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ENVIRONMENTAL AND OPTICAL SIGNATURES OF *COCHLODINIUM*
POLYKRIKOIDES IN NARRAGANSETT BAY, RHODE ISLAND

By

JESSICA CARNEY

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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MASTER OF SCIENCE THESIS
OF
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Abstract

In the summer of 2018, a dinoflagellate bloomed in the open waters of Narragansett Bay, Rhode Island. The dinoflagellate, *Cochlodinium polykrikoides*, is a potentially toxic phytoplankton that has appeared in the Bay sporadically over the past few decades. It was first recorded in the Narragansett Bay Time Series in 1979, but its appearance was infrequent. In the summers of 2016 and 2018 *C. polykrikoides* bloomed in the bay at densities high enough to form rust colored patches on the surface due to the dark pigmentation of the cells, which was distinct enough to be seen from shore, and from small boats. The 2018 bloom was captured by an Imaging FlowCytobot deployed from the URI GSO Pier, which recorded a sample approximately every 20 minutes continuously throughout August. This provided a unique opportunity to study the bloom at fine-scale temporal resolution. The goals of this thesis were to assess the relationship between *C. polykrikoides* and surrounding environmental parameters, assess the community composition before, prior to, and after the bloom period, and measure the inherent optical properties of this dinoflagellate.

Two environmental parameters, salinity and dissolved oxygen, were found to have significant relationships with the number of images containing *C. polykrikoides* in the Imaging FlowCytobot. A time series of additional community data from the Imaging FlowCytobot allowed for a community composition analysis that resulted in a significant difference between four periods within the time series. *C. polykrikoides* was among the top contributors to the dissimilarity within these periods. Lastly, absorption and scattering of *C. polykrikoides* cultures revealed double peaks in absorption in the blue wavelengths and a single peak in the red wavelength.

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Preface

This thesis has been prepared in manuscript format to meet the criteria set by the Graduate School of the University of Rhode Island.

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1. Introduction

Cochlodinium polykrikoides (Margalef, 1961¹) is a photosynthetic, athecate, and often catenate dinoflagellate. This coastal species has a global distribution in temperate and tropical oceans (Kudela & Gobler, 2012, Matsuoka et al., 2008) and has formed blooms off the coastlines of North and Central America, Western India, Southwestern Europe, and Eastern Asia. Its wide geographic range is due in part to being euryhaline, meaning *C. polykrikoides* can survive in a wide range of salinities (Kudela et al., 2008). This dinoflagellate is of particular interest due to its toxic nature and ability to form harmful algal blooms (Tang & Gobler, 2009).

Cochlodinium cells lack cellulose plates, or armor, thus they are referred to as athecate. They have a cingulum that encircles the cells twice, and a sulcus that encircles once (Matsuoka et al., 2008). A single ovoidal cell measures approximately 30-50 micrometers in length, and 25-30 micrometers in width, and contains many rod-shaped chloroplasts. The anterior part of the cell, called the epicone, is pointed conically, and the posterior part of the cell, the hypocone, is rounded (Matsuoka et al., 2008). Each cell has a reddish-orange eyespot that can detect light and the cells exhibit positive phototaxy; they swim towards the light (Gómez et al., 2017; Iwataki et al., 2010; Y. S. Kim et al., 2010). Along the periphery of the cell are trichocysts, which exude rod-shaped filaments that can clog gills of filter-feeding organisms (Iwataki et al., 2010).

C. polykrikoides can be found living as single cells, but it often forms chains that range in length from short 2 cell chains to long 8 cell chains. The length of these chains can vary due to the presence of grazers and vitamins. When grown with the copepod

¹Recently changed to *Margalefidinium polykrikoides* Margalef (Gómez et al., 2017). *Cochlodinium polykrikoides* is still accepted as a synonym (Guiry, M.D. & Guiry, G.M. 2020).

Acartia tonsa, *C. polykrikoides* formed chains as an effective defense mechanism (Jiang et al., 2010) and the longer chains were found to reduce the mortality from grazing. Jiang et al. (2010) also demonstrated that the presence of vitamins B₁, B₇, and B₁₂ had a positive relationship with chain length.

Adjacent cells in a chain are joined by a cytoplasmic connection between opposite sides of each cell (Gómez et al., 2017; Iwataki et al., 2010). This connection is delicate and easily severed, resulting in the separation of long chains into short 2-cell chains or single cells. The morphology of single cells and the chain-forming cells that are seen during bloom events differ slightly. The anterior cell in the chain has a slightly flattened hypocone, the posterior cell has a truncated epicone, and the intermediate cells are longitudinally compressed (Matsuoka et al., 2008). Due to this difference in morphology, a single cell is slightly larger than a compressed interior cell in a chain.

Both single cells and chains of *C. polykrikoides* exhibit diel vertical migration (Park et al., 2001) This migration occurs when the dinoflagellate actively rises towards the surface during daylight hours and swims back down during the night. The vertical migration allows dinoflagellates to access nutrients at depth and get light at the surface and throughout the water column (Smayda, 1997) and therefore, may be a strategy to increase nutrient uptake. Additional access to nutrients is helpful to *C. polykrikoides* because it is slow growing.

The growth rate of *C. polykrikoides* is slow, at only 0.3 divisions per day (D. Il Kim et al., 2004) compared to diatoms which can undergo multiple divisions per day (Furnas, 1990). In order to compete with faster growing phytoplankton, this dinoflagellate produces allelopathic chemicals that inhibit the growth of other plankton

taxa (Tang & Gobler, 2009; Yamasaki et al., 2007). This strategy is effective in aiding bloom-formation because the inhibition of others' growth means there are more resources available for *C. polykrikoides* to utilize.

In addition to producing allelochemicals that negatively impact other phytoplankton, this dinoflagellate is of interest because it can form toxic algal blooms. It secretes a hydrogen peroxide-like toxin that is lethal to fish and shellfish. Cultures of *C. polykrikoides* proved lethal at densities as low as 330 cells mL⁻¹ (Tang & Gobler, 2009). Harmful blooms have formed off the coast of Korea for decades that have had severe impacts to their fisheries. A bloom of *C. polykrikoides* in 1995 in the South and East Seas of Korea lasted eight weeks, had a maximum density of 30x10⁶ cells L⁻¹, and resulted in \$95 million U.S. dollars in damages to South Korea's fisheries (Kang et al., 2002; NIFS, 2003).

Harmful blooms of *C. polykrikoides* have also formed off the eastern coast of the United States. The waters around Long Island, NY, have seen several large blooms over the past few decades. Griffith et al. (2019) studied the effects of *C. polykrikoides* on scallops and oysters in two bays in Long Island, NY, and found high shellfish mortality during short, intensive blooms. The Chesapeake Bay also experienced an intensive bloom of *C. polykrikoides* in the summer of 2007 (Mulholland et al., 2009) with cell counts exceeding 11,000 cells mL⁻¹.

Across Rhode Island, *Cochlodinium polykrikoides* is most commonly found in coastal ponds and coves; particularly Pettaquamscutt and Greenwich coves. In the summer of 1981, a bloom in Pettaquamscutt Cove occurred with a maximum density of 3.4 x10⁶ cells L⁻¹ (Tomas & Smayda, 2008). This density in the cove was far greater than

anything that had been seen in the open bay during the Narragansett Bay Time Series NABATS survey (1959-1997), which was a weekly survey from a site in the west passage of the bay that identified the microplankton through microscopy.

Cochlodinium polykrikoides has appeared sporadically in Narragansett Bay, RI, since in 1979 in the NABATS. Since that time, it increased in frequency of detection, being observed only a single week in the 1970s to a total twelve weeks throughout the 1990s (Smayda et al., 1959-1997). In 2016 and 2018 blooms of *C. polykrikoides* occurred in Narragansett Bay at a density high enough to form rust colored patches on the surface due to the dark pigmentation of the cells, which was distinct enough to be seen from shore in the wake of ships (Figure 1).

Figure 1. (a) Rust colored water in Coweset Marina (Warwick, RI) on 2019-08-10. (b) *Cochlodinium polykrikoides* images captured by the IFCB on the GSO Pier on 2019-08-22 (http://phyto-optics.gso.uri.edu:8888/GSODock/dashboard/http://phyto-optics.gso.uri.edu:8888/GSODock/D20180822T165007_IFCB120)

Weekly monitoring of the phytoplankton community has continued from 1999 to present day as the Narragansett Bay Long-Term Plankton Survey (LTPS). This survey has a sampling resolution of once per week with samples collected from the same location (Fox Island, 41.570°N and -71.390°W) every Monday at 8:00 a.m. (EST).

Several hours transpire between sample collection and observation, and only one milliliter of whole seawater is counted in a Sedgewick/Rafter counting chamber. This survey reported *Cochlodinium polykrikoides* only once in 2001 after which it was not seen again until it bloomed over the course of a five-week period in both 2016 and 2018. *C. polykrikoides* was also counted anecdotally by David Borkman (RI Department of Environmental Management, personal communication) at 6 additional stations throughout Narragansett Bay during the 2018 bloom, indicating a wide spatial distribution of the cells. Here, we will focus on the 2018 bloom due to the existence of extensive temporal sampling during this timeframe.

In addition to the long-term plankton survey, the 2018 bloom was also captured by a new technology not previously used in Narragansett Bay. A McLane Laboratories Imaging FlowCytobot (IFCB) was deployed at the GSO Pier in 2018. The IFCB continuously observes the phytoplankton community (within the size range of ~5-150 μm) approximately every 20 minutes and was active prior to and during this bloom. This new technology allowed us to track the bloom on an hourly basis instead of the weekly samples afforded by the LTPS; something that was not possible during the 2016 bloom. Coincident with the IFCB, a YSI Sonde recorded temperature, salinity, chlorophyll, and dissolved oxygen every 15 minutes. These high temporally resolved parameters are used to achieve greater insight into the environmental conditions that contributed to the bloom's success. Additional parameters not measured at the GSO Pier but observed nearby that may lend insight into bloom formation include wind speed and water level (recorded by a buoy at Quonset Point, approximately 9 km north) and precipitation and solar radiance (recorded with a light sensor in Kingston, RI; approximately 11 km west).

Environmental factors that have previously found to contribute to bloom proliferation include temperature, salinity, rain, and irradiance. To determine the optimal ranges of these environmental parameters for *C. polykrikoides*, D. Il Kim et al. (2004) manipulated each of these variables to induce maximum growth in laboratory cultures. They found that the ideal temperature and salinity ranges were between 21°C-26°C and 30-36 PSU, respectively, with maximum growth occurring at 25 °C and 34 PSU (D. Il Kim et al., 2004). The range for irradiance was much larger; *C. polykrikoides* was able to grow well at all irradiances tested ($30 \mu\text{mol m}^{-2}\text{s}^{-1}$ to $230 \mu\text{mol m}^{-2}\text{s}^{-1}$) and did not experience photoinhibition at the maximum irradiance (D. Il Kim et al., 2004). Lastly, rain decreases salinity and increases water column stratification. Margalef (1978) designed a model that looked at how turbulence and nutrient concentration determine phytoplankton bloom strategies. He found that dinoflagellates can thrive in calmer, stratified conditions because they are able to move independently of the water to areas with higher nutrient concentrations.

These optimal temperature and salinity ranges have been reported in Narragansett Bay during the summer months. Oviatt (2004) found that the average temperature between 1957 to 1998 for August at the GSO Pier was 22°C. Average surface temperatures in Narragansett Bay have increased by approximately 1.6 °C between 1959 to 2005 (Collie et al., 2008) and in August 2018 the average water temperature at the pier was 23.3°C. The average salinity in the bay ranged narrowly from 29.5 to 30 PSU during the previous decade, sitting at the lower end of the optimal salinity range for *C. polykrikoides* blooms (D. Il Kim et al., 2004; Oviatt, 2004).

In addition to the quantification of *C. polykrikoides* from the IFCB, its pigments and optical characteristics may allow for it to be detected with remote sensing methods. All dinoflagellates contain the pigments chlorophyll *a* and peridinin (Jeffrey et al. 1975). These pigments are responsible for the phytoplankton's ability to absorb light and convert it into energy through the process of photosynthesis. While individual *Cochlodinium* cells cannot be seen with the naked eye, when the species blooms in high densities the pigments present within their cells cause discoloration of the water that can be detected by the human eye and remotely via satellite radiometers.

To be able to develop satellite algorithms for the detection of phytoplankton groups of particular species, it is very helpful to understand the species inherent optical properties (IOPs). These are the absorption and scattering properties that are independent from the ambient light field. Y. Kim et al. (2016) found that *C. polykrikoides* displays a similar spectral absorption patterns to many other red HAB dinoflagellates; a high peak in absorption in the blue wavelengths and a secondary peak in the shorter wavelengths. They compared the phytoplankton absorption spectra of three other red tide dinoflagellates that can form co-blooms and found it difficult to distinguish between the four species (Y. Kim et al., 2016). Here, in addition to quantifying absorption, attenuation was also observed on laboratory cultures, that further allows scattering to be calculated. These additional IOPs will provide a more holistic understanding of *C. polykrikoides'* optical properties.

The focus of this research is to understand the environmental drivers of the 2018 bloom as it coincided with the deployment of an Imaging FlowCytobot (IFCB). The

IFCB data is utilized to examine shifts in community composition as it captures high resolution data and allows a greater depth of analysis compared to single weekly samples that were previously available. We investigate the community composition prior to, during and post the 2018 *C. polykrikoides* bloom. Additionally, the optical properties of this species are characterized to inform remote sensing efforts into the future. Specifically, the following questions will be addressed:

- Which environmental parameter(s) had the greatest influence on the 2018 *Cochlodinium polykrikoides* bloom?
- How did the phytoplankton community shift during this bloom?
- What are the specific absorption and scattering properties of this species?

2. Methods

2.1 *In situ* observations

In August 2018, the phytoplankton community was continuously sampled every 20 minutes at the Pier (41.492°N and -71.419°W) at the University of Rhode Island (URI) Graduate School of Oceanography (GSO) using an Imaging FlowCytobot (IFCB) (Figure 2). The IFCB combines a flow cytometer with a camera that triggers on chlorophyll fluorescence to capture images of the phytoplankton (within a size range of ~5 to 150 μm) as they pass through the flow cell and samples ~5mL of seawater (Olson & Sosik, 2007, Campbell et al., 2016, <http://phyto-optics.gso.uri.edu:8888/GSODock>). A subset of manually classified images were used to train a Random Forest based automated classifier (Sosik & Olson, 2007) that was then applied to the entire dataset. The IFCB has been used to identify and track harmful algal species, allowing for detection on a finer time scale than what is typical when manually counting live samples (Campbell et al., 2010, 2013). It also enhances the accurate enumeration of fragile taxa, which are often destroyed by other sampling procedures. The image classifications were used to identify the phytoplankton community prior to, during, and after the *C. polykrikoides* bloom.

Due to the catenate nature of *Cochlodinium*, each IFCB image can contain between one and four cells. Assuming all images contain a single cell, a conservative estimate directly relates the number of images to the number of cells present. However, many of the images contain multiple cells (Figure 1b). The interior cells of a chain are longitudinally compressed, making them shorter than the anterior and posterior cell (Matsuoka et al., 2008). This compression within the chain makes it impossible for the IFCB to distinguish individual cells, and the inconsistent cell length makes it impractical

to calculate the number of cells in each image based on the length of the chain. Therefore, image counts are used as a proxy for cell counts in this study.

Environmental parameters were measured using a YSI Sonde adjacent to the IFCB, a NOAA buoy at Quonset Point, and a NOAA sensor in Kingston, RI, (Figure 2). The Sonde recorded temperature, salinity, dissolved oxygen, and chlorophyll every fifteen minutes and was provided by Heather Stoffel on behalf of Dr. Candace Oviatt's group at URI GSO (personal communication, Stoffel 2018). Thirteen kilometers north of the pier, the Quonset Point buoy (41.5983 N, -71.40417 W) recorded wind speed (m/s) and water level every 6 minutes (NOAA's National Ocean Service, 2019). Ten kilometers west of the Pier, the NOAA sensor in Kingston, RI, (41.4918 N, -71.538 W) measured precipitation (mm) and solar radiance (Watts m⁻²) every five minutes (NOAA National Centers for Environmental Information, 2019). The LTPS was used for historical context of the occurrence of *C. polykrikoides* and the station is located at Fox Island (41.570°N and -71.390°W).

Figure 2. Environmental parameters were sampled at these four stations in Rhode Island. QP is Quonset Point, FI is the Fox Island station where the long-term plankton survey occurs, KS is the site of the NOAA light sensor, and GP is the location of the IFCB.

2.2 Quality control of *in situ* data

The different instruments recorded data at different frequencies: the IFCB recorded every twenty minutes, the YSI every fifteen, and the NOAA buoy and light sensor every five. All data was averaged per hour to accommodate the different sampling frequencies. For example, any data recorded between 9:00 and 10:00 was averaged and reported as occurring at 9:00.

Gaps in the *in-situ* data accounted for less than 5% of the overall environmental dataset. In order to approximate the missing values, we estimate an autoregressive integrated moving average (ARIMA) model using a state-space representation (Kalman filter). The missing values are imputed using the mean of the observational equation, obtained with estimated values of the latent state process. The `auto.arima` function is in the `imputeTS` package of the software R (Kalman, 1961; Moritz & Bartz-Beielstein, 2017). The R package selects the order for the ARIMA model using the Akaike Information Criterion, which quantifies the goodness of fit and simplicity of a statistical model into a single statistic. Cell counts from the IFCB of *Cochlodinium polykrikoides* were also imputed with this method to account for a 37-hour gap from 0100 August 19th to 1400 August 20th, 2018.

The physical properties of the Bay were influenced by the tide, causing the temperature and salinity data to contain a lot of noise. Tidal variability was removed in MATLAB using a moving mean filter that was based on the 33-hour tidal cycle in Narragansett Bay. Removing the tidal signal revealed a daily cycle in the temperature and salinity values.

2.3 Culture maintenance

Cultures of *C. polykrikoides* were grown to characterize the species' inherent optical properties. A 30 mL starter culture of *Cochlodinium polykrikoides* ARC_169 was obtained from the Algal Resources Collection at University of North Carolina Wilmington CREST Research Park. These cells were transferred with 1mL pipettes into test tubes and were grown in L1 -Si medium, which has a filtered seawater base and contains nitrate, phosphate, vitamins, additional trace metals, and no silicate to promote growth of coastal flagellate species (Andersen 2005). The media was made with 0.2 μm filtered seawater collected from Narragansett Bay, which had a salinity of 32.5 PSU and kept in polycarbonate carboys which were stored in the dark at 12°C. The starter culture was grown in water with a salinity of 36 PSU, so approximately 3.5 grams of sodium chloride was added to the liter of L1 -Si media to increase its salinity from 32.5 PSU to 36 PSU. The starter culture was grown in a 20°C incubator with 14:10 light dark cycle, and the transferred cultures were maintained on a 14:10 light dark cycle in a 19°C incubator. The incubator had cool white bulbs and an average light output of 99.3 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

I inoculated 6 borosilicate glass test tubes with 2mL of starter culture into 10mL of media and one 200mL polycarbonate bottle with 5mL of starter culture into 30mL of culture. Batch cultures of *C. polykrikoides* in the tubes were maintained from November 2019 through May 2020, and the tubes were transferred every two weeks with 2mL of inoculate into 10mL of media. These batch transfers were filtered onto 0.22 μm Whatman

glass fiber/filter (GF/F) pads for use in a particulate absorption analysis and stored in a cryofreezer at -80°C until they were analyzed.

In addition to the batch cultures, a continuous culture was also maintained to grow a large volume for analysis with a SeaBird Scientific hyperspectral absorption and attenuation sensor (AC-S), which measures these parameters at 80 wavelengths with 4 nm resolution from 400 to 730 nm. Two 125mL Erlenmeyer flasks were inoculated from the 200mL polycarbonate bottle with 5mL of culture into 50mL of media. Every two weeks, I doubled the volume in the flasks; for example, the initial volume of 55mL was doubled to 110 mL. On the next transfer, because the flask was full, I split it in half between two flasks and doubled the volume with fresh media. This allowed me to grow larger volumes in shorter periods of time. When 1 liter of culture was achieved, I prepared for measuring absorption and attenuation with the AC-S (described in section 2.4.2).

2.4 Inherent optical properties

Six inherent optical properties (IOPs) of *C. polykrikoides* were measured in this study: total absorption (a_{total}), total attenuation (c_{total}), non-water absorption (a_{nw}), CDOM absorption (a_{g}), particulate absorption (a_{p}), and non-algal particulate absorption (a_{nap}). From these measured IOPs the phytoplankton absorption (a_{ph}) and chlorophyll *a* specific absorption (a_{ph}^*) of *C. polykrikoides* were calculated. The IOPs were measured with two different methods. First, a_{p} and a_{nap} were measured on a Perkin Elmer Lambda 650 benchtop spectrophotometer from filtered cultures of *C. polykrikoides* (section 2.4.1). Then a_{total} , c_{total} , and a_{nw} were measured from live dilute cultures with the AC-S (section

2.4.2). After these live dilute cultures were measured by the AC-S, one replicate from each set was saved to measure a_g with the spectrophotometer (section 2.4.3).

The total absorption and total attenuation were measured with the AC-S. The particulate absorption (a_p) for the samples run through the AC-S was calculated by subtracting the CDOM absorption measured on the benchtop spectrophotometer from the absorption due to water (known from temperature and salinity). Non-algal particulate absorption (a_{nap}) was not measured for the cultures of *C. polykrikoides* that were run through the AC-S. Instead, the mean a_{nap} of all the samples measured on the benchtop spectrophotometer were used as a proxy.

The samples measured on the spectrophotometer were not diluted. In order to compare them with the dilute cultures in the AC-S, an artificial dilution was applied to the mean a_{nap} . The mean a_{nap} was multiplied by 0.133 in order to achieve the same dilution of the samples measured in the AC-S. This mean dilute a_{nap} was then subtracted from the calculated a_p from the AC-S samples in order to calculate the phytoplankton absorption. Lastly, the total scattering (b) of the cultures measured on the AC-S were calculated with the equation

$$b = c - a$$

where c is the total attenuation and a is the total absorption.

2.4.1 Particulate absorption analysis with benchtop spectrophotometer

Particulate absorption ($a_p(\lambda)$) and non-algal particulate absorption ($a_{nap}(\lambda)$) of the cultures was measured in accordance to the NASA Ocean Optics Protocols for Satellite Ocean Color Sensor Validation (Mitchell et al. 2003). Six sets of culture samples were

filtered onto Whatman GF/F pads in triplicates. Samples were collected on different dates spanning the course of 6 months to account for any variability in absorption of the living culture over time. Filtration volumes and dates for the six sets can be found in Table 1. $a_p(\lambda)$ and $a_{nap}(\lambda)$ were read using a PerkinElmer Lambda 650 UV/Vis spectrophotometer with the transmittance-reflectance method that utilizes an integrating sphere (Lohrenz, 2000; Lohrenz et al., 2003; Tassan & Ferrari, 2002). Filters were placed on a quartz slide at the entrance (transmittance) and exit (reflectance) of the sphere and scanned at a rate of 120 nm min^{-1} with a slit width of 2 nm both before ($a_p(\lambda)$) and after ($a_{nap}(\lambda)$) exposure to methanol. Phytoplankton absorption ($a_{ph}(\lambda)$) was calculated by the equation:

$$a_{ph}(\lambda) = a_p(\lambda) - a_{nap}(\lambda) \quad (1)$$

and chlorophyll-specific phytoplankton absorption ($a_{ph}^*(\lambda)$) was calculated with the equation:

$$a_{ph}^*(\lambda) = \frac{a_{ph}(\lambda)}{chl} \quad (2)$$

There was one replicate removed from sets 2, 3, and 4, due to errors in removing the phytoplankton contribution with methanol.

Table 1. Six sets of triplicates were filtered throughout the course of this study and labelled with the collection date. Sample sets were collected on different dates to account for any variability in the absorption with time.

Date	Number of replicates	Volume filtered (ml)
2019-12-18	3	50
2020-03-25	3	30
2020-04-15	3	24
2020-04-29	3	24
2020-05-18	3	24
2020-05-29	3	24

2.4.2 Collecting AC-S measurements

The absorption and attenuation of *C. polykrikoides* was measured by running dilute live cultures through an AC-S. The setup was designed to be a mix of static and flow-through to minimize the amount of dilute culture needed for each measurement and maximize the ability of the cells to remain in suspension. The culture was poured into a funnel and delivered into the AC-S via a darkened tube attached to the intake ports. The tube had a split Y-connector at the end to allow the absorption and attenuation channels to fill simultaneously. The AC-S was mounted on a metal stand and raised to a 45-degree angle to allow bubbles to flow easily out of the exhaust ports via a darkened tube (McFarland, 2014). Once the bubbles were out, the end of the exhaust tube was capped to prevent excessive waste. The cap had a small hole to allow approximately 30mL of dilute culture to be exchanged during the two minutes of measurements. While the measurements were being recorded, the AC-S was gently rocked to keep the culture suspended. Appendix A contains photos of the set up.

The set-up required 300mL of dilute culture to ensure both chambers of the AC-S were entirely filled, and to allow enough extra volume to accommodate the exchange of 30mL from the slow drip during the measurement period. An Erlenmeyer flask containing a *C. polykrikoides* culture was placed in a dark 20°C lab one hour prior to the experiment to allow it to acclimate to room temperature. The filtered seawater used in the dilution was taken from a 15°C walk-in incubator and was placed in a water bath at 20°C to increase its temperature to match the culture.

I measured six triplicate sets of 13.3% dilute cultures over the course of three days. The cultures were diluted using the same filtered seawater they were grown in. The dilution step was necessary to optimize efficient use of the cultures and to fall within the sensitivity of the AC-S. Prior to each set of measurements, the AC-S was calibrated with milli-Q and the absorption and attenuation of just the filtered seawater was measured. Odd numbered sets were measured in the morning, and even numbered sets were measured in the afternoon. Approximately two hours were needed between the morning and afternoon sets to clean the equipment and to allow the next flask of culture to acclimate to room temperature. The first set had triplicates that consisted of 45mL of culture and 255mL of filtered seawater. Sets two through six had triplicates with 40mL of culture and 260mL of culture. The last two sets of measurements were discarded from analysis due to the impact of bubbles skewing the data within those two sets.

The particulate absorption ($a_p(\lambda)$) for the sets run through the AC-S was calculated by subtracting the CDOM absorption measured on the benchtop spectrophotometer from the absorption due to water (known from temperature and salinity). Non-algal particulate absorption ($a_{nap}(\lambda)$) was not measured for the cultures of *C. polykrikoides* that were run through the AC-S. Instead, the mean $a_{nap}(\lambda)$ of all the samples measured on the benchtop spectrophotometer were used as a proxy. In order to make a direct comparison, the mean $a_{nap}(\lambda)$ measured on 100% culture was artificially diluted to match the dilution factor of the live dilute cultures.

2.4.3 Colored dissolved organic matter absorption analysis

Ten dilute culture samples were filtered through 0.22μm millipore filters for CDOM absorption analysis. Each CDOM sample consisted of 150mL of filtrate from which three replicate measurements were taken. The filtrate was stored in clear borosilicate bottles (Qorpak; GLC-01151) in a 4°C refrigerator for CDOM absorption analysis on a benchtop spectrophotometer. The CDOM samples were filtered and analyzed in accordance to NASA Ocean Optics Protocols for Satellite Ocean Color Sensor Validation (Mitchell et al. 2003). The samples were read using a PerkinElmer Lambda 650 UV/Vis spectrophotometer with 10cm cuvettes for wavelengths between 300-800 nm using a slit width of 2 nm and a scan rate of 240 nm min⁻¹. I'm confused. Are these the same spectrophotometer, or are there 2 different ones?

2.4.4 Processing AC-S observations

The AC-S data were extracted using the manufacturer's software (WET Labs 2011). Absorption ($a(\lambda)$) and attenuation ($c(\lambda)$) were corrected for temperature and salinity:

$$a_m^{TS} = a_m - [(T_m - T_r) \cdot \Psi_{Ta}] - [S_m - \Psi_{Sa}] \quad (3)$$

$$c_m^{TS} = c_m - [(T_m - T_r) \cdot \Psi_{Tc}] - [S_m - \Psi_{Sc}] \quad (4)$$

where a_m^{TS} and c_m^{TS} are corrected a and c (m⁻¹), a_m and c_m are extracted values from the AC-S (m⁻¹), T_m is the recorded temperature, T_r is the reference temperature (15°C), Ψ_{Ta} and Ψ_{Tc} are the temperature correction coefficients (m⁻¹ °C⁻¹) and Ψ_{Sa} and Ψ_{Sc} are the salinity correction coefficients (m⁻¹ PSU⁻¹) (Mouw et al., 2017). Temperature and salinity coefficients came from Sullivan et al. (2006).

The AC-S was calibrated with pure water prior to measuring the cultures. The pure water signal was removed from the culture measurements with the equations:

$$a = a_m^{TS} - a_{wcal} \quad (5)$$

$$c = c_m^{TS} - c_{wcal} \quad (6)$$

where a_{wcal} and c_{wcal} are the median of three pure water calibrations made before each set of measurements.

The data were corrected for scattering using the variable scattering error method (Pegau et al., 2002; Zaneveld et al., 1994). Particulate absorption and attenuation were calculated by subtracting the CDOM measured for each set from the scattering-corrected absorption and attenuation. Six sets of AC-S measurements were taken; set 6 had evidence of bubbles and was removed from analysis. The data were corrected for absorption due to water with pure water and the mean of three replicates was taken for each sample. Due to the presence of bubbles in AC-S set 6, the corresponding set of CDOM data was not considered for analysis.

2.5 Statistical Analysis

2.5.1 Impact of environmental parameters on *C. polykrikoides*

The influence of environmental parameters on the abundance of *Cochlodinium polykrikoides* (cells mL⁻¹) was assessed using a Generalized Linear Model (GLM) with Poisson distribution. The environmental parameters considered for this analysis were: water temperature (°C), salinity (PSU), dissolved oxygen (µg L⁻¹), chlorophyll (µg L⁻¹), solar radiation (Watts m⁻²), precipitation (mm), wind speed (m s⁻¹), and water level height (m). Prior to analysis, all outliers in the dataset that exceeded three standard deviations of the mean were removed (Tukey, 1962). A test for colinearity revealed temperature had

high collinearity with salinity and therefore temperature was removed from further analysis. Temperature was removed because it had less variability throughout the time series (23.5 ± 0.37) compared to salinity (31.35 ± 0.44).

Prior to analysis, each variable was standardized by subtracting the mean and dividing by the standard deviation. The standardized dataset was centered by subtracting the mean of all variables. All standardized image counts of *Cochlodinium* had +1 added to prevent negative numbers in the GLM analysis.

Parameters shown to be significant by the GLM analysis were examined further by establishing a Non-bloom period and a Bloom period. The Non-bloom period ran from 2018/08/01-2018/08/07 and captured the first week of August when all mean hourly *Cochlodinium* image counts were $<1 \text{ image mL}^{-1}$. The Bloom period (2018/08/17-2018/08/23) captured the peak of the *Cochlodinium* bloom on 08/21 and the surrounding dates (Figure 3). The range of image counts during the Bloom period was 0-115.8 images. A Wilcoxon rank sum test was performed to determine if the parameters were significantly different before and during the bloom.

Figure 3. The full time series began August 1st, 2018 and ended September 17th, 2018. The time series was divided into four periods: Non-bloom (2018/08/01-2018/08/07), Pre-Bloom (2018/08/08-2018/08/16), Bloom (2018/08/17-2018/08/23), and Post-bloom (2018/09/11-2018/09/17). Periods were named in relation to the week that *C. polykrikoides* bloomed. A gap in the time series exists from 2018/08/24-2018/09/10 due to maintenance on the IFCB.

2.5.2 Assessing phytoplankton community composition

The phytoplankton community was continuously sampled during August and September 2018 by the IFCB at the GSO Pier. During this time, there were approximately 8.5 million images of phytoplankton cells taken from seawater samples. The IFCB has the ability to capture plankton within the size range of 5 to 150 μ m (Olson & Sosik, 2007). 88% of the images taken were less than 20 μ m in diameter; plankton that are between 2 μ m and 20 μ m fall within the nanoplankton group (Yamaguchi et al., 2002), and their small size makes them almost impossible to identify without scanning electron microscopy, which is outside the scope of this project. Therefore, all images that were less than 20 μ m in size were removed prior to analysis, allowing me to focus on the community composition of the microplankton.

The Random Forest classifier developed for our IFCB dataset was trained on

roughly 22,000 manually identified images of phytoplankton cells representing 84 different classes. The classifier has an overall error rate of 13%. Much of the uncertainty is due to the inability to classify nanoplankton accurately. *C. polykrikoides* classification has an error rate of 8%, meaning it is accurate 92% of the time. Within the subset of the time series considered here, 57 classes were represented. Of these 57 classes, twelve classes represent images that do not contain phytoplankton and two represent images that cannot be classified and are labeled unknown. A full list of the classes can be found in Appendix B. Images that were classified as the twelve non-phytoplankton classes or two unknown classes were removed prior to analysis. Image counts for each species were calculated by dividing the number of images per species by the number of milliliters that the IFCB actively sampled. Hourly averages were calculated to create temporal stations for community comparison.

Temporal distribution patterns of the phytoplankton community captured by the IFCB during August and September 2018 at the GSO Pier were assessed using non-metric multidimensional scaling (NMDS) on a Bray-Curtis dissimilarity matrix. NMDS is used to assess community composition across stations, and the Bray-Curtis dissimilarity matrix calculates the most appropriate distance to use for nonparametric cell count data (Collie et al., 2008; Anglès et al., 2019).

The time series was then broken into four periods: Non-bloom, Pre-bloom, Bloom, and Post-bloom (Figure 3). The Non-bloom and Bloom periods represent the same time periods as the Non-bloom and Bloom periods introduced in section 2.5.1. The Non-bloom period includes all hourly samples from 2018/08/01 through 2018/08/07 and represents a week-long period before the *C. polykrikoides* bloom when the hourly image

counts of *C. polykrikoides* were all <1 image mL^{-1} . The Pre-bloom period runs from 2018/08/08 through 2018/08/16 and represents the nine days between the Non-bloom period and the Bloom period. During the Pre-bloom all image counts were <20 cells mL^{-1} (Figure 3). The Bloom period (2018/08/17 through 2018/08/23) represents the week that the bloom occurred. The Bloom period is centered on the day the bloom peaked and includes the four days leading up to the bloom (08/17-08/19) and two days following the peak (08/22-08/23). The IFCB was not actively sampling between 2018/08/24-2018/09/10. The Post-bloom period runs from 09/11-09/17 and contains the first week that the IFCB was back online.

An analysis of similarity (ANOSIM) was used to calculate the percent dissimilarity between each time period (Anglès et al., 2019). Additionally, a similarity percentages analysis (SIMPER) was performed to determine which species drove the dissimilarity between each period. Both analyses were performed using the Fathom Toolbox in MATLAB (Jones, 2014).

3. Results and Discussion

A bloom of *Cochlodinium polykrikoides* occurred in the open waters of Narragansett Bay, Rhode Island, in August 2018. The bloom peaked on August 21st with a mean image count of 116 images mL⁻¹ (Figure 4a). Assuming all images contain a single cell, a conservative estimate puts the peak of the bloom at 116 cells mL⁻¹. However, many images contained 2-cell or 4-cell chains, so it is likely that the number of cells per milliliter is greater than 116. All image counts and environmental parameters were measured sub-hourly and were averaged by hour to determine if any played a significant role in the *C. polykrikoides* bloom proliferation.

Throughout August, the mean hourly water temperature ranged from 22.6°C-24.4°C, peaked on August 17th and immediately trended down to 23°C by the end of August 23rd. The mean hourly salinity was highest at the beginning of the time series and began trending downwards on August 10th through the rest of the time series, decreasing from a maximum of 31.86 PSU on August 5th to a minimum 30.40 PSU on August 21st (Figure 4b). Dissolved oxygen and chlorophyll concentrations had no trend throughout August, although both did have small peaks when *C. polykrikoides* was abundant. Chlorophyll was highest on August 21st at 21.50µg L⁻¹ and dissolved oxygen was highest on August 20th with a mean hourly value of 8.53mg L⁻¹ (Figure 4c). Water height and solar radiation experienced daily maxima but did not vary throughout the month (Figure 4d&e). Precipitation was rare and never exceed 0.3mm per hour and wind speed had no trend (Figure 4f&g).

Figure 4. a) *Cochlodinium polykrikoides* was most abundant between 2018-08-19 and 2018-08-23 in Narragansett Bay, Rhode Island. b) Salinity was the lowest during the height of the *C. polykrikoides* bloom. c) Chlorophyll and dissolved oxygen both had maximum concentrations during the height of the bloom. d) Water level was tidally dominated. The spring tide occurred in the middle of the month, and neap tides occurred during the beginning of August and during the week that *Cochlodinium* was most abundant. e&f) Solar radiation experienced daily minima when precipitation occurred. g) Wind speed had no discernible trend during this time series.

3.1 Impact of environmental parameters on *C. polykrikoides*

The coefficients of the GLM were plotted to show the relationship between each parameter and the image counts (Figure 5). Two of the seven parameters, salinity and dissolved oxygen, had a significant relationship with the image counts ($p < 0.05$). Salinity had a negative relationship and dissolved oxygen had a positive relationship with the number of images containing *C. polykrikoides*. Chlorophyll also had a positive relationship with increasing *C. polykrikoides* image counts, but it was not statistically significant ($p = 0.107$, Figure 6).

Figure 5. Salinity and dissolved oxygen were the only parameters with a significant relationship to *Cochlodinium* image counts ($R^2 = 0.2263$; $p = 5.16 \times 10^{-15}$, $p = 0.0152$, respectively).

Figure 6. *Cochlodinium* image counts compared to the environmental parameters with which it had the strongest relationships. The colorbar represents how the data fall within one bin; dark blue bins contain relatively few data points and yellow bins contain relatively many data points. a) Salinity had a negative relationship with *Cochlodinium* image counts; as salinity decreased there were more images of *Cochlodinium* present per sample. b) Dissolved oxygen increased with *Cochlodinium* image counts. c) Chlorophyll values increased with image counts.

The time series was split into ‘Non-bloom’ and ‘Bloom’ periods to analyze changes in the significant parameters prior to and during the bloom (described in section 2.5.2). The Non-bloom period captured the first week of August 2018 (8/01-8/07) when *Cochlodinium* image counts were less than one image per mL per hour. The Bloom period captured the week of the *C. polykrikoides* bloom (08/17-08/23). The difference in salinity from the first week of August 2018 to the week of the bloom was significant (Wilcoxon rank sum test, $p=1.1 \times 10^{-53}$) (Figure 7). The mean salinity in the Non-bloom period was 31.77 PSU and the mean salinity in the Bloom period was 30.82 PSU; a difference of 0.95 PSU. When the mean dissolved oxygen in the Non-bloom was compared to the mean dissolved oxygen during the bloom, the increase from 6.70 mg L⁻¹ during the Non-bloom to 6.79 mg L⁻¹ during the Bloom periods was not significant (Wilcoxon rank sum test, $p=0.35$).

Figure 7. The center line represents the median of the variable, the bottom and top of the box represents the first and third quartiles, and the whiskers represent the minimum and maximum values. a) Mean salinity was significantly higher during the first week of August (Non-bloom) than during the Bloom period ($p=1.1 \times 10^{-53}$). The Non-bloom period had several outliers (red crosses) below the minimum. b) Mean dissolved oxygen was slightly higher in the Bloom period, but the difference was not significant ($p=0.35$).

3.1.1 Interpreting the results of the GLM

Salinity was one of two environmental parameters that had a significant relationship with *Cochlodinium polykrikoides* image counts ($R^2=0.2263$, $p=5.16 \times 10^{-15}$). Salinity had a negative relationship with *Cochlodinium* indicating that as salinity decreased, the abundance of the dinoflagellate increased (Figures 5, 6). *C. polykrikoides* is a euryhaline species (Kudela et al., 2008) and can grow at a variety of salinities ranging from 20 PSU to 36 PSU. A study by D.II Kim et al. (2004) found that the optimum growth of this species occurred within a salinity range of 30-36 PSU. Conditions in Narragansett Bay were within this range and during the Bloom period average salinity was 30.82 PSU.

The salinity in the Bloom period was found to be significantly different from the Non-bloom period at the start of August (Figure 7, $p=1.1 \times 10^{-53}$). The salinity during the Non-bloom period averaged 31.77 PSU. It began decreasing on August 10th during the Pre-bloom period; by the Bloom period the salinity had decreased by 0.95 PSU (Figure 4b). A common driver in the decrease of salinity is an increase in precipitation. However, precipitation did not play a significant role throughout this time series. There was very little precipitation throughout August 2018 (Figure 4f). The average precipitation for the time series was 0.005mm per hour and the range was 0-0.2917mm per hour. When total precipitation was summed by day, both August 4th and August 13th had the greatest amount of precipitation at 0.67mm per day, each. It did not have a significant relationship with *Cochlodinium* image counts (Figure 5), nor did precipitation change significantly between the Non-bloom and Bloom periods (Wilcoxon rank sum test, $p=0.21$).

Another possible explanation for the decrease in salinity could be contributed to freshwater input from tributaries. For example, the salinity of the upper bay is lower than that of the mid-Bay due to influence from the Providence River, a major freshwater source (Hess, 1976). Increases in freshwater input in the vicinity of the GSO Pier may account for the decrease in salinity during the Bloom period. However, proving the influence of tributaries is beyond the scope of this project.

In addition to salinity, dissolved oxygen also had significant relationship with *Cochlodinium* image counts ($R^2=0.2263$, $p=0.0152$). Unlike salinity, dissolved oxygen displayed a positive relationship with the image counts (Figures 5, 6). *C. polykrikoides* is a mixotrophic species, meaning it can both produce energy through photosynthesis and produce energy through the consumption of other organisms (Jeong et al., 2004). Oxygen

is a byproduct of photosynthesis, and when there are more photosynthesizers present, they are likely to drive the dissolved oxygen concentration upwards.

Although there was a significant relationship between the increase in *C. polykrikoides* images and dissolved oxygen concentration, there was not a significant difference between the mean dissolved oxygen in the Non-bloom period versus the Bloom period ($p=0.350$, Figure 7). This may be due to other photosynthetic plankton such as *Skeletonema* spp. or *Eucampia zodiacus* being present in high abundance during the Non-bloom period—section 4.2 elaborates on the different plankton present in the time series. Production of dissolved oxygen in estuaries tend to be dominated by biological processes such as photosynthesis, with physical processes playing only a minor role (Kemp & Boynton, 1980).

3.2 Assessing phytoplankton community composition

Throughout August and leading up to the peak of the *Cochlodinium* bloom on August 21st, the abundance and species composition of microplankton present in the IFCB shifted. A time series of the phytoplankton community began on August 1st, 2018 and ran through the bloom and beyond to September 17th, 2018 to analyze the shift in community composition around the bloom event. Ordination of the phytoplankton community using nonmetric multidimensional scaling (NMDS) on a Bray Curtis dissimilarity matrix in 3 dimensions revealed shifts in the community composition (Figure 8).

These shifts clustered within the four time-periods previously established in section 2.5.2: Non-bloom (2018/08/01-2018/08/07), Pre-bloom (2018/08/08-2018/08/16), Bloom (2018/08/17-2018/08/23), and Post-bloom (2018/09/11-2018/09/17) (Figure 3).

Each period was one week-long except for the Pre-bloom period, which was extended to ensure coverage of all days in the time series.

Figure 8. NMDS performed in 3 dimensions on the number of taxa present and their respective image counts from 2018/08/01-2018/09/17 (stress=0.06). The NMDS was performed on 641 sequential temporal stations where each station represents one hour during the time series. The data was then colored by period, which was defined as the Non-bloom, Pre-bloom, Bloom, and Post-bloom periods with respect to the *C. polykrikoides* bloom. The colors revealed a clustering pattern around the specific time periods. Appendix C contains weighted biplot produced from the NMDS relating the 43 taxa to each other.

An analysis of similarity (ANOSIM) found these four periods significantly different from each other ($R=0.3936$, $p=0.005$). Following the ANOSIM, a similarity percentage (SIMPER) analysis was performed to determine which species drove the dissimilarity between the four time periods. The SIMPER analysis revealed high dissimilarity between each period as well as calculated the individual percent contribution to dissimilarity by each of the 43 species. The top seven drivers of dissimilarity were phytoplankton that were present in every period and contributed to >5% dissimilarity in at least one period. Their percent individual percent contributions to dissimilarity are reported in Table 2. These seven phytoplankton were responsible for 83-89% dissimilarity within the periods. *Cochlodinium polykrikoides* was one of the top

seven contributors to dissimilarity, especially among comparisons that contained the Bloom period.

Table 2. SIMPER analysis results for the 4 periods in the time series. Total average dissimilarity indicates how dissimilar each period was based on abundance and species composition. The percent contribution to dissimilarity represents how much each species contributed to the dissimilarity between periods, and the cumulative percent contribution provides a running total of the individual contributions. Seven phytoplankton were top contributors to dissimilarity in each period and are listed below. The cell color of each name matches the color that represents each of these phytoplankton in Figure 9.

	Non-bloom vs. Bloom		Non-bloom vs. Pre-bloom	
	Total Average Dissimilarity: 63%		Total Average Dissimilarity: 67%	
Name	% Contribution to dissimilarity	Cumulative % Contribution	% Contribution to dissimilarity	Cumulative % Contribution
<i>Skeletonema</i>	27	27	35	35
<i>Cylindrotheca</i>	24	51	30	65
<i>Eucampia</i>	10	61	12	77
<i>Cryptophyte</i>	10	71	2	79
<i>Cochlodinium</i>	7	78	1	80
<i>Leptocylindrus</i>	6	84	7	87
<i>Pennate</i>	2	86	3	90
	Non-bloom vs. Post-bloom		Pre-bloom vs. Bloom	
	Total Average Dissimilarity: 79%		Total Average Dissimilarity: 60%	
<i>Skeletonema</i>	32	32	33	33
<i>Cylindrotheca</i>	30	62	19	52
<i>Eucampia</i>	11	73	1	53
<i>Cryptophyte</i>	2	75	14	67
<i>Cochlodinium</i>	<1	75	10	77
<i>Leptocylindrus</i>	9	84	7	84
<i>Pennate</i>	3	87	3	87
	Bloom vs. Post-bloom		Pre-bloom vs. Post-bloom	
	Total Average Dissimilarity: 73%		Total Average Dissimilarity: 66%	
<i>Skeletonema</i>	31	31	23	23
<i>Cylindrotheca</i>	18	49	34	57
<i>Eucampia</i>	<1	49	1	58
<i>Cryptophyte</i>	14	63	4	62
<i>Cochlodinium</i>	9	72	2	64
<i>Leptocylindrus</i>	9	81	14	78
<i>Pennate</i>	3	84	5	83

Intercomparing the periods with a SIMPER analysis found that the Non-bloom vs. Post-bloom periods had the highest average dissimilarity (79%) followed by the Bloom vs. Post-bloom periods (73%) (Table 2). All periods were dominated by diatoms (Figure 9, Table 4), however the dominating diatom in the Non-bloom period was *Skeletonema* spp. and the dominating diatom of the Post-bloom period was *Leptocylindrus* spp. (Figure 9). The Bloom vs. Post-bloom periods had the second highest dissimilarity. This dissimilarity was driven by the rise in the flagellate Cryptophytes and the dinoflagellate *C. polykrikoides*. Cryptophytes and *C. polykrikoides* made up 16.7% and 12.3% of the Bloom period population, respectively (Table 3).

Figure 9. Diatoms (shades of blue) dominated the micro-phytoplankton community at the GSO Pier. The diatoms *Skeletonema* spp. and *Cylindrotheca closterium* were the most abundant phytoplankton throughout the entire time series. *Cochlodinium* (black) was present at low concentrations in the Non-bloom, Pre-bloom, and Post-bloom periods, and made up over 12% of the community during the Bloom period. *Heterocapsa* (red) was the only dinoflagellate other than *Cochlodinium* that was present during this time series with a contribution >1%. Other phytoplankton (shades of green) were most abundant in the Bloom period. <1% Contributors (yellow) represent the combined contribution of 30 phytoplankton that individually contributed to <1% of the community in every period.

Table 3. The top seven contributors were present in different abundances throughout the time series. Below are their percent contributions to the community composition within each period.

Name	Non-bloom	Pre-bloom	Bloom	Post-bloom
<i>Skeletonema</i>	34.4	35.5	30.0	8.2
<i>Cylindrotheca</i>	32.9	45.7	16.8	29.7
<i>Eucampia</i>	12.7	0.9	0.6	0.7
Cryptophytes	1.6	2.6	16.7	4.5
<i>Cochlodinium</i>	0.0	1.3	12.3	0.0
<i>Leptocylindrus</i>	7.6	4.5	7.1	28.8
Pennate	2.4	4.5	3.1	6.6

Table 4. Diatoms dominated the microplankton community throughout the time series. Their presence dropped below 90% only during the week of the *C. polykrikoides* bloom, when both dinoflagellates and other phytoplankton (specifically Cryptophytes) experienced an increase in abundance. Overall, there were 28 diatoms, 9 dinoflagellates, and 6 ‘Other’ phytoplankton (Cryptophytes, *Dinobryon*, *Phaeocystis*, *Dictyocha*, *Chrysochromulina lanceolata*, and *Pyramimonas longicauda*). Appendix D contains a list of the species’ belonging to each group.

Group	Percent (%) of Community Composition			
	Non-bloom	Pre-bloom	Bloom	Post-bloom
Diatom	97.5	94.1	61	91.1
Dinoflagellate	0.2	2.4	13.6	1.5
‘Other’ Phytoplankton	2.1	3.4	25.5	9

The Non-bloom vs. Pre-bloom periods had 67% dissimilarity and were both dominated by diatoms (Table 2, 4). *Eucampia zodiacus* was the third most dominant diatom in the non-bloom period and then was almost entirely absent in the Pre-bloom period. *Eucampia zodiacus* never rebounded and was present only in low concentrations for the rest of the time series (Figure 9). The Pre-bloom vs. Bloom periods were the least dissimilar at only 60% dissimilarity (Table 2). Dissimilarity was driven by *Skeletonema* spp. and *Cylindrotheca closterium* along with the presence of Cryptophytes and *C. polykrikoides* in the Bloom period. Lastly, the 66% dissimilarity in the Pre-bloom vs. Post-bloom was mainly driven by *Skeletonema* spp. abundance decreasing while *Leptocylindrus* spp. abundance increased (Figure 9, Table 2).

Diatoms such as *Skeletonema* spp. and *Cylindrotheca closterium* dominated the time series; the phytoplankton community consisted of >90% diatoms in the Non-bloom, Pre-bloom, and Post-bloom periods (Table 4). However, during the Bloom period when *C. polykrikoides* bloomed, diatoms only represented approximately 61% of the community. Dinoflagellates such as *C. polykrikoides* constituted 13.6% of the community, and other phytoplankton represented the remaining 25%. This time period had the greatest representation of dinoflagellate and other non-diatom phytoplankton (Table 4, Figure 9).

Out of 43 phytoplankton classifications present during this time series, 13 genera/species each contributed to >1% each of the total microplankton population (Figure 9). The other 30 groups that were present individually contributed to <1% of the total population and were combined into one group for display in Figure 9 (<1% Contributors). A list of the species and their percent contribution to total population can be found in Appendix E. *Skeletonema* spp. and *Cylindrotheca* were the two most abundant phytoplankton during this time series. *Skeletonema* spp. dominated the population during August and experienced a decline from making up 30% of the community during the *Cochlodinium* bloom, to only making up 8% of the population in the Post-bloom period, which occurred approximately two weeks following the bloom (Figure 9).

3.2.1 Interpreting the community composition results

The phytoplankton community was sampled from a fixed location at the GSO Pier. This allowed for the observation of changes in the community over time, but did not account for changes in community due to advection within the bay or from offshore waters. While it is understood that Narragansett Bay is a tidally dominated estuary and

therefore the water mass surrounding the GSO Pier is different at different times of the day, accommodating change in composition due to advection was outside the scope of this study.

Sampling the phytoplankton community with an IFCB has several advantages when compared to more traditional sampling. It can sample a greater volume of 4-5mL every 20-40 minutes; the same volume counted with a light microscope would take several hours. It can also sample continuously, allowing for 24/7 coverage of the local phytoplankton community, which cannot be achieved by one person at a light microscope. All the images taken by the IFCB are saved and can be automatically classified through machine-learning, allowing it to identify a wide range of images, occasionally exceeding 10,000 images per sample. This high-resolution sampling and automatic classification allow for the collection of data at an unprecedented scale. It also takes images immediately in-situ, so the cells are more likely to survive than if they were bottled and left in a lab for several hours before processed.

The SIMPER analysis of the IFCB community data revealed that five of the top seven dissimilarity drivers were diatoms. These diatoms were: *Skeletonema* spp., *Cylindrotheca closterium*, *Eucampia zodiacus*, *Leptocylindrus* spp., and the group pennates. Only one dinoflagellate, *Cochlodinium polykrikoides*, was abundant enough to have a high contribution to dissimilarity. The final contributor to dissimilarity being considered between the four periods were the Cryptophytes, which has been added to the group 'Other Phytoplankton' because they are neither a diatom nor dinoflagellate.

Skeletonema spp. had the greatest contribution to dissimilarity in five out of six period comparisons. The only comparison in which *Skeletonema* spp. was not the number

one driver was in the Pre-bloom versus Post-bloom comparison, when it ranked second behind *Cylindrotheca closterium* (Table 2). The findings of *Skeletonema* spp. being the top driver are not surprising; it is considered to be the most dominant diatom in Narragansett Bay (Karentz & Smayda, 1984). Its dominance in the bay makes it likely to be present in many of the samples. *Skeletonema* exhibits allelopathic effects on other phytoplankton, inhibiting their growth through the release of allelochemicals (Pratt, 1966; Hulot & Huisman, 2004; Nagasoe et al., 2006; Yamasaki et al., 2007). This effective strategy in suppressing competitor's growth allows it to sustain its presence throughout the time series. *Skeletonema* spp. was present in high abundance during the first three periods before it experienced a sharp decline by the Post-bloom period (Figure 9). As *Skeletonema* decreased, *Leptocylindrus* spp. increased in the Post-bloom period to fill the recently opened niche (Figure 9).

The second most abundant diatom that contributed to dissimilarity was *Cylindrotheca closterium*. This pennate diatom also releases allelochemicals that are capable of suppressing other phytoplankton's growth (Xu et al., 2019). Its presence from the Non-bloom period to the Pre-bloom period rose sharply, seemingly at the expense of *Eucampia zodiacus* (Figure 9). *Eucampia zodiacus* had high contributions ($\geq 10\%$) to dissimilarity when any period was compared to the Non-bloom period, but it had low contributions ($\geq 1\%$) in the Pre-bloom vs. Bloom, Pre-bloom vs. Post Bloom, and Bloom vs. Post-bloom comparisons (Table 2). There is no evidence of *Eucampia zodiacus* producing allelochemicals to suppress the growth of other phytoplankton. This lack of competitive strategy may explain how it went from highly abundant in the Non-bloom period where it made up almost 13% of the phytoplankton community, to almost non-

existent in the following three periods (Figure 9). It is also possible that they were victims of predation, parasitism, or viral infection.

Only one dinoflagellate had a large contribution to the dissimilarity between periods: *Cochlodinium polykrikoides* (Table 2). It was almost entirely absent from the Non-bloom period (Figures 4a, 9). Its abundance began to increase during the Pre-bloom period, but all samples in this period contained less than 20 images per mL and it only made up 1.3% of the total community composition (Figure 9, Table 3). In the first two periods diatoms comprised 97.5% and 94.1% of the micro-phytoplankton community, respectively (Table 4). By the Bloom period, the percent of diatoms decreased to 61% while the dinoflagellates increased to 13% and the ‘other phytoplankton’ increased to 25.5% of the community. This decrease in diatoms during the Bloom period was driven by two taxa: *Cochlodinium polykrikoides* and Cryptophytes (Table 2, Figure 9).

C. polykrikoides was the most abundant dinoflagellate during the Bloom period and accounted for 12.3% of the total community composition (Table 3). In addition to the decrease in salinity discussed in section 4.1, its quick rise in abundance can potentially be attributed to its allelopathic nature (Tang & Gobler, 2010; Yamasaki et al., 2007). *C. polykrikoides* has a very slow division rate of only 0.3 divisions per day (D. Il Kim et al., 2004) and utilizes allelopathy to inhibit the growth of other phytoplankton to compensate for its own slow growth. During the *C. polykrikoides* bloom there was a sharp reduction in the abundance of *Cylindrotheca* from 45% of the community during the Pre-bloom period to 16.8% during the Bloom period. *Skeletonema* spp. abundance dropped by 5% in the transition from the Pre-bloom period to the Bloom period, and another 22% decline from the Bloom period to the Post-bloom period (Table 3).

A potential contributor to the bloom of *C. polykrikoides* is the increase in the Cryptophytes during the Bloom period (Figure 9). Cryptophytes were present throughout the time series but were most abundant during the Bloom period. The strong co-occurrence of the Cryptophytes and *C. polykrikoides* indicates a relationship between the two taxa, and Cryptophytes have been documented as a food source for the mixotrophic *C. polykrikoides* (Mullholland et al. 2009, Jeong et al. 2004). Their short-lived increase in abundance may have provided the extra energy required and/or nutritional supplements for *C. polykrikoides* to proliferate. By the Post-bloom period, Cryptophyte numbers quickly dropped and their percent contribution to the community composition resembled that of the Non-bloom and Pre-bloom periods again.

As the IFCB becomes more present in coastal laboratories, it will open up a new era of time series data that provide sub-hourly insight to the invisible world of phytoplankton. These time series will contribute to the understanding of specific species interactions and behaviors *in situ*—something that has not been done on a wide scale. For example, my research with the IFCB highlighted the strong co-occurrence of a dinoflagellate and cryptophyte that may not have been noticed if sampling was less often.

3.3 Optical properties of *Cochlodinium polykrikoides*

The absorption of *C. polykrikoides* cultures was measured two ways: with a benchtop spectrophotometer and with an AC-S. The benchtop spectrophotometer method measured filtered cultures for particulate absorption ($a_p(\lambda)$), non-algal particulate absorption ($a_{nap}(\lambda)$) and by difference, phytoplankton absorption ($a_{ph}(\lambda)$) (Figure 10 a-c). Chlorophyll *a* specific absorption ($a_{ph}^*(\lambda)$) was calculated from $a_{ph}(\lambda)$ (Figure 10d). CDOM ($a_g(\lambda)$) was also measured on the spectrophotometer; however, it was not

measured from the same samples as the filtered cultures and was instead measured from the live dilute cultures that were run through the AC-S.

Figure 10. Particulate absorption and non-algal absorption were measured from filtered *C. polykrikoides* cultures on six separate dates ($n=6$). Each set is labeled with the date it was taken on and is the average of three replicates taken. Mean a_p and a_{nap} were calculated as the average from the respective measurements and are represented by black dashed lines. It is important to note that each subplot has a different scale on the y-axis, which is due to the different magnitude of contributions from each absorption type. a) Particulate absorption (a_p) is the combination of non-algal particulate absorption and phytoplankton absorption. b) Non-algal particulate absorption (a_{nap}) had higher contributions in the short wavelengths and low contribution in the long wavelengths. c) Phytoplankton absorption (a_{ph}) was calculated from a_p and a_{nap} . d) Chlorophyll *a* specific absorption (a^*_{ph}) peaked twice in the blue wavelengths and once in the red wavelengths. Mean a^*_{ph} (black dashed line) peaked at 439 nm and 462 nm, and again around 672 nm.

The particulate absorption was much greater than the non-algal particulate absorption within these cultures (Figure 10a&b). $a_{\text{nap}}(\lambda)$ was greatest at 400nm (0.02 m^{-1}) and decreased exponentially with increasing wavelengths. The small contribution of $a_{\text{nap}}(\lambda)$ led the shape of the particulate absorption spectra to be greatly influenced by the phytoplankton absorption (Figure 10a&c).

C. polykrikoides phytoplankton absorption had two consecutive peaks in the blue wavelengths at 439 nm and 462 nm and one in the red wavelengths at 672nm. The dual peaks in the blue wavelengths are similar to a previous study on the phytoplankton absorption of *C. polykrikoides* (Y. Kim et al., 2016). These peaks were conserved when phytoplankton absorption was normalized by chlorophyll *a* (Figure 10d). The chlorophyll *a* specific absorption ($a_{\text{ph}}^*(\lambda)$) also peaked at 439nm (mean $a_{\text{ph}}^*(\lambda) = 0.0146 \text{ m}^{-1}$) and 462 nm (mean $a_{\text{ph}}^*(\lambda) = 0.0132 \text{ m}^{-1}$). The $a_{\text{ph}}^*(\lambda)$ absorption of *C. polykrikoides* calculated by Kim et al. (2016) was similar to the $a_{\text{ph}}^*(\lambda)$ calculated in this study. Y. Kim et al. (2016) found that the mean $a_{\text{ph}}^*(\lambda)$ of *C. polykrikoides* at 440 nm was 0.0328, compared to the mean $a_{\text{ph}}^*(\lambda)$ I calculated from the spectrophotometer measurements (mean $a_{\text{ph}}^*(\lambda) = 0.014$) and the $a_{\text{ph}}^*(\lambda)$ calculated from the AC-S measurements (mean $a_{\text{ph}}^*(\lambda) = 0.0226$).

In addition to the measurements made on the benchtop spectrophotometer, the AC-S measured the total non-water absorption ($a_{\text{nw}}(\lambda)$) and the total non-water attenuation ($c_{\text{nw}}(\lambda)$) of dilute culture (Figure 11a&b). This method was particularly necessary in order to calculate the scattering of the *C. polykrikoides* cultures, of which the spectrophotometer was not capable. Particulate absorption of these live dilute cultures was then calculated by subtracting the $a_{\text{g}}(\lambda)$ present in each dilute culture from $a_{\text{nw}}(\lambda)$ (Figure 11c&d).

Figure 11. Each subplot contains measurements from 4 sets of dilute culture (solid lines) and their mean (black dashed line), labelled with the date that they were taken ($n=4$). a) Non-water absorption (a_{nw}) measured with the AC-S represents the total absorption sans absorption due to water. b) Non-water attenuation (c_{nw}) of the dilute cultures measured with the AC-S. c) CDOM absorption (a_g) of each dilute culture, measured by the spectrophotometer. d) Particulate absorption (a_p) is difference between a_{nw} and a_g . e) Phytoplankton absorption (a_{ph}) is the difference between the AC-S-measured a_p and the calculated dilute mean a_{nap} from the spectrophotometer data. g) Chlorophyll *a* corrected phytoplankton absorption (a^*_{ph}) peaked at 439 nm and again at 670 nm.

The $a_{ph}^*(\lambda)$ calculated from the spectrophotometer measurements differed slightly from the $a_{ph}^*(\lambda)$ calculated from the AC-S measurements (Figure 12). Values for both spectrophotometer and AC-S $a_{ph}^*(\lambda)$ can be found in Appendix F. The increased $a_{ph}^*(\lambda)$ from the AC-S may be due to a lack of measuring the $a_{nap}(\lambda)$ directly from each dilute culture and instead relying on an artificially diluted $a_{nap}(\lambda)$ calculated from the spectrophotometer data. It could also be due to the method of measurement; the AC-S measured live cells in culture whereas the benchtop spectrophotometer measured cells on a filter. The cells may have lysed upon filtration, leading to a decrease in the $a_{ph}^*(\lambda)$. This difference in methodology may also account for the double peak in the blue wavelengths being more evident in the benchtop spectrophotometer than the AC-S. However, it is important to note that both had similarly shaped spectra. This indicates that even though the magnitude is different, the $a_{ph}^*(\lambda)$ measured from both methods peaks at similar wavelengths.

Figure 12. The a_{ph}^* calculated from the AC-S measurements (blue) was higher than the a_{ph}^* calculated by the spectrophotometer (red), although both had similar spectral shapes.

Figure 13. An absorption budget for each contributor to the total absorption of the live dilute cultures measured with the AC-S. Phytoplankton absorption (a_{ph}) is green, the mean dilute non-algal particle absorption (a_{nap}) is red, CDOM absorption (a_g) is yellow, and absorption due to water (a_w) is blue.

Figure 14. The mean non-water attenuation, absorption, and scattering of the *C. polykrikoides* cultures calculated from the AC-S measurements. Mean scattering was greatest in the blue wavelengths and experienced a dip when absorption increased in the red wavelengths.

The greatest mean non-water scattering occurred at 400nm and was 1.3m^{-1} .

Scattering slowly decreased with increasing wavelength before experiencing a dip at

670nm (Figure 14). Scattering is the difference of attenuation and absorption; the attenuation maintained an exponential decrease with increasing wavelengths, whereas absorption experienced a peak at 670nm. Therefore, the dip in non-water scattering was influenced by the corresponding peak in non-water absorption.

3.3.1 Interpreting the IOP data

CDOM had the highest contribution to absorption in the shorter wavelengths and almost no contribution to absorption in the longer wavelengths, constituting almost 70% of the absorption at 400nm and <1% at 700nm (Figure 13). Its spectral shape displayed in Figure 11c is typical of CDOM and displays exponential decay with increasing wavelength. The CDOM of the dilute cultures at 400 nm (0.5m^{-1}) was greater than that of average coastal Atlantic waters (0.08m^{-1}) (Babin et al., 2003). This difference can be explained by the concentration of the dilute cultures; even with the dilution, they were most likely denser with phytoplankton than average seawater. The plankton also produced CDOM as they died and lysed within the culture, so the culture media acted as a sink for CDOM.

Juxtaposed to $a_g(\lambda)$, the absorption due to water ($a_w(\lambda)$) (blue) was highest in the longer wavelengths where it constituted >90% of the total absorption (Figure 13). $a_{\text{nap}}(\lambda)$ (red) had a very small contribution to total absorption across all wavelengths (Figure 13). The $a_{\text{nap}}(\lambda)$ was the mean dilute $a_{\text{nap}}(\lambda)$ measured by the spectrophotometer, and it was measured on a phytoplankton culture that did not contain any non-algal particles such as sediments. The largest contributor to $a_{\text{nap}}(\lambda)$ would be the filaments exuded by *C. polykrikoides* or lysed cells. *C. polykrikoides* is athecate and lacks cellulose plates

(Matsuoka et al., 2008), therefore when the cells lysed, the majority of the detritus produced would be organic matter.

Phytoplankton absorption ($a_{ph}(\lambda)$) accounted for >40% of the total absorption when it peaked between 450-500nm (Figure 13). It also peaked again in the red wavelengths, contributing to 15% of the total absorption around 675nm. The $a_{ph}(\lambda)$ spectra of these cultures (Figure 10c) displays the two characteristic peaks of phytoplankton absorption, with the larger peak occurring in the blue wavelengths (Roessler & Perry, 1989).

Understanding the different IOPs of *C. polykrikoides* is imperative to detecting harmful algal blooms via satellite remote detection. Presently, *C. polykrikoides* can be detected from satellites (Y. Kim et al., 2016) but only if the bloom is monospecific. If there are multiple red HAB species blooming, it will be impossible to distinguish the absorption spectra of *C. polykrikoides* from the other red HAB dinoflagellates because *C. polykrikoides* does not have unique pigments. However, previous attempts neglect to measure the scattering coefficients of *C. polykrikoides*. Due to its size, catenate nature, and lack of cellulose armor (thecate), it is likely to scatter light differently than a much larger, thecate, single celled dinoflagellates such as *Ceratium* spp. This study began to lay the groundwork for calculating the scattering of *C. polykrikoides* so that future studies in remote sensing can utilize the spectral shape of the scattering of *C. polykrikoides* cultures.

4. Conclusions

In the summer of 2018, the toxic dinoflagellate *Cochlodinium polykrikoides* bloomed in Narragansett Bay, Rhode Island. The bloom was captured by an Imaging FlowCytobot deployed from the GSO Pier at the URI Bay Campus. During the peak of the bloom on August 21st, 2018, the number of images containing *C. polykrikoides* exceeded 115 images mL⁻¹. Coincident with the IFCB, a YSI sonde, NOAA Buoy, and NOAA light sensor measured eight environmental parameters. Of these eight parameters, only salinity and dissolved oxygen had a significant relationship with the *C. polykrikoides* image counts.

Non-metric multidimensional scaling and an analysis of similarity (ANOSIM) of the micro-phytoplankton community composition surrounding the *C. polykrikoides* bloom found that there were four distinct communities in the six-week time series. These communities clustered within pre-determined periods in relation to the week of the bloom: Non-bloom, Pre-bloom, Bloom, and Post-bloom. A similarity percentages (SIMPER) analysis found the *C. polykrikoides* was one of the top drivers of dissimilarity between the communities of the pre-determined periods.

Lastly, the inherent optical properties of *C. polykrikoides* were measured. The chlorophyll *a* specific phytoplankton absorption spectrum was found to be similar to previous studies, and the calculated scattering of the *C. polykrikoides* cultures will be useful for future studies on the topic of detecting *C. polykrikoides* blooms via satellite remote sensing.

5. Appendices

Appendix A. The final set-up for recording absorption and attenuation of *C. polykrikoides* with an AC-S.

Appendix B. All classes in the current IFCB classifier that were represented in the August and September 2018 community.

Name	
<i>Akashiwo</i>	<i>Pleurosigma</i>
<i>Asterionellopsis glacialis</i>	<i>Polykrikos</i>
<i>Bacillaria</i>	<i>Prorocentrum</i>
<i>Cerataulina pelagica</i>	<i>Proto-peridinium</i>
<i>Ceratium</i>	<i>Pseudo-nitzschia</i>
<i>Chaetoceros lorenzianus</i>	<i>Pyramimonas longicauda</i>
<i>Chaetoceros lorenzianus</i> form3	<i>Rhizosolenia</i>
<i>Chrysochromulina lanceolata</i>	<i>Skeletonema</i>
<i>Corethron hystrix</i>	<i>Striatella</i>
<i>Coscinodiscus</i>	<i>Strobilidium</i>
<i>Cylindrotheca</i>	<i>Strombidium morphotype2</i>
<i>Dactyliosolen blavyanus</i>	<i>Thalassionema</i>
<i>Dictyocha</i>	<i>Thalassiosira</i>
<i>Dinobryon</i>	<i>Tintinnida</i>
<i>Dinophysis</i>	bad
<i>Ditylum brightwellii</i>	bubble
<i>Eucampia</i>	ciliate
<i>Guinardia delicatula</i>	cryptophyta
<i>Guinardia striata</i>	detritus
<i>Gyrodinium</i>	detritus clear
<i>Heterocapsa rotundata</i>	Group <i>Chaetoceros</i> helical
<i>Laboea strobila</i>	group <i>Chaetoceros</i> single cell
<i>Leptocylindrus</i>	group <i>Chaetoceros</i> straight
<i>Licmophora</i>	group pennate
<i>Cochlodinium</i>	pollen
<i>Mesodinium</i>	unclassified
<i>Odontella</i>	unknown1
<i>Paralia sulcata</i>	zooplankton
<i>Phaeocystis</i>	

Appendix C. Weighted biplot displays the relationship between the 43 phytoplankton taxa. Arrow number 4 represents *Cylindrotheca closterium* and arrow number 19 represents *Skeletonema* spp.

Appendix D. List of all species in the analysis and the group that they belong in.

Group	Name	
Diatoms	<i>Asterionellopsis glacialis</i>	group pennate
	<i>Bacillaria</i>	<i>Guinardia delicatula</i>
	<i>Cerataulina pelagica</i>	<i>Guinardia striata</i>
	<i>Chaetoceros lorenzianus</i>	<i>Leptocylinthus</i>
	<i>Chaetoceros lorenzianus form3</i>	<i>Licmophora</i>
	<i>Corethron hystrix</i>	<i>Odontella</i>
	<i>Coscinodiscus</i>	<i>Paralia sulcata</i>
	<i>Cylindrotheca</i>	<i>Pleurosigma</i>
	<i>Dactyliosolen blavyanus</i>	<i>Pseudo-nitzschia</i>
	<i>Ditylum brightwellii</i>	<i>Rhizosolenia</i>
	<i>Eucampia</i>	<i>Skeletonema</i>
	group <i>Chaetoceros</i> helical	<i>Striatella</i>
	group <i>Chaetoceros</i> single cell	<i>Thalassionema</i>
	group <i>Chaetoceros</i> straight	<i>Thalassiosira</i>
Dinoflagellates	<i>Akashiwo</i>	<i>Heterocapsa</i>
	<i>Ceratium</i>	<i>Polykrikos</i>
	<i>Cochlodinium</i>	<i>Prorocentrum</i>
	<i>Dinophysis</i>	<i>Protoperidinium</i>
	<i>Gyrodinium</i>	
Other phytoplankton	Cryptophyte	<i>Phaeocystis</i>
	<i>Dictyocha</i>	<i>Pyramimonas longicauda</i>
		<i>Chrysochromulina</i>
	<i>Dinobryon</i>	<i>lanceolata</i>

Appendix E. List of all other species present in the time series (minus the top 7 contributors) and their percent (%) contribution to the phytoplankton community during each period.

<i>Name</i>	<i>Non-bloom</i>	<i>Pre-bloom</i>	<i>Bloom</i>	<i>Post-bloom</i>
<i>Akashiwo</i>	0.00	0.02	0.01	0.18
<i>Asterionellopsis glacialis</i>	0.11	0.06	0.05	1.14
<i>Bacillaria</i>	0.06	0.02	0.01	0.06
<i>Cerataulina pelagica</i>	0.22	0.30	0.59	0.21
<i>Ceratium</i>	0.01	0.01	0.00	0.01
<i>Chaetoceros lorenzianus</i>	0.01	0.00	0.01	0.05
<i>Chaetoceros lorenzianus form3</i>	0.03	0.01	0.03	0.10
<i>Chrysochromulina lanceolata</i>	0.02	0.09	0.34	0.38
<i>Corethron hystrix</i>	0.01	0.05	0.06	0.07
<i>Coscinodiscus</i>	0.00	0.01	0.01	0.02
<i>Dactyliosolen blavyanus</i>	0.02	0.02	0.01	0.02
<i>Dictyocha</i>	0.00	0.01	0.00	0.06
<i>Dinobryon</i>	0.34	0.49	7.90	1.90
<i>Dinophysis</i>	0.03	0.10	0.06	0.19
<i>Ditylum brightwellii</i>	0.00	0.01	0.01	0.17
group <i>Chaetoceros</i> helical	0.52	0.15	0.18	6.59
group <i>Chaetoceros</i> single cell	0.24	0.05	0.01	0.21
group <i>Chaetoceros</i> straight	5.25	1.37	1.06	1.53
<i>Guinardia delicatula</i>	0.04	0.07	0.03	0.67
<i>Guinardia striata</i>	0.01	0.00	0.00	0.09
<i>Gyrodinium</i>	0.00	0.00	0.02	0.02
<i>Heterocapsa</i>	0.08	0.70	0.64	1.01
<i>Licmophora</i>	0.02	0.02	0.01	0.15
<i>Odontella</i>	0.00	0.00	0.00	0.02
<i>Paralia sulcata</i>	0.01	0.06	0.05	0.26
<i>Phaeocystis</i>	0.00	0.00	0.00	0.00
<i>Pleurosigma</i>	0.16	0.10	0.05	0.19
<i>Polykrikos</i>	0.00	0.00	0.00	0.00
<i>Prorocentrum</i>	0.06	0.35	0.59	0.27
<i>Protoperidinium</i>	0.00	0.01	0.00	0.01
<i>Pseudo-nitzschia</i>	0.59	0.38	0.60	3.47
<i>Pyramimonas longicauda</i>	0.13	0.25	0.46	2.12
<i>Rhizosolenia</i>	0.03	0.13	0.03	0.09
<i>Striatella</i>	0.00	0.00	0.00	0.01
<i>Thalassionema</i>	0.43	0.23	0.37	0.25
<i>Thalassiosira</i>	0.00	0.01	0.01	0.10

Appendix F. Chlorophyll *a* specific absorption values of *C. polykrikoides* calculated from the spectrophotometer measurements. Wavelength is represented by λ .

λ	a^*_{ph}	λ	a^*_{ph}	λ	a^*_{ph}	λ	a^*_{ph}	λ	a^*_{ph}	λ	a^*_{ph}
400	0.0109	450	0.0130	500	0.0085	550	0.0028	600	0.0015	650	0.0021
401	0.0109	451	0.0130	501	0.0083	551	0.0027	601	0.0015	651	0.0021
402	0.0108	452	0.0130	502	0.0081	552	0.0026	602	0.0014	652	0.0022
403	0.0109	453	0.0130	503	0.0079	553	0.0025	603	0.0014	653	0.0023
404	0.0110	454	0.0130	504	0.0078	554	0.0024	604	0.0014	654	0.0024
405	0.0111	455	0.0130	505	0.0076	555	0.0023	605	0.0014	655	0.0025
406	0.0112	456	0.0130	506	0.0074	556	0.0023	606	0.0014	656	0.0026
407	0.0114	457	0.0131	507	0.0072	557	0.0022	607	0.0015	657	0.0028
408	0.0115	458	0.0131	508	0.0070	558	0.0021	608	0.0015	658	0.0030
409	0.0116	459	0.0132	509	0.0069	559	0.0020	609	0.0015	659	0.0032
410	0.0118	460	0.0132	510	0.0067	560	0.0020	610	0.0016	660	0.0035
411	0.0120	461	0.0132	511	0.0065	561	0.0019	611	0.0016	661	0.0038
412	0.0121	462	0.0132	512	0.0064	562	0.0018	612	0.0016	662	0.0041
413	0.0122	463	0.0132	513	0.0062	563	0.0018	613	0.0017	663	0.0044
414	0.0123	464	0.0132	514	0.0061	564	0.0017	614	0.0017	664	0.0047
415	0.0124	465	0.0131	515	0.0060	565	0.0017	615	0.0017	665	0.0050
416	0.0125	466	0.0130	516	0.0058	566	0.0016	616	0.0018	666	0.0054
417	0.0126	467	0.0129	517	0.0057	567	0.0016	617	0.0018	667	0.0057
418	0.0126	468	0.0128	518	0.0056	568	0.0016	618	0.0018	668	0.0059
419	0.0127	469	0.0127	519	0.0055	569	0.0016	619	0.0018	669	0.0062
420	0.0127	470	0.0125	520	0.0054	570	0.0016	620	0.0019	670	0.0064
421	0.0128	471	0.0124	521	0.0053	571	0.0015	621	0.0019	671	0.0065
422	0.0128	472	0.0122	522	0.0052	572	0.0015	622	0.0019	672	0.0067
423	0.0129	473	0.0120	523	0.0051	573	0.0015	623	0.0019	673	0.0067
424	0.0130	474	0.0118	524	0.0050	574	0.0015	624	0.0019	674	0.0067
425	0.0131	475	0.0116	525	0.0049	575	0.0015	625	0.0019	675	0.0066
426	0.0132	476	0.0114	526	0.0049	576	0.0015	626	0.0019	676	0.0065
427	0.0134	477	0.0112	527	0.0048	577	0.0015	627	0.0019	677	0.0064
428	0.0136	478	0.0110	528	0.0047	578	0.0015	628	0.0019	678	0.0061
429	0.0137	479	0.0109	529	0.0046	579	0.0015	629	0.0019	679	0.0059
430	0.0139	480	0.0107	530	0.0045	580	0.0015	630	0.0020	680	0.0056
431	0.0141	481	0.0105	531	0.0044	581	0.0015	631	0.0020	681	0.0052
432	0.0143	482	0.0104	532	0.0044	582	0.0015	632	0.0020	682	0.0049
433	0.0144	483	0.0103	533	0.0043	583	0.0016	633	0.0019	683	0.0045
434	0.0145	484	0.0102	534	0.0042	584	0.0016	634	0.0019	684	0.0041
435	0.0146	485	0.0101	535	0.0041	585	0.0016	635	0.0019	685	0.0037

436	0.0146	486	0.0100	536	0.0040	586	0.0016	636	0.0019	686	0.0033
437	0.0146	487	0.0099	537	0.0039	587	0.0016	637	0.0019	687	0.0029
438	0.0146	488	0.0098	538	0.0039	588	0.0016	638	0.0019	688	0.0025
439	0.0146	489	0.0098	539	0.0038	589	0.0016	639	0.0019	689	0.0022
440	0.0144	490	0.0097	540	0.0037	590	0.0016	640	0.0019	690	0.0019
441	0.0143	491	0.0096	541	0.0036	591	0.0016	641	0.0019	691	0.0016
442	0.0142	492	0.0095	542	0.0035	592	0.0016	642	0.0019	692	0.0014
443	0.0140	493	0.0095	543	0.0034	593	0.0016	643	0.0020	693	0.0011
444	0.0138	494	0.0093	544	0.0033	594	0.0016	644	0.0020	694	0.0009
445	0.0137	495	0.0092	545	0.0032	595	0.0016	645	0.0020	695	0.0008
446	0.0135	496	0.0091	546	0.0031	596	0.0015	646	0.0020	696	0.0007
447	0.0134	497	0.0090	547	0.0030	597	0.0015	647	0.0020	697	0.0005
448	0.0132	498	0.0088	548	0.0030	598	0.0015	648	0.0020	698	0.0004
449	0.0131	499	0.0087	549	0.0029	599	0.0015	649	0.0020	699	0.0004

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