Determining the Effects of Ocean Perturbations on Marine Phytoplankton Assemblages

by

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Abstract

The ocean provides a dynamic, constantly changing environment for marine phytoplankton. Understanding the effects of these changes on phytoplankton assemblages is fundamental when trying to anticipate future responses of primary producer's community to long-term environmental changes. In this study, artificial perturbation experiments were successfully performed on natural assemblages of phytoplankton to simulate conditions consistent with ocean acidification and North Pacific Subtropical Gyre (NPSG) upwelling patterns. These experiments demonstrated the feasibility and value of such perturbations in studying phytoplankton responses to environmental forcing.

Acknowledgements

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Table of Contents

List of Figures

1. Introduction

Phytoplankton are responsible for roughly half of photosynthesis on earth (Field et al., 1998), and are estimated to remove as much carbon dioxide from the atmosphere as all terrestrial plants combined (Richardson et al., 2004). It is thought that as much as 30% of this carbon eventually reaches the deep ocean, where it is effectively sequestered for hundreds, or even thousands, of years (Falkowski et al., 1998). Just as importantly, phytoplankton resides at the base of the ocean food web, and contributes to the diversity of marine ecosystems.

The world's oceans, which cover more than 70% of Earth, provide a habitat for thousands of species of phytoplankton. Although the overwhelming size and diversity of phytoplankton populations may indicate high level of stability, the conditions that the oceans provide are far from constant. Instead, natural perturbations create a dynamic, unpredictable environment that leads to periodic upheaval within microbial communities. Examining the effects of these perturbations is necessary to gain knowledge about phytoplankton communities and to comprehend community interactions with the environment. This information in turn plays a vital role in predicting ecosystem responses to changing ocean conditions, a necessity for scientists and policymakers alike.

1.1 Ocean Acidification

Ocean acidification is one of the most well-known ocean perturbations. Ocean acidification occurs as a result of increased atmospheric carbon dioxide (CO_2) concentrations. As it equilibrates with the ocean, atmospheric CO_2 reacts with water to form carbonic acid, releasing hydrogen ions. This results in an overall decrease in pH and increase in acidity of the oceans.

Over the past 150 years, anthropogenic consumption of fossil fuels has resulted in increases in atmospheric concentrations of CO_2 of roughly 100 ppm, resulting in an oceanic pH decrease of roughly 0.1 units (Caldeira and Wickett, 2003). Levels of atmospheric CO_2 are conservatively predicted to continue increasing over the next 100 years, eventually reaching concentrations of 800 ppm (Caldeira and Wickett, 2003). This increase will shift pH down by an additional 0.3 units, which is likely to have dramatic effects on chemical and biological processes in the ocean.

Changes in biological activity as a result of ocean acidification have been detected already. The excess of hydrogen ions produced by increased CO_2 dissolution reacts with dissolved calcium carbonate to form bicarbonate ions, decreasing the amounts of biologically available carbonate. This in turn hinders the growth of calcareous organisms, as has been seen in declining coral populations (Hoegh-Guldberg et al., 2007).

The effects of pH change on phytoplankton populations have just recently begun to be studied, in part because of the diversity of phytoplankton species and in part because the effects are less predictable (Balch and Fabry, 2008). This study provides preliminary data regarding the effects of ocean acidification on phytoplankton assemblages' photosynthetic rates in an attempt to characterize these effects on the oceanic energy cycle. Acquiring a better understanding of phytoplankton community response to ocean acidification conditions is crucial in determining future policy regarding global CO_2 emissions.

1.2 Localized Phytoplankton Blooms in the North Pacific Subtropical Gyre

Perturbations are not limited to anthropogenic causes; natural perturbations often occur on local scales and result in temporary alterations to microbial community

populations. Localized phytoplankton blooms within oligotrophic regions of the oceans represent one such example, and are important in understanding the dynamics and processes within plankton communities (Takahashi and Kishi, 1984; Karl and Letelier, 2008)

The North Pacific Subtropical Gyre (NPSG) is one of the most actively studied regions for open ocean phytoplankton blooms. The NPSG extends over an area of roughly 2 x 10^7 square kilometers and extends from 15°N to 35°N latitude and 135°E to 135°W longitude (Dore et al., 2008). Miniscule nutrient concentrations and low measured stocks of standing biomass characterize this region of the Pacific. As recently as twenty years ago it was considered a static and homogeneous ecosystem, supporting little life. Recently, however, studies have revealed that despite the presence of strongly stratified nutrient-depleted surface waters in the summer months, short-lived phytoplankton blooms are an annual summer feature in NPSG (Dore et al., 2008). It is thought that regions of local upwelling provide the nutrients and impetus for blooms to take place. Understanding the cause and mechanics of these blooms is critical in understanding the ecosystem dynamics of the entire NPSG.

1.3 Perturbation Simulations: Strategies and Methods

Although naturally occurring ocean perturbations can be observed and interpreted, it is difficult to be present at the occurrence of aperiodic events such as plankton blooms because of their unpredictable and rapidly evolving character. In addition, the ever-changing nature of these events requires an extremely flexible sampling strategy that must continuously adapt to capture the temporal and spatial characteristics of these blooms.

For these reasons, artificial perturbation experiments are often performed to replicate natural phenomena. They allow a larger degree of control over experimental location and environmental conditions. As a result, it is possible to investigate a variety of phenomena with scales ranging from shipboard laboratory and deck experiments to mesocosm and mesoscale experiments. The versatility of such experiments has resulted in heightened interest in perturbation experiments within the scientific community (Gattuso and Lavigne, 2009); this paper aims to demonstrate the utility of these experiments in exploring natural phenomena and microbial community interactions.

1.4 Fluorescence Induction

The use of chlorophyll fluorescence to determine photosynthetic properties of photosystem II provides a non-destructive, real time form of observation that can be used in the laboratory and *in situ*. Of the many techniques that rely on fluorescence signals, one of the most useful is fast repetition rate (FRR) fluorometry.

FRR fluorometry uses brief pulses of light, termed flashlets, to both stimulate the photosynthetic activity of phytoplankton and to measure the resulting changes in the fluorescence signal. The intensity, duration, and interval of these flashlets can be selectively altered to manipulate the reduction state of the primary electron acceptor (Q_A) and the plastoquinone pool (PQ) within the electron transport chain (Kolber et al., 1998). This allows for the assessment of the kinetics of the photosynthetic electron transport between Q_A and PQ pool, and between PQ pool and the photosystem I. Just as importantly, the absorption cross-section of photosystem II(σ_{PSII}) and the energy transfer between reaction centers can be calculated by examining the kinetics of single turnover fluorescent transients (Kolber et al., 1998). Altogether, these measurements allow for a

quick and accurate assessment of electron transport kinetics that can be correlated with photosynthetic activity and primary production.

2. Materials and Methods

2.1 Variable Fluorescence

A Fast Repetition Rate Fluorometer (FRRF, MBARI, Monterey, California) was used to determine the maximal fluorescence (F_m), photochemical quantum yield (F_v/F_m), and absorption cross-section (σ_{PSII}) of discrete dark-adapted samples from on-deck incubations and of batch cultures kept in the laboratory. The F_m signal was used as a proxy for biomass, while photochemical quantum yield and absorption cross-section were used as indicators of physiological state.

2.2 Laboratory Incubation – Ocean Acidification

Two 2-liter seawater samples were taken from a depth of 45 meters and were incubated in batch culture in MBARI's chemostat apparatus. Samples were exposed to a 13 hour/11 hour light/dark sinusoidal diel cycle and were kept at a constant temperature corresponding to the 45 m depth. 100 ml/min of air was bubbled through each sample, with air in one of the samples enriched with additional 0.5 ml/min of a 10% CO_2 to simulate future increases in atmospheric CO_2. Samples were analyzed continuously using a dedicated FRR instrument.

2.3 Deck Incubation – Ocean Acidification

Four 20-liter samples were taken from a depth of 45 meters and kept in batch culture. Samples were exposed to a natural light cycle and were kept at ambient water temperature by circulating surface water through the on-deck incubator. Light entering the incubator was attenuated through blue acrylic to achieve 40% transmittance. Compressed air containing approximately 380 ppm of CO_2 was bubbled through each sample at a rate of 1 L/min, with two samples enriched with 0.4 L/min CO_2 to simulate

future increases in atmospheric CO_2. Each set of duplicate samples were analyzed twice daily using a FRRF.

Additionally, four 18-liter samples were taken from a depth of 45 meters. These samples were subsequently 'enriched' with 2-liter water samples taken from a depth of 300 meters. Compressed air containing approximately 380 ppm of CO_2 was bubbled through each sample at a rate of 1 L/min, with two samples enriched with 0.4 L/min CO_2 to simulate future increases in atmospheric CO_2. Each set of duplicate samples was analyzed twice daily using a FRRF.

2.4 Deck Incubation – North Pacific Subtropical Gyre

18-liter water samples taken from 45 meters were enriched with 2 liters of water acquired from depths of 200, 300, and 500 meters. Two 20-liter samples without enrichment were incubated as a control. Samples were exposed to a natural light cycle and kept at ambient water temperature by circulation of surface water through the on-deck incubator. Light entering the incubator was attenuated through blue acrylic. Duplicate samples were kept at 40% and 16% light transmittance levels in respective incubators using sheets of blue acrylic. Samples were analyzed twice daily using a FRRF.

2.5 Sampling Locations

Deck incubation experiments were performed three times. Samples for the on-deck and laboratory experiments were taken from three different locations (Fig. 1). Locations were chosen so as to span a range of upwelling and downwelling conditions.

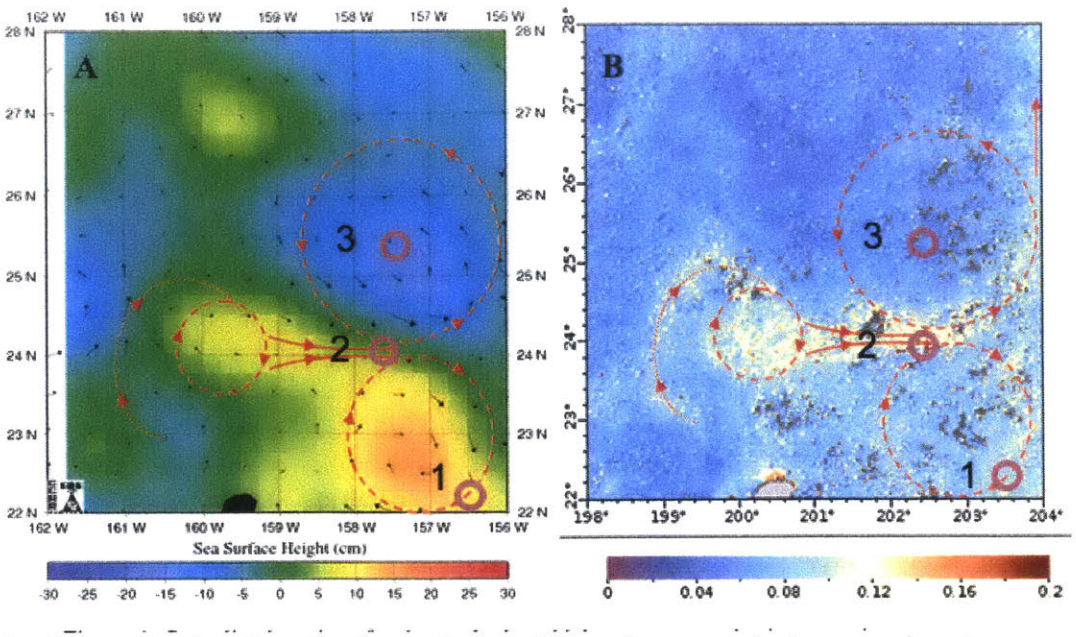


Fig. 1 Sampling locations for the on-deck and laboratory perturbation experiments. Locations are presented in relation to sea surface height (A) and satellite-determined chlorophyll concentration (B). Position 2 is located at the convergence zone between the upwelling and downwelling eddies, and shows evidence of increased chlorophyll concentrations characteristic of a phytoplankton bloom event.

2.6 Statistical Treatment

Chemostat data were normalized to a single start point for the laboratory incubation experiment. Data for each of three on-deck incubations were compiled from duplicate samples for each treatment and are presented as an average with standard deviation error bars for each parameter.

3. Results

3.1 Laboratory Incubation - Ocean Acidification

The F_m signal for the CO_2-enriched batch culture taken at location 2 decreased slightly compared to that of the control. The observed F_v/F_m and σ_{PSII} values appear to be similar between the CO_2-enriched and control samples. Both samples' physiological responses were strongly influenced by the artificial diel cycle induced by the chemostat. (Fig. 2).

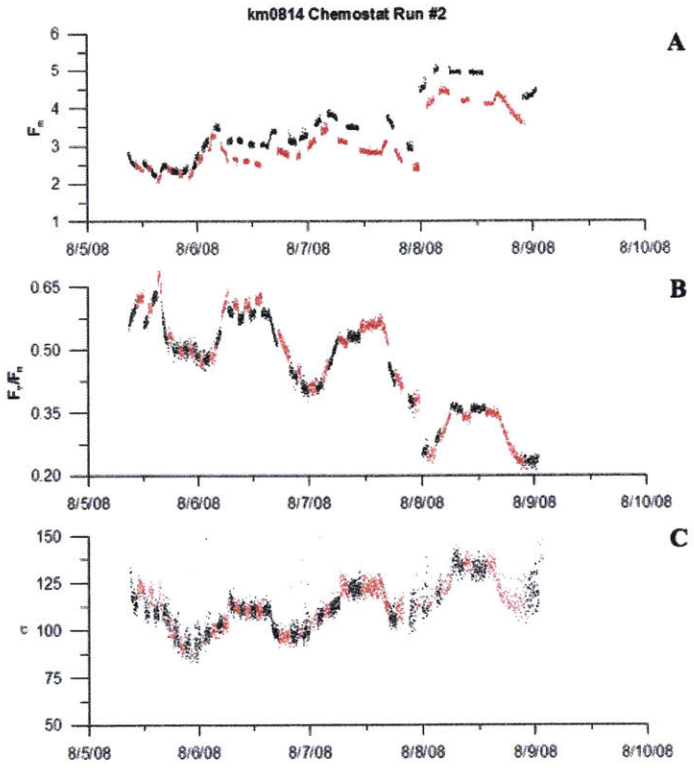


Fig. 2 Physiological parameters of CO_2 enriched batch culture samples as measured continuously by Fast Repetition Rate Fluorometry. CO_2-enriched sample measurements are depicted in red. Shown are the maximum fluorescence, a proxy measurements for biomass (A), photosynthetic quantum yield (B) and absorption section of PSII (C)

3.2 Deck Incubation – Ocean Acidification

A significant difference in F_m, F_v/F_m, and absorption cross-section was observed between samples enriched with seawater taken from 300 meters depth and the controls. The enriched samples demonstrated higher F_m, F_v/F_m, and σ_{PSII} signals in each of the three experiments, as depicted for location 2 below (Figure 3). All of the samples also exhibited strong diel trends in F_v/F_m and σ_{PSII} signals. Complete data for each location can be found in Appendix A.

No noticeable trend existed in F_m, F_v/F_m, or σ_{PSII} signals between the CO_2-enriched and control samples from comparable depths. In each of the three experiments F_m signals were roughly equal for the CO_2-enriched and control samples taken from 45 meters and for the CO_2-enriched and control samples enriched with water taken from 300 meters (Fig. 4). Similarly, F_v/F_m and σ_{PSII} demonstrated no consistently observable difference between CO_2-enriched and control treatments in the three experiments (Appendix A).

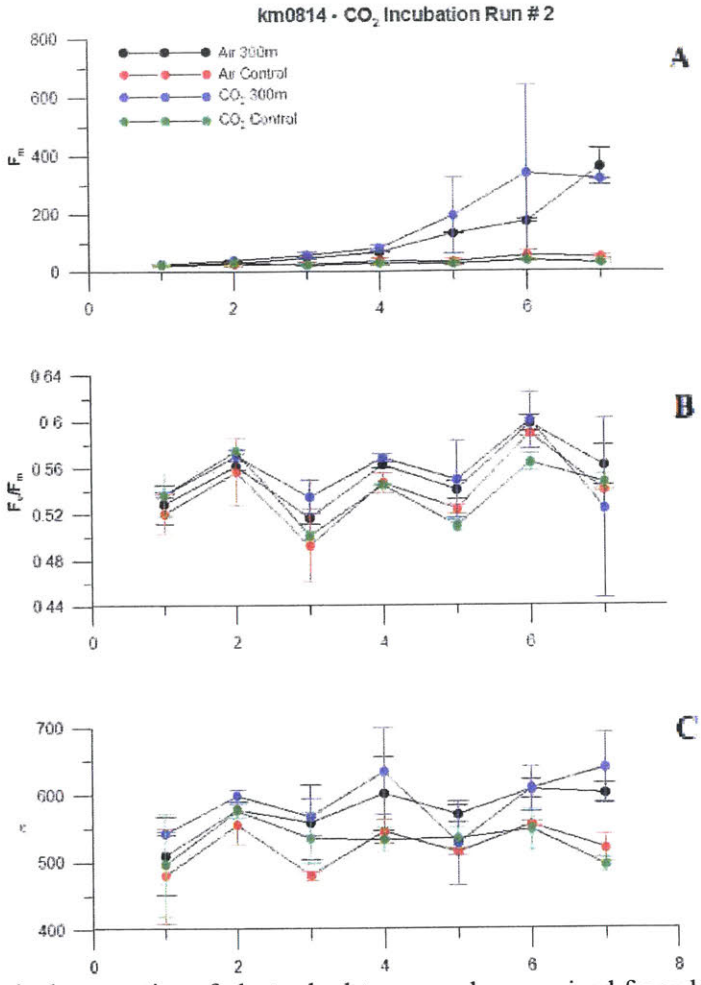


Fig. 3 Physiological properties of phytoplankton samples acquired from location 2 as measured by Fast Repetition Rate Fluorometry. Samples denoted by CO_2 were enriched with a mixture of air and 400 ppm CO_2. Samples labeled as 300m were enriched with 2 liters of water from 300 meters depth. Control samples were taken from 45 meters depth. Shown are proxy measurements for biomass (A) and physiological state (B, C). Circles represent averages of duplicate samples with vertical error bars representing standard errors for each pair of duplicates.

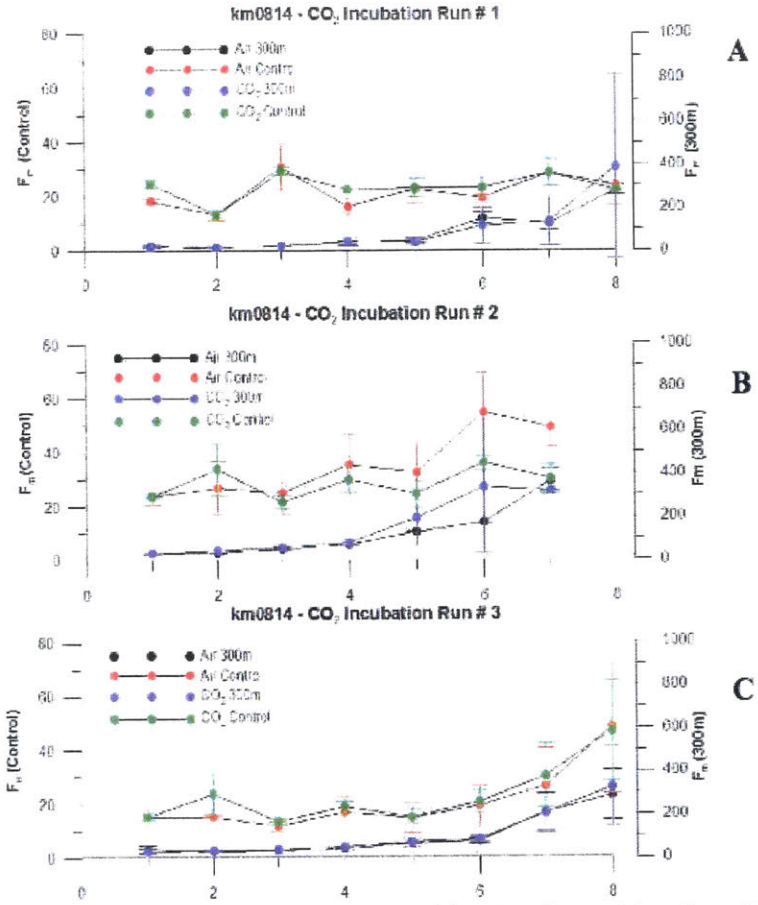


Fig. 4 Biomass proxy measurements as measured by Fast Repetition Rate Fluorometry. Samples denoted by CO_2 were enriched with a mixture of air and 400 ppm CO_2. Samples labeled as 300m were enriched with 2 liters of water from 300 meters depth. Control samples were taken from 45 meters depth. Samples labeled as 'control' and '300m' correspond to the 'control' and '300m' axes on the left and right of the graphs, respectively. Proxy measurements for biomass are depicted for location 1 (A), location 2 (B), and location 3 (C).

3.3 Deck Incubation – North Pacific Subtropical Gyre

A considerable disparity in F_m, F_v/F_m, and absorption cross-sections were observed between each of the different enrichment samples, depicted below for location 2 (Fig. 5) and in full in Appendix B. F_m signals were higher in samples enriched with water from depth compared to controls in each of the three experiments (Fig. 5, Appendix B). F_m signals consistently increased with the depth of the enrichment; samples enriched with 500-meter water had higher F_m signals than those enriched with 300-meter or 200-meter water (Fig. 5, Appendix B).

A noticeable physiological response also existed between deep-water-enriched and control samples, as the enriched samples had much higher F_v/F_m and absorption cross-section signals. These signals did not, however, demonstrate the same correlation between signal strength and enrichment depth as was seen for the Fm signals.

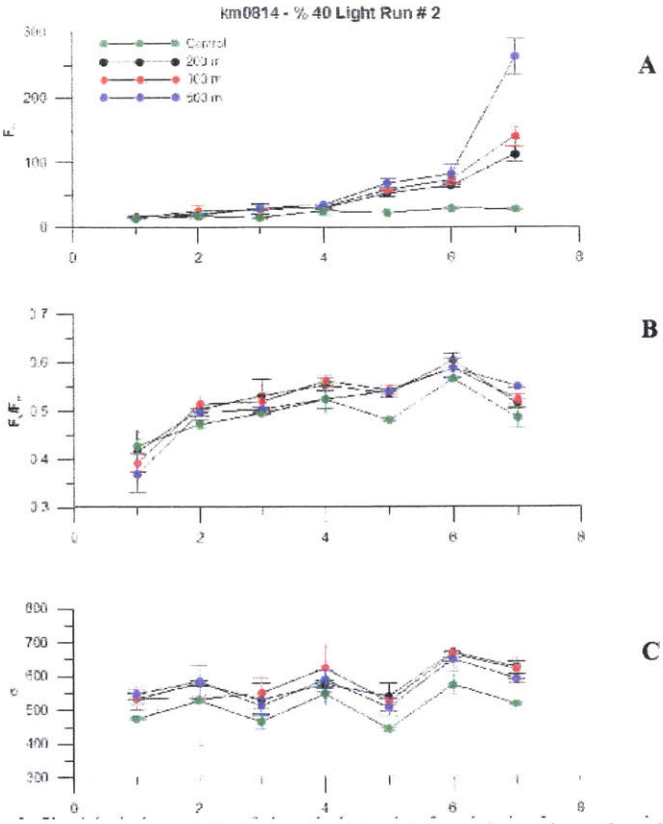


Fig. 5 Physiological parameters of phytoplankton taken from location 2 as measured by Fast Repetition Rate Fluorometry. Samples labeled as 200m, 300m, and 500m were enriched with 2 liters of water from 200 meter, 300 meter, and 500 meter depths respectively. Control samples were taken from 45 meters depth. All samples were kept at 40% light transmittance. Shown are proxy measurements for biomass (A) and physiological state (B, C). Circles represent averages of duplicate samples with vertical error bars representing standard errors for each pair of duplicates.

Interestingly, both the enrichment and the control samples did not exhibit a significant difference in responses between the 16% and 40% light transmittance levels for each of the three parameters (Fig. 6). A strong diel cycle response was observed in the absorption cross-section parameter in each sampling location for both the 40% and 16% incubation experiments (Appendix A, Appendix B).

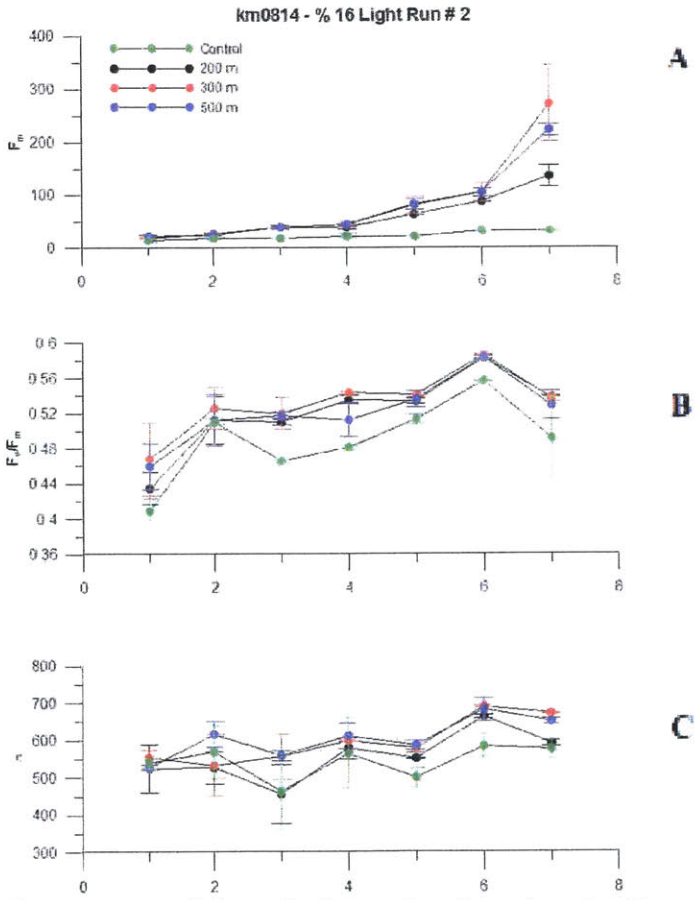


Fig. 6 Physiological parameters of phytoplankton taken from location 2 as measured by Fast Repetition Rate Fluorometry. Samples labeled as 200m, 300m, and 500m were enriched with 2 liters of water from 200 meter, 300 meter, and 500 meter depths respectively. Control samples were taken from 45 meters depth. All samples were kept at 16% light transmittance. Shown are proxy measurements for biomass (A) and physiological state (B, C). Circles represent averages of duplicate samples with vertical error bars representing standard errors for each pair of duplicates. These results strongly mirror those measured for the 40% light transmittance samples.

4. Discussion

The strong diel signal observed in both the laboratory and deck experiments is similar to that experienced by natural phytoplankton communities. The existence of this cycle indicates that the perturbation experiments adequately simulated natural light conditions. Other conditions such as temperature were also monitored and adjusted in order to best simulate the natural conditions of the upper water column at 45 meters depth.

Initial results indicate a limited effect of ocean acidification on the phytoplankton populations of the NPSG. The overall decrease in F_m signal of the CO_2-enriched sample relative to that of the control indicates a small decline in the overall biomass in the CO_2-enriched treatment. It appears unlikely, however, that the addition of the CO_2 had a significant effect upon the physiological status of the phytoplankton populations within the sample, as the quantum efficiency and absorption cross-section parameters for the affected samples displayed little or no difference from the controls.

This preliminary conclusion is supported by the deck incubation experiments. The increased levels of CO_2 had little effect upon the phytoplankton populations as a whole, as neither the rate of biomass increase nor the physiological processes involved with phytoplankton populations showed any apparent differences between the CO_2 enrichment and control experiments.

The results of the deep-water enrichment experiment indicate that local upwelling may indeed promote phytoplankton blooms. In each of three experiments, strong increases in biomass, photosynthetic efficiency, and absorption cross-section were observed following the enrichment of surface water with water from depth. The increase

in biomass appeared to correlate with the enrichment depth, indicating that the growth rates responded to increased nutrients/trace metals at depth. Further analysis of deep-water samples should allow for the identification of the particular nutrients responsible for this effect.

5. Conclusion

The NPSG blooms that are commonly observed in the summer months are likely fueled by the local supply of deep, nutrient-rich waters to the euphotic zone. This excess causes a rapid increase in biomass due to accelerated growth of one or more species of the phytoplankton community, which then dies off quickly (Dore et al., 2008). The ephemeral nature of the NPSG bloom makes it difficult to observe the initiation and evolution of a bloom; instead, it is much more common to observe the elevated biomass at the final stage of the bloom development. Any observations of this nature, however, provide little insight into the cause of the bloom, with only limited information regarding the size and location of the bloom. As a result, little evidence for bloom-promoting agents in the NPSG has previously been available due to a lack of direct sampling and observation (Dore et al., 2008).

Surface phytoplankton blooms, as observed during OPEREX experiment, are closely related to the activity of the local eddy field (Fig.1). Ship-deck incubation experiments demonstrate one of the mechanisms by which the NPSG eddy system influences the formation and persistence of these blooms. What remains unknown is whether these fields will intensify or weaken as a result of the increasing thermal loading to the upper ocean. In either case, strong relationship between eddy fields and photosynthetic activity will, to large extent, define the future responses of oceans' productivity to climate forcing.

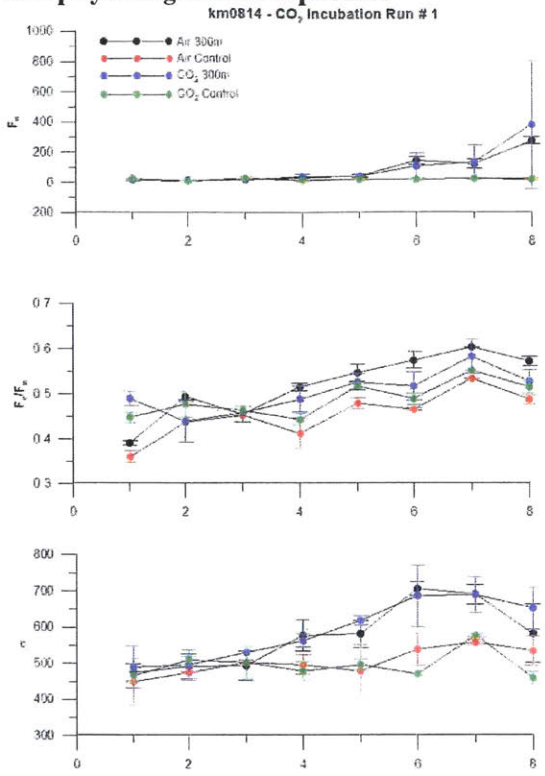
Similarly, ocean acidification experiments performed on deck and in the laboratory provided accurate simulations of ocean conditions one hundred years from now. These simulations demonstrated no noticeable effect of acidification upon the

phytoplankton communities sampled. This result helps provide a baseline expectation for the NPSG plankton communities that may prove useful in both a policy and scientific context when considering future climate scenarios.

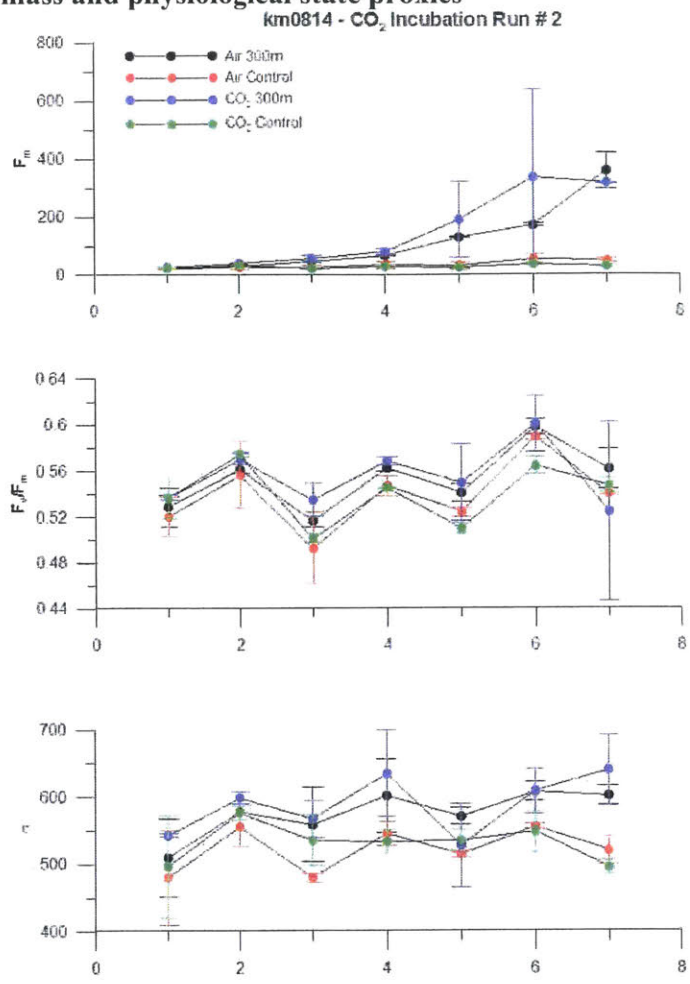
Both deck and laboratory incubations produced valuable information about phytoplankton assemblages otherwise unavailable through *in situ* observation and sampling. These experiments demonstrate the feasibility of small-scale perturbation experiments by simulating natural out-of equilibrium conditions over selected temporal and physical scales. Such experiments allow for testing of a range of hypotheses regarding future oceanic responses to climate forcing. Although short-term incubation experiments cannot fully resolve the question of long-term adaptation to changing climate conditions, they can indicate which of these adaptations are likely to occur.

Appendix A – Ocean Acidification Deck Incubation Perturbation Data

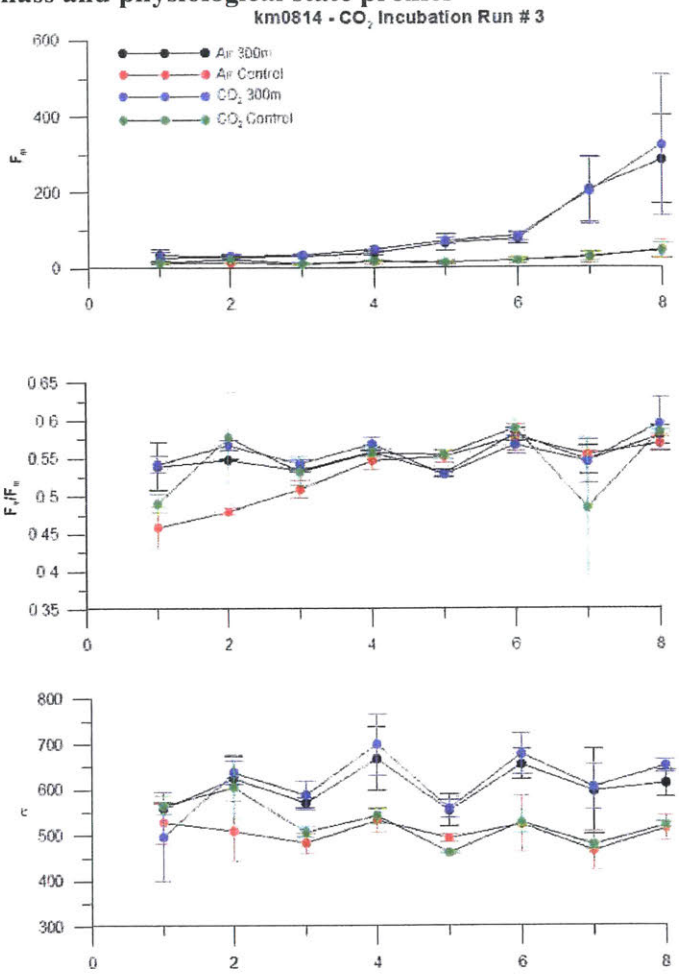
Location 1: Biomass and physiological state proxies



Location 2: Biomass and physiological state proxies

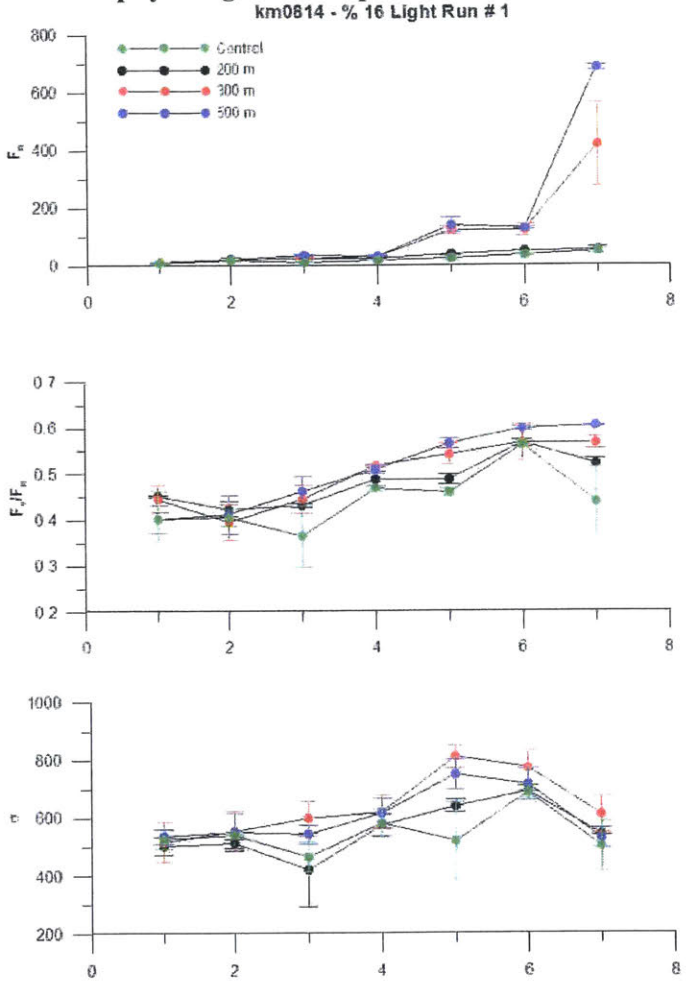


Location 3: Biomass and physiological state proxies

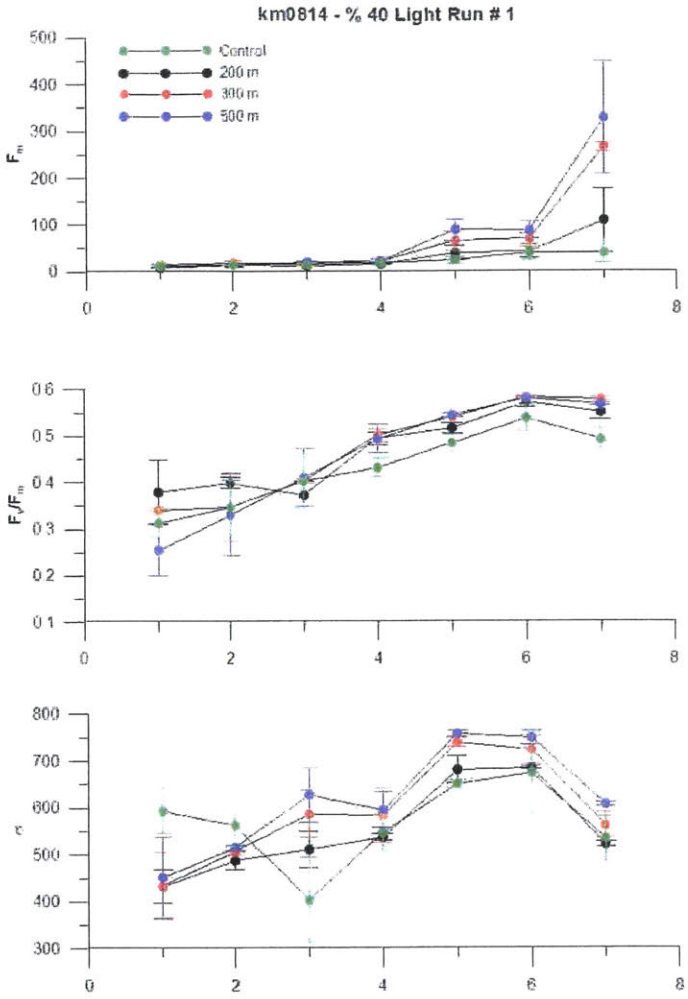


Appendix B – NPSG Regional Upwelling Deck Incubation Perturbation Data

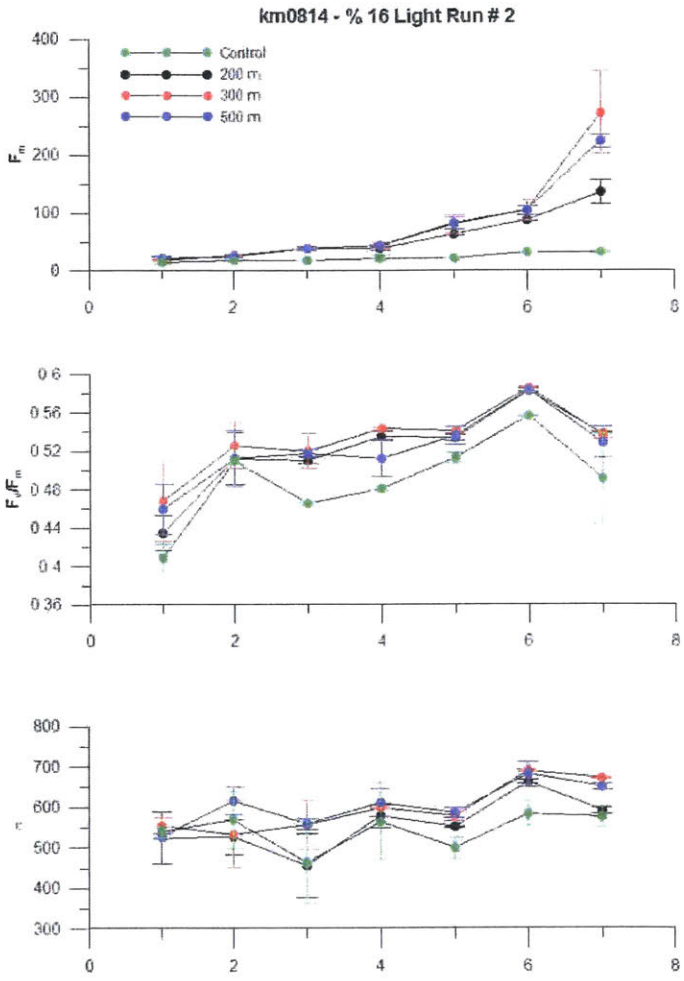
Location 1: Biomass and physiological state proxies for 16% transmittance data



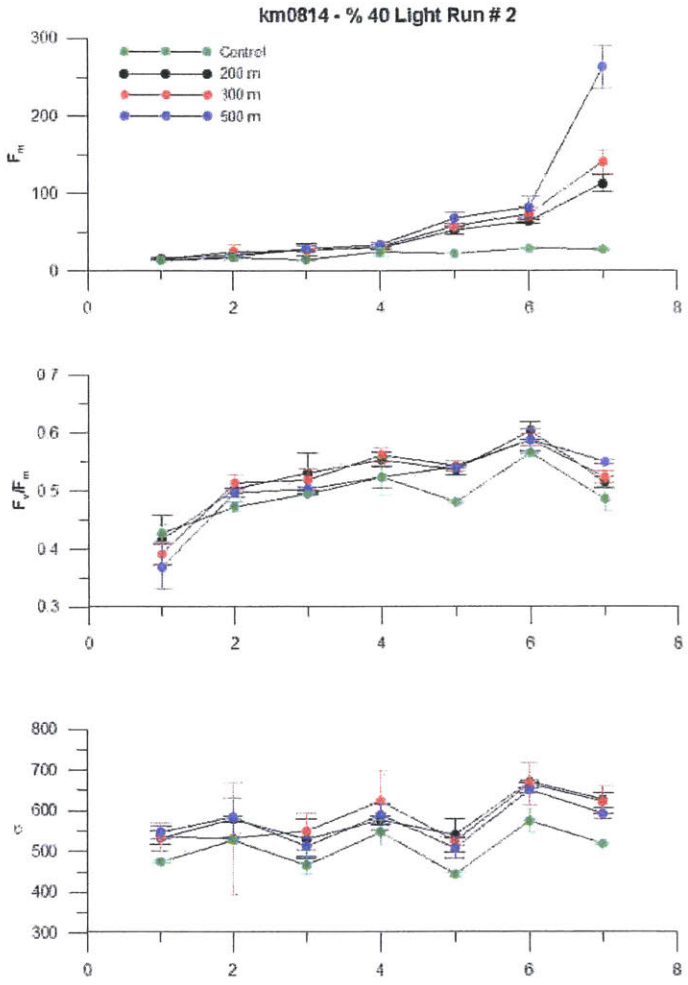
Location 1: Biomass and physiological state proxies for 40% transmittance data



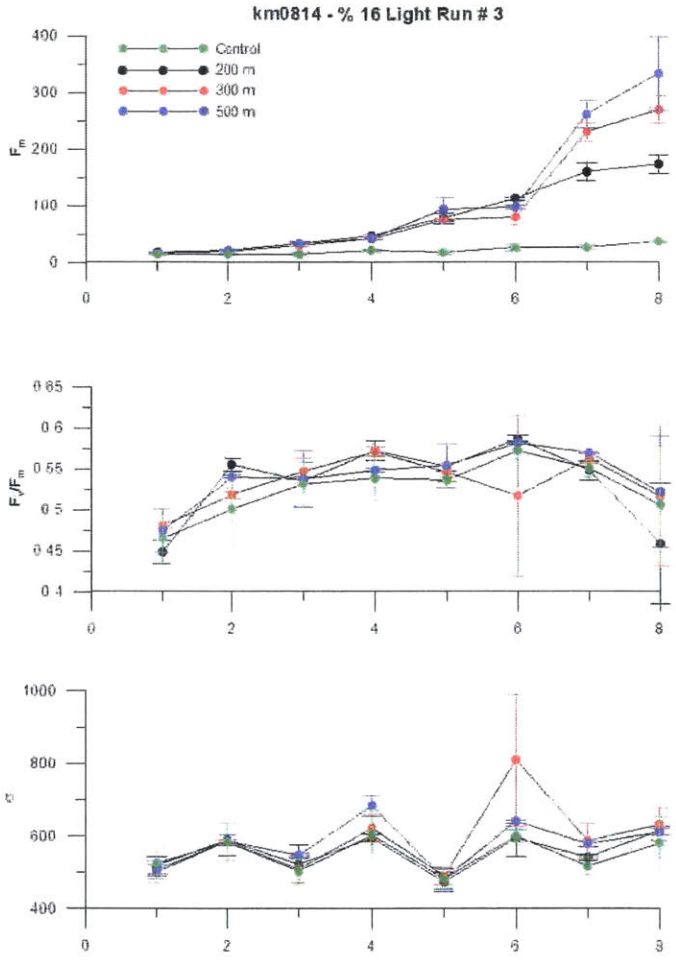
Location 2: Biomass and physiological state proxies for 16% transmittance data



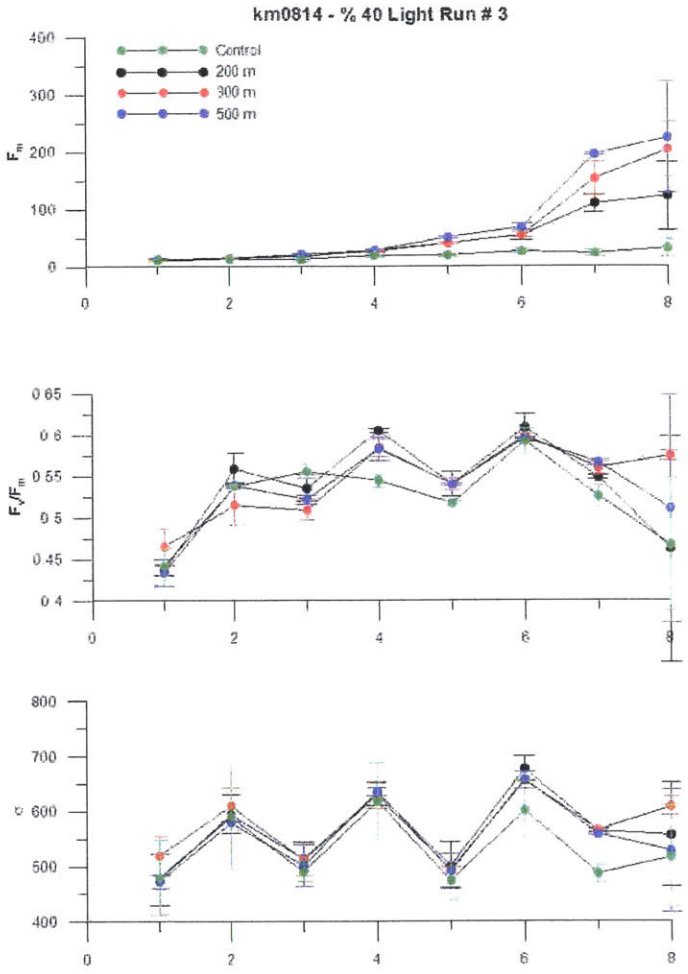
Location 2: Biomass and physiological state proxies for 40% transmittance data



Location 3: Biomass and physiological state proxies for 16% transmittance data



Location 3: Biomass and physiological state proxies for 40% transmittance data



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