Three-Channel Fluorescence to Characterize Harpswell Sound Phytoplankton Community Dynamics

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Abstract:

Chlorophyll fluorescence is a common method of estimating in-situ phytoplankton biomass. Unlike terrestrial plants, phytoplankton evolved the ability to synthesize different pigments for different parts of the light spectrum, depending on their depth. The presence or absence of certain pigments in different species will affect their absorption of varying wavelengths of light. Each wavelength is absorbed in varying amounts, which directly relates to the fluorescence response. Comparing the relative fluorescence response of the three wavelengths gives insight into the species makeup at a current time. Previous work by Luke Carberry (Bowdoin '18) uncovered how higher biomass can occur at both high and low tide, and my work this summer was focused on analyzing these populations to determine how species makeup varies with tides.

Objectives:

My goal this summer was to analyze how the phytoplankton species makeup changes in Harpswell Sound as a result of tidal influence. I used a three-channel fluorometer on the Land-Ocean Biogeochemical Observatory (LOBO) buoy. I also wanted to gain field and lab experience, and improve at using MATLAB for data visualization and interpretation.

Methods:

A 3-excitation 1-emission (3X1M) fluorometer on Dr. Roesler's Land-Ocean Biogeochemical Observatory (LOBO) buoy in the Harpswell Sound has taken hourly observations since its installation in 2014, along with tidal velocity, irradiance, temperature, and salinity. Raw data is taken in millivolts, so I therefore applied a separate calibration to each wavelength to convert fluorescence response to a chlorophyll concentration in µg/L. Each fluorometer must be calibrated individually due to small variations in their build: In the lab, a dilution series with diatoms was set up with linearly increasing cell concentrations. Due to the diatom's pigments, each wavelength of fluorescence response increases at a different linear rate as cell concentration increases, yielding different slopes (fig. 1). Now, say we're looking at a diatom in-situ (one species of phytoplankton); since the calibration slopes were set up with a diatom, the fluorescence response for each wavelength will yield the same Chl concentration (x-axis). Or, the ratios of FChl 470/FChl 440 (fluorescence-derived Chl, specific to each wavelength) and FChl 532/FChl 440 are both 1. However, a chlorophyte, for example, has a different set of pigments that allow it to absorb more from the 532 nm wavelength only. As a result, the fluorescence response from 532 nm will be higher, yielding a greater Chl concentration according to the calibration slope. The ratio of FChl 532/FChl 440 is now >1, so this ratio is a proxy for identifying the presence of different species. Note: Because Chl a is found in all phytoplankton and its peak absorption is at 440 nm, FChl 440 is decided to be the denominator to make comparison between species easier.

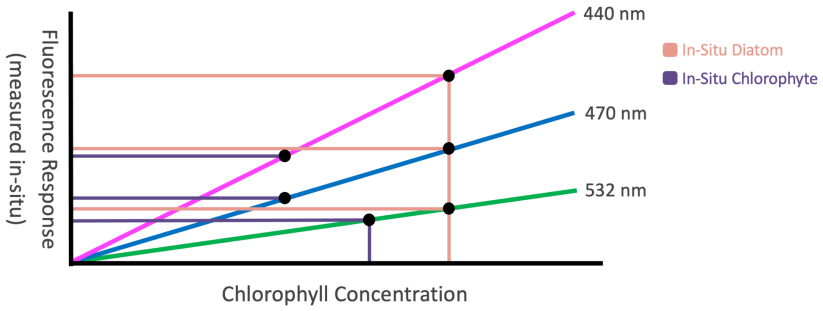


Fig. 1. Example of how calibration works for three wavelengths

Due to the spatial variability in Harpswell Sound caused by tides, quenching cannot be corrected by simply interpolating between night-time fluorescence values (this works for only areas with little tidal movement). Instead, the model I used to correct for NPQ uses LOBO's tide and irradiance data to find all the fluorescence values at night-time tidal maxima and minima, where quenching is not a factor (Carberry et al. 2019). It then uses a cosinusoidal model to interpolate between these data points and tidally resolve them, with two fluorescence maxima and minima per day, following tidal advection. I now have the opportunity to apply this model to the 3X1M fluorometer instead of a single wavelength, allowing me to obtain insights about phytoplankton composition instead of only concentration.

Results:

- Higher fluorescence, and therefore phytoplankton concentration (or biomass), occurs at both high or low tide (fig. 3a).
- Tidal influence on 3-channel fluorescence between high and low tide can be caused by both a varying amount of phytoplankton and a changing species composition (fig. 2b).
    - There are times at which the concentration at high and low tide hardly change, yet the species of phytoplankton changes distinctly (figs. 3b & 3c). From July 21 – 23, fluorescence ratios during high tide continued returning to the same values, indicating a recurring population. However, the low tide population fluorescence ratios changed for each cycle, indicating each low tide brought a different set of species.
    - July 17 likely saw a bloom of a particular phytoplankton; while biomass continued to vary between high and low tide, both fluorescence ratios remained constant.
- Fluorescence maxima and minima don't necessarily occur at exactly high and low tide. A negative residual indicates a correction for quenching, yet a positive residual means biomass was higher at a time other than high or low tide.
- Throughout summer 2021, spring tides tended to align with smaller changes in concentration between high and low tide and neap tides brought larger variation.

Discussion:

Harpswell Sound's rare reverse estuarine circulation, with the head being the saltiest, leads to interesting phytoplankton community dynamics. The sound is not closed, with gaps to adjacent bays throughout. Low tide having a higher biomass indicates a high population at the salty head of the sound, or in adjacent bodies of water that get sucked into Harpswell. A higher biomass at high tide indicates the fresh Kennebec river water or surface ocean layer contained a larger population, but we need to know the location of the tidal mixing front to determine the corresponding water mass (the point at which salty and fresh water is no longer stratified due to tidal and wind mixing). During this summer's sampling cruises, we identified the tidal mixing front to be north of LOBO some days, and south of it on other days. Whether phytoplankton in one water mass have mixed with another will have significant effects on both composition and concentration observed by LOBO. During July 2021, spring tides (full & new moon) tended to align with times of less variation in biomass between tides. Maybe, a spring tide brings phytoplankton (of multiple compositions) closer to the head of the sound, so when the tide goes out the population isn't moved fully past LOBO, keeping biomass relatively constant.

Although the focus of my project was on finding the tidal effect of three wavelength fluorescence, mid-tide populations are still very much affected by water movement yet are ignored by

the tidal correction model I used. Much of the correction residual was negative, indicating quenching correction, but the positive regions indicate a higher biomass at mid-tide rather than at tidal maxima. Resolving for tidal influence may ignore significant peaks in fluorescence from other water masses that may reflect additional populations. Because of the complexity of the coastline, there are multiple distinct water masses that all mix together and certain significant populations may never be at LOBO at tidal extrema. However, using this model was essential in correcting for quenching as the daily drop in fluorescence at solar noon would've prevented me from seeing a true tidal influence time series. Determining the exact taxonomy from solely 3-wavelength fluorescence in-situ isn't viable because the 3X1M looks at a volume of water in front of the sensor and measures its entire fluorescence response, not just a single phytoplankton. Instead, I would need to combine my data with the Imaging FlowCytobot (IFCB) at the Coastal Studies Center to identify the exact species of phytoplankton and see how fluorescence reacts to blooms, grazing, etc.

Because I used LOBO data from 2021, since the 3G modem was recently disabled, I was unable to look at immediate weather influences on fluorescence.

Figures:

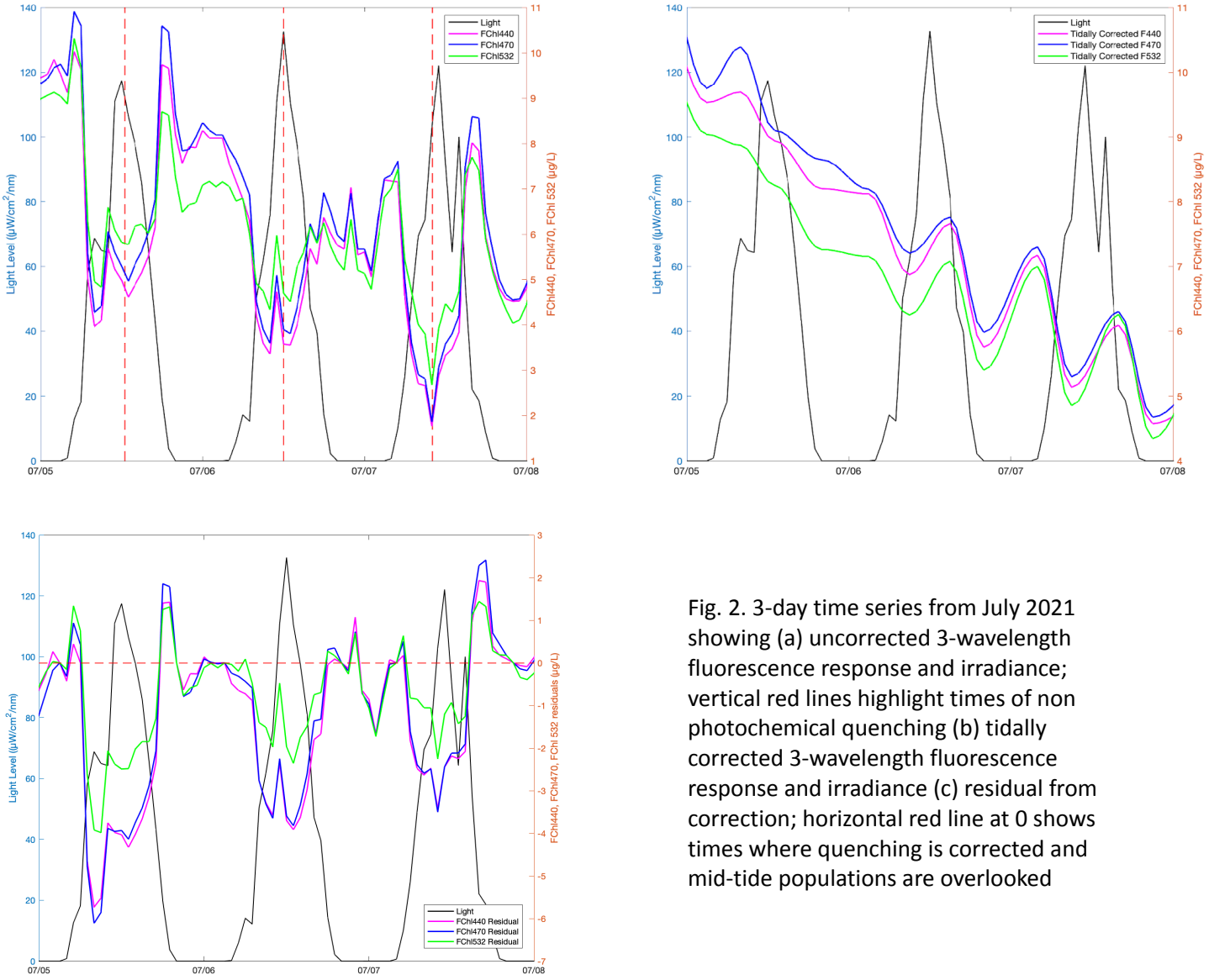
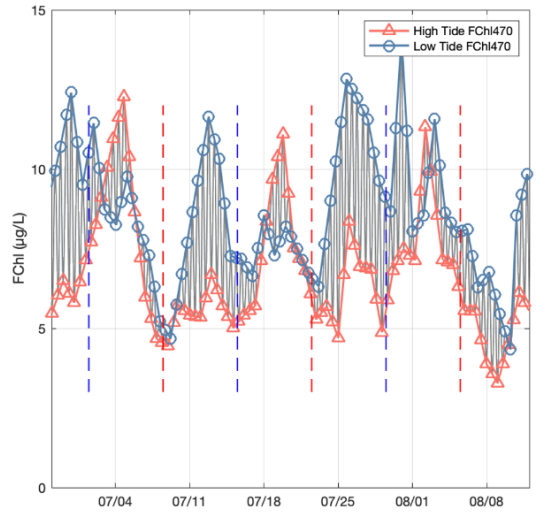
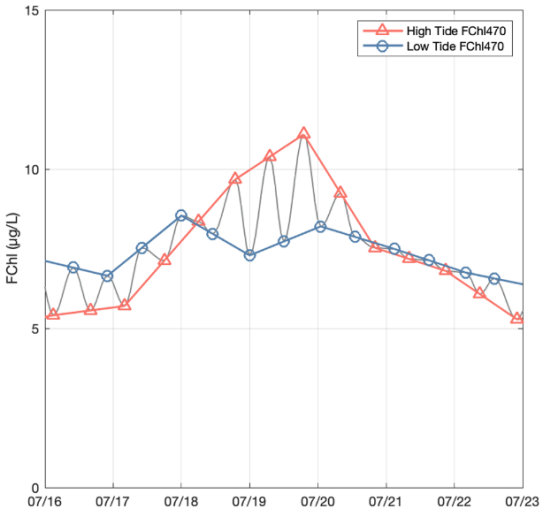


Fig. 2. 3-day time series from July 2021 showing (a) uncorrected 3-wavelength fluorescence response and irradiance; vertical red lines highlight times of non photochemical quenching (b) tidally corrected 3-wavelength fluorescence response and irradiance (c) residual from correction; horizontal red line at 0 shows times where quenching is corrected and mid-tide populations are overlooked





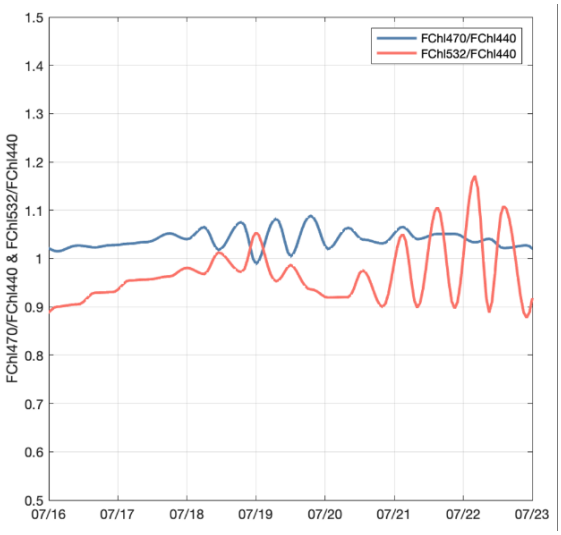


Fig. 3. Time series from July 2021 showing (a) 6 weeks of tidally corrected Fchl at tidal maxima; spring tides are red dashed lines, neap tides are blue (b) 1 week of tidally corrected Fchl at tidal maxima (c) 1 week of Fchl ratios, showing taxonomic variation

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References:

Carberry, L., Roesler, C., & Drapeau, S. (2019). Correcting in situ chlorophyll fluorescence time-series observations for nonphotochemical quenching and tidal variability reveals nonconservative phytoplankton variability in coastal waters. Limnology and Oceanography: Methods, 17(8), 462-473.