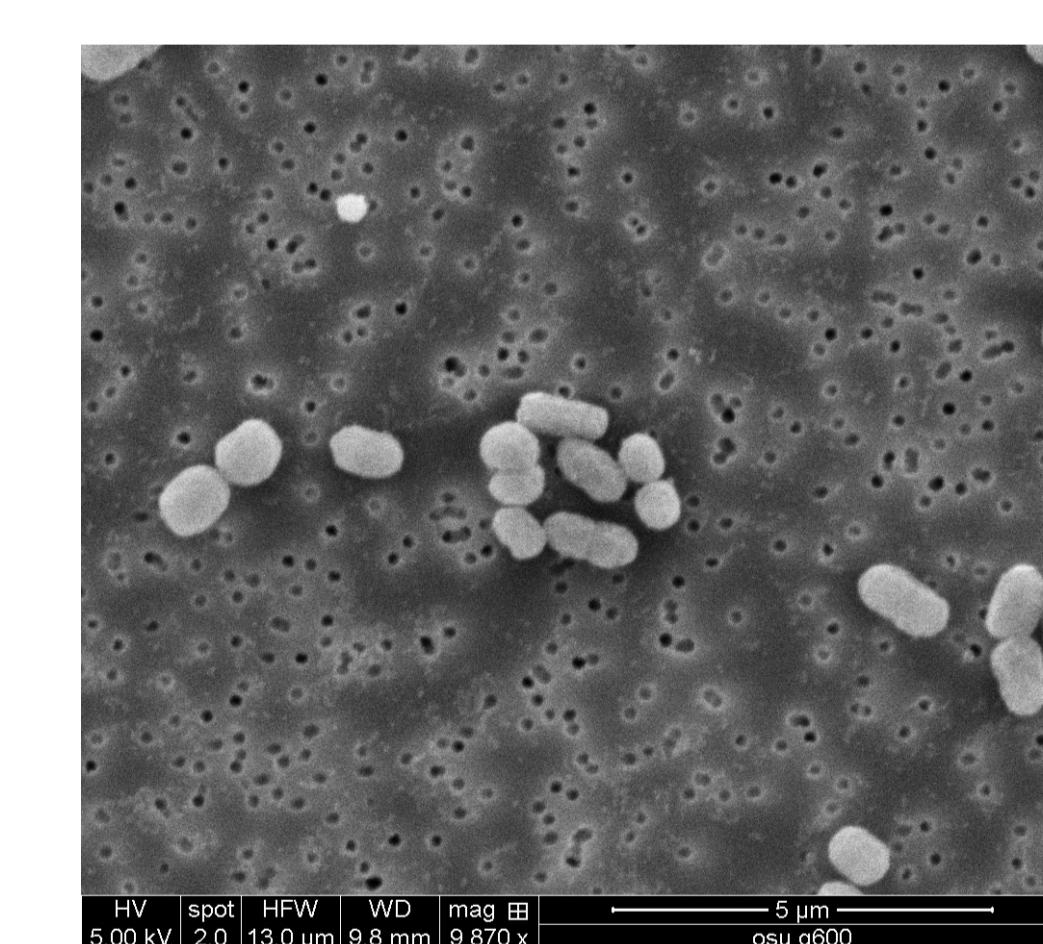
**Figure 2.** Cultures in incubation room (left) and sampling set up of optical instruments (right).

## Diel Cycles in Optical Properties



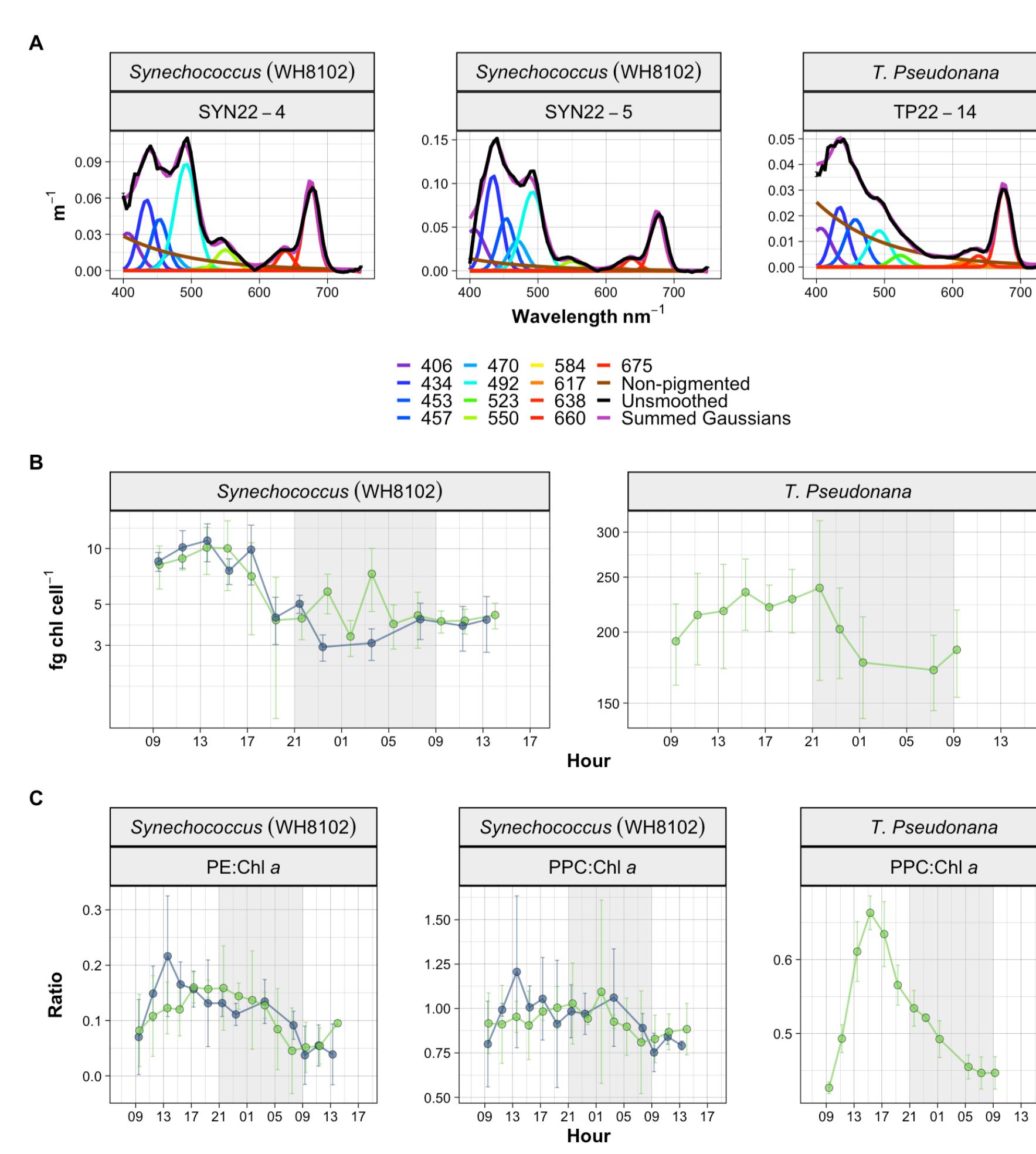
**Figure 4.** Bulk (A) and cell concentration-normalized (B) properties of  $a_p$ ,  $b_{bp}$ , and  $c_p$ , (C) Backscattering efficiency ( $b_{bp}/b_p$ ). Error bars denote the standard deviation from mean of biological replicates for each experiment.

- Bulk optical properties showed diel variability for only TP, while normalized properties show similar patterns between species.
- Coefficients are maximal prior to cell division during the day and then 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 05:00.

## $a_p$ 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 (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 h to 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.