UNIVERSTIY OF CALIFORNIA, SAN DIEGO

Assessment of Phytoplankton Community Dynamics using Bead Array Technology

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Asako Yamamoto

Committee in charge:

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Professor Eric Allen

2010

The Thesis of Asako Yamamoto is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Co-Chair

Chair

University of California, San Diego

2010

“Twenty years from now you will be more disappointed by the things that you didn't do than by the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails. Explore. Dream. Discover.”

~ Mark Twain

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LIST OF ABBREVIATIONS

Diatoms:

Chaet	*Chaetoceros*
Cylin	*Cylindrotheca*

Dinoflagellates:

Aka	*Akashiwo*
Cerat	*Ceratium*
Ling	*Lingulodinium*
Proro	*Prorocentrum*
Scrip	*Scrippsiella*

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ACKNOWLEDGEMENTS

I would like to thank Lani Gleason and Elaine Denny for their help in collecting pier samples, and Michael Latz for providing me with phytoplankton cultures. I would also like to show my gratitude to Xavier Mayali, Vera Tai, and Emy Daniels for their technical assistance, and Sean Schoville and Felipe Barreto for their advice on data analysis.

Brian Palenik, Melissa Carter and Mary Hilbern were generous in providing their invaluable insight and expertise into the world of phytoplankton as well as sharing unpublished data. I would like to acknowledge the Southern California Coastal Ocean Observing System (SCCOOS) for providing data from the manual shore stations.

A special thank you to my supervisor Ronald Burton, Ph.D., for his encouragement and guidance throughout this research and also to the rest of my committee members, Lorraine Pillus and Eric Allen. Funding for this project has been provided by the National Science Foundation.

ABSTRACT OF THE THESIS

Assessment of Phytoplankton Community Dynamics using Bead Array Technology

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Professor Ronald Burton, Chair
Professor Lorraine Pillus, Co-Chair

Molecular methods are becoming increasingly popular in the field of microbial ecology for the characterization of phytoplankton communities at the taxonomic level. However, many techniques lack the potential for large scale spatiotemporal studies due to limitations in their methodology. In this study, a high-throughput, rapid and cost effective

hybridization-based bead assay was applied to two sets of samples: (1) a yearlong time series (March 2009-2010) of surface seawater samples taken off the Scripps Pier, and (2) a three month long series of samples taken during a *Lingulodinium polyhedrum* bloom in June 2010. The presence and abundance of seven phytoplankton taxa known to occur in the La Jolla, CA coastal water community were measured using taxon-specific probes, and results showed significant temporal variability throughout the sampling period. The diatoms *Chaetoceros* and *Cylindrotheca* and the dinoflagellate taxa *Prorocentrum* and *Scrippsiella* exhibited similar temporal abundances, suggesting that commonalities in traits allow for the coexistence of these phylogenetically divergent taxa. The dinoflagellates *Akashiwo* and *Lingulodinium* also shared similar peak distributions during the bloom following peak abundances of diatoms in early spring, indicating biological succession of these taxa. Our study demonstrated the potential application of the Luminex bead array assay as a valuable tool to assess phytoplankton community dynamics. Further analyses of the ecological strategies of the seven taxa are necessary for a more comprehensive evaluation of the biotic and abiotic factors structuring phytoplankton communities.

INTRODUCTION

Marine phytoplanktonic communities play a vital role in ecosystem function by regulating carbon flux through the microbial loop as a result of complex interactions between zooplankton, phytoplankton, bacteria and viruses (Azam 1983, 1998; Suttle 2007). It is therefore of great interest to understand the processes underlying microbial community dynamics by exploring interspecific relationships among the component taxa (Haruta et al. 2009). In recent decades, there has been a rapid development of techniques that assess the presence and diversity of microbes including advances in molecular approaches (Grossart 2010). Traditional methods for assessing phytoplankton communities have relied upon microscopy; although this method is time consuming and demands a high level of expertise to distinguish among individual taxa (Miller and Scholin 2000). Techniques that utilize optical signatures are quantitative and support high throughput (Dubelaar and Jonker 2000). The specificity of these methods is however limited by the number of taxonmically unique pigments and cell size classifications (Rutten et al. 2005).

Utilizing molecular signatures allow for a more in depth examination of specific taxa within marine communities. Some recent methods used to study phytoplankton assemblages include constructing gene clone libraries (Jones and Mikulski 2010), quantitative PCR (Moorthi et al. 2006; Tai and Palenik 2009), denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (TRFLP) (Cunliffe et al. 2009a, 2009b). These methods lack quantitative assessment or are limited by their multiplex capabilities. Another more recent method utilizes a hybridization-based bead array platform to target organisms across a broad range of

taxonomic levels (Ellison and Burton 2005; Scorzetti et al. 2009; Mayali et al. 2010). This method contains features that are highly favorable in assessing microbial communities at high spatial and temporal scales: it is high-throughput, multiplexed, cost and time-effective, as well as sensitive and replicative (Deshpande 2010). Among bead array platforms, Luminex xMAP technology (Luminex Corp.) has had wide applications for pathogen detection in healthcare, water quality and food industries with some recent advances in the field of microbial ecology (Dunbar 2006; Diaz et al. 2006; Baums et al. 2008).

Multiplex bead arrays use taxon-specific probes that are assigned and coupled to a uniquely colored microsphere. These beads may be purchased in up to 100 different colors thus allowing for multiplexed analysis of up to 100 different taxa. The coupled beads are then hybridized to sample genomic DNA (or PCR amplicons); the Luminex 100 flow cytometer draws up one bead at a time and uses two lasers to detect both the color of the bead and the fluorescent intensity of the reporter fluorophore (coupled to sample DNA) from each bead. This allows determination of the presence and abundance of each particular taxon in an environmental sample containing a mixed assemblage of phytoplankton. With the formation of a standard curve using pure phytoplankton cultures, fluorescent signal intensity values may be converted to cell concentration values. The rapid, affordable and high throughput features of this assay are favorable for large-scale ecological studies that examine phytoplankton communities over large spatial and temporal scales.

One application for this assay is the detection of harmful algal bloom (HAB) forming species. Blooms appear to be increasing in frequency and intensity around the

globe as a result of complex interactions of various factors leading to dynamic responses in microbial interactions (Van Dolah 2000; Hallegraeff 2010). Monitoring programs such as the Southern California Coastal Ocean Observing System (SCCOOS) have stations along the coastline to sample water and perform cell counts of HAB species using light microscopy. This requires researchers with a high level of expertise in order to distinguish among different species, but it is also difficult to make direct comparisons of phytoplankton presence and abundance when inconsistent measurement standards associated with individual measurement values across the many shore stations must be taken into consideration (Shirono et al. 2010). The existence of a monitoring method that is high throughput and can cover a large spatiotemporal range is critical for the management of HABs and their negative implications on the environment as well as on humans (Hallegraeff 2010). This type of multiplexed molecular assay also has the capability of uncovering some of the complex interactions that underlie the formation and collapse of blooms.

This study builds on the work of Mayali et al. (2010) using eukaryotic universal primers and seven taxon-specific probes that were designed and validated for their analysis of phytoplankton communities in La Jolla, CA (Table 1). Mayali et al. observed dynamic positive and negative interactions in community structure among prokaryotic and eukaryotic microbes in a 37-day time series. Our goal has been to optimize the quantitative abilities of the Luminex assay and to apply it to a yearlong time series and a 3-month time series during an *L. polyhedrum* bloom, in order to assess phytoplankton community dynamics in La Jolla, CA coastal waters. In collaboration with Melissa Carter and Mary Hilbern who perform weekly monitoring of the SIO Pier water, comparisons

were made with cell count values obtained by light microscopy versus cell concentration values calculated by the molecular bead array method for three species in which cell cultures were available. Water measurement data were also taken from the SCCOOS website (http://www.sccoos.org) in order to perform correlation analysis between cell abundance and various seawater characteristics. Pairwise correlations between eukaryotic phytoplankton taxa were also performed to gain insight on interspecific relationships.

MATERIALS AND METHODS

Sample Collection

Surface seawater samples were collected from the Scripps Institution of Oceanography (SIO) Pier in La Jolla, California once to twice a week between 8:00 and 10:00 am from March 2, 2009 to March 25, 2010 for a yearlong time series and from April 26, 2010 to June 10, 2010 for a *Lingulodinium polyhedrum* bloom time series. 500 ml to 1 L of seawater were filtered through 5 μm polycarbonate filters (Millipore) and each sample was replicated to obtain duplicate filters. Filters were stored at -80°C for later use. DNA was extracted from the filters using the Qiagen DNEasy® Tissue Kit.

Phytoplankton cultures of *Akashiwo sanguinea*, *Lingulodinium polyhedrum* and *Scrippsiella trochoidea* were grown in Provasoli-Guillard f/2 media at 20°C to generate standard curves of Luminex fluorescent intensity as a function of cell concentration. Cell concentrations from cultures were determined by counting all of the cells in a defined volume using shallow depression slides under 200-250x magnification using a stereo microscope. Known quantities of cells were filtered and DNA was extracted using the same protocol as the field samples.

Asymmetric PCR Amplification

Universal eukaryotic primers developed by Mayali *et al*. (2010) were used to amplify a region of the 18S ribosomal subunit. Asymmetric PCR was performed to preferentially amplify the biotin-labeled DNA strand complementary to the probe. The 25 μl reaction mixtures contained final concentrations of Promega GoTaq® 1X Green Master Mix, 0.4 μM Euk1193-F, 0.133 μM Euk1380-R, and the extracted DNA (which

was diluted by 10-fold, resulting in target DNA concentrations ranging from 0.55 to 10.1 ng depending upon the DNA extracted from the field sample). The forward primer was labeled with a 5' biotin tag permitting later coupling with the reporter fluorophore. PCR cycling conditions consisted of an initial denaturation at 95 °C for 5 minutes followed by 25 cycles of denaturation at 95 °C for 45 seconds, annealing at 52 °C for 45 seconds, extenstion at 72 °C for 1 minute, and a final extension at 72 °C for 15 minutes. Products were verified by electrophoresis on a 1.8% agarose gel with ethidium bromide staining.

Probe Design and Microsphere Coupling

Phytoplankton probes were developed by Mayali *et al*. (2010) from clone libraries generated from SIO Pier surface water DNA obtained during August and October 2004. A unique external standard probe (arbitrarily designed for the house cricket *Acheta domesticus*) and its biotinylated complement oligonucleotide were used to correct the fluorescence signal across plates and control for day-to-day variation in sample processing. Luminex xMAP® polysterene beads were coupled to their respective probes following the protocol set forth by Lowe *et al*. (2004). The probes were modified with a 5' C6-amino linker and the reactions took place in EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide HCl) which allowed for the formation of amine bonds to the carboxylated microspheres. One microliter of 1mM capture oligonucleotide was coupled to approximately one million beads per reaction. Coupled beads were stored in Tris-EDTA buffer [1.0 M Tris-HCl (pH 8.0) and 0.1 M EDTA].

Hybridization and Data Acquisition

The hybridization procedure followed the published protocol by Mayali *et al*. (2010) with some minor modifications. The reaction was performed in 1X TMAC buffer [3 M tetramethylammonium chloride, 0.1% SDS, 50 mM Tris-HCl (pH 8.0) and 4 mM EDTA (pH 8.0)] with approximately 500 beads of each color and 4.5 µl of PCR product and 1 pmol of the cricket complement. Two negative controls were used: one containing the PCR negative control and 1 pmol of the complement to the external standard probe (labeled "BL1") and another containing the PCR negative control and 1 µl of nuclease free water (labeled "BL2"). Samples were run in triplicates and the average of the median fluorescence was used for subsequent analyses. The reaction mixture was denatured at 95 °C for 5 minutes followed by a 2 hour incubation step at 52 °C. After the incubation, 35 µl of 1x TMAC buffer was added to the reaction mixture. The beads were then pelleted at 4400 rpm at 22°C for 3 minutes and resuspended in a streptavidin, R-phycoerythrin conjugate mixture (Invitrogen; 4 µg/ml in 1x TMAC buffer) and incubated at 52°C for 10 minutes to allow reporter fluorophore binding to the biotinylated amplicons hybridized to the beads. The beads were then washed for the second time with 35 µl of 1x TMAC buffer and resuspended in 50 µl of 1x TMAC buffer for data acquisition through the Luminex100 instrumentation. Forty beads of each color were analyzed per sample.

Signal Normalization and Statistical Analysis

Raw median fluorescence data were corrected for well-to well and plate-to-plate variation introduced by the hybridization and fluorescence detection procedures using the

signal of the external standard added to each sample (which should be equal across all wells). The correction was made by first calculating the external standard signal in the blank (E_a, see below) and comparing it to the external standard signal in given sample (E_b) to calculate ratio E_a: E_b. This ratio was then applied to the signals for each taxon to obtain the corrected signal. The overall calculation is as follows:

BL1 = PCR negative control and 1pmol external standard complement
BL 2 = PCR negative control and nuclease free water
F_c = corrected signal fluorescence
F_s = field sample signal – BL1 signal for that particular taxon
E_a = external standard signal from BL1 – external standard signal from BL2
E_b = external standard signal from field sample – external standard signal from BL2

$$F_c = F_s (E_a : E_b)$$

Corrected fluorescence data were then normalized for the correlation analysis for the yearlong and bloom time series. This procedure was necessary to account for the differences in relative intensity because the individual bead types require a separate coupling reaction. The normalization method converts signals across all taxa to a common scale with an average of zero and a standard deviation of one, for direct comparisons of abundance patterns throughout the sampling period. The mean of the corrected signals across all of the samples for a taxon was subtracted from the signal for each sampling date and then divided by the standard deviation. These normalized values were then used for pairwise Pearson's correlation analysis using JMP v.8.0 to reveal taxa with similar or dissimilar temporal patterns. A time lag and time lead was applied to the correlation analysis to investigate relationships between the taxa by shifting the sampling date by 2-3 days for each taxon, separately. The time shift analyses followed the procedure from unpublished work by Xavier Mayali with some minor modifications..

Bonferroni's correction for multiple comparisons was applied to determine significance of correlation coefficients (Bonferroni 1936; Miller 1981).

RESULTS

Standard Curve

Pure cultures of *A. sanguinea, L. polyhedrum*, and *S. trochoidea* were used to generate standard curves that relate signal fluorescence to cell concentration (Fig. 1). Rather than performing serial dilutions of DNA extracted from an initial cell concentration, DNA was extracted from a known number of cells at each concentration to eliminate differences in extraction efficiency due to varying cell concentrations. Complex polynomial equations for each standard curve indicating the relationship between the log of the cell number as a function of normalized fluorescence were generated using the statistical software package JMP v.8.0. The equations are as follows:

A. sanguinea: $Y = 66.09\ (\log X-2)^3 + 348.82\ (\log X-2)^2 + 942.28\ \log X - 844.26$

L. polyedrum: $Y = -1.01\ (\log X-2)^4 + 104.33\ (\log X-2)^3 + 484.64\ (\log x-2)^2 + 737.92\ \log X - 1023.02$

S. trochoidea: $Y = 21.03\ (\log X-2.5)^5 + 30.13\ (\log X-2.5)^4 - 134.1\ (\log X-2.5)^3 - 89.97(\log X-2.5)^2 + 416.6\ \log X - 655.34$

A range of PCR cycle numbers were also tested; the dynamic range of the system was optimal at 25 cycles. The Luminex instrumentation was able to distinguish a broad range of cell concentrations, ranging from a single cell to 100,000 cells depending on the taxon.

Fluorescence Correction with External Standard

The goal of correcting the raw fluorescent output was to account for experimental and technical error introduced throughout the assay. The use of an external standard, as applied here, appears to reduce experimental error. For example, when applied to raw fluorescence data from 50 environmental samples obtained from March 2, 2009 to

August 20, 2009, the mean standard error improved from 31.6 to 21.9, decreasing well-to-well variation by 30%.

To investigate the effects of non-target DNA on fluorescent output for individual target taxa, a standard curve of *L. polyhedrum* was spiked with 1.4 ng of *A. sanguinea* DNA to represent background DNA as would be observed in an environmental sample representing a diverse assemblage of organisms. A reduction in hybridization efficiency of the target-taxon due to the presence of non-target DNA within the sample was observed. However, correcting the signal using the external standard probe effectively eliminated this problem (Fig. 2).

Duplicate filters for each sampling date labeled "A" and "B" were also examined for possible errors in sample preparation. Results from comparing corrected fluorescence data for the duplicate samples during the yearlong and bloom time series showed high positive correlations when examined across all seven taxa (Table 2). This indicates that seawater sample preparation is an insignificant source of error on the final results. Averages and standard deviations of the duplicate filters were used for the time series analyses.

Microscopy and Bead Array Method Comparison

Corrected Luminex signals were converted to cell concentration values for taxa for which standard curves were constructed. Results were compared with microscopy counts performed on the same water samples for the one-year time series, and the correlations were significant ($p < 0.0033$) across all three species. Although correlations were significant, in some cases abundance values differed by 2 to 3 orders of magnitude

between the two methods. Only one significant correlation was observed in the bloom time series (Table 3).

Time Series

The goal of applying the bead array method to a time series was to assess phytoplankton temporal dynamics in the La Jolla coastal water community. A yearlong time series during March 2009 to March 2010 was created and dynamic trends in temporal distributions were observed. Significant pairwise correlations among the taxa and water variables were determined (Table 4 and 5) with the goal of revealing taxa with similar or opposite temporal trends. A high positive correlation was observed between the diatoms *Cylindrotheca* and *Chaetoceros.* Many taxa were positively correlated with *Prorocentrum* including the diatom *Chaetoceros* and the dinoflagellates *Akashiwo*, *Ceratium*, and *Scrippsiella*. Other significant positive correlations were observed between *Chaetoceros* and the dinoflagellaltes *Akashiwo* and *Scrippsiella*. Pearson's correlations between the taxa and water variables from the SCCOOS data sets were also assessed. All taxa except for *Ceratium* and *Scrippsiella* showed a significant positive correlation with chlorophyll. Significant negative correlations were found in *Lingulodinium* in both temperature and salinity. *Scrippsiella* on the other hand showed a positive correlation with salinity.

Water samples from a *L. polyhedrum* bloom during April to June 2010 were also analyzed (Tables 6 and 7). The results indicate little change in community structure during the bloom, with the majority of the correlations observed from the yearlong time series being maintained. There was a newly observed positive association between

Lingulodinium and *Akashiwo.* Some signficant correlations from the annual time series were not apparent in the bloom time series, including the positive correlation between *Akashiwo* and *Chaetoceros* or *Prorocentrum.* When comparing the taxa with the water variables, significant correlations between chlorophyll and many of the taxa were lost, with an increase in correlation coefficients in *Lingulodinium* and *Akashwio.* Negative correlations between temperature and *Lingulodinium* and *Akashiwo* were observed. Correlations between salinity and *Scrippsiella* were maintatined from the yearlong time series, though these were no longer apparent for *Lingulodinium.* Normalized signal fluorescence data for the seven taxa and water variables are plotted with respect to time in Figures 3 to 7.

A time lag analysis was performed on normalized data from both the yearlong and bloom time series with the goal of assessing temporal dynamics among taxa in response to one another. As expected, results from the 2-3 day time lag were consistent with results from the 2-3 day time lead. For example, a 2-3 day time lag in *Lingulodinium* would cause the other taxa to be 2-3 days in lead. More losses in significant correlations were observed than gains when compared to correlation results with no time shift. Significant correlations were maintained between *Lingulodinium* and *Akashiwo* and between *Chaetoceros* and *Cylindrotheca* or *Prorocentrum* when a 2-3 day lead was placed on *Chaetoceros* whereas a 2-3 day lag on *Chaetoceros* led to loss in correlations. Another association that was maintained was between *Prorocentrum* and *Scrippsiella* when a lag was placed on *Scrippsiella.* The 2-3 day lead and time lag series resulted in a significant positive correlation between *Prorocentrum* and *Cylindrotheca*, which may imply that these taxa respond positively to the presence of another. *Lingulodinium* and

Cheatoceros also gained positive correlation when a lead was placed on *Lingulodinium.* Significant correlations that were lost between taxa may suggest similarities in temporal dynamics that were lost when a shift in the time series was introduced. *Prorocentrum* and *Ceratium* lost their positive correlation as well as *Scrippsiella* and *Chaetoceros.* Cross-correlation results for the time shift analyses are summarized in Tables 8 and 9 and presented as network diagrams in Figure 8.

DISCUSSION

Despite the obviously important roles phytoplankton play in marine ecosystems, relatively little is known about the mechanisms underlying their community structure. The data presented in this study show that Luminex bead arrays can offer affordable, multiplex and high-throughput capabilities combined with the specificity and sensitivity for target taxa needed for high spatiotemporal studies on microbial communities. Here, the approach was applied to environmental samples from La Jolla, CA coastal waters with the goal of better understanding the interactions within the phytoplankton community.

Tests using pure cultures of *L. polyhedrum, S. trochoidea,* and *A. sanguinea* have shown that the Luminex has a dynamic range of about 4.5 logs and can detect target cells within a mixed assemblage. By employing an external standard, we were able to correct for reduced signal intensities resulting from the presence of background DNA as well as eliminate much of the variability observed between replicate samples. Standard curves constructed to calibrate signal intensities to cell concentration values were compared to microscopy counts performed on the same samples. Pairwise correlations of cell abundance measurements comparing both methods were relatively high for the yearlong time series; however, it is important to note that these values may be an artifact of low overall cellular abundance throughout the sampling period. Instances in which high levels of variation were observed between the methods (up to 2-3 orders of magnitude) are likely due to extreme extrapolations by the microscopy method in which low abundances of cells can lead to an overestimation or underestimation of extrapolated cell counts due to chance events (Schartau et al. 2010). Significant correlations were lost in *Akashiwo*

and *Lingulodinium* when comparing both methods in the bloom samples, which may be due to the small sample size spanning only 14 days. *Lingulodinium* cell counts from the microscopy method were consistently higher by an order of magnitude compared to the Luminex cell concentration values. Intraspecific variation in *L. polyhedrum* may reduce specificity of the probe; however, this is unlikely due to the highly conservative nature of the ribosomal gene as was shown in previous work on *L. polyedrum* isolates from the same sampling site during 1998 and 2003 (Frommlet and Iglesias-Rodríguez 2008).

Field applications of the assay revealed temporal dynamics in phytoplankton composition and abundance. Seasonal variation and temporal segregation were expected between the diatoms and dinoflagellates as physiological traits, resource requirements, and behavior are thought to be important factors determining species selection and biological succession within a community (Smayda et al. 2001; Narwani et al. 2009). Furthermore, commonalities in these attributes may allow for coexistence of species despite phylogenetic divergence (Smayda et al. 2001).

Not surprisingly, the diatoms showed high positive correlation with one another suggesting similar temporal dynamics across the sampling period. An interesting result observed was the strong positive correlation found between the abundances of the diatom *Chaetoceros* and the dinoflagellates *Prorocentrum* and *Scrippsiella.* Explanations for their close association may be found by examining commonalities in their traits. Many studies have aimed to understand the nitrogen uptake and nitrogenous substrate preference of bloom forming phytoplankton, and previous research has shown that *Chaetoceros* and *Cylindrotheca,* or diatoms in general, favor nitrate as their nitrogen source (Alvez-de-Souza 2008). However, studies have also exhibited that *Prorocentrum*

and *Scrippsiella* have high nitrate uptake values comparable to that of diatoms (Lomas and Glibert 2000) and some of their blooms have been associated with high nitrate levels (Martínez-López et al. 2008). Similar preferential maximum uptake rates of ammonium and urea over nitrate have been observed in *Lingulodinium* and *Akashiwo* which may explain their high correlation during the bloom (Kudela et al. 2008). More data on nutrient composition over the sampling period is needed to infer nutrient limitation effects on phytoplankton abundance. Nutrient preference and uptake is just one possible factor that may explain that shared ecophysiological traits among taxa allow for their co-existence.

The *L. polyhedrum* bloom occurred in late April to beginning of June of 2010, and Luminex results showed peak fluorescence in *Lingulodinium* and *Akashiwo* during the sampling period as well as significantly high correlations with chlorophyll whereas other taxa that previously showed high correlations in the yearlong time series showed no correlation with chlorophyll during the bloom time series. The dominance of these taxa could be attributed to biological succession following peak diatom abundances in March 2010 which is consistent with many studies that have observed diatom bloom occurences in late winter to early spring and dinoflagellate blooms throughout the summer (Broekhuizen 1999; Smayda et al. 2001; Badylak and Phlips 2004; Thompson et al. 2008). Dinoflagellates are known to favor increased light attenuation and diatoms tolerate fluctuating irradiance, which allows to them to have a competitive edge during the winter season (Thompson et al. 2008). Moreover, upwelling events are commonly observed in La Jolla Bay in early spring which increases nutrient levels favored by diatoms, allowing for the subsequent succession of dinoflagellates as diatoms deplete surface water

nutrients; dinoflagellates are able to better exploit nutrient-low conditions through their increased motility and regulation of depth (Kamykowski 1974; Broekhuizen 1999). It is important to note that temporal variability in the hydrographic dynamics of the region has been commonly observed from yearly comparisons of phytoplankton community composition in La Jolla Bay (Reid et al. 1970). More knowledge of exogenous variables such as upwelling events, stratification, light intensity as well as the physiological adaptation of each taxa are required to discern some of the factors affecting phytoplankton growth and abundance.

A 2-3 day time shift analysis was performed with the goal of assessing the dynamic interactions between taxa by observing the response of a taxon's temporal distribution in relation to another. Results obtained were not consistent with expected results considering temporal segregation of similar taxonomic groups. Taxa that were highly correlated without the time shift showed mixed results of maintained correlations as in the case with *Lingulodinium* and *Akashiwo* or showed loss of correlations as was observed in *Chaetoceros* and *Cylindrotheca*. Choosing the appropriate time lag is critical in utilizing time lag analysis to discern taxon interactions. More sets of time lags must be tested in order to find the time lag (if any) that best represents response effects: commonly 25% of the time series duration is used and/or simulation models (Olden and Neff 2001; Angeler et al. 2009).

This study has demonstrated the potential use of bead array technology to characterize phytoplankton communities through simultaneous detection of various taxa within a mixed assemblage. The high throughput capabilities of the assay holds great promise for microbial dynamics studies at large spatial and temporal scales and at

different taxonomic levels from genus to strains, in order to better comprehend interactions between taxa that structure phytoplankton communities.

FURTHER WORK

I plan to analyze samples from the March 2009 to March 2010 yearlong time series and the April to June 2010 bloom time series for prokaryotic taxa using primers and probes designed by Xavier *et al.* (2010) to assess prokaryote and eukaryote interactions within the La Jolla, CA coastal water microbial community. The filtrate of water filtered through the 5.0 μm filters was subsequently filtered through 0.2 μm filters to capture the prokaryotes. Genomic DNA has already been extracted and are stored at -80°C for future analyses.

FIGURES

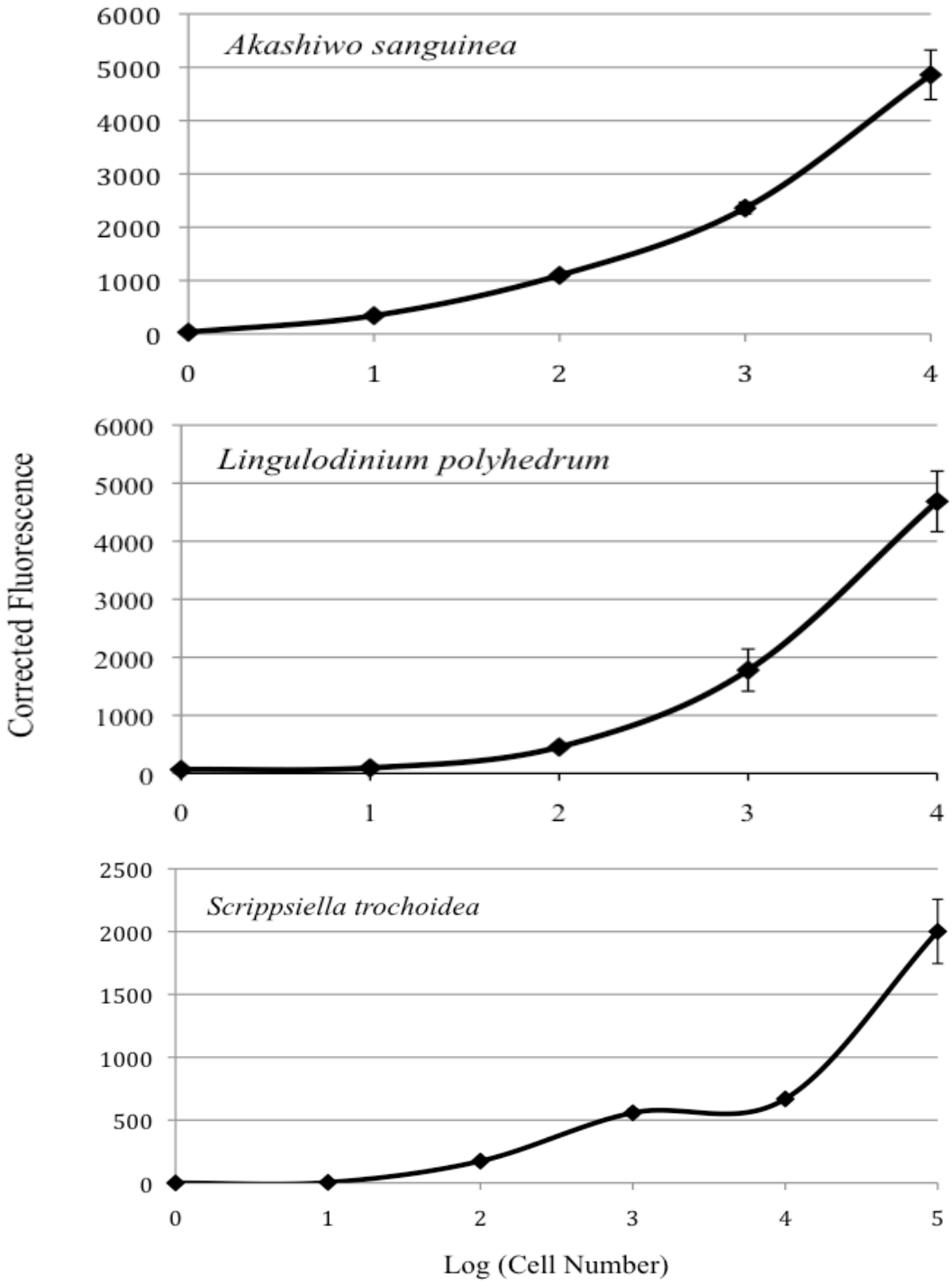

Fig. 1. Standard curves of *A. sanguinea, L. polyhedrum* and *S. trochoidea* relating corrected fluorescence to the log of the cell concentration. Mean ± 1 SE

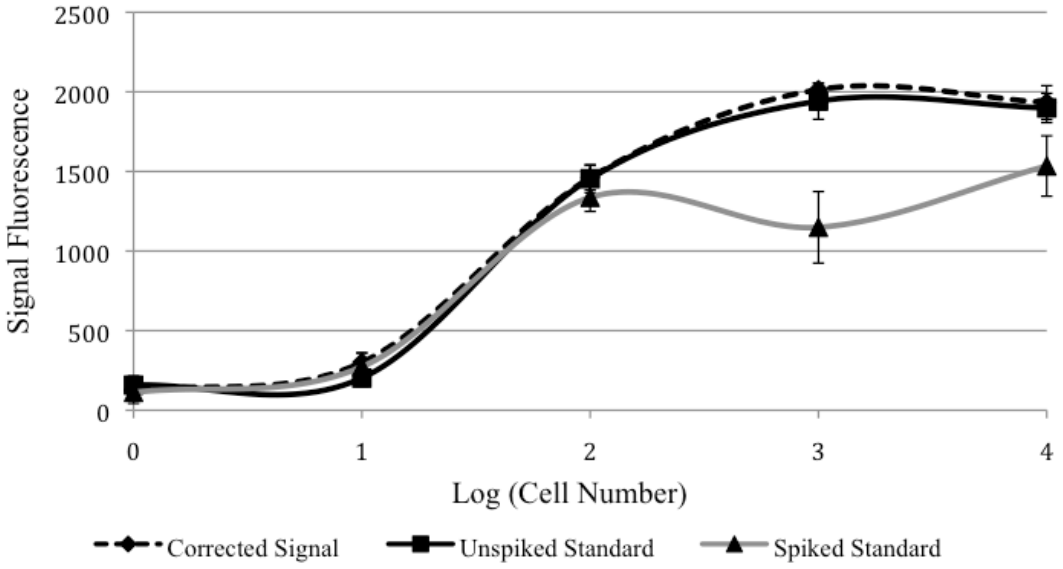

Figure 2. Standard curves for *Lingulodinium polyhedrum* applying the correction method. Spiking the standard with *Akashiwo sanguinea* DNA reduced hybridization efficiency (spiked standard); however, when applying the correction method to the spiked standard, signal intensities closely represented unspiked standard intensities. Mean ±1 SE

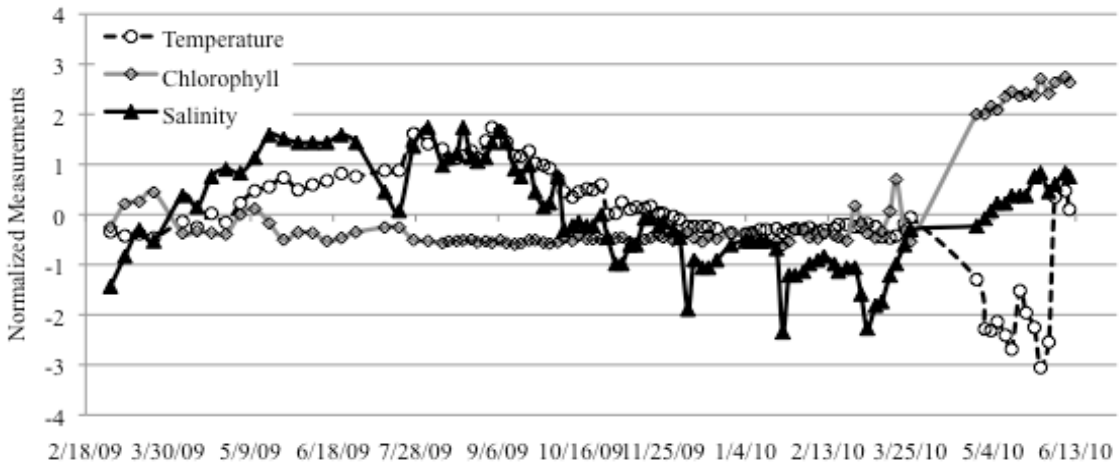

Figure 3. Normalized measurements from SCCOOS data sets during March 2, 2009 to March 25, 2010 and from April 26 to June 10, 2010.

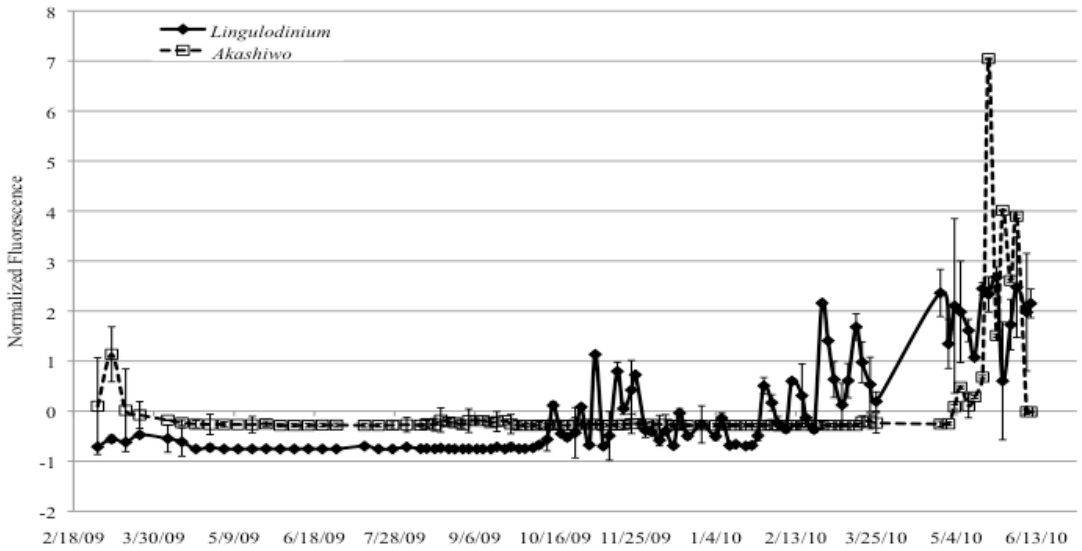

Figure 4. *Lingulodinium* and *Akashiwo* normalized signals from March 2, 2009 to March 25, 2010 and from April 26 to June 10, 2010. Mean ±1 SE

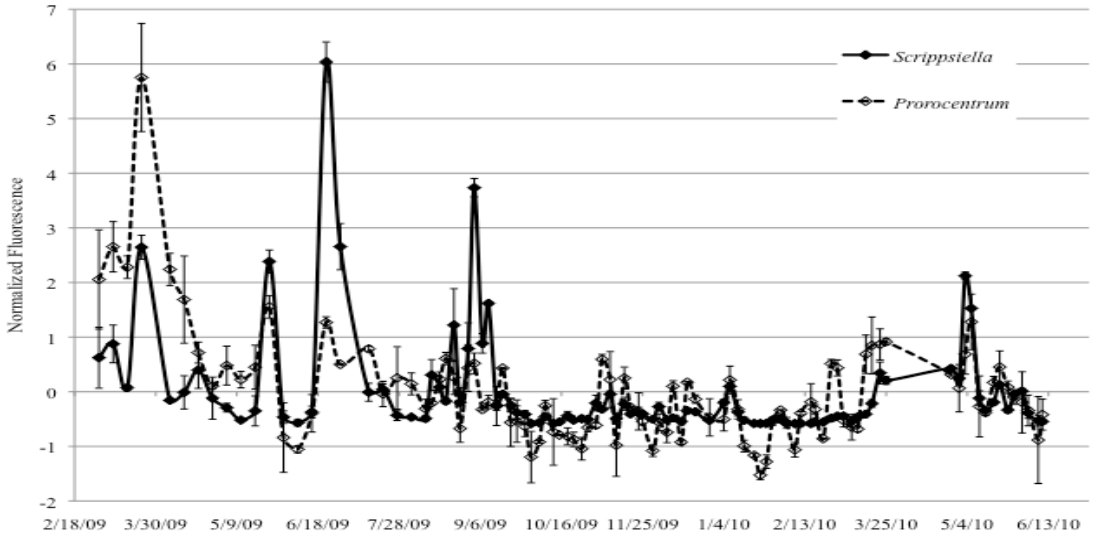

Figure 5. *Scrippsiella* and *Prorocentrum* normalized signals from March 2, 2009 to March 25, 2010 and from April 26 to June 10, 2010. Mean ±1 SE

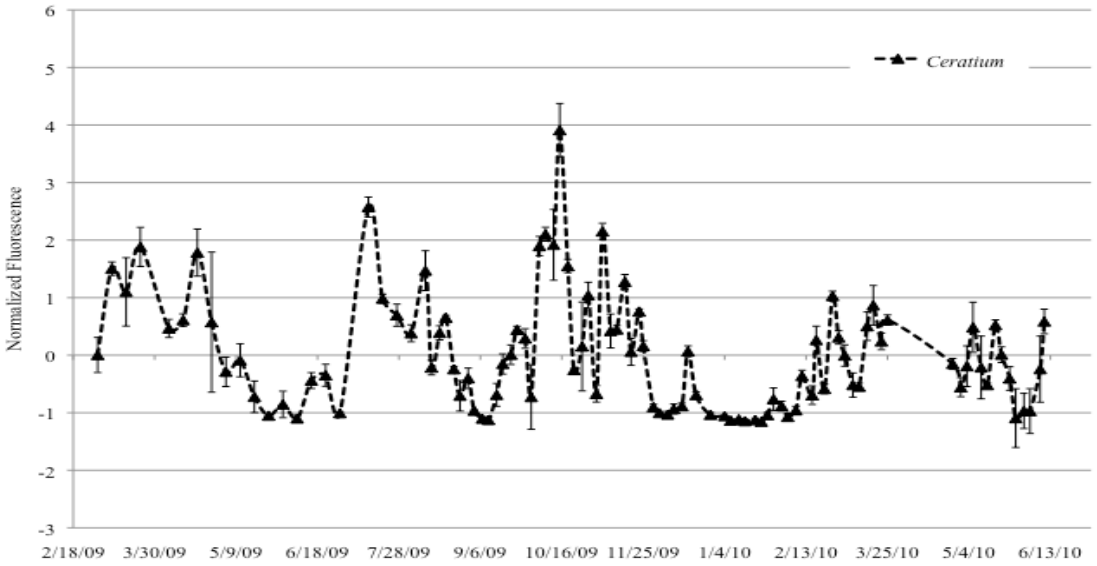

Figure 6. *Ceratium* normalized signals from March 2, 2009 to March 25, 2010 and from April 26 to June 10, 2010. Mean ±1 SE

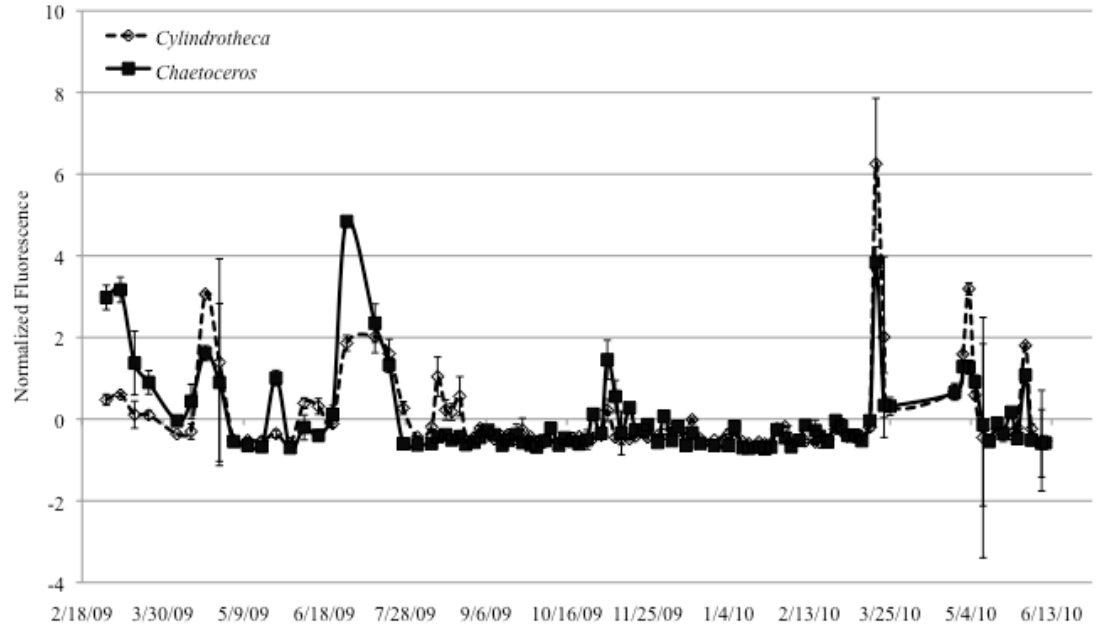

Figure 7. *Cylindrotheca* and *Chaetoceros* normalized signals from March 2, 2009 to March 25, 2010 and from April 26 to June 10, 2010. Mean ±1 SE

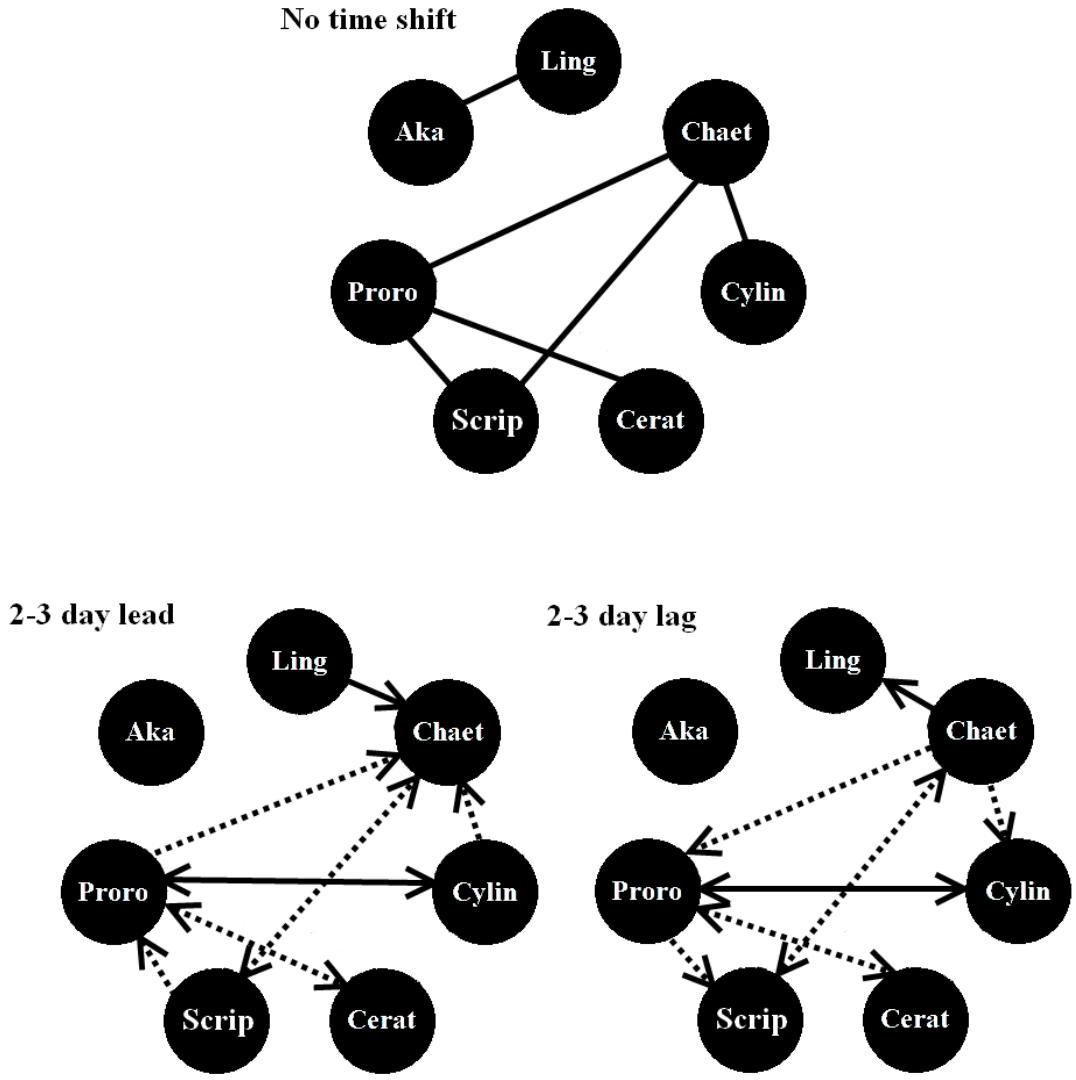

Figure 8. Network diagrams of the time lag analysis. Dashed arrows depict lost correlations and solid arrows depict gained correlations compared to the analysis with no time shift. The taxon for which the lag or lead was applied is shown as the arrow pointing away from that taxon.

TABLES

Table 1. List of primers and probes.

Universal Eukaryotic Primers	**Sequence 5'- 3'**	**Bead Color**
Euk 1193F	AAC AGG TCT GTG ATG CCC	
Euk 1380R	GTG TAC AAA GGG CAG GGA	
Probes		
Akashiwo	CCT GCC GGA CCA GGC AGA AAC TCG T	*56*
Ceratium	CCT TCC CAG GAC AGG TTA AAG ACT C	*65*
Chaetoceros	AAC ACG CGT GCG GTT CAG AAC ATC T	*31*
Cylindrotheca	GGC CAA GGT AGA ACT CGT TGA ATG C	*10*
Lingulodinium	CTT GTT GAT CAC GTC AGT GTA GCG C	*25*
Prorocentrum	GAT TTA AAA AGA TTA CCC AAC CCT A	*78*
Scrippsiella	ACC CTG CCG GGC AAG CTC ATA AAC T	*36*
Acheta domesticus	ATC AGC GGG AAG TAA TGA TTC CCG C	*95*

Table 2. Pearson's correlation coefficients comparing duplicate filter samples "A" and "B" for each taxon using all of the 99 environmental samples. Significance after Bonferonni correction ($p < 0.00055$) is identified with an asterisk.

Taxon	**Pearson's r**
Akashiwo	0.9848*
Ceratium	0.9377*
Chaetoceros	0.9178*
Cylindrotheca	0.8595*
Lingulodinium	0.9802*
Prorocentrum	0.9053*
Scrippsiella	0.9694*

Table 3. Pearson's correlation coefficients comparing cell concentration per liter of sea water by the microscopy method and the bead array method. Significance after Bonferonni correction ($p < 0.00333$) is identified with an asterisk.

Taxon	**Yearlong time series**	**Bloom time series**
Akashiwo	0.7457*	0.3059
Lingulodinium	0.9645*	0.4385
Scrippsiella	0.8056*	0.9272*

Table 4. Pearson's correlation coefficients between normalized signals for the phytoplankton and normalized water variable measurements for the yearlong time series. Significance after Bonferonni correction ($p < 0.0011$) is identified with an asterisk.

Taxon	Chlorophyll a	Temperature	Salinity
Akashiwo	0.3839*	-0.1067	-0.0456
Ceratium	0.2694	0.0728	0.0338
Chaetoceros	0.5139*	-0.1429	-0.0122
Cylindrotheca	0.4806*	-0.0175	0.0870
Lingulodinium	0.3681*	-0.4731*	-0.5405*
Prorocentrum	0.6043*	-0.0881	0.1245
Scrippsiella	0.0834	0.3039	0.3907*

Table 5. Correlation coefficients between normalized signals for the phytoplankton for the yearlong time series. Significance after Bonferonni correction ($p < 0.0024$) is identified with an asterisk.

	Akashiwo	*Ceratium*	*Chaetoceros*	*Cylindrotheca*	*Lingulodinium*	*Prorocentrum*
Ceratium	0.1857					
Chaetoceros	0.4485*	0.3018				
Cylindrotheca	0.1297	0.2640	0.7152*			
Lingulodinium	-0.0902	0.1967	0.0876	0.0924		
Prorocentrum	0.4814*	0.3373*	0.5325*	0.2965	0.0340	
Scrippsiella	0.1953	-0.0649	0.3403*	0.1665	-0.2217	0.5141*

Table 6. Correlation coefficients between normalized signals for the phytoplankton and normalized water variable measurements for the bloom time series. Significance after Bonferonni correction ($p < 0.0011$) is identified with an asterisk.

Taxon	Chlorophyll a	Temperature	Salinity
Akashiwo	0.6114*	-0.4459*	0.1190
Ceratium	-0.0519	0.1370	0.0134
Chaetoceros	0.1474	-0.1711	-0.0259
Cylindrotheca	0.1979	-0.1557	0.0683
Lingulodinium	0.8054*	-0.6531*	-0.2117
Prorocentrum	0.1306	-0.0966	0.1061
Scrippsiella	0.0563	0.0885	0.3512*

Table 7. Correlation coefficients between normalized signals for the phytoplankton for the bloom time series. Significance after Bonferonni correction ($p < 0.0024$) is identified with an asterisk.

	Akashiwo	*Ceratium*	*Chaetoceros*	*Cylindrotheca*	*Lingulodinium*	*Prorocentrum*
Ceratium	-0.0906					
Chaetoceros	0.0342	0.2751				
Cylindrotheca	0.0222	0.2053	0.7259*			
Lingulodinium	0.4592*	0.0468	0.0991	0.1459		
Prorocentrum	0.0891	0.3354*	0.5346*	0.3007	0.0391	
Scrippsiella	0.0249	-0.0553	0.3717*	0.2405	-0.0766	0.5293*

Table 8. Correlation coefficients for the 2-3 day time lead analysis. The taxon with the lead applied to it is placed in the first column in bold. Significance after Bonferonni correction ($p < 0.0024$) is identified with an asterisk.

	Cylin	*Ling*	*Chaet*	*Scrip*	*Aka*	*Cerat*	*Proro*
Cylin		0.2312	0.2274	0.3237	0.0980	0.0265	0.458*
Ling	0.2754		0.3489*	0.0267	0.5023*	-0.0535	0.2566
Chaet	0.3811*	0.2946		0.2520	0.1501	0.0399	0.3727*
Scrip	0.0739	0.0225	0.1103		0.0529	-0.1787	0.2502
Aka	0.0424	0.5211*	0.0965	-0.0248		-0.1352	0.0481
Cerat	0.0578	-0.0870	0.0642	-0.0562	0.0513		0.0441
Proro	0.3655*	0.1829	0.3204	0.4148*	0.1772	-0.0728	

Table 9. Correlation coefficients for the 2-3 day time lag analysis. The taxon with the lag applied to it is placed in the first column in bold. Significance after Bonferonni correction ($p < 0.0024$) is identified with an asterisk.

	Cylin	*Ling*	*Chaet*	*Scrip*	*Aka*	*Cerat*	*Proro*
Cylin		0.2754	0.3731*	0.0739	0.0424	0.0578	0.3548*
Ling	0.2312		0.2761	0.0225	0.5211*	-0.0870	0.1769
Chaet	0.2774	0.3489*		0.1103	0.0965	0.0642	0.3135
Scrip	0.3237	0.0267	0.2427		-0.0248	-0.0562	0.4046*
Aka	0.0980	0.5023*	0.1497	0.0529		-0.0513	0.1775
Cerat	0.0265	-0.0535	0.0252	-0.1787	-0.1352		-0.0726
Proro	0.4804*	0.3086	0.3961*	0.2714	0.0418	-0.0427	

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APPENDIX

SCCOOS data set.

Date	*Lingulodinium polyedrum*	*Akashiwo sanguinea*	*Prorocentrum spp.*	*Scrippsiella spp.*	Water Temp (°C)	Chl a (mg/m^3)	Salinity (PSU)
3/2/09	303	909	56667	0	14.9	2.43	33.28
3/9/09	0	5455	186970	0	14.5	4.99	33.36
3/16/09	400	0	233200	0	14.7	5.24	33.43
3/23/09	606	303	223030	0	14.3	6.27	33.40
4/6/09	0	0	57576	0	15.9	1.77	33.52
4/13/09	0	0	29394	0	15.3	1.99	33.49
4/20/09	0	0	13333	0	16.7	1.82	33.57
4/27/09	0	0	8788	0	15.8	1.66	33.59
5/4/09	0	0	11818	0	17.7	3.82	33.58
5/11/09	0	0	37576	0	18.9	4.45	33.62
5/18/09	0	0	22121	0	19.3	2.88	33.68
5/25/09	0	0	4545	0	20.2	1.06	33.67
6/1/09	0	0	10606	0	19.0	1.86	33.66
6/8/09	0	0	1818	0	19.5	1.80	33.66
6/15/09	0	0	0	0	19.9	0.91	33.66
6/22/09	0	0	606	909	20.6	1.26	33.68
6/29/09	606	0	1818	606	20.3	1.88	33.66
7/13/09	303	0	7879	0	20.9	2.41	33.53
7/20/09	0	0	1200	0	20.9	2.41	33.48
7/27/09	0	0	9697	0	24.5	1.04	33.65
8/3/09	0	0	606	0	23.5	0.93	33.70
8/10/09	0	0	303	0	23.0	0.69	33.60
8/17/09	0	0	1515	0	22.1	0.93	33.63
8/24/09	0	0	1818	0	22.8	1.08	33.62
8/31/09	0	0	2424	0	23.8	0.91	33.62
9/7/09	0	0	4242	0	24.7	1.01	33.69
9/14/09	0	0	2727	0	22.3	0.57	33.59
9/21/09	0	0	4242	0	22.8	1.01	33.60
9/28/09	0	0	606	0	21.4	0.76	33.49
10/5/09	0	0	2121	0	20.1	0.79	33.57
10/12/09	1515	0	5152	0	18.3	0.95	33.44
10/19/09	909	0	0	0	19.1	1.16	33.44
10/26/09	1818	0	3333	0	19.5	1.07	33.47
11/2/09	606	0	7576	0	16.7	1.24	33.34
11/9/09	303	0	2424	0	17.1	0.78	33.39
11/16/09	3030	0	3939	0	17.2	1.09	33.46
11/23/09	4242	0	3030	0	16.7	1.51	33.44
11/30/09	606	0	0	0	16.4	0.97	33.43
12/7/09	303	0	303	0	15.4	1.97	33.22
12/14/09	0	0	909	0	15.4	0.83	33.33
12/21/09	1515	0	909	0	15.2	1.16	33.35
12/28/09	1515	0	909	0	14.8	1.73	33.39
1/4/10	303	0	2424	0	14.7	1.75	33.40
1/11/10	0	0	0	0	15.1	0.81	33.40
1/19/10	606	0	0	0	15.2	0.89	33.38
1/25/10	2424	0	606	0	15.0	0.84	33.31
2/1/10	3333	0	1515	0	15.1	2.22	33.32
2/8/10	1818	0	1515	0	14.9	1.21	33.35
2/16/10	7273	0	8182	0	15.2	1.54	33.34
2/22/10	303	0	1515	0	15.6	0.95	33.33
3/1/10	18182	0	9091	0	15.4	2.92	33.26
3/8/10	3030	0	303	0	15.4	1.24	33.23
3/15/10	35152	0	31818	303	14.3	8.36	33.31
3/18/10	17059	0	23529	0	14.5	7.66	33.34
3/22/10	3333	0	16667	0	15.7	2.12	33.39
4/26/10	233333	0	90303	0	14.8	20.46	33.44
5/3/10	183200	400	88400	5200	15.7	10.46	33.48
5/10/10	109200	400	39200	1600	16.7	9.53	33.50
5/17/10	210400	400	28400	1200	16.8	18.26	33.52
5/24/10	200000	400	30400	0	16.9	11.08	33.57
5/31/10	37600	400	9600	0	17.1	4.11	33.53
6/8/10	273200	400	76000	0	18.9	18.91	33.58