2001 Annual Progress Report for NOAA Grant #NA06OP0517

The Importance of Organic Phosphorus in Promoting Cyanobacterial Blooms in Florida Bay: Competition Between Bacteria and Phytoplankton

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Introduction

The occurrence of extensive phytoplankton blooms in central Florida Bay has precipitated a significant amount of public, political, and scientific concern. Most vexing is the fact that these blooms are dominated by cyanobacteria, specifically *Synechococcus elongatus*. None of the current physical/chemical or nutrient limitation models accurately predict or explain these occurrences. There are significant differences in physiology between cyanobacteria and algae which may explain their dominance. Cyanobacteria have a much higher cell-specific production of alkaline phosphatase than do algae. Alkaline phosphatase is an enzyme which cleaves organic P making it available for cellular uptake. This enhanced enzyme capacity gives cyanobacteria an advantage over algae under P limited conditions in the presence of labile organic matter. These are the very conditions which are commonly found in central Florida Bay.

Objectives

We initially proposed a two tiered approach to elucidating a mechanism for cyanobacterial bloom development in Florida Bay. First, we proposed to determine the relative contribution of bacteria to the total community production. We hypothesized that spatial patterns in primary production of bacteria and phytoplankton in Florida Bay were a product of inorganic N and organic P availability. We used a running 10 year database of nutrients and phytoplankton biomass (chlorophyll a) combined with new measurements of primary production (pulse amplitude modulated (PAM) fluorometry), bacterial production, bacterial numbers, and enzyme assays to develop statistical models of substrate and nutrient competition among the major components of the phyto- and bacterioplankton community.

The second approach was to test the hypothesis that the enhanced alkaline phosphatase activity of cyanobacteria combined with a terrestrial source of labile DOM would favor the initiation of cyanobacterial blooms in central Florida Bay. From these experiments we expected to quantify: 1) the effect of DOM on phytoplankton community structure; 2) the bacterial contribution to ambient community production; 3) the bacterial contribution to DOM amended community production; and 4) the effect of bioavailability of ambient and amended DOM on community structure.

Work Accomplishments

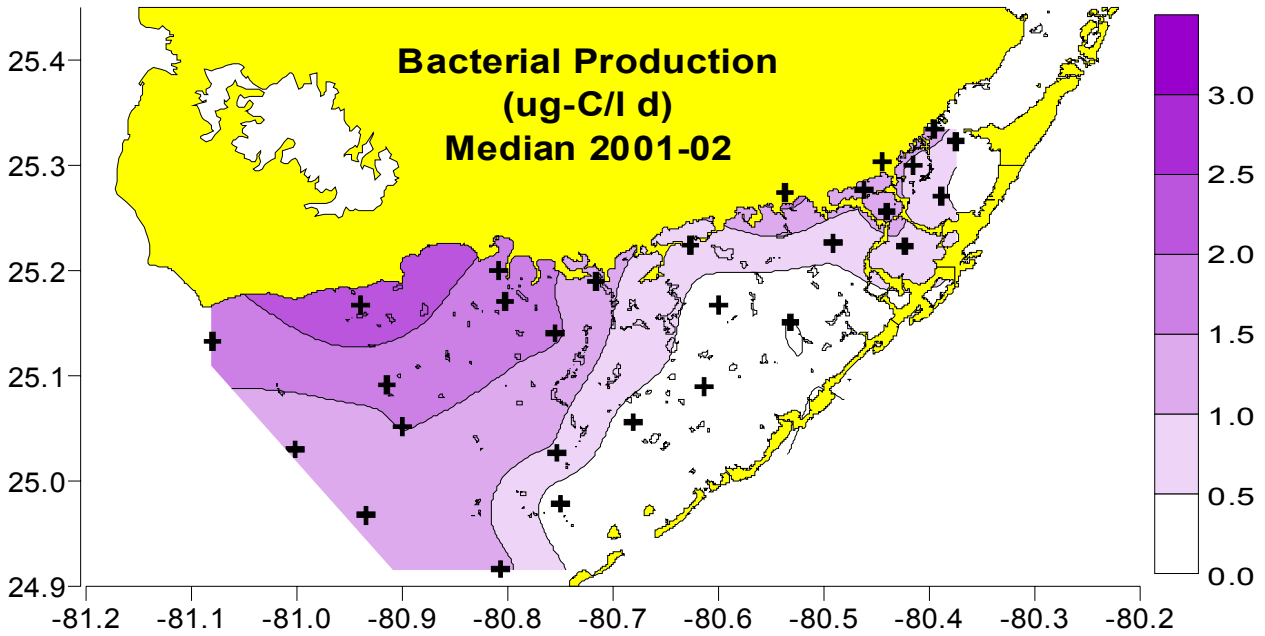
Results Obtained to Date

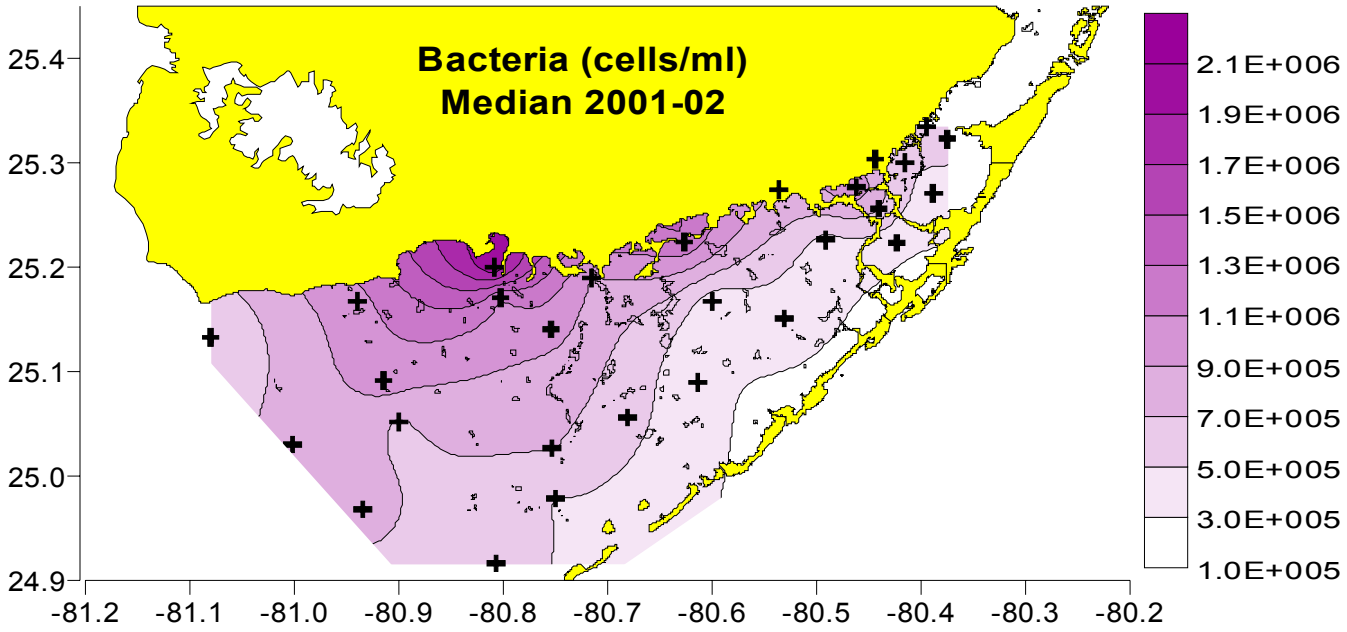
As this project involved use of new approaches to studying phytoplankton dynamics, our first efforts were put into methods development. Phytoplankton primary production was measured using pulse amplitude modulated (PAM) fluorometry. The advantages of PAM fluorometry include quick production measurements (no light/dark incubation assays), discrimination of major phytoplankton groups, and independence from using radioisotopes. This technique permits rapid measurement of: 1) the absorption cross section of Photosystem II (quantum yield); 2) the rate of photosynthetic electron transport and; 3) the level of photochemical quenching. PAM fluorometry was also used to discriminate chlorophyll *a* (CHLA) and quantum yield among the three major groups of phytoplankton: cyanobacteria, green algae, and diatoms. We compared PAM fluorometric determinations of CHLA with the standard acetone extraction method and found reasonable agreement. One of the problems we found with PAM fluorometry was that of instrument sensitivity. Sometimes the very low ambient phytoplankton biomass precluded the generation of acceptable light curves resulting in noisy data. We have addressed this problem with the addition of a new sensor having better sensitivity.

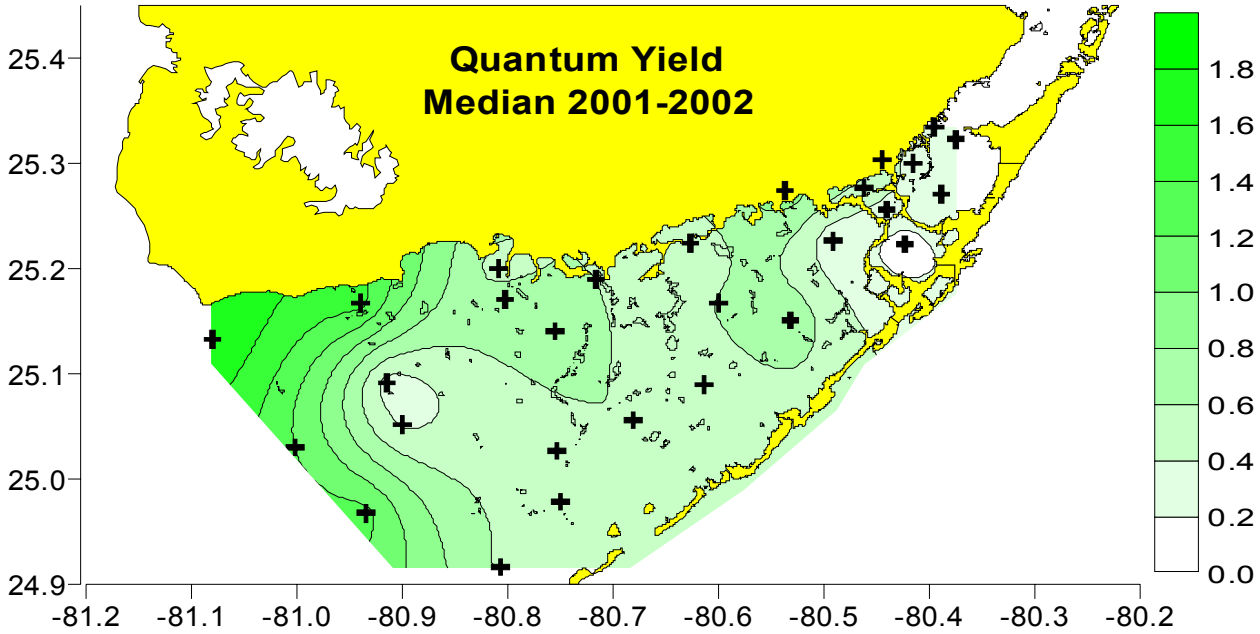
We tested new stains for epifluorescence microscopy of bacterial counts, specifically SYBR Green, Pico Green, and SYBR Gold. Our comparisons with the acridine orange and DAPI techniques were originally very favorable for the SYBR stains: less fading of the sample and lower background fluorescence. The result was that smaller bacteria were more easily counted using SYBR Green and Gold. Part of the reason for testing this stain was that we hoped to use flow cytometry to characterize microbial communities in the near future. Unfortunately, the laboratory comparisons among stains did not hold up for environmental samples. We found that there were permeability problems with SYBR dyes resulting in anomalously low bacterial counts. We have therefore switched back to using DAPI for all bacterial enumeration.

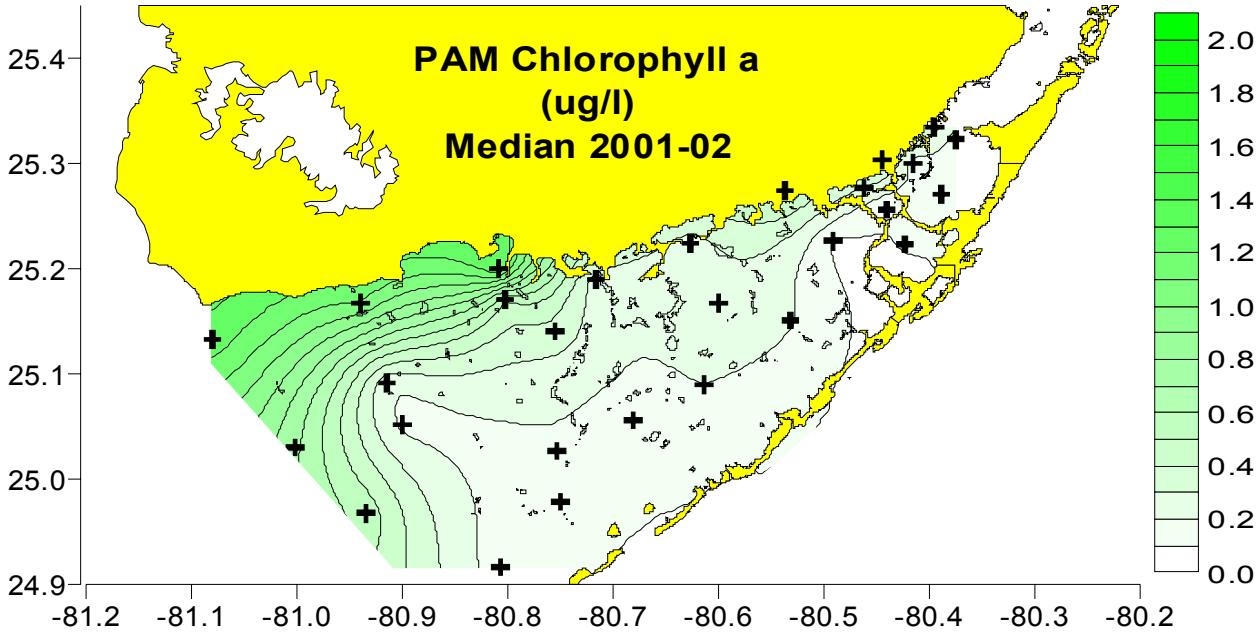
Data from these measurements were used to develop functional response curves of microbial activities with physical/chemical water quality variables. We are relating enzyme activities with bacterial production and cell numbers in an effort to better characterize the microbial loop. Relationships among variables are also being developed using multivariate statistics.

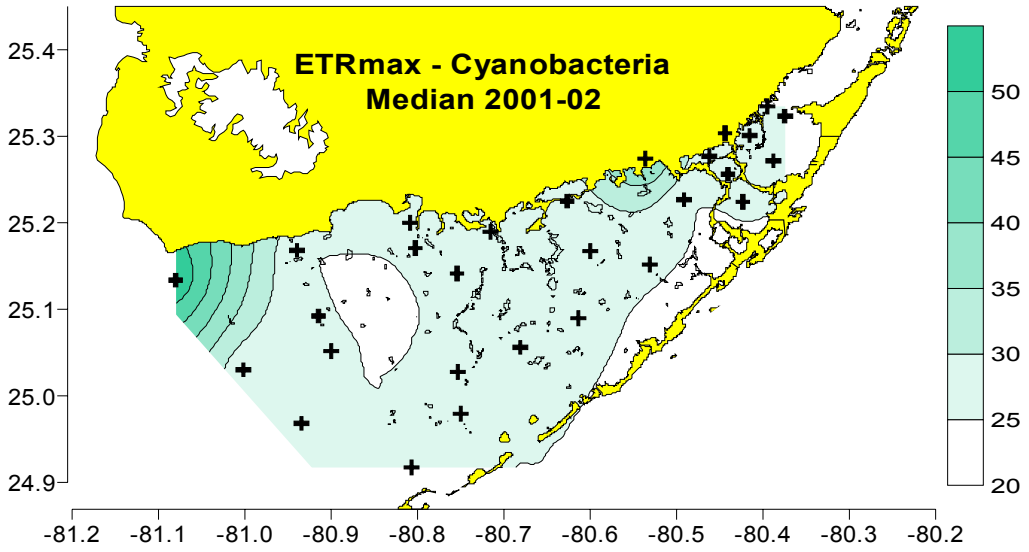
<u>*Monthly Surveys*</u> - Sample results from the surveys are shown in the following figures. The spatial pattern in median bacterial production (Fig. 1) was similar to that of bacterial numbers (Fig. 2) as expected. What was surprising was the dearth of biomass and activity in the central region of the Bay. A similar spatial distribution of phytoplankton quantum yield (Φ_m) and chlorophyll *a* (CHLA) concentration was also observed with extremely low biomass and activity occurring in the central Bay.

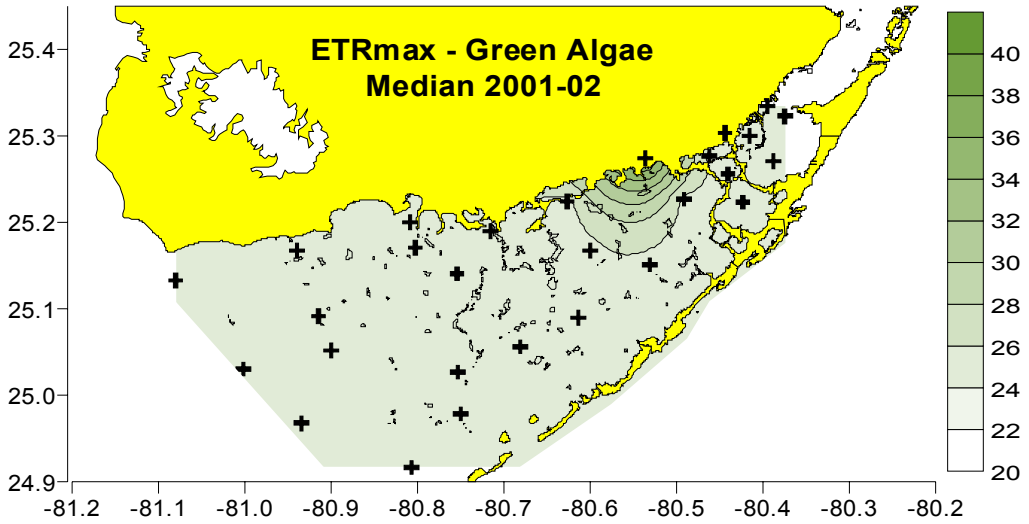

Bacterial Production
(ug-C/l d)
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
3.0
2.5
2.0
1.5
1.0
0.5
0.0

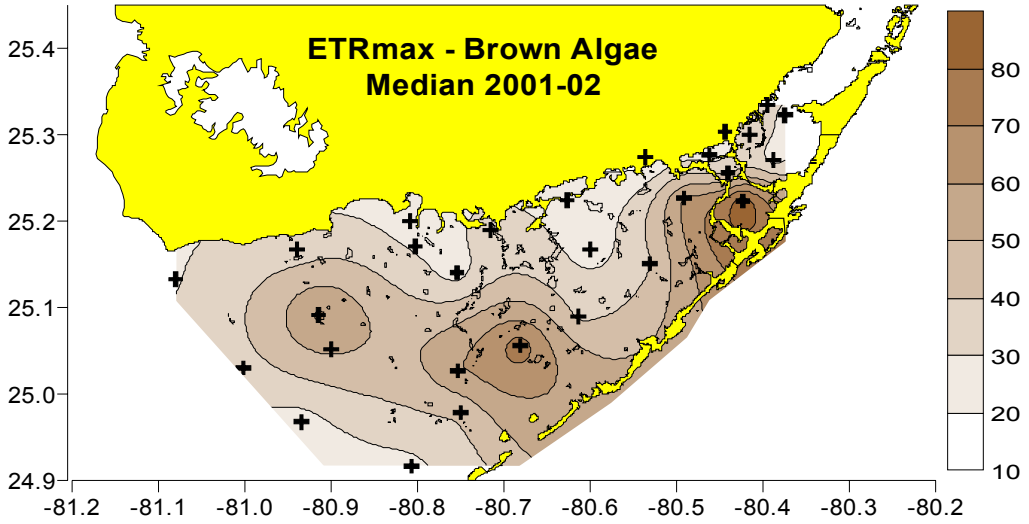

Bacteria (cells/ml)
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
2.1E+006
1.9E+006
1.7E+006
1.5E+006
1.3E+006
1.1E+006
9.0E+005
7.0E+005
5.0E+005
3.0E+005
1.0E+005

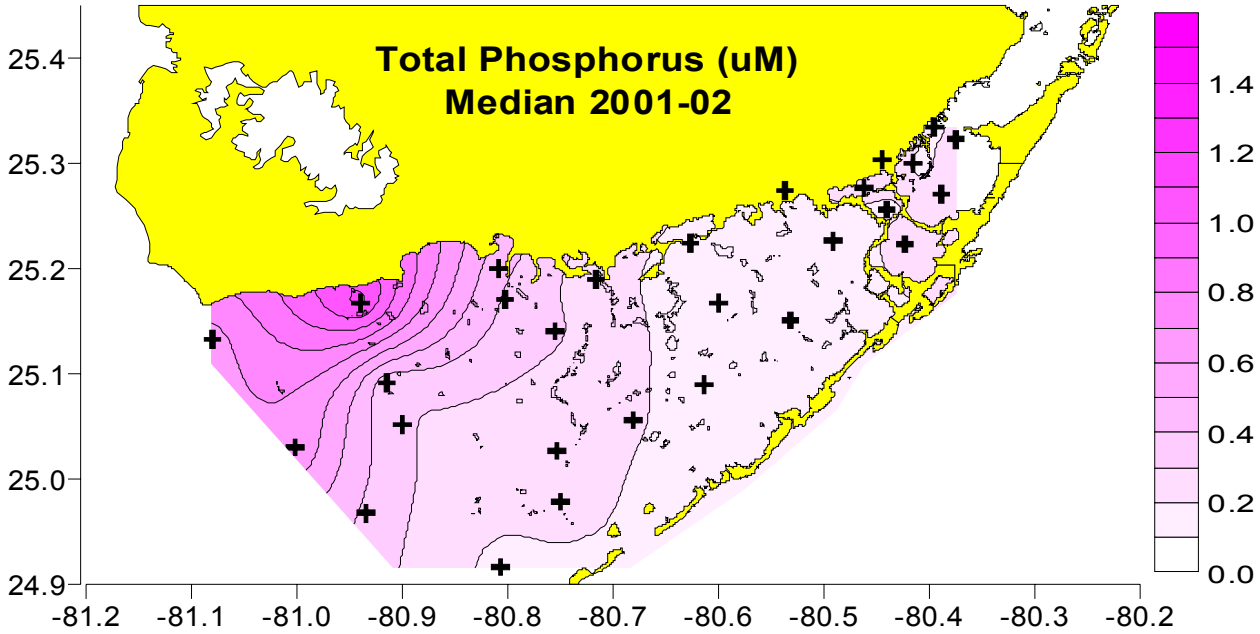

Quantum Yield
Median 2001-2002
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0

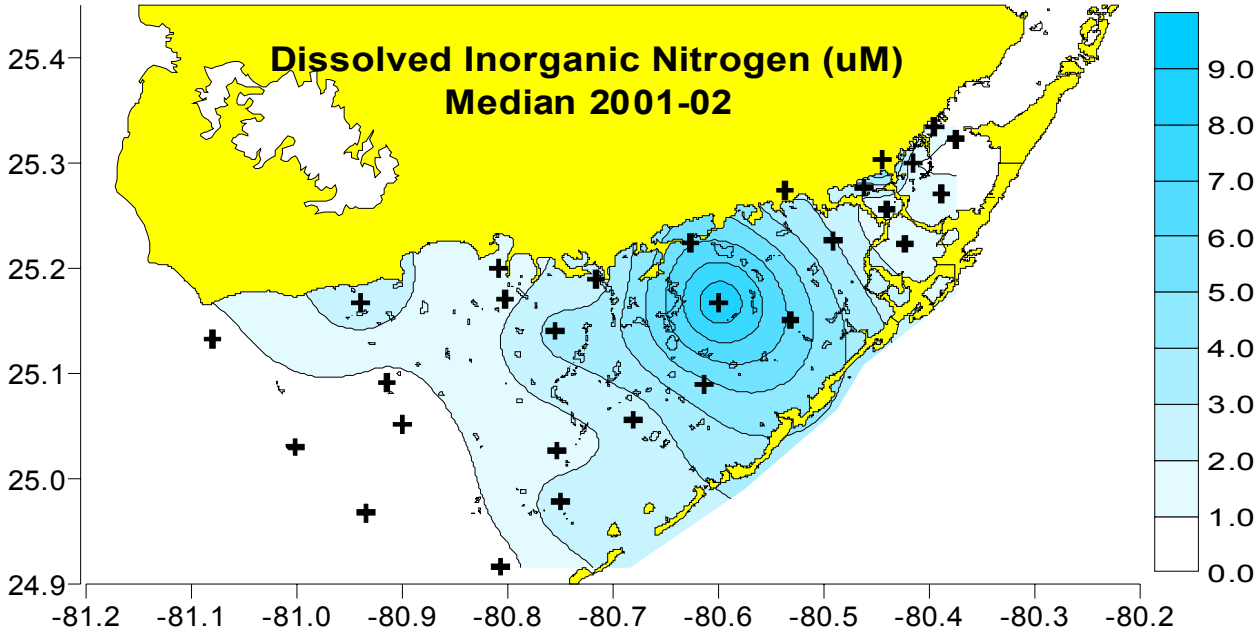

PAM Chlorophyll a
(ug/l)
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0


ETRmax - Cyanobacteria
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
50
45
40
35
30
25
20


ETRmax - Green Algae
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
40
38
36
34
32
30
28
26
24
22
20


ETRmax - Brown Algae
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
80
70
60
50
40
30
20
10


Total Phosphorus (uM)
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0


Dissolved Inorganic Nitrogen (uM)
Median 2001-02
25.4
25.3
25.2
25.1
25.0
24.9
-81.2
-81.1
-81.0
-80.9
-80.8
-80.7
-80.6
-80.5
-80.4
-80.3
-80.2
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

Principal component analysis (PCA) was used to extract composite variables (principal components) from data of the16 monthly surveys. This dataset included the following variables: bacterial numbers (BACT, ml^{-1}), bacterial production (BP, µg-C l^{-1} d^{-1}), PAM chlorophyll *a* (CHLA-PAM, µg l^{-1}), photosynthetic quantum yield (Φ_m), NO_3^-, NO_2^-, NH_4^+(µM), total organic nitrogen (TON, µM), total phosphorus (TP, µM), soluble reactive phosphorus (SRP, µM), alkaline phosphatase activity (APA, µM h^{-1}), extractive chlorophyll *a* (CHLA, µg l^{-1}), total organic carbon (TOC, µM), salinity (SAL), dissolved oxygen (DO, mg l^{-1}), and turbidity (TURB in NTU). Data were standardized (Z-scores) prior to analysis to reduce artifacts of magnitude. The PCA solution was rotated (VARIMAX) in order to facilitate the interpretation of the principal components and the factor scores saved for each data record.

PCA identified four composite variables (PC1, PC2, etc.) which passed the rule N for significance at $P<0.05$ (Table 1), indicating that there were four separate modes of variation in the data. PC1 had high factor loadings for bacterial cell counts, TOC, TON, and APA. PC2 was composed of bacterial cell counts, bacterial production, PAM-CHLA, phytoplankton quantum yield, TP, CHLA, and turbidity. SRP and salinity were inversely related in PC3. PC4 was composed of NO_2^-, NO_3^-, and NH_4^+. These four principal components accounted for 79.6 % of the total variance of the original variables.

Table 1.

	Principal Component			
Parameter	PC1	PC2	PC3	PC4
BACT	**0.481**	**0.426**	0.108	-0.053
TON	**0.837**	-0.030	0.144	-0.067
TOC	**0.897**	-0.179	0.132	0.065
APA	**0.867**	**-0.038**	0.046	-0.052
BP	-0.304	**0.586**	-0.128	-0.373
PAM-CHLA	0.313	**0.635**	-0.020	0.030
Φ_m	-0.234	**0.652**	0.209	-0.076
TP	0.126	**0.593**	-0.215	-0.039
CHLA	0.098	**0.769**	-0.070	0.030
TURB	0.001	**0.759**	0.162	0.032
SRP	0.186	-0.112	**0.883**	0.001
SAL	-0.006	-0.135	**-0.890**	-0.173
NO_3^-	-0.015	0.068	0.403	**0.786**
NO_2^-	-0.429	-0.008	-0.011	**0.765**
NH_4^+	0.260	-0.165	-0.147	**0.737**
%Variation Explained	39.4	20.0	11.2	9.0

The interpretation of this PCA is valuable in that it provides insight into the dynamic relationship between bacteria and phytoplankton. First, PC1 shows that bacterial numbers in the water column are partially related to distribution of DOM in the estuary and also correspond to the measured alkaline phosphatase exoenzymatic activities in these areas. This was expected from our previous work but had never been verified. However, the fact that no phytoplankton parameters were included with APA in PC1 was a surprise as we expected some sort of relationship to be evident. PC2 shows that both bacterial and phytoplankton biomass and activity are related to TP and turbidity. A similar relationship among CHLA, TP and TURB was previously observed in Florida Bay. This implies that, overall, both bacteria and phytoplankton are P-limited in the system and probably compete for this nutrient.

To address the second approach, we conducted quarterly sampling of ambient water from each of the three zones of Florida Bay and incubated samples under three treatments: light/dark incubation, DOM amendments, and inorganic nutrient amendments.

Anticipated Final Activities

During the final portion of the grant period (Apr. 15 – July 31), we will continue the monthly assays of phytoplankton primary production, bacterial production and bacterial enumeration at 28 sites in Florida Bay. We will also continue to test the hypothesis that both the addition of labile DOM (both P and N) and the enhanced alkaline phosphatase activity of cyanobacteria favor the initiation and dominance of cyanobacterial blooms in central Florida Bay. We will perform one more quarterly incubation experiment using ambient water from each of the three zones of Florida Bay under three treatments: light/dark incubation, DOM amendments, and inorganic nutrient amendments.

Applications

- Presentation by Boyer on Florida Bay phytoplankton production and nutrient mass balance modeling at 2001 Estuarine Research Federation Meeting.
- Presentation by Susan Dailey (postdoc on project) at 2001 Estuarine Research Federation Meeting.
- Presentation by Boyer on assessing bacterial community structure and bioavailability of DOM in Florida Bay at the Everglades LTER Annual All Scientists meeting.
- Presentation by Boyer on application of Phyto-PAM to assessing phytoplankton community structure and activity in Florida Bay at the Everglades LTER Annual All Scientists meeting.
- Presentation by Dailey at the Everglades LTER Annual All Scientists meeting.
- This project is directly integrated with our ongoing NSF Long Term Ecological Research program in the Florida Coastal Everglades (http://fcelter.fiu.edu/). Three of the Florida Bay sites in this proposal are part of the FCE-LTER and are complimenting our efforts therein by providing a bigger picture of microbial activity in Florida Bay.
- Data is being integrated into Southeast Environmental Research Center water quality database http://serc.fiu.edu/wqmnetwork to produce spatial maps of primary production, bacterial production, bacterial biomass, and DOM bioavailability much like those currently available on the website.