THE EFFECTS OF CRUDE OIL ON THE MARINE DIATOM, *PHAEODACTYLUM TRICORNUTUM,* GROWN IN SILICA-ENRICHED AND SILICA-LIMITED CONDITIONS

An Undergraduate Research Scholars Thesis

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ABSTRACT

The Effects of Crude Oil on the Marine Diatom, *Phaeodactylum tricornutum,* Grown in Enriched and Silica-Limited Conditions

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This experiment was designed to determine the effects that crude oil and dispersants have on a marine diatom, *Phaeodactylum tricornutum*. This species was chosen as it has a siliceous frustule, which may increase its resilience to pollutant exposure. We hypothesized that *P. tricornutum* grown without their siliceous frustule would be more susceptible to pollutants compared to those grown with their siliceous frustule. We analyzed estimated oil equivalents, growth, photosynthetic efficiency, and macromolecular composition to examine the effects of oil and oil and dispersant exposure. *P. tricornutum* exhibited a high level of robustness in response to WAF and DCEWAF and a high sensitivity to CEWAF. Silica-limitation proved to be a major factor in the sensitivity of *P. tricornutum* to the oil and dispersants, which can be explained by significant differences in treatments with and without the presence of silica. We found that the effect of oil and dispersants on phytoplankton vary based on the environmental conditions and oil concentrations and that the effects of oil exposure are not always detrimental. These data provide an understanding of the response of this phytoplankton following an oil spill. In future studies, it would be beneficial to expand the parameters being tested to gain more insight into the physiological changes in phytoplankton cells resulting from crude oil exposure.

DEDICATION

This thesis work is dedicated to my parents, who have been a constant source of support and encouragement throughout my life. Thank you for always helping me realize my full potential and for inspiring me to achieve greatness. I am so thankful for all of the sacrifices you have made for me to be where I am today and am grateful that we can share these victories and successes as a family.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Quigg, for believing in me and providing me with all of the resources, support, and encouragement necessary to achieve my goals as a budding scientist. I would also like to thank Dr. Bretherton and Dr. Kamalanathan for their guidance and assistance as I encountered some trials and tribulations throughout my research.

Big thanks also go to my colleagues, friends, and the faculty in the Department of Marine Biology for making my experience at Texas A&M University at Galveston truly unforgettable. I would also like to thank the GOMRI Aggregation and Degradation of Dispersants and Oil by Microbial Exopolymers (ADDOMEx) consortium for funding this research.

Last but certainly not least, I would like to thank the Abe and Peggy Levy Foundation Fellowship for providing me with the means and support necessary to pursue my dreams and make the most of my time at Texas A&M University at Galveston. Without their support, I would not be where I am today.

NOMENCLATURE

CEWAF	chemically enhanced water accommodated fraction
CEWAF-Si	chemically enhanced water accommodated fraction without silica added to the media
DCEWAF	dilute chemically enhanced water accommodated fraction
DCEWAF-Si	dilute chemically enhanced water accommodated fraction without silica added to the media
DCM	Dichloromethane
DwH	Deepwater Horizon
EOE	estimated oil equivalents
F_o	minimum fluorescence
F_m	maximum fluorescence
F_v	variable fluorescence
F_v/F_m	maximum quantum yield of photosystem II (PSII)
GoM	Gulf of Mexico
PAH	polycyclic aromatic hydrocarbon
PSII	photosystem II
WAF	water accommodated fraction
WAF-Si	water accommodated fraction without silica added to the media
ρ	energy transfer between photosynthetic units
σ_{PSII}	functional absorption cross section of PSII

τ	time constant for relaxation kinetics of fluorescence following single turnover flash

CHAPTER I

INTRODUCTION

In April 2010, the Deepwater Horizon (DwH) wellhead experienced a blowout which resulted in the release of >4 million barrels of oil into the waters of the Gulf of Mexico (GoM) (Crone and Tolstoy, 2010; McNutt et al., 2012). Chemical dispersants were applied to the ruptured wellhead near the seafloor as well as to the surface ocean to mitigate the consequences of the spill. This volume of dispersant applied was unprecedented in terms of remediating the high outflow of crude oil from the well (Kujawinski et al., 2011). Scientists are interested in studying the short and long-term effects of crude oil as well as dispersants on the ecosystems near the spill as well as throughout the GoM.

Following the DwH oil spill, several laboratory groups dedicated their time to exploring and monitoring the effect of polycyclic aromatic hydrocarbons (PAHs), on the growth and development of marine megafauna (Allan et al., 2012). In a study conducted by Xia et al. (2012), one finfish species and three invertebrate species presented PAH levels that were below the Level of Concern set forth by the Federal Drug Administration. Despite the extensive findings of this study and those like it, there is still a lot that needs to be learned about other components of the food web. The recovery and composition of phytoplankton communities following an oil spill is of particular interest given their role as a food source at the base of the food web.

Oil is known to have adverse effects on the growth and photosynthetic efficiency across many different phytoplankton species (González et al., 2009). These adverse effects of oil can be found in multiple studies across many species of phytoplankton (Bretherton et al., 2018). Dispersants, which are used to accommodate more oil into the water column, have been shown

to also have adverse effects on the growth and functionality of the photosynthetic systems of phytoplankton (Hook and Osborn, 2012).

Though there are many studies available on oil toxicity on these phytoplankton, these findings are still limited in comparison to other more well-developed fields of study. The limited amount of data available on oil toxicity on phytoplankton species is due in part to the recent standardization of methodology used for exposure (Singer et al., 2000). In recent years, methodology has been created that exposes algal cells to only water accommodated fractions of oil opposed to adding oil to media and exposing the phytoplankton cells this way. These older methods had the potential of adding some unknown variables such as shading and limiting gas exchange that could have skewed observed changes in phytoplankton physiology instead of showing the effects of the soluble components of oil on these species.

In this experiment, we exposed a marine diatom typically found in the GoM, *Phaeodactylum tricornutum,* to water-accommodated fractions (WAF) of oil in control and silica-limited artificial seawater media. The water soluble components of oil in this series of experiments led to oil concentrations that resembled the oil concentrations found after the spill (Wade et al., 2016). We also exposed *P. tricornutum* to a chemically-enhanced water accommodated fraction (CEWAF) that consisted of the dispersant Corexit added to WAF in a 1:20 ratio. CEWAF was then used to make a diluted CEWAF, which was one of the most environmentally relatable treatment conditions given the GoM post oil spill (Bretherton et al., 2018).

We used artificial seawater instead of ambient water from the GoM, because we wanted to be able to limit the pollutants that may be present in media made with ambient water. In doing so, we can attribute the changes in phytoplankton biomass and oil concentration to the activity of

the algal cells and not foreign presences in the media. Furthermore, artificial seawater allowed us to limit the amount of contaminants that may be smaller than the 0.2µm mesh that is typically used to filter ambient seawater in natural seawater media. We anticipated that the CEWAF treatments would invoke the greatest physiological changes in relation to treatments with lower oil concentrations. Additionally, we expected to see a more pronounced physiological response in the treatments without silica added to the media due to the siliceous nature of *P. tricornutum* frustules.

CHAPTER II

METHODS

Experimental setup

Making Artificial Seawater

Artificial seawater was used as the medium for the phytoplankton culture as well as the basis for water accommodated fraction (WAF) of oil, CEWAF, and DCEWAF. We used the artificial seawater recipe adapted from Berges et al. (2001), who modified that of Harrison et al. (1980). This recipe was used because it has been tested with many species and classes of open ocean phytoplankton (Harrison et al., 1980). A 20L carboy was filled to 10L of deionized water. The designated amounts of anhydrous salts were added to this initial volume of water, followed by an additional amount of 5L (15L total) before mixing. The hydrated salts were measured and added to a 2L flask filled to 1200mL with DI water. After the addition of these hydrated salts, the flask was topped off to 2000mL and allowed to stir at medium speed for one hour alongside the 20L carboy. After the hour-long mixing period, the hydrated salts were added and allowed to mix in the 20L carboy for another hour.

The pH of the artificial seawater was monitored using a pH probe. After thoroughly rinsing the pH probe with DI water, the probe was placed inside the carboy. To increase the alkalinity of the artificial seawater, 10% Trisma base was slowly added to the carboys and stirred vigorously on a stir plate until the pH reached 8.2. If the measured pH was already at the correct level, then no additions were made.

Cultivation of phytoplankton

The strain of *Phaeodactylum tricornutum* UTEX 646 was obtained from The Culture Collection of Algae at The University of Texas at Austin (UTEX). *P. tricornutum* was aseptically cultured in the above medium. Silica-limited media was prepared as described above with one exception, no addition of any siliceous components. The *P. tricornutum* strain was inoculated into 1L glass bottles with equal volume of media and phytoplankton. All cultures were grown in a constant 19±1°C with light intensity of 100-130 µmol m^{-2} s^{-1}. To best simulate light conditions, the cultures were exposed to a 12:12h light:dark cycle.

Half of the total volume of phytoplankton cultures was used to form a silica-limited culture. 50mL of the existing phytoplankton were transferred into a falcon tube and centrifuged for 10 minutes at 2000g. After this first round of centrifugation, the supernatant liquid was removed leaving the pellet of phytoplankton remaining. The phytoplankton were rinsed thoroughly with silica-limited media and centrifuged for another 10 minutes at 2000g. Once again, the supernatant liquid was removed leaving a few milliliters of media in the falcon tube with the pellet. This condensed pellet of phytoplankton was poured into an autoclaved 1L glass bottle with silica-limited media and grown in the same conditions as those listed above.

Preparation of treatments

The WAF treatments were created using the CROSERF method described by Singer et al. (2000). To begin this process 1L of f/2 media, created in the steps outlined above, was added to each aspirator. Each glass aspirator was equipped with a bottom spigot and a glass stopper. After the addition of this initial volume of water, 400µL of MC252 Louisiana crude oil was added to each aspirator using a positive displacement pipette. CEWAF was prepared by mixing the

dispersant, Corexit 9500A, with the crude oil in a 1:20 dispersant:oil ratio. After vortexing for one minute, 400µL of this mixture were added to the 1L aspirators already filled with the appropriate media. The aspirators were then covered and allowed to stir for 24 hours at a speed that resulted in a vortex that occupied the upper quarter of the aspirator.

After 24 hours of mixing, each of the 1L aspirators was filtered through a 20µm nylon mesh into 9L glass aspirators, one for each stock solution taking special care to exclude the oil slick remaining in each of the 1L aspirators.

The DCEWAF was created from the CEWAF stock solution. CEWAF is inherently higher in oil concentration than WAF without the addition of dispersant. 5mL of both the WAF and CEWAF stock solutions were removed and the EOE value was found for each. The CEWAF stock solution was then diluted with f/2 media made from artificial seawater until the EOE matched that of the WAF.

Starting cell concentrations

Cell densities of the existing phytoplankton culture were determined using hemocytometers. These cell counts were then used to find a volume of the cultures that would equate to 50,000 cell/mL for the 1000mL of the glass bottles for a total cell concentration of 50×10^6 cells/L. This calculated volume was then used to find the amount of media that needed to be added by treatment. Each of the treatment bottles was filled with the appropriate volume and type of media by treatment. Non-biological controls were created for the media, WAF, CEWAF, and DCEWAF by pouring about 700mLs of each in separate 1L glass bottles. The glass bottles were then transferred to the incubation room where they remained for the duration of the experiment in conditions like the culturing conditions outlined above.

Experimental design

The experiment was performed in two parts. The first part of the experiment involved the exposure of *P. tricornutum,* both the silica-enriched and silica-limited cultures, to WAF. Part two of the experiment included the exposure of silica-enriched and silica-limited cultures of *P. tricornutum* to CEWAF and DCEWAF. Each treatment was completed in triplicate excluding non-biological controls.

Measurements

Estimated Oil Equivalents

The Estimated Oil Equivalents (EOE) was calculated for each treatment every day of the experiment. 10mL aliquots were removed from each bottle and transferred to a 20mL scintillation vial. Samples were preserved with 10mL of dichloromethane (DCM) also added to the scintillation vials. 3mL aliquots were carefully removed from the bottom of each scintillation vial and transferred into a 4mL quartz cuvette. The maximum intensity of each sample was found on the Shimadzu spectrofluorometer (RF-5301PC, Shimadzu, Houston, TX, USA) at excitation and emission wavelengths of 322 and 376, respectively.

A calibration curve was created using a series of dilutions ranging from 0.001075g/L to 0.086g/L. The equations yielded from this curve were then used to translate the maximum intensities recorded into actual oil concentrations for each sample. Equation 1 was used to calculate the EOE for the first WAF treatments, and Equation 2 was used to calculate the EOE for the CEWAF and DCEWAF treatments.

$$\text{EOE} = 1\text{E-}05 \times (\text{Spectrofluorometer Fluorescence Intensity}) - 0.0007 \quad (1)$$

$$\text{EOE} = 0.0512 \times (\text{Spectrofluorometer Fluorescence Intensity}) - 1.1131 \quad (2)$$

Chlorophyll a and calculation of growth rates

Measurements of chlorophyll *a* were made using a benchtop fluorometer (10AU Turner Designs). 4mL aliquots were removed from each treatment bottle every day of experimentation (7 days) for the WAF experiment and every other day in the CEWAF and DCEWAF experiments. The samples were dark acclimated for a period of at least 15 minutes prior to placing them in the fluorometer. In addition to running the samples, blanks of the f/2, WAF, CEWAF, and DCEWAF as well as their silica-limited counterparts were also sampled to account for background fluorescence. Intensity of chlorophyll fluorescence was measured for each sample and was used to calculate the chlorophyll *a* concentration (µg/L) using a calibration curve. Aliquots of f/2 medium, WAF, CEWAF, and DCEWAF non-biological controls were used to account for background fluorescence (Cullen and Davis, 2003). Additionally, a standard curve was created using a chlorophyll standard extracted from *Anacystis nidulans* (Sigma-Aldrich) and the Environmental Protection Agency Method 445.0 (Arar and Collins, 1997).

Chlorophyll specific growth rates (µ, d^{-1}) were calculated using the following equation:

$$\mu = \frac{ln C_t - ln C_0}{t} \quad (3)$$

where µ is the average specific growth rate, C_t is the chlorophyll concentration at time *t*, C_0 is the chlorophyll concentration at beginning of the exponential phase, and *t* is the time considered (days).

Fluorescence induction and relaxation parameters

The Fluorescence Induction and Relaxation (FIRe) fluorometer system (Satlantic, Halifax NS, Canada) was used to measure the chlorophyll fluorescence of the *P. tricornutum* cells in the various treatments using the single turnover protocol (Kolber et al. 1998, Kromkamp and Forster

2003). 4mL aliquots of each treatment were removed for each day of the 7-day experiment. These aliquots were transferred into 4mL disposable cuvettes and left to dark-acclimate for 15 minutes. Following this period of dark-acclimation, the samples were placed into the fluorometer and the minimum fluorescence (F_o), maximum fluorescence (F_m) were measured and automatically recorded. From these values, the variable fluorescence (F_v) was calculated [F_o-F_v]. Additionally, F_v/F_m (maximum quantum yield of photosystem II (PSII) photochemistry, σ_{PSII} (functional absorption cross section of PSII, Å 2 ·quanta^{-1}), ρ (energy transfer between photosynthetic units), and τ (time constant for relaxation kinetics of fluorescence following single turnover flash, µsec) were used from the exponential phase of *P. tricornutum* growth.

Fourier Transform Infrared spectroscopy

Samples were obtained on day 1 of the experiment and day 7 of both the WAF and CEWAF/DCEWAF experiments. At both time points, 50mL of culture from each treatment were transferred into centrifuge tubes. The suspension was centrifuged at 2000 × g for 10 minutes to form a pellet. Supernatant liquid was decanted from the tubes and the samples were immediately stored at -4ºC in the dark. To sample, the pellets were allowed to defrost and a sample was removed and dried using a hand-held dryer. Spectra were obtained using a Varian 3100 FTIR Excalibur series spectrometer running Varian Resolution-Pro 4.0 software. The absorbance spectra were collected between 3650cm^{-1} and 600cm^{-1} at a spectral resolution of 8cm^{-1} with 50 scans coadded (Giordano et al., 2001; Sackett and Cox, 2013). Background spectra were obtained prior to each sample measurement, and each sample measurement was taken in triplicate. ATR correction for the diamond crystal was performed and the spectra were exported

in GRAMS format for multivariate analysis using The Unscrambler X v 10.4 (Camo Inc., Oslo, Norway) using a method described by Kamalanathan et al. (2018).

Statistical Analysis

Data represented means of the triplicated measurements taken for each experiment as well as the standard deviation. An analysis of variance (ANOVA) test was used to determine the presence of significant differences between treatments. P-values less than 0.05 were defined as being statistically significant values.(Kolber et al., 1998; Kromkamp and Forster, 2003).

CHAPTER III

RESULTS

Estimated Oil Equivalents

WAF treatments

The EOE for all treatments for the WAF treatments were below detection limits for the entirety of the experimental time period.

CEWAF and DCEWAF treatments

Oil concentrations had completely declined in the CEWAF and CEWAF-S treatments by day 3 (Figure 1). Additionally, from day 5 onward of experimentation, the oil concentration had reached a plateau. In all of the biological controls, both control and silica-limited, the EOE was below detection limits. CEWAF treatments always showed the highest starting EOE values, with an average oil concentration of 15 mg/L for CEWAF and 14.38 mg/L for CEWAF-Si on day 1. These treatments also displayed the lower percentage decrease between day 1 and day 5 with CEWAF and CEWAF-S showing percent changes of 32.85 and 53.59%, respectively. Percent decrease of EOE values for DCEWAF and DCEWAF-S were higher than those for the CEWAF treatment, with decreases of 86.36 and 94.53%, respectively. There were significant differences (1-way ANOVA, $p<0.05$) between Control and CEWAF, CEWAF and DCEWAF, CEWAF and CEWAF-Si, Control-Si and CEWAF-Si, and CEWAF-Si and DCEWAF-Si.

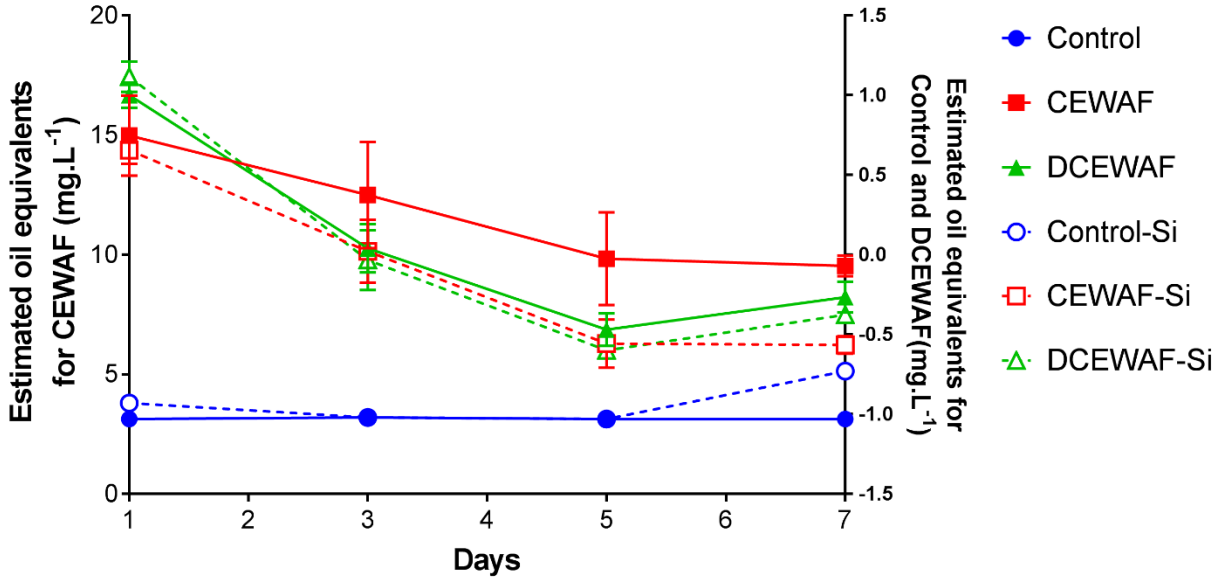

Figure 1. Estimated Oil Equivalents (EOE) of the biological controls, WAF, CEWAF, DCEWAF, and silica-limited biological controls, WAF, CEWAF, and DCEWAF. The solid lines represent the silica-enriched treatments, and the dotted lines represent the silica-limited. The error bars represent standard deviation of the means. The vertical axis on the left is used for the CEWAF treatments while the vertical axis on the right is used to convey EOE of WAF and DCEWAF.

Chlorophyll *α* and changes in biomass

WAF Treatments

Chlorophyll *α*, a proxy for biomass increase, in the biological control and corresponding WAF treatment increased steadily from day 4 onward, while Control-Si and the WAF-Si cultures peaked at day 5 followed by a rapid decline (Figure 2). There were significant differences between Control and Control-Si, Control and WAF-Si, WAF and Control-Si, and WAF and WAF-Si (1-way ANOVA, $p<0.05$). There were no significant differences between Control and WAF or Control-Si and WAF-Si. During the phase of exponential growth for the WAF and Control treatments (day 4 to 6) the growth chlorophyll specific growth rates were calculated to

be 1.19 µg/L and 1.48 µg/L, respectively. These growth rates are higher than those observed in the Control-Si and WAF-Si treatments (0.77 µg/L and 0.79 µg/L, respectively).

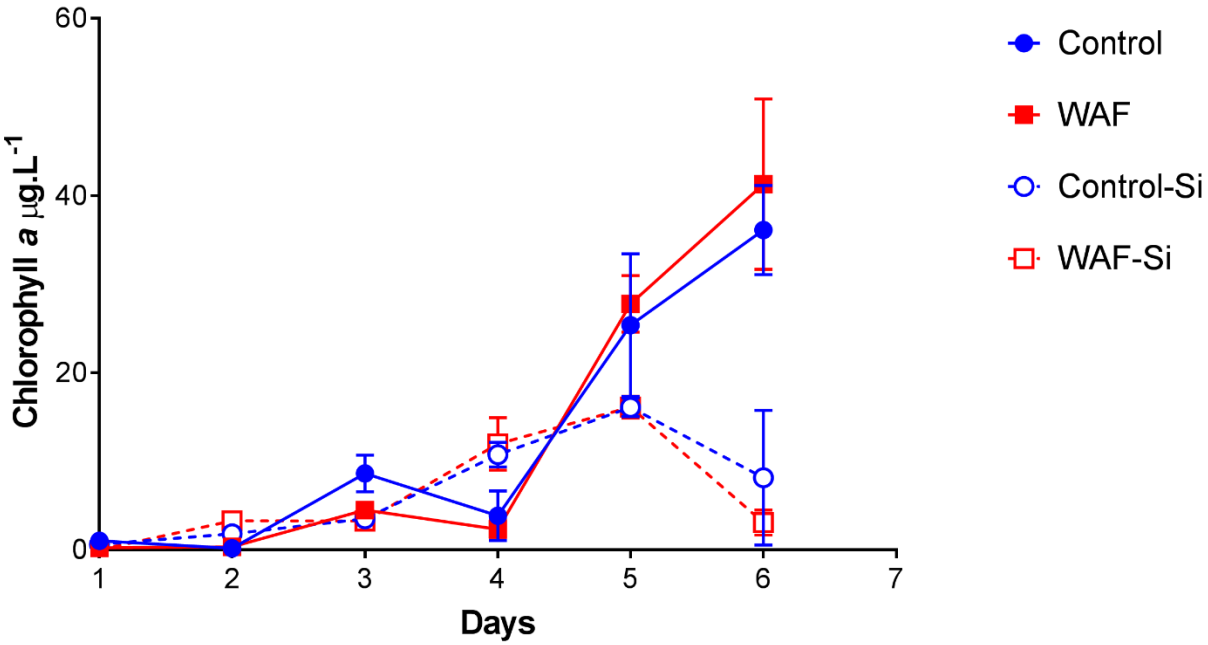

Figure 2. Relative chlorophyll *a* concentration of the biological controls, WAF, and their silica limited counterparts. The solid lines represent the silica-enriched treatments, and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

CEWAF and DCEWAF treatments

Chlorophyll *a* concentrations across all six treatments of the second part of the experiment increased with time (Figure 3). There were no noticeable differences in chlorophyll concentration until day 5. On day 6, there were significant differences (1-way ANOVA, $p<0.05$) observed between Control and Control-Si, CEWAF and CEWAF-S, DCEWAF and CEWAF-S, and CEWAF-Si and DCEWAF-Si. The greatest growth rate during the exponential phase (day 5 to 7) was observed in the DCEWAF-Si cultures (0.55µg/L) while the smallest growth rate was

shown in the Control-Si treatment (0.388 µg/L). All of the chlorophyll specific growth rates are shown in Table 1.

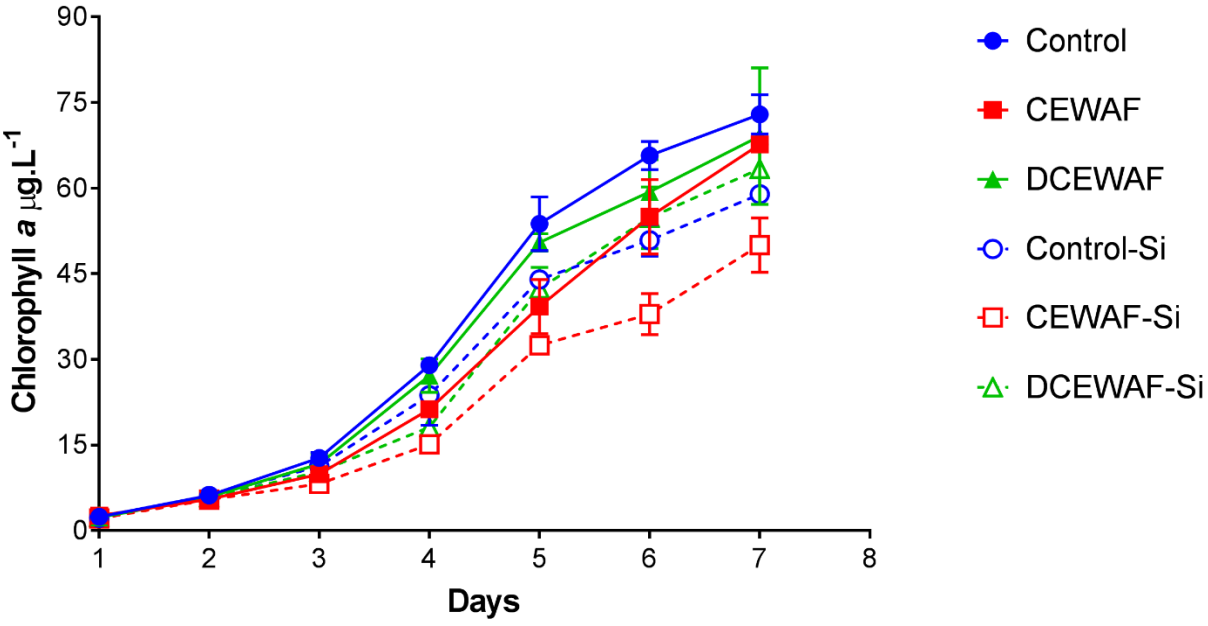

Figure 3. Chlorophyll concentrations (µg/L) of the biological controls, CEWAF, and DCEWAF as well as their silica-limited counterparts. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

Table 1. Chlorophyll *a* concentrations across all treatments. Standard deviations are calculated from the triplicate measurements taken for each treatment.

Treatment	Chlorophyll concentration (µg/L)	Standard deviation (±)
Control	0.41	0.035
CEWAF	0.47	0.09
DCEWAF	0.39	0.05
Control-Si	0.38	0.09
CEWAF-Si	0.45	0.05
DCEWAF-Si	0.55	0.08

Photosynthetic efficiency and photophysiology

WAF Treatments

The maximum quantum yield of PSII photochemistry (F_v/F_m) of all the treatments exposed to WAF and control conditions did not show any significant variation over time. As shown in Figure 4, F_v/F_m showed no variation between treatments until day 6, when the cultures presented a slight separation, though these differences were not found to be significant. Additionally, no significant change was observed in τ (Figure 5), or ρ (Figure 6), σ (Figure 7). The largest change in F_v/F_m was observed in the WAF-Si treatment at 0.02 day^{-1}. The smallest change in F_v/F_m was observed in the WAT treatment, with a rate of decrease of 0.008 day^{-1}.

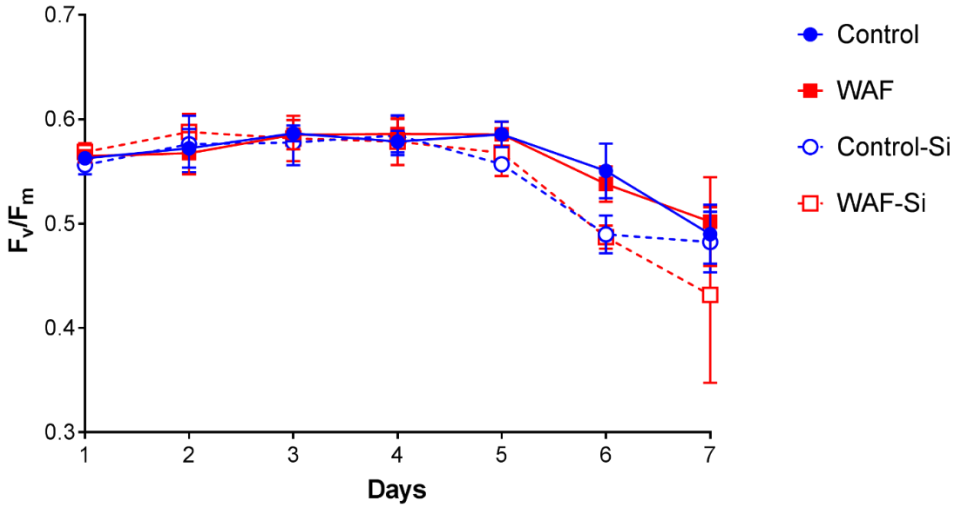

Figure 4. Fv/Fm for WAF of Control, WAF, Control-Si, and WAF-Si. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

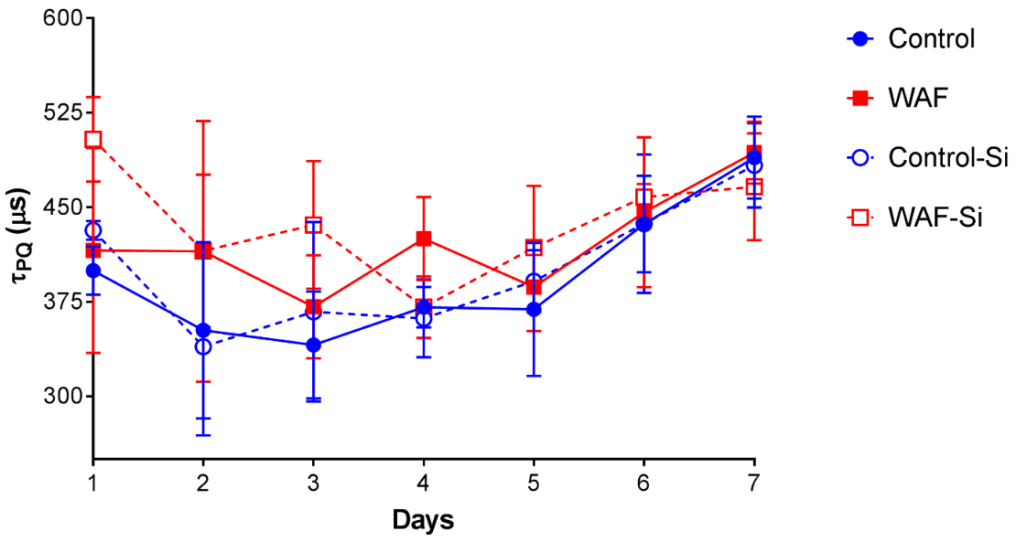

Figure 5. Change in τ over time for Control, WAF< Control-S, and WAF-S. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

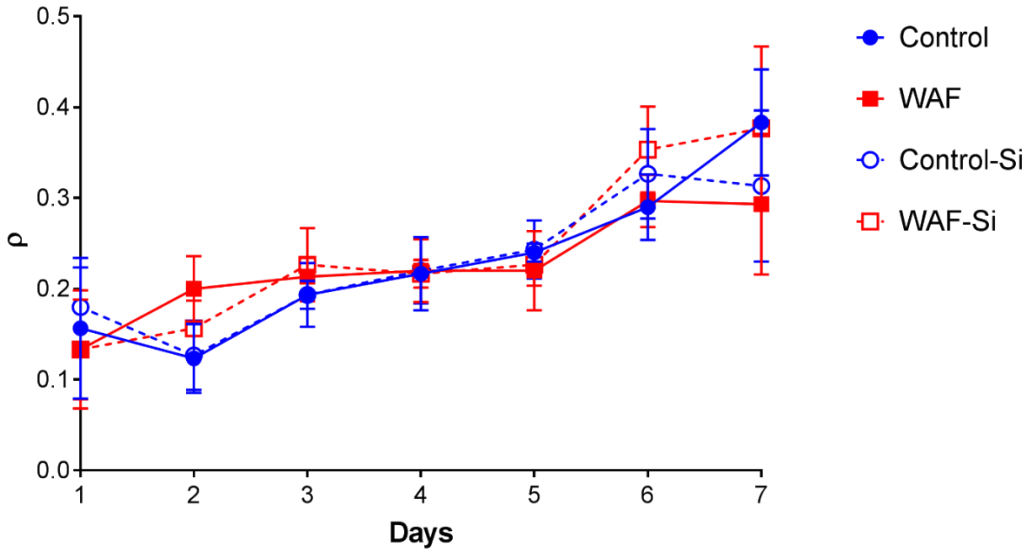

Figure 6. Change in ρ over time for *P. tricornutum* exposed to Control, WAF, Control- Si, and WAF-Si. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

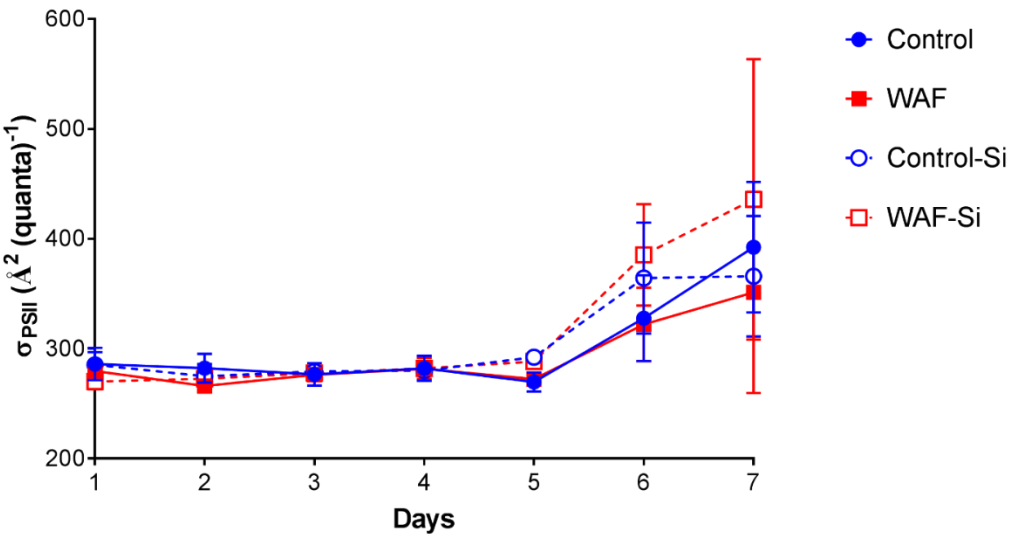

Figure 7. Change in σ_{PSII} over time for Control, WAF, Control-S, and WAF-S. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

CEWAF and DCEWAF Treatments

Upon exposure to CEWAF and DCEWAF, the greatest difference between treatments was observed within the first two days of experimentation. F_v/F_m values in the CEWAF treatments was severely lowered (<0.3) but recovered by day 3. There were several significant differences observed on the first day of experimentation (1-way ANOVA, $p<0.05$) (Figure 8)(Appendix Table 2). Silica limitation presented significant differences between CEWAF and CEWAF-Si, and DCEWAF and DCEWAF-Si. Additionally, the effect of oil concentration presented significant differences between Control and CEWAF, CEWAF and DCEWAF, Control-Si and CEWAF-Si, as well as Control-Si and DCEWAF-Si. Though there were no significant differences observed in τ (Figure 9), ρ (Figure 10). There were significant differences observed in σ_{PSII} on day 1 (1-way ANOVA, $p<0.05$) between the biological control and

CEWAF-Si as well as between DCEWAF and CEWAF-Si (Figure 11). On day 3, there were no significant differences between any of the treatments (Figure 11).

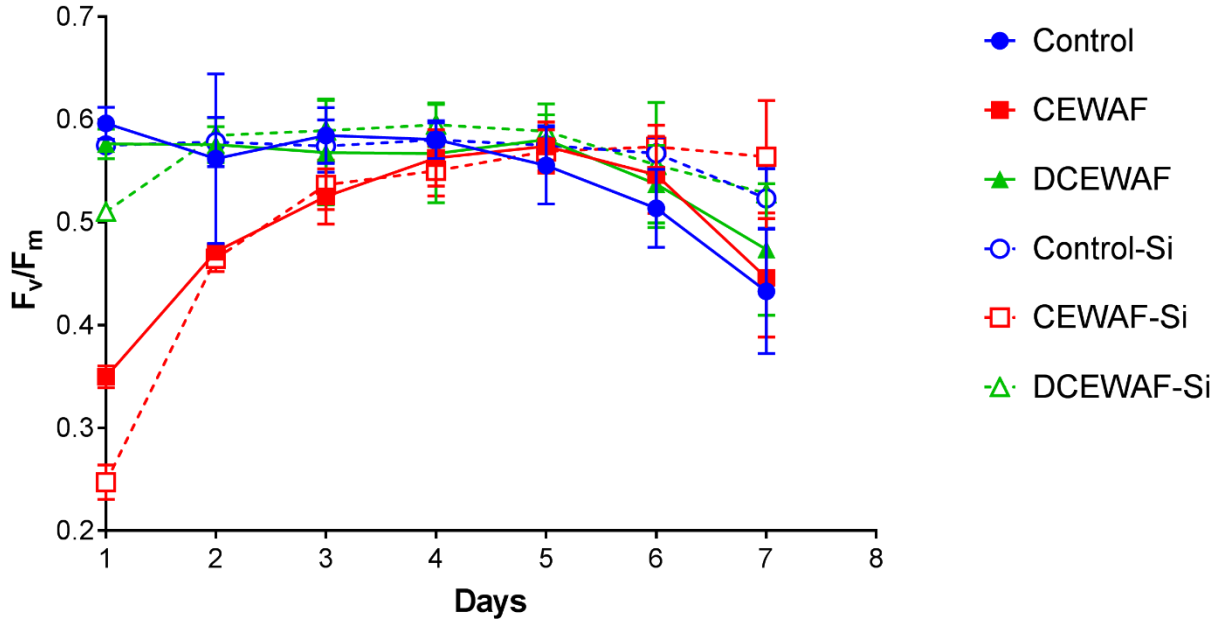

Figure 8. F_v/F_m for Control, CEWAF, DCEWAF, Control-Si, CEWAF-Si, and DCEWAF-Si. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

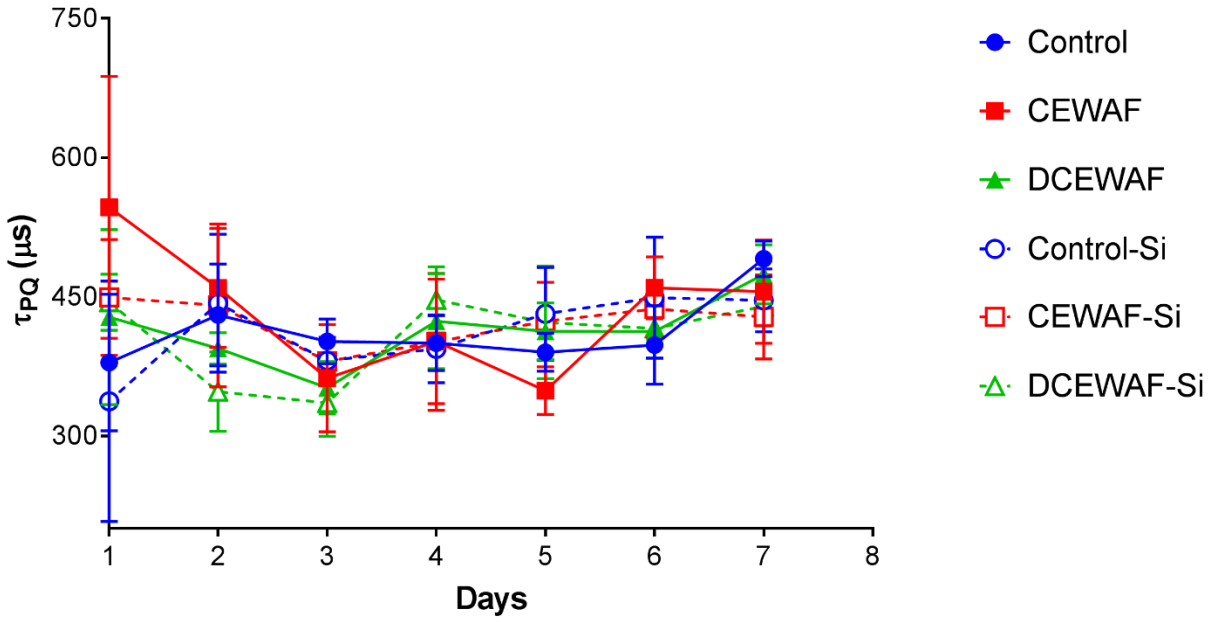

Figure 9. Change in τ over time for Control, CEWAF, DCEWAF, Control-Si, CEWAF-Si, and DCEWAF-Si. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

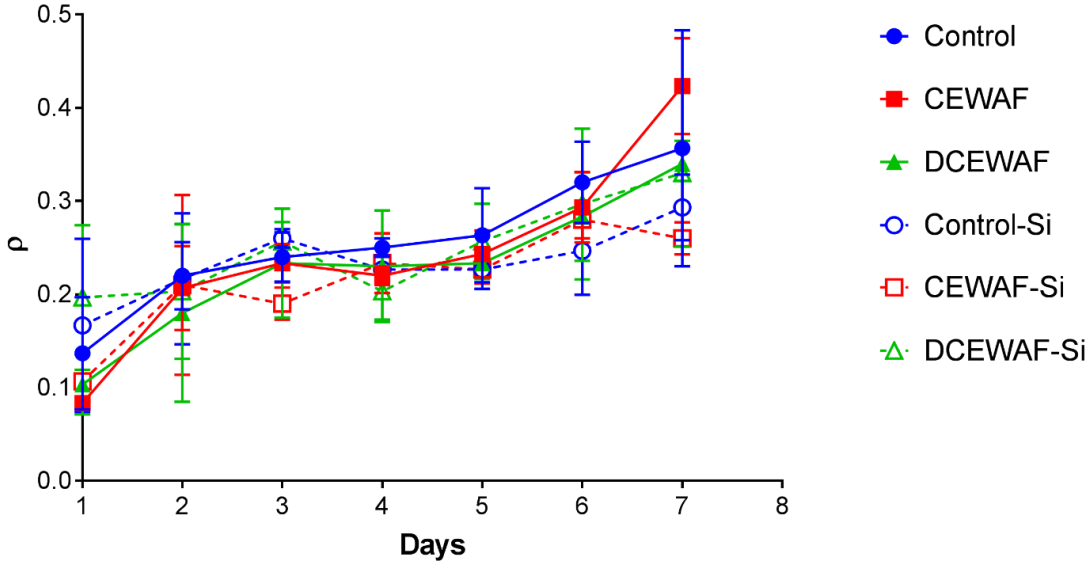

Figure 10. Change in ρ over time for Control, CEWAF, DCEWAF, Control-Si, CEWAF-Si, and DCEWAF-Si. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

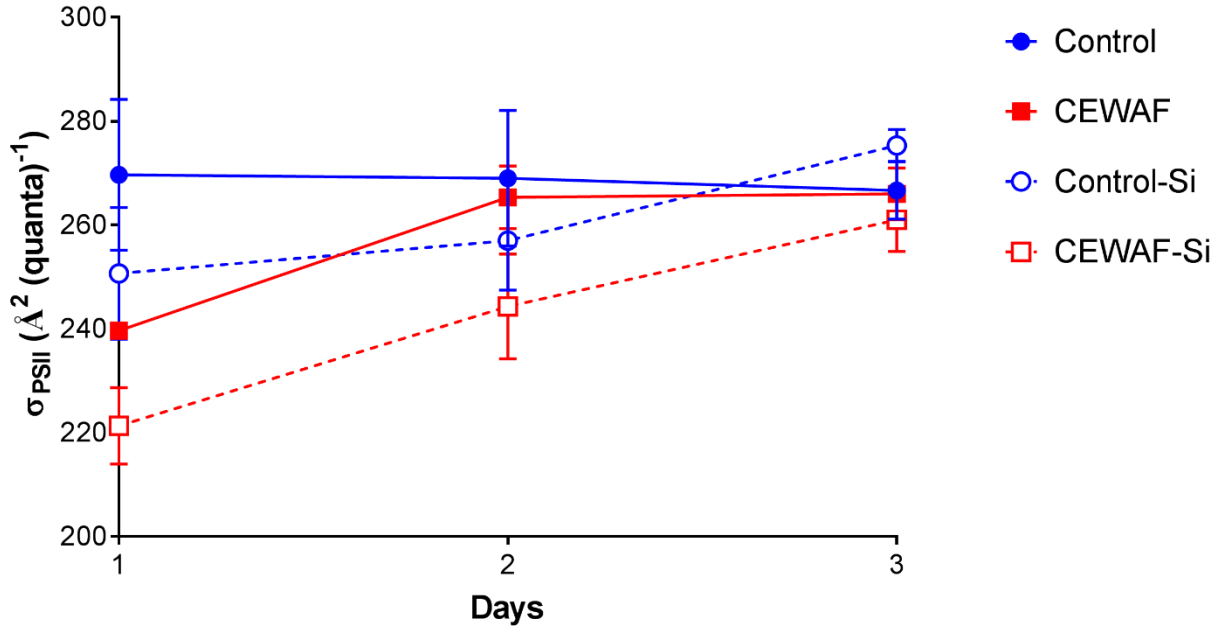

Figure 11. σ_{PSII} from day 1 to day 3 for Control, CEWAF, DCEWAF, Control-Si, CEWAF-Si, and DCEWAF-Si. The solid lines represent the silica-enriched treatments and the dotted lines represent the silica-limited treatments. The error bars represent the standard deviation of the mean calculated from the triplicate measurements.

Changes in macromolecular composition

The macromolecular composition of *P. tricornutum* exposed to WAF and control conditions did not show any significant variation between treatments (Figure 12). No differences were observed in the presence or relative concentrations of carbohydrates, silicates, Amide I or Amide II for the WAF or CEWAF and DCEWAF portions of the experiment (Figure 13).

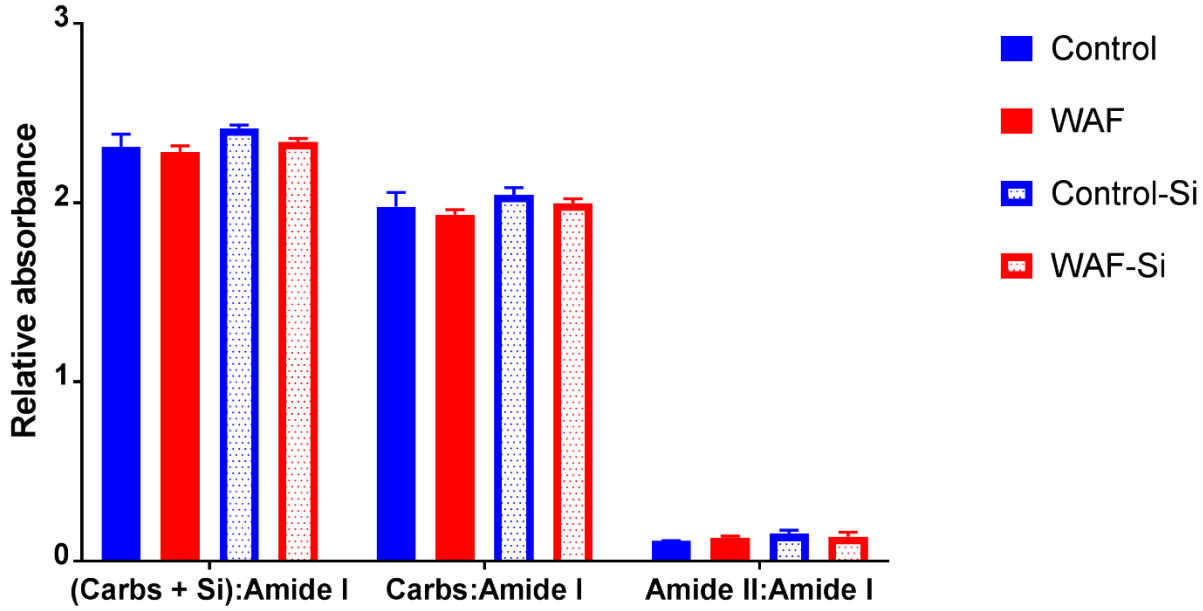

Figure 12. Macromolecular changes observed in Control, WAF, Control-Si, and WAF-Si. The solid bars represent the silica-enriched treatments, and the dotted bars represent the silica-limited treatments.

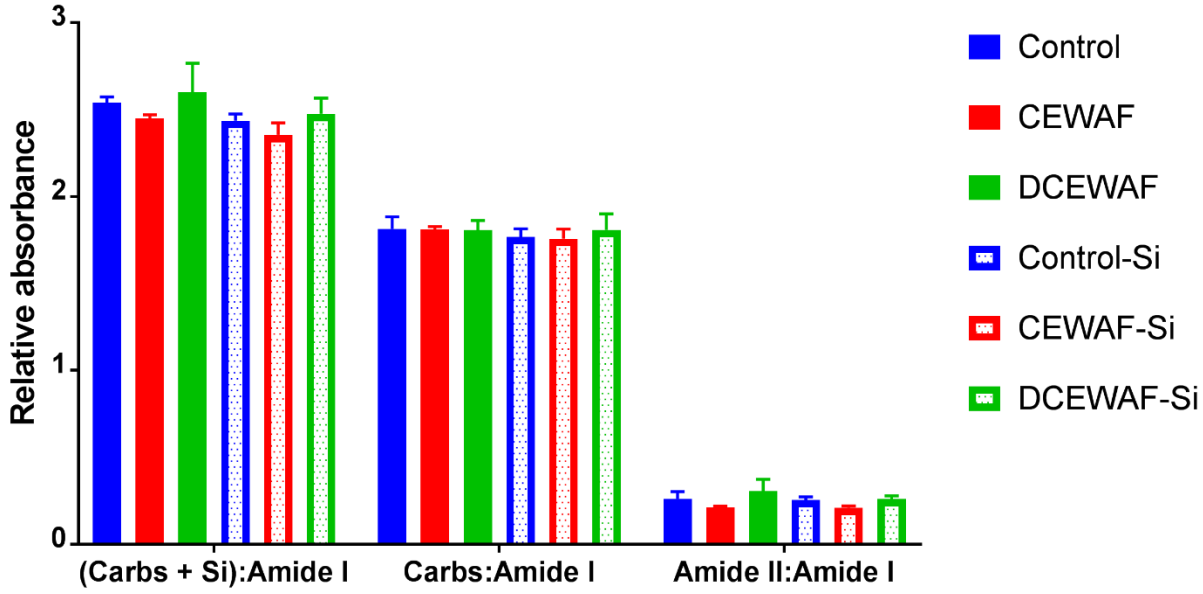

Figure 13. Macromolecular changes observed in Control, CEWAWF, DCEWAF, Control-Si, CEWAF-Si, and DCEWAF-Si. The solid bars represent the silica-enriched treatments, and the dotted bars represent the silica-limited treatments.

CHAPTER IV

DISCUSSION

Cultures exposed to both silica-limited conditions and oil and dispersants presented the greatest physiological responses. To our knowledge, this is the first study to analyze the effects of silica-limitation and oil and dispersants on the physiological responses of *P. tricornutum.* Diatoms grown in silica-limited conditions have been shown to respond adversely (Bucciarelli and Sunda, 2003). Similarly, diatoms exposed to several different types of dispersants have also been shown to respond negatively (Hook and Osborn, 2012). However, with the parameters that we measured in this experiment, it is difficult to discern whether the greatest portion of the effects observed are in response to strictly silica-limitation, which are due to oil and dispersant exposure, and which are possibly due to a compounding impact of both treatments.

Various components of crude oil have been shown to have detrimental effects on the photosynthetic capabilities, growth, and biomass accumulation of several phytoplankton species (Bretherton et al., 2018; Carrera-Martínez et al., 2010). Though there have been adverse effects found for phytoplankton exposed to crude oil fractions, little is known about the effect of oil and oil and dispersant on major phytoplankton species. Of the studies performed on marine diatoms, it has been found that the siliceous frustule may play a role in the resilience of diatoms to exposure from oil and other pollutants (Macintyre et al., 2011; Mulholland et al., 2006; Strom and Strom, 1996). Based on the premise of the study by Macintyre et al. (2011), it is presumed that *P. tricornutum* cells grown in silica-enriched media would present a greater amount of robustness than those grown in silica-limited media (Harrison et al., 1986). Exposure experiments have also shown that WAF, CEWAF, and DCEWAF lead to varying effects on the

phytoplankton-there are two possible explanations. First, the varied accommodated fractions of oil and/or oil plus dispersant that are present in each of the treatments (Bretherton et al., 2018). Second, species specific responses based on their differential physiology and characteristics such as the presence of an outer wall or shell (Bretherton et al., 2018). These studies led us to believe that there may be a compounding effect of silica-limitation and increased oil concentration that could explain the differing responses of the *P. tricornutum* to the different treatments.

In our study, *P. tricornutum* grown in silica-enriched and silica-limited media presented a high level of robustness when exposed to WAF alone. In these treatments, there were virtually no changes in biomass, photosynthetic capabilities, or changes on macromolecular composition. This lack of an observed change could indicate that the fractions of oil accommodated into the water due to mixing alone are not influencing the response of *P. tricornutum.* This observation is consistent with findings of Bretherton et al. (2018). However, when considering the estimated oil equivalents of the WAF experiments there may be another factor leading to the lack of an impact observed in *P. tricornutum.* Throughout the WAF experiment the oil concentrations were below detection limits, which indicates that the oil components were not high enough in concentration to have any effect on the physiology of the phytoplankton.

Relative chlorophyll a concentrations, used as a proxy for biomass accumulation, were lowest in the CEWAF treatments without silica added. There was a relatively higher reduction in biomass in CEWAF in the absence of silica compared to those with silica-added. The silica component of the adverse reactions observed is not surprising, however, because centric diatoms such as *P. tricornutum* require silica to form their frustules, a defining attribute of these algal cells. Diatoms also exhibit a greater amount of robustness to oil supposedly due to the presence

of this feature (Bretherton et al., 2018). The possible compounding effect of silica and oil and dispersant, however, is still under investigation.

The low F_v/F_m levels in the CEWAF and CEWAF-Si treatments on the first day of experimentation suggests that those particular treatments were undergoing immediate stress from exposure to the oil and dispersants. However, by day 3 the F_v/F_m values of these treatments had completely recovered. This rapid acclimation of the cultures in these treatments hinted at a rapid adaptation in their photosynthetic systems. Upon further analysis, it was found that there increases in σ_{PSII} or the functional absorption cross section of PSII. The significant differences observed between the control and CEWAF-Si and between the DCEWAF and CEWAF-Si treatments shows that the high oil concentration in conjunction with silica limitation is applying a stress to the photosynthetic systems that is leading to a significant change in the absorption cross-sectional area of PSII that is available to capture light used for photosynthesis. Adaptations made to the functional absorption cross section represented one way that this marine diatom can not only withstand but recover from exposure to oil and dispersants. This change in photosynthetic physiology was observed an different algal species exposed to less than ideal environmental conditions (Berges et al., 1996; Kamalanathan et al., 2017).

The robustness of *P. tricornutum* and other pennate diatoms have been used in various oil exposure studies where they have exhibited little response to WAF and DCEWAF treatments. In previous studies, diatoms were found to be more sensitive to dispersants than WAF alone and exhibited more sensitivity to CEWAF (Bretherton et al., 2018). It has been established that this increased sensitivity is due to membrane damage which takes place as a result of the dispersants in CEWAF (Hook and Osborn, 2012). Prior records of sensitivity to dispersants Rial et al. (2013) provide some explanation as to why our own CEWAF cultures showed not only the greatest

change in photosynthetic physiology, but also a significantly lower rate of biomass accumulation.

The physiological responses observed in this study support the tested idea that phytoplankton show an adverse physiological response with respect to oil and dispersant exposure (González et al., 2009; Jung et al., 2012; Ozhan and Bargu, 2014). Effects of high oil concentration are also evident in the lack of stress and adaptation exhibited by the WAF and DCEWAF cultures. This study also adds a component of silica-limitation that has not been explored in conjunction with oil and dispersant exposure. Silica-limitation did not increase the number of physiological parameters being altered in response to stress. Instead, this added stressor intensified the physiological responses that were already shown in previous studies performed on *P. tricornutum* (Bretherton et al., 2018). Silica-limitation, which could result from diatom blooms, could further complicate remediation situations after a major oil spill. Marine food web structure depends very heavily on phytoplankton for sheer energetic requirements and production of organic material. This importance means that we must strive to better out understanding of the population dynamics of phytoplankton in general as well as determining responses of specific species to the soluble components of oil in a variety of environmental conditions.

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APPENDIX

Table 2. Differences between F_v/F_m values in CEWAF, DCEWAF, and control treatments on day 1. Significance is denoted by ***. All significance values reported are from a 1-way ANOVA, and significance is defined as $p<0.05$.

Treatment	Significance
Control v. CEWAF	***
Control vs. DCEWAF	
Control vs. Control-Si	
Control vs. CEWAF-Si	***
Control vs. DCEWAF-Si	***
CEWAF vs. DCEWAF	***
CEWAF vs. Control-Si	***
CEWAF vs. CEWAF-Si	***
CEWAF vs. DCEWAF-Si	***
DCEWAF vs. Control-Si	
DCEWAF vs. CEWAF-Si	***
DCEWAF vs. DCEWAF-Si	***
Control-Si vs. CEWAF-Si	***
Control-Si vs. DCEWAF-Si	***
CEWAF-Si vs. DCEWAF-Si	***