An analysis of phytoplankton fluorescence and photosynthetic pigments in Harpswell Sound

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Phytoplankton are integral to aquatic ecosystems because they are positioned at the bottom of the oceanic food web. They are thus responsible for feeding marine ecosystems. As photosynthesizing organisms, all phytoplankton contain chlorophyll a, a pigment synthesized within the chloroplast that allows phytoplankton to absorb energy from the sun and undergo photosynthesis. Because the spectrum of sunlight varies in the ocean, phytoplankton species evolved accessory pigments that work in conjunction with chlorophyll a to make use of the part of the spectrum to which they are exposed. As a result, some pigments serve as taxonomical markers of the phytoplankton in which they occur. Because pigments affect which wavelengths phytoplankton can absorb, they are also reflected in fluorescence. My research this summer focused on how phytoplankton community structure in Harpswell Sound varies temporally–focusing on a long-term data set of two years and a short-term data set of eight weeks–using fluorescence and pigments as a proxy for phytoplankton taxonomy.

Two key groups of phytoplankton common to Maine coastal waters are diatoms and dinoflagellates. Diatoms tend to bloom early in the year and can survive in turbulent waters. Dinoflagellates, on the other hand, prefer calm, stratified waters. Thus, diatoms tend to outcompete dinoflagellates when there are enough nutrients for both to exist. However, diatoms require silica to form their cell walls, and seasonally use up all the available silica. Once silica becomes a limiting nutrient, diatoms die off and dinoflagellates begin to enter the waters. This change in phytoplankton community structure poses an issue because several genera of dinoflagellates produce a marine biotoxin that accumulates in shellfish and can prove dangerous to humans. One such dinoflagellate is *Karenia brevis*, which produces brevetoxin, a toxin that hinders certain neurological processes. The ability to discern the transition of the phytoplankton community from diatoms to dinoflagellates would be a tremendous achievement for those agencies that monitor brevetoxin and is imperative to ensuring the safety of Maine residents. This is the phenomenon I investigated this summer, using fluorescence and pigments to understand the changing phytoplankton community structure in Harpswell Sound.

We sampled at four stations along Harpswell Sound on seven weekly cruises between May 31st and July 19th, 2022 (Fig. 1). Harpswell Sound is a reverse estuary located in Cumberland County and receives freshwater input from the Kennebec River. Saltier, denser ocean water, originally located at the surface, subducts beneath this freshwater during high tide. Harpswell Sound experiences semidiurnal tides, with two low and two high tides occurring each day. The tidal cycle of Harpswell Sound is crucial because it influences phytoplankton community structure, as distinct species are present in distinct water masses. We used a Niskin Bottle to obtain all water samples. At Station 0, we collected samples at 1 m and one other depth (usually at the fluorescence peak). For all the other stations, we collected samples at 1 m, 5 m, 10 m, and 20 m. Station 1 was Bowdoin's LOBO, a moored buoy collecting continuous data.

When light of a specific wavelength shines on phytoplankton, phytoplankton fluoresce in response to the wavelengths that they absorb. I analyzed the hourly observations from the 3X1M fluorometer that has been deployed at the Schiller Coastal Studies Center (SCSC) since 2020. This fluorometer measures fluorescence resulting from excitation channels at 440 nm, 470 nm, and 532 nm. Chlorophyll a, for example, fluoresces most at 440 nm. All our fluorescence data is corrected for nonphotochemical quenching (whereby phytoplankton adapt to high light conditions by dissipating excess energy) and calibrated to fit a diatom (with all channels being absorbed at a 1:1 ratio). Because fluorescence data is directly related to absorption, it can be used as a proxy for chlorophyll concentration. More specifically, we calculate chlorophyll concentration using the equation $C = (F – D)/S$ where C is chlorophyll concentration, F is fluorescence raw data, D is fluorescence of the instrument in

the dark, and S is calibration slope. Chlorophyll concentration can, in turn, be used as an indication of phytoplankton biomass.

High-performance liquid chromatography (HPLC) is a technique used to separate distinct components within a mixture. In the context of phytoplankton pigments, this technique is useful in identifying the various pigments in a filtered and extracted water sample. Our HPLC machine passes a water sample through a silica column made up of chains of 8 carbon atoms bonded to silica. Because different pigments have different polarities, they pass through this column at different rates. The time it takes for each pigment to flow through the silica column is known as the retention time. These retention times appear in a chromatogram, a graphical depiction of the results of the HPLC analysis (showing absorbance as a function of time). Retention times allow us to discern which pigments are present in our sample. Knowing pigments, in turn, allows us to deduce which phytoplankton groups may be present. Because some pigments are unique to the species in which they occur, we can conclude with relative certainty that these species are present. Peridinin, for example, occurs in all dinoflagellates. Gyroxanthin occurs in the toxic dinoflagellate, *Karenia brevis*. Water samples collected on our weekly cruises were filtered through a Whatman gf/f filter, extracted in 95% acetone, and then loaded into the HPLC machine.

In my investigation of the fluorescence time series beginning in September of 2020, when the 3X1M fluorometer was deployed at the SCSC, I observed a major phytoplankton bloom in February of 2021 (Fig. 2). This bloom was present in both high and low tide communities. A smaller time series from January 2021 to February 2021 indicates that fluorescence resulting from all three excitation channels peaked during this bloom, with the 532 nm channel indicating the highest chlorophyll concentration (Fig. 3a). After around February 7th, 2021, where the bloom begins (Fig. 3a), the ratio of chlorophyll concentration calculated from the 532 nm wavelength excitation channel over the chlorophyll concentration calculated from the 440 nm wavelength excitation channel increases (Fig. 3b). Analyzing a water sample collected on February 9th, 2021 using HPLC methods indicated that chlorophyll c2, fucoxanthin, diadinoxanthin, and alloxanthin were all present during this time (Fig. 4).

During my eight weeks of sampling this summer, I observed variation in the presence of chlorophyll a and accessory pigments in the water collected at LOBO based on date and depth. Chlorophyll a was highest during our second sampling trip and greatest at depth (Fig. 5a). Fucoxanthin and diadinoxanthin, characteristic of diatoms, similarly peaked on June 7th, 2022 (Fig. 5b). Gyroxanthin, unique to *Karenia brevis*, was also present at this time (Fig. 5c). Chlorophyll a spiked again during our last sampling trip (Fig. 5a). This spike corresponded with increases in diadinoxanthin, diatoxanthin, peridinin, and gyroxanthin (Fig. 5b, Fig. 5c).

The peak chlorophyll concentrations in February of 2021 can be attributed to a bloom of diatoms and cryptophytes. According to Kramer and Siegel (2019), alloxanthin is always present in cryptophytes and does not appear in any other phytoplankton taxonomic groups that we would expect to find in Harpswell Sound. Additionally, they observed that diatoms always contain diadinoxanthin and fucoxanthin. Due to the relative absence of other accessory pigments, we conclude that diatoms and cryptophytes were the primary taxonomic groups present in this bloom. This knowledge is important because, when combined with nutrient, tidal, temperature, salinity, light, and dissolved oxygen data, it gives insight into the conditions that allow for these phytoplankton groups to be present in the waters. Not only that, but a diatom bloom can indicate that (harmful) dinoflagellates may enter the waters soon, once the diatoms use up all of the available silica. In other words, these results help us to better understand and predict phytoplankton community structure in Harpswell Sound.

The data I collected from the HPLC instrument this summer witnessed the rise and fall of a small summer diatom bloom as well as the onset of a diatom and dinoflagellate bloom. Because chlorophyll a is good proxy for phytoplankton biomass, and it peaks and dips alongside fucoxanthin and diadinoxanthin around June 7th, we can conclude that a diatom bloom began and ended during this

time. The increasing chlorophyll a concentration on June 28th coincides with an increase in the accessory pigments present in diatoms and dinoflagellates, suggesting that both these taxonomic groups were beginning to bloom. As with the long-term data set, this recent data also gives us a better sense of the conditions that lead to phytoplankton blooms. Finally, the presence of gyroxanthin on June 7th, 15th, and 28th, suggest that *Karenia brevis* were present in Harpswell Sound during this time. Because *Karenia brevis* possesses a harmful biotoxin, this information is crucial for matters of public health.

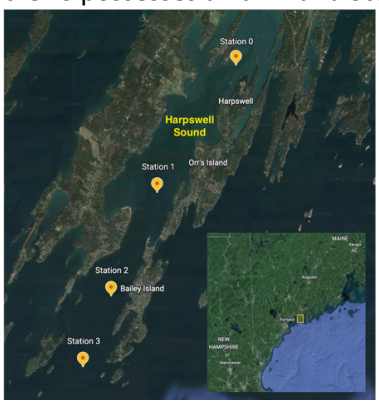

Figure 1. Map of sampling sites.

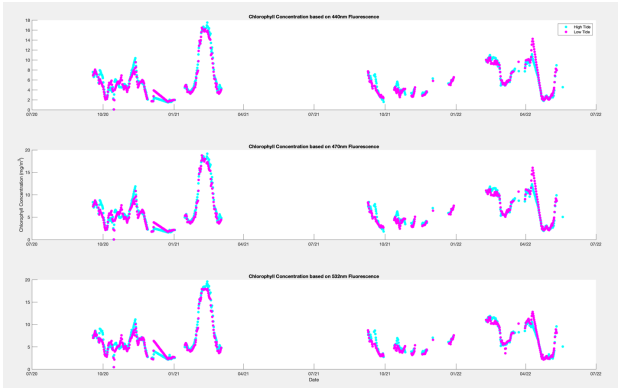
Figure 2. All available data of chlorophyll concentrations at high and low tide based on fluorescence data from the 3X1M at the SCSC since the fluorometer was deployed in 2020. Corrected for quenching and scaled to a diatom.

a) b)

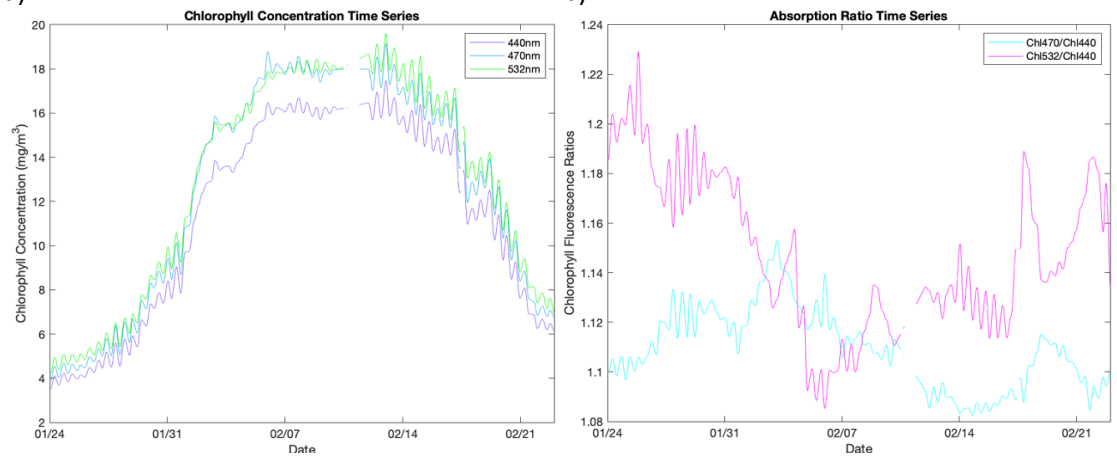

Figure 3. a) Chlorophyll concentrations and b) chlorophyll ratios from January 24th, 2021 through February 23rd, 2021 based on fluorescence data from the 3X1M at the SCSC. Corrected for quenching and scaled to a diatom.

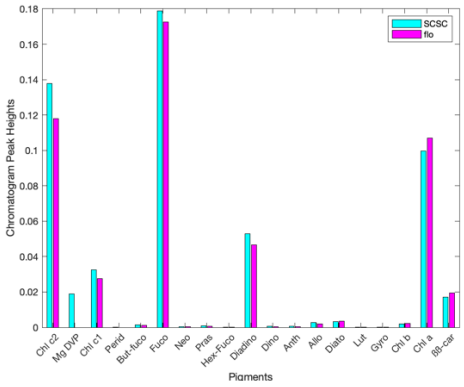

Figure 4. HPLC pigment peak heights from samples collected on February 9th, 2021.

a) b) c)

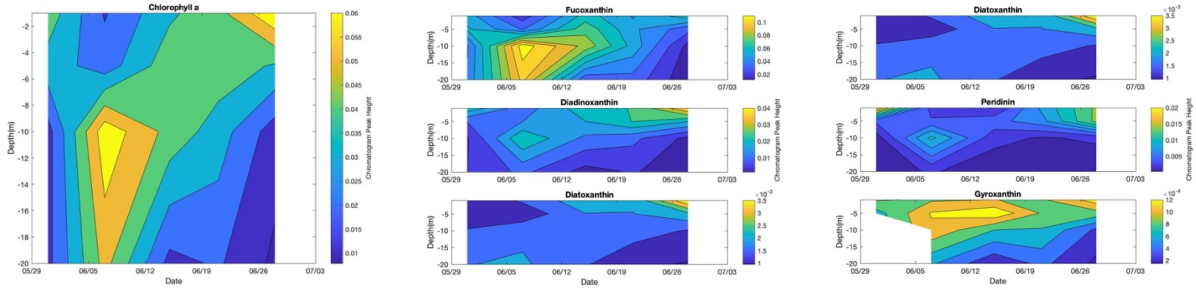

Figure 5. Contour plots based on HPLC pigment peak heights at LOBO for a) chlorophyll a, b) accessory pigments found in diatoms, and c) accessory pigments found in dinoflagellates.

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