The increase in cyanobacterial blooms worldwide has generally been attributed to increased nutrient inputs. In the oligotrophic, P-limited Florida Bay, episodic cyanobacterial blooms have been dominated by members of the genus Synechococcus, often without any observed increase in nutrients. It is well known that cyanobacteria can access dissolved organic P (DOP) while eukaryotic phytoplankton cannot. DOP can be present in many forms: the most common are compounds based on phosphatase esters (P-O) such as DNA, RNA, and ADP. Anther is the phosphatase bond (P-O) as found in plant and animal membranes, as synthetic chelators, peroxide bleach stabilizers, and organophosphate pesticides such as glyphosate (Roundup™). Only bacteria can metabolize phosphonates however many synthetic forms are not readily bioavailable. Inositol-1-P is a special case of ester-P based on an carbon ring used by plants as primary P storage compound.

Our aim was to determine if the cyanobacterial community structure might be responding to differential P sources.

**Materials and Methods**

Bloom samples were collected from three sites located in the internal-central region of Florida Bay while a cyanobacterial bloom was occurring. The non-bloom samples were taken from three FCE-LTER sites in Florida Bay which are dominated by diatoms (TS/Ph-9 (FB9), TS/Ph-10 (FB21) and TS/Ph-11 (FB27)) (Fig. 1). Three one-liter samples from surface water were collected in each site. Once in the laboratory, three microcosms of 20 ml by each treatment were done (Fig. 2). Treatments were performed with two different concentrations (0.1 mM and 1 mM) of the following compounds: KH2PO4, KH2HPO4, Na2HPO4, urea, glycine, guanine, an organophosphonate-P source (2-aminoethylphosphonic acid, AEP) on ester-P (ADP), and inositol (phytate). A control with no nutrient addition was added. Samples were incubated in the light for 28 days.

Chi-4 concentrations were determined in each microcosm using PAM fluorescence. In these microcosms where a significant increase of Chi-4 was observed (p<0.05, one-way ANOVA), cyanobacteria counts and molecular analyses were performed. For both analyses, 20 milliliter of water were collected on a 0.22 µm pore size filter. Cyanobacteria counts were done by auto-fluorescence microscopy. For molecular analyses three samples from bloom areas as well as replicates for each treatment were pooled. DNA was extracted directly from bloom filter using the Mobio UltraSoil soil DNA extraction kit amplification of the cyanobacterial 16S rRNA gene was performed using specific primers (CYA106F and CYA781R). A clone library was generated from the bloom sample and three clones libraries were constructed from FB27 (control, 0.1 mM ADP and 1 mM AEP). For this purpose, PCR products were ligated into pGEM-T easy and transforming them into E. coli JM109 competent cells (Promega). A clone library was generated from the bloom sample and three clones libraries were constructed from FB27 (control, 0.1 mM ADP and 1 mM AEP). For this purpose, PCR products were ligated into pGEM-T easy and transforming them into E. coli JM109 competent cells (Promega) according to the manufacturer’s protocol (Fig. 2). The sequences were searched against the GenBank database by BLAST-N. Only sequences with a percentage of sequence identity higher than 90% were included in the phylogenetic analysis.

**Results**

1. Chi-4 concentrations were significantly higher in samples treated with ADP and AEP (Fig. 3). Also, an increase in Synechococcus-type cells number was observed in these treatments (Fig. 4).

2. Clone libraries showed an increase in number of DNA sequences related to Phormidium sp. and Limnothrix lineolaris in microcosms supplemented with ADP. While sequences related to Synechococcus sp. and Limnothrix neilei were increased in microcosms amended with ADP as well as in AEP-amended microcosms (Fig. 5).

3. Clones related to Synechococcus sp. made up virtually 100% of clone library in bloom sample (n=42) and 71% of AEP treatment (n=31) but only 15% and 16% of clones were Synechococcus sp. in the control (n=34) and ADP treatment (n=31), respectively (Fig 5).

4. Of the Synechococcus sp. recovered from bloom samples, 88% were Synechococcus sp. KORDI-63, 10% Synechococcus sp. WH8102, and 2% Synechococcus sp. KORDI-65. Only a 20% of the sequences from non-bloom sample were related to Synechococcus sp. KORDI-63. The AEP treatment showed a shift in cyanobacterial community structure towards 50% Synechococcus sp. KORDI-63, while ADP treatment changed to 80% Synechococcus sp. KORDI-63 and 20% Synechococcus sp. KORDI-65 (Fig 6 and table 1).

**Discussion**

These preliminary findings suggest that, under P-limitation, growth of cyanobacteria is favored by organophosphate additions, especially some Synechococcus strains which have also been observed as a dominant strain in cyanobacterial blooms occurred in Florida Bay. It is possible that natural or anthropogenic inputs of organophosphate compounds estuaries and marine waters may be the trigger which shifts phytoplankton community structure to that of cyanobacteria and promotes bloom formation.

**Acknowledgements**

We wish to thank Sandoor Stumpf, Jeff Absten and the Microbial Ecology field crew. This research was supported by the National Science Foundation through the FCE LTER program.