SFP2006: MEASURING AND MODELING NUTRIENT UPTAKE IN FLORIDA BAY

Revised Work Plan

APPROACH AND METHODOLOGY

General Description

This study will use a multi-faceted approach which combines synthesis with targeted field and laboratory studies. The dynamics of different sources of nutrients and their fate in the ecosystem will be explored through collaboration of modeling (Madden) with experimentation (Glibert, Heil and Seitzinger). The components of the research, each described in more detail below include: 1) field surveys of chemical and biological parameters and physiological experimentation; 3) targeted culture experiments; 4) application of these results to ecosystem models.

Field sites

Sampling efforts will focus on intensive process measurements during the wet (late summer/early fall) and dry (spring) seasons during each of 2 years. The research facilities at the Key Largo Ranger station or the Keys Lab will be used as the base for field sampling. The latter is preferred due to restrictions on the use of ^{33}P isotopes at other available laboratory facilities and we will move our operation to this facility when reconstruction from the devastation of Hurricane Wilma allows.

We have established a series of 6 sites within Florida Bay which will continue to be used for representative coverage of the different recognized regions within Florida Bay. Our protocol is to sample and conduct all experiments on water from one station per day during field efforts that generally run 10 days in duration. The sites give representation by region (eastern, central, western), and by transect (east-west and north-south). They include:

Eastern basin- north	Little Madeira
Eastern basin- middle	Duck Key
Central basin- north	Rankin Bight
Central basin-south	Barnes Key
Western basin	Rabbit Key
Gulf of Mexico end me	Sprigger Bank

Additional sites in the Everglades, Taylor Slough and Shark Slough, will also be sampled to provide characterization of source organic nutrient and for extracted DOM for use in the bioassay experiments.

As a baseline, a fixed suite of parameters will be characterized for each station during each sampling visit. These measurements are the same as we have made for the past several years at these same sites. Chemical measurements will include both dissolved and particulate forms of N,P, C and Si. N forms have been further subdivided into measurements of different organic fractions (urea, dissolved free amino acids, and bulk DON) and inorganic (NO_3^-, NO_2^- and NH_4^+) forms, as has phosphorus (TDP, DOP, PO_4^{3-}). Particulate and dissolved C and Si will also be measured, as will bacterial abundance, size-fractioned (total and <3 μm) chl *a*, and accessory pigments (Table 1). Biological measurements will include rates of N, and P uptake as well as productivity (Table 1).

Table 1. Samples that will be taken during the proposed research and methodology reference.

Parameter	Reference
Baseline Assessment Program	
Chemical Measurements	
NH_4, NO_3, NO_2, SiO_4, PO_4	Autoanalyzer
Total Dissolved P, Dissolved Organic P	Solorzano & Sharp 1980
Hydrolysable Organic P	Strickland & Solorzano 1966
Total Dissolved N, Dissolved Organic N	Bronk et al. 2000
Urea	Parsons et al. 1984
Dissolved Free Amino Acids	Cowie & Hedges 1992; Flynn 1998
Dissolved Organic Carbon	High Temperature Oxidation
Particulate Carbon, Nitrogen	Elemental Analyzer
Particulate Phosphorus	Solorzano & Sharp 1980
Biogenic Silica	Paasche 1980
DOM characterization	ESI-MS (Seitzinger et al. 2005)
Biological Measurements	
Chlorophyll *a* (Pelagic, size fractioned)	Holm-Hansen et al. 1965
Bacterial Abundance	Noble & Fuhrman 1998
Phytoplankton Community Composition	
Chemotaxonomic	HPLC & Chemtax Program
Microscopic	Gluteraldehyde, Microscopic observation
DON (urea, 2 amino acids) uptake	Uptake ofδ^{15}N and δ^{13}C labeled compounds
DIN (NH_4, NO_3, NO_2) uptake	Uptake of δ^{15}N labeled compounds
$H_2{}^{13}CO_3$ fixation	Mass spectrometry
$^{33}PO_4$ uptake	Ammerman 1993

Year 1

Field efforts

The first set of experiments will be in support of the modeling activities. These experiments are designed to provide the necessary kinetic constants of uptake for each nutrient form for the model. To date we have been measuring the uptake of different forms of N (as NO_3^-, NH_4^+, urea, and amino acids glycine and glutamic acid) at a range of concentrations (<0.5 to >20 μmol N L^{-1}) using ^{15}N stable isotope techniques. However, our existing data has raised some questions with respect to appropriately formulating the derived constants in the model. For example, our existing data does not show full saturation of NO_3^- uptake at certain times of year. We thus want to repeat these experiments at a broader concentration range to determine if the kinetic follow a saturating hyperbolic model, or a linear model (e.g. Lomas and Glibert 1999). Rate measurements will be initiated at the same time of day for each station. Samples will be dispensed into small volume polycarbonate containers and ^{15}N substrates will be added at a range of concentrations (n=10 concentration levels). Incubations will be carried out under simulated in situ conditions (dock incubators), for not longer than 1 hr, and then filtered. Samples will be analyzed by mass spectrometry (Glibert and Capone 1993). Kinetics of P uptake have not been previously measured along a sufficiently detailed concentration gradient in order to resolve the parameters of P uptake. Lack of access to a facility allowing radioisotope work for P limited our P uptake measurements to chemical disappearance. We will now be able to use ^{33}P at the Keys Lab, and thus a detailed gradient of P uptake rates along a concentration range can now be measured. We will conduct these studies during 2 field seasons (wet and dry) using methodology described by Ammerman (1993) with a concentration range encompassing the range previously observed at these stations. Activity of all samples will be determined immediately on site using a probale Beckman BeatScout scintillation counter. Silicic acid uptake has previously been measured at several concentrations during 4 seasons, providing sufficient data for initial modeling purposes, given that the focus of this effort is to resolve N and P, inorganic and organic uptake.

Year 1 also provides the opportunity to further characterize the DOM from the source waters, Taylor Slough and Shark Slough. In our past studies we have characterized this material through stable isotopic abundance analysis and through some chemical characterization (general molecular weight fractions, humic and fulvic content, etc). Here, we will use the opportunity for source water collection to fully characterize the chemical composition through ESI-MS analysis (see below). This will require concentration of roughly 200 l from each source to obtain DOM concentrates that are salt-free. Removal of salts has been a major stumbling block to many analytical procedures in the past. However, the majority of DOM can now be isolated using a combination of ultrafiltration and C_{18} solid phase extraction (SPE; Simjouw et al. 2004).

Culture Experiments

Using established cultures of the Florida Bay species, *Synechococcus elongatus* and *Cyclotella* spp., the kinetics of uptake of N and P will be measured and compared to the field data. These species are of particular interest because they represent two potentially extreme responses of previously problematic bloom-forming genera in Florida Bay: that of the common bloom-forming cyanobacterium and that of a common diatom. Cultures will be grown in batch culture, under N-limiting conditions and immediately upon N depletion aliquots of the cultures

will be removed for experimentation. Replicate cultures will be grown with NO_3^- and with urea as the growth N substrate. For N uptake rates, multiple aliquots will be dispensed into polycarbonate tubes to which ^{15}N –labeled isotopes will be added in a gradient of concentrations. Experiments will be repeated with a range of inorganic and organic N substrates. For P uptake rates, cultures will be grown to P-limitation, aliquots will be removed and enriched with the radioisotope ^{33}P across a concentration range. These experiments will provide uptake constants for these species which can then be compared to those derived from the field during the periods in which phytoplankton were dominated by one major phytoplankton group or another.

Analytical methods: ESI-mass spectrometry

Compound-level analysis of DOM will use a quadrupole mass spectrometer (MS) with an atmospheric pressure electrospray ionization system (ESI-MS [Agilent 1100]; Seitzinger et al. 2003 and 2005) to chemically characterize DOM. Samples (20 µl) are injected into the ESI-MS by an autosampler after salt removal. The mobile phase is 50 : 50 v : v methanol : water (0.05% formic acid in E-pure water [pH 3.5]) with a flow rate of 0.22 ml min^{-1}. The drying gas is N_2 (3508C, 10 L min21, 1.76 kilograms per centimeter). The capillary voltage is 3 kV; the fragmentor voltage is 40 V. Samples will be analyzed in both ESI-positive and ESI-negative ion modes. For each sample, replicate injections (6) will be used to establish a solid statistical basis for interpreting the m/z (full-scan mass spectra m/z 50-3000) and ion abundance of each m/z. Therefore, we can follow the change in ion abundance of each mass species (m/z) and determine if there has been a statistically significant increase or decrease in concentration. While this analysis will not tell the exact concentration of each mass species (because of differences in ionization efficiencies among compounds), it can be used to determine whether the concentration of any particular compound is increasing or decreasing (as in Fig. 5C-E above). Mixed standards (known concentrations of authentic standards) will be used to spike samples to determine if there are any changes in ionization efficiencies between runs or due to matrix effects. Our established procedures result in low procedural blanks, and maintain sample integrity during storage. A number of caveats are applied to the interpretation of the ESI spectra for mixtures of unknown compounds as reviewed by Kujawinski (2002a,b) and summarized by Seitzinger et al. (2005). For example, our ESI-MS has unit mass resolution, and therefore, more than one compound may be included in any particular peak. Although the delineation of the complete elemental formula for the semi-labile DOM pool is beyond the scope of this project, future ultra high resolution ESI-(FTICR-MS) studies could be conducted on specific m/z's targeted from the results of this study (e.g. Kim et al. 2003). We have developed Microsoft Excel and Access macros to efficiently handle the large data stream. This greatly facilitates statistical analysis of replicate injections, comparisons among samples of m/z occurrence, and determination of statistically significance differences in ion abundance (concentration) for the same m/z among samples (e.g., over time in bioavailability experiments; Seitzinger et al. 2005). This includes tracking each m/z and its ion abundance over time in a bioavailability experiment as well as comparing the occurrence and ion abundance of each m/z among experiments.

Modeling Approaches

An existing ecological model will be used as the platform for developing the phytoplankton module. The existing model is a mechanistic unit model of seagrass community dynamics, calibrated for representative areas Florida Bay. The model has a dt=3 h and provides much of the basic shell within which the phytoplankton kernal will be developed, including sediment geochemistry, environmental forcings and seagrass biology. Under this proposed project expansion, during Year 1 the model code for phytoplankton will be developed in collaboration with the experimental components of this project. Conceptual model components will be developed in STELLA, an object-oriented programming language suitable for proof-of-concept heuristics, scaling, and hypothesis testing. Model experiments will be performed to simulate the experimental results of nutrient kinetics and nutrient and carbon mass-balance measured in the experimental work. The formal model development, based on these initial model experiments, will be done in MATLAB so as to be compatible with, and to facilitate incorporation into, the existing Florida Bay Seagrass Model. Translation between the two platforms is facile. Nutrient kinetic relationships will be derived from data previously generated by Glibert under previous SFP efforts, as well as new experiments to be conducted herein. Model algorithms will use single-stage or two-stage Michaelis-Menten functions as well as existing light and photosynthesis-irradiance (P-I) relationships developed for Florida Bay (Carlson 2003; Madden 2001) to calculate phytoplankton growth for several species in the model.

Work Plan- Year 2

Field Experiments

The second set of experiments, which will constitute Year 2 efforts, will be to fully characterize both the responses of the Florida Bay microbiota to DOM extracted from the various sources and the changes in specific compounds of the DOM itself. To do so, we will conduct bioassay experiments using DOM from different sites. Using the highly characterized DOM (extracted as described above), a suite of bioassay experiments will be conducted. These bioassays will be similar to those we have previously conducted, but we will include treatments with the natural DOM concentrates, and through ESI-MS we will **follow the utilization of these compounds, not just the change in biota**. Bioassays will be conducted in duplicate with water from 5 stations (Sprigger Bank, Little Madeira, Rabbit Key, Rankin Bight and Duck Key) which will be prescreened through 154 μm screening to eliminate large grazers). The treatments are listed in Table 2:

Table 2. Proposed bioassay treatments

1. control- no addition
2. 10.0 μM NH_4^+
3. 10.0 μM NO_3^-
4. 2.0 μM PO4
5. 2.0 μM DOP (as glycerophosphate)
6. DOM extracted from Taylor Slough at known concentration
7. DOM extracted from Shark Slough at known concentration

Treatments 1-5 are comparable to those previously conducted, while treatments 6-7 will allow characterization of the bioavailability of the natural source DOM. Incubations will be conducted in "cubitainers" at ambient field temperature and light intensities. Although we have used a 48 hr time period for our previous studies, we will extend the duration of these incubations to 96 hrs to be sure we have tracked a significant change in DOM compounds. The amount of DOM added will be the same for all flasks (based on N content). We will determine which DOM compounds in the complex mixture are being utilized by the plankton community using ESI-MS. Response of the phytoplankton communities to the DOM additions will be quantified based on changes in phytoplankton biomass and composition using concurrent pigment analyses (VanHeukelem et al. 1994, 2001), size- fractioned chl *a* analysis, microscopic analysis (identification and cell counts; Hasle 1978), and bacterial enumerations (flow cytometry; Table 1). The general phytoplankton community responses will guide us in selecting up to 3 sites for which a complete suite of analyses by ESI-MS will be conducted. **These data will thus allow us to determine the extent to which different components of the biota responds to DOM, which compounds they are using, and whether all biotic components are responding to the same or different DOM compounds.**

Sample, analytical and data analysis will continue though Year 2.

Model Approaches

Year 2 modeling activities will be an extension of those begun in Year 1, refining and updating model structure and model parameters as information becomes available from the experimental components of the study. There will be a certain amount of reverse information flow as well, as model experiments may suggest experimental approaches or treatment levels. As kinetic data is developed experimentally in Year 1, refinements to Michealis Menten parameters will be undertaken in Year 2. Furthermore, in this year, the phytoplankton module will be fully inserted into the ecological model. Nutrient mass balance and phytoplankton competition with seagrasses for nutrients will be enabled. Algorithms for water column light attenuation based on phytoplankton species and concentration will be established. Model code will be fully developed in MATLAB and finally ported to FORTRAN for compatibility with the emerging 3-D hydrodynamic water quality model at a landscape scale to be developed for Florida Bay (Hamrick and Moustafa 2003). During Year 2, linkage of the phytoplankton-seagrass model will be made to the EFDC hydrodynamic transport framework, and a 3-D landscape-level water quality model.

Manuscript preparation will also be a high priority for Year 2.