BIOLOGICAL AVAILABILITY OF ORGANIC NITROGEN IN FLORIDA BAY

Final Report to the South Florida Water Management District
(Contract No. C-C20306A)

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Final Report for SFWMD Agreement C-C20306A

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Executive Summary

A better understanding of the biogeochemical cycling of nutrients entering Florida Bay is a key issue regarding the restoration of the Everglades. In addition to precipitation, the other major source of freshwater to Florida Bay is from Taylor Slough and the C-111 Basin in the northeast section of the Bay. While it is known that these areas deliver significant amounts of N to the Bay, a significant portion of this is in the form of dissolved organic N (DON). The sources, environmental fate and bioavailability to microorganisms of this DON are however, not known. Should this DON be readily available, any increased load as a function of restoration changes might have an impact on internal phytoplankton bloom dynamics. This set of experiments was designed to address the issue of chemical characterization of DON entering the bay and its bioavailability.

Experiment 1. Overall, the first experiment performed showed that no significant flocculation or precipitation of DOM occurred with increase in salinity. This means that the DOM does not partition into the sediments but stays in the water column where it subjected to photolysis and advective transport. In other words, DON from the Taylor Slough or C-111 panhandle does not precipitate out at the entrance into the bay, but stays in the more mobile, soluble phase.

This experiment also showed that physical changes in the DOM occurred along a salinity gradient. The decrease in average molecular weight and increase in polydispersity may be explained by two different models: the disaggregation/dissociation of HMW complexes into many LMW components or the physical contraction of DOM structure. The exact nature of this physical process is not well understood at this time, but may affect photoreactivity and bioavailability of the DOM. Other researchers have noted the increased degradation of terrestrial DOM at elevated salinities. This effect was attributed to three possibilities: 1) the enhanced ability of marine bacteria to degrade macromolecules, 2) increased activity of exoenymes with ionic strength, and 3) flocculation and/or contraction of DOM with increased ionic strength. We now add another factor to this list: disaggregation of macromolecular structure of DOM.

Experiment 2. Sunlight has a significant effect on the chemical characterization of DOM. While the DOC levels did not change significantly during photo-exposure, the optical characteristics of the DOM were modified. The environmental implications of this are conflicting: photo-induced polymerization may stabilize the DOM by reducing its bioavailability while photolysis may make the DOM more labile.

Experiment 3. In general, DON bioavailability was relatively low in this region. Bioavailable DON (BDON) in the freshwater wetlands (9.2% over 8 days) was higher than the

mangrove (4.8%) and estuary sites (3.5%). The bacterial contribution to the DON pool was low but significant and was accounted for in the assays. Although bacterial abundances at TS/PH 6 and 9 were similar, the biovolume was much greater at TS/PH 9. This suggests that there is a potential for bioavailable DON from the marsh and mangrove to be transported to Florida Bay. However, we also showed that the composition of the DOM at each site is more distinct than we originally thought.

Elevated NH_4^+ levels observed were due to a sequential processing of DON by estuarine bacteria. First the labile DON fraction was converted to biomass and the excess released as NH_4^+. After the BDON was exhausted, the bacteria assimilated the previously released NH_4^+.

Experiment 4. A great amount of variability was introduced into the DON analysis because we did not filter samples from the bottles prior to measurement. We do not recommend performing this type of assay in the future. Overall, the trends in nutrient/biological variables were similar to the water column, but of greater magnitude.

Implications for the Nutrient Budget. These experiments show that, although the amount of DON loaded to the bay may be large, the fraction of DON available for microbial cycling is much smaller. However, since the median DIN concentration for TS/Ph 9 was 4.6 μM while BDON was 1.6 μM, the amount of recycled DON may indeed be a significant portion of the total DIN pool. We should add that more slowly decomposed DON that is not readily detected, and that might decompose over time periods exceeding that of the experiment, could also be a component of the BDON. Thus the 1.6 μM value for BDON would be a minimum estimate.

All this must be considered in context with the proposed CERP modifications to flows. As of the latest Initial CERP Update, the flows to Taylor Slough and C-111/Panhandle Basis are not predicted to change very much from base conditions. Therefore we do not expect any great increases in TN loading in this region. In contrast, the proposed flow increases to the Shark River Slough are large and may have significant effects on transport of DOM to the Southwest Florida Shelf. We believe that future efforts in DON characterization and bioavailability should be concentrated in this area.

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INTRODUCTION

Background

Florida Bay is a wedge-shaped estuary separated from the Straits of Florida by the Florida Keys, which form a nearly continuous barrier (Fig. 1). Freshwater inputs to Florida Bay are dominated by direct precipitation (~98 cm yr^{-1}) with smaller inputs (~9 cm yr^{-1}) from flow through the Taylor Slough/C111 Canal basin (Nuttle et al. 2000). Freshwater flows through the Taylor Slough and C-111 canal are controlled by local water management activities and can directly influence salinities in the eastern portion of the Bay (Boyer and Jones 1999). Groundwater is not considered to be a significant freshwater source but there is continuing debate as to its true contribution (Corbett et al. 1999; Price and Swart 2001; Top and Brand 2001).

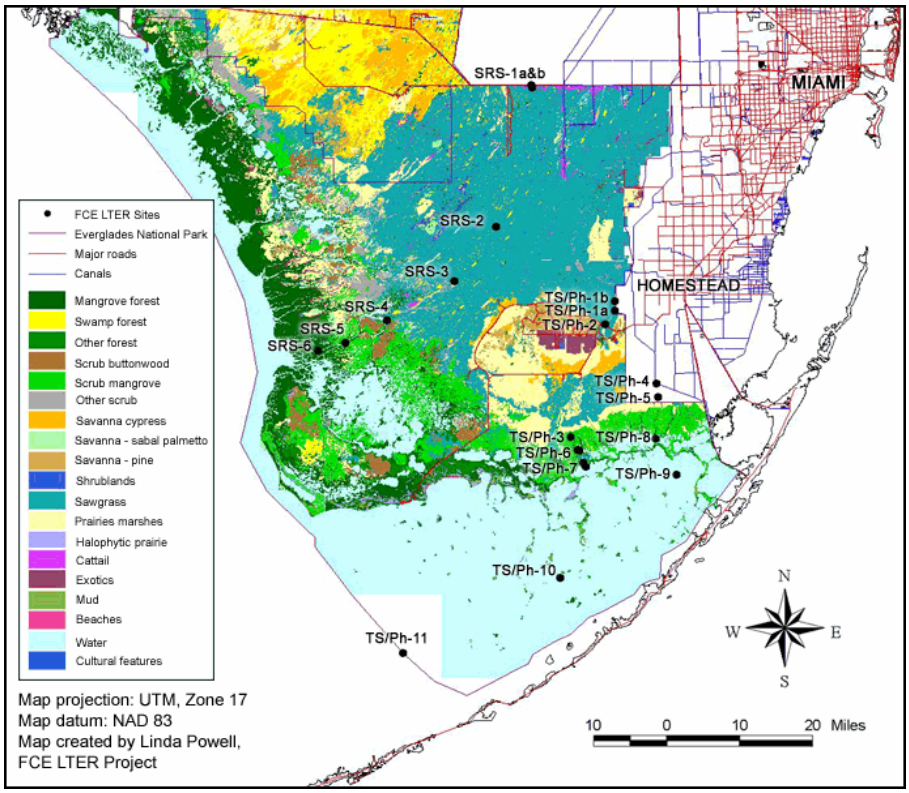


Figure 1. *Map of Florida Coastal Everglades including sampling sites in Taylor Slough, C-111 canal basin, and Florida Bay.*

The first steps in the hydrologic restoration of South Florida have already begun (http://www.evergladesplan.org). The redistribution of water within the terrestrial ecosystem will necessarily affect the water entering the marine ecosystem. It is expected that sheet flow to Florida Bay will increase, in fact it already has with the removal of the southern berm on the C-111 canal in 1997 (Fig. 2, Parker 2000). Along with greater flow there is some concern that increased nutrient loading might occur (Brand 2001).

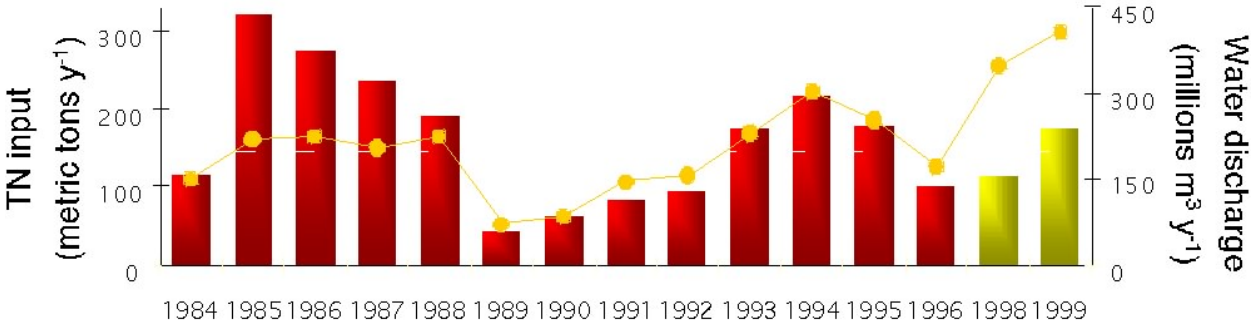


Figure 2. *Water discharge (points) and total nitrogen mass load (bars) from the C-111 canal before (red) and after (yellow) the berm had been removed (Rudnick et al. 1999, Parker 2000).*

Phytoplankton biomass in eastern Florida Bay is strongly P-limited (Fourqurean et al., 1993; Phlips and Badylak, 1996; Boyer et al. 1997; Lavrentyev et al., 1998), however other resources (e.g. light, N, Si) have been shown to partially control productivity in the central and western Bay (Lavrentyev et al. 1998; Brand 1999). In addition to dissolved inorganic nutrients (DIN and DIP) there is a significant loading of DON from the eastern marshes (Walker 1998, Boyer and Jones 1999, Rudnick et al. 1999). DON concentrations in eastern Florida Bay are typically 5-10 times the DIN levels (Fig. 3) and therefore represent a possible N source to phytoplankton and bacteria (Carlsson and Graneli 1993). The question that we propose to answer is: what are the sources of this material, what is its molecular composition and how much of the DON pool is available to the biota for growth and development?

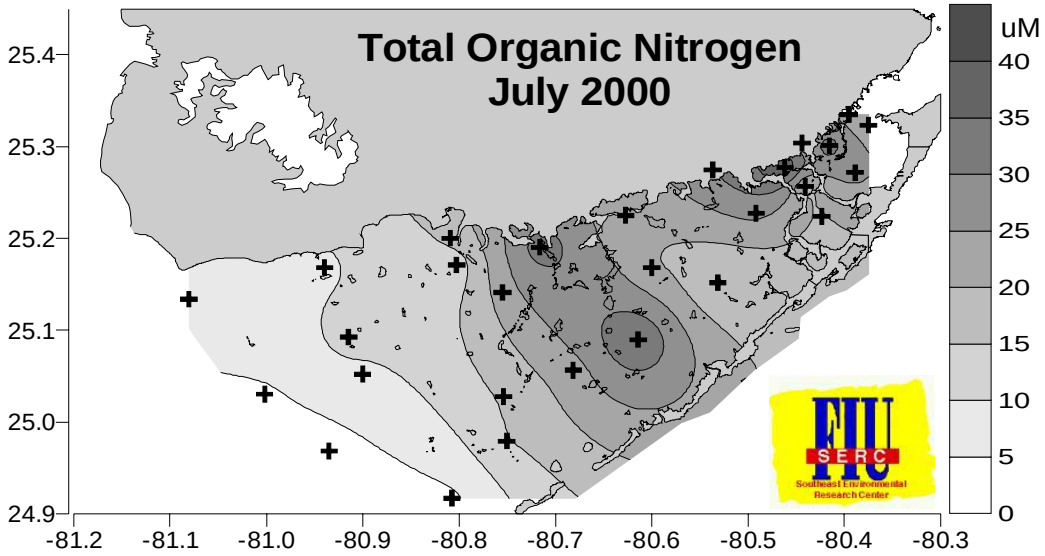


Figure 3. *Concentrations of DON in surface waters of Florida Bay during July 2000. Note the higher levels in the northeast boundary.*

Most nutrient and phytoplankton studies to date have been primarily concerned with the amounts of DIN and DIP, as these fractions are directly available for phytoplankton uptake (Tomas 1998, Brand 1999). However, a significant portion of the DOM may be remineralized by microbes. Bacterial utilization of DOM, and subsequent grazing on these bacteria by protists and microzooplankton (the microbial loop), is an important alternative pathway in many aquatic food webs (Azam et al. 1983, Stepanauskas et al. 1999). Little is known about the microbial loop in Florida Bay as few measurements of bacterial productivity have ever been published. Lavrentyev et al. (1988) concluded that nutrient resource gradients were the predominant factor determining planktonic community structure. Cotner et al. (2000) reported bacterial production estimates of 8-31 μg-C l^{-1} d^{-1}. Previous results (Boyer unpublished) are summarized as follow:

1. Synchronous fluorescence measurements of DOM in incubations show that labile DOM increases with time in the dark but declines rapidly in the light.
2. Bacterial production is enhanced by addition of DOM to a greater degree than by the addition of nutrients.
3. CHLA increased with nutrient addition relative to DOM addition.
4. The change in DOC concentration over time is greatest in the DOM amended treatments; addition of N & P had a smaller effect.

These results imply that the microbial loop may be uncoupled to phytoplankton production in Florida Bay as a result of DOM input from other sources.

Bioavailability of DON

Assessments of bioavailable DON (BDON) have been hampered by methodological constraints. There are basically two different approaches to determine DON bioavailability: 1) chemical proxies (Benner 2002) and 2) bacterial re-growth assays (Transvik 1988).

Chemical proxy methods attempt to characterize the "usability" of DON by relating its degradation to some telltale chemical characteristic (see review Benner 2002). The simplest proxy uses stoichiometry (C:N:P ratio) of the source material to predict its bioavailability (Tezuka, 1990). If N is limiting, then addition of low C:N material will promote remineralization of N. The microbial loop may also play a significant role in nutrient remineralization when C:N and C:P ratios are low. Conversely, when nutrient concentrations are limiting, bacterioplankton compete with phytoplankton for inorganic nutrients (Caron, 1994). Therefore, the bioavailability and C:N:P ratio of the DOM can determine whether the microbial loop is a source or sink for dissolved nutrients.

Another approach includes size distribution analysis (Benner at al. 1992, Guo et al. 1995). Size fractions have been operationally defined by the development of tangential flow ultrafiltration. It is generally accepted that the HMW fraction is more bioavailable than LWM (Amon and Benner 1996, Boyer and Groffman 1996). Enzymatic and photochemical degradation generally leads to decreases in size and molecular weight of DOM, and therefore, reactivity.

A third method is to use molecular characterization to predict the chemical bioavailability. These include components such as neutral sugars (Amon et al. 2001), amino acids (Hubberton et al. 1995), and amino sugars (Kaiser and Benner 2000). The higher the ratio of these biochemicals relative to bulk C and N, the more "fresh" and/or bioreactive organic matter.

Finally, as described below, we are working on developing optical/fluorescent methodologies to assess DOM bioavailability.

Bacterial re-growth assays, or batch incubations, are routinely used to assess bioavailability of DOM in environmental samples (Servais et al 1989, Transvik 1998). The usual protocol is to

filter the water first to remove bactivores, add an inoculum, then incubate in the dark for two weeks or more. An important prerequisite, often overlooked, is the need to ensure N limitation of the incubation bottles. Bacteria will use the most cost-effective N source available, inorganic N, rather than express exoenzymes necessary to break down DON.

For BDON assays, activity is usually measured using three general approaches.

1) O_2 consumption and relate decline to DON mineralization (Moran et al. 1999). The main problem with this approach is that oxygen consumption is not specific for DON conversion: nitrification (conversion of NH_4 to NO_3) also consumes O_2.
2) Decrease in DON pool over time (Wickner et al. 1999). This method is hampered by low sensitivity of the DON analysis (generally 2-3 µM MDL), however, newer analytical methodology (HTC w/ chemiluminescence detection) has improved precision. The largest problem is the contribution of bacterial biomass to the operationally-defined DON pool. The amount of DON converted to bacterial biomass is masked by the inclusion of bacteria in the DON pool. Filtration of samples (0.2µm) before and after incubation may get around this problem but also requires PN analysis of the filter. Flocculation/precipitation may also interfere with interpretation.
3) Bacterial biomass conversion (Stepanauskas et al. 1999.). This approach measures bacterial numbers over time and relates carrying capacity to amount of DON converted. This requires knowledge of biovolumes, assumes constant N:biomass (no change in community structure), assumes constant growth efficiency, and is affected by flocculation effects. Stepanaukas et al. (1999) used a spike of DIN to calibrate the N:cell which provides a measure of improvement with concomitant increase in complexity of the assay.

We chose to perform a modification of methods 2 & 3 wherein the DON pool was measured as well as bacterial numbers. We assessed change in bacterial size over the incubation and adjusted N content accordingly.

Chemical Characterization of DON

The quality/lability of DOM is a function of its chemical characteristics, molecular weight, elemental ratio (C:N:P:other), its age and/or degree of diagenetic transformation. Both DOC and DON concentrations are being determined across the southern Everglades landscape as part of the Florida Coastal Everglades LTER program (FCE, http://fcelter.fiu.edu) in an attempt to better understand key biogeochemical cycles. However, detailed information on the molecular characterization of the DOM in this system is still lacking. While bulk DOM measurements are without a doubt a key to the understanding of nutrient dynamics in wetland, estuarine and coastal systems, they do not provide critical information as to the source, chemical composition, potential bioavailability and diagenetic transformations of these materials. During the past two years our research group has generated some preliminary, but rather useful molecular information on DOM in several environments of the FCE. This information was based on a series of specific chemical analysis methods, which are described in more detail in the experimental section of this proposal. The power of these techniques in the assessment of DOM sources, and particularly in the study of DON is described below.

Spectroscopic techniques such as fluorescence have been widely used in the characterization and assessment of DOM sources in aquatic environments (Coble, 1996; Battin, 1998; McKnight et al., 2001; de Sousa Sierra et al., 1997; Lu and Jaffe, 2001). We have studied the optical properties of DOM in water samples from the estuarine and coastal areas of the western Everglades (Ten Thousand Islands and Whitewater Bay) as well as from Florida Bay. These

studies were focused on applying spectroscopic techniques such as UV-visible and fluorescence spectroscopy to the assessment of terrestrially vs. marine derived DOM in the region. While absolute DOM determinations seem to indicate a conservative mixing of fresh and marine waters with a dilution of the terrestrially-derived DOM, our fluorescence studies have shown that while this is true, there is also a very clear signal of marine derived DOM that is being mixed with a ever lower terrestrial signal in the estuaries of the western Everglades. While this marine-derived DOM is less abundant in the near-shore zone, it is composed of primarily non-humic material, most likely of carbohydrates and proteins, and may therefore be more bioavailable than the presumably more degraded terrestrially derived DOM being transported from the Everglades freshwater marshes and fringe mangrove forests. This marine derived DOM becomes dominant at salinities above 30 (Fig. 4). Surface water samples from Florida Bay show an elevated amount of this non-humic, protein-like material compared to the freshwater sites and most of the Ten Thousand Island and Whitewater Bay sites. However, no correlation with salinity was observed for Florida Bay waters. While this research in presently on-going, these preliminary results seem to suggest that a significant amount of the proteins in Florida Bay may be derived from autochthonous sources (e.g. seagrass beds and marine plankton) and are not derived from the freshwater Everglades and mangrove forests in Taylor Slough.

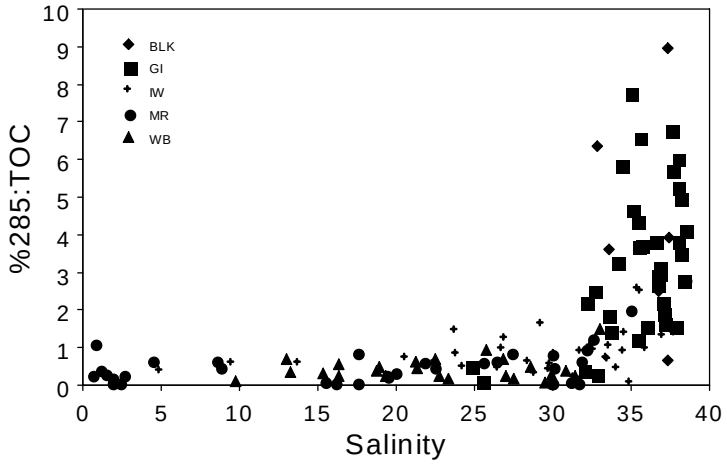


Figure 4: *Relationship between surface water salinity and relative abundance (%285) of protein-like material normalized to TOC concentration for sites from the Florida coastal Everglades (Ten Thousand Islands and Whitewater Bay; Lu et al., 2001).*

Water quality data from the FCE LTER Program show that the freshwater wetlands of the Southern Everglades are a consistent source of total N, (unpubl.LTER data) while the mangrove wetlands between these marshes and Florida Bay take up TN much of the year (Davis et al., 2000a,b). Fluorescence studies of this TN, which is mostly DON, suggest that the relative concentration of the synchronous fluorescence based protein like materials (relative abundance of the peak at 285 nm) are much lower that humic materials such as fulvic and humic acids (peaks at higher wavelengths; see Fig. 5). While this is an expected result for wetland waters, we have found evidence, based on fluorescence (Fig. 5), FTIR, ^{13}C-NMR and pyrolysis-Gas Chromatography/Mass Spectrometry (py-GC/MS; Fig. 6) results, that the amount of proteins and carbohydrates in surface waters increases with distance from the canal, suggesting generation of biogenically derived DON as the water flows from the canal through the freshwater marsh (C-111 Basin example). This information suggests that a significant fraction of the DON in this

system is not derived from allochthonous and possibly anthropogenic sources (i.e. water input from the canals) but is of an autochthonous source. Furthermore, this DON probably makes up most of the TN being supplied by the freshwater marshes. As to possible sources for this DON, both DOM leached from mangroves, sawgrass and periphyton showed a significant amount of protein-like material (based on synchronous fluorescence measurements; Lu and Jaffe, 2001). This freshly leached DOM is quite labile, as it is sensitive to biodegradation and to a lower degree, photochemical degradation (Fig. 7). Its presence in surface water samples therefore, seems indicative of relatively recently produced DOM, and points at the Everglades freshwater marshes and mangrove forests as a potential source of DON to Florida Bay.

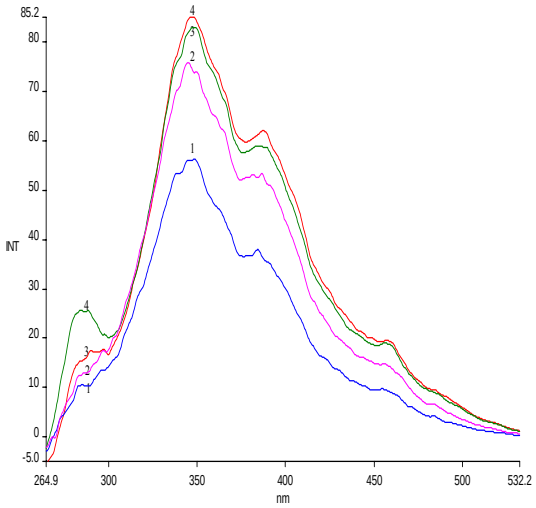


Figure 5: *DOM synchronous fluorescence spectra of surface water samples from a transect in the C-111 Canal basin depicting a transect from the canal (1) through the adjacent freshwater marsh (2 to 4) with increasing distance from the canal. Relative abundance of protein-like materials are provided by peak at 285 nm. Bands at higher wavelengths are due to humic substances.*

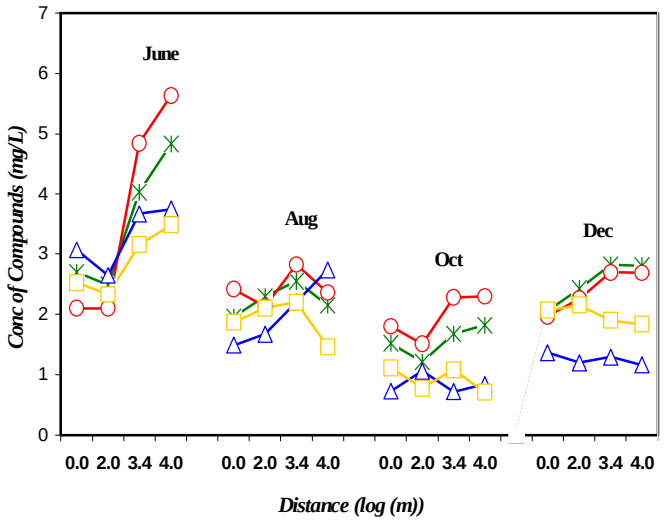


Figure 6: *Pyrolysis-GC/MS results from transect of C-111 basin indicating distance from canal at different times: ✳) proteins; ○) carbohydrates; Δ) lignins: and □) poly-aromatics (seasonal data for year 1999).*

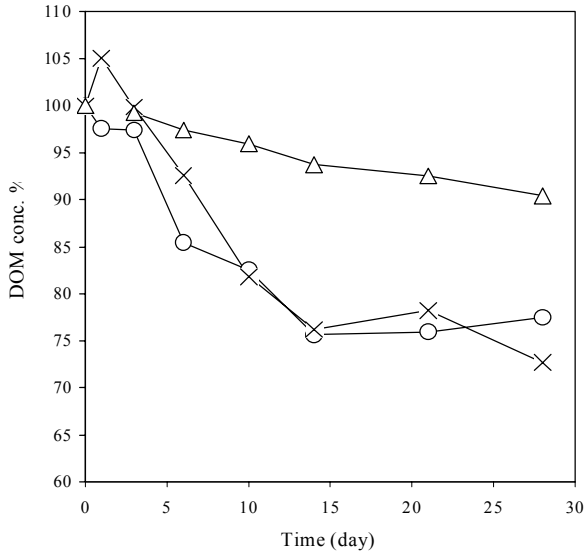


Figure 7: *Relative concentrations (%) of the DOM samples (fresh leachate from Rhizophora mangle) incubated: x) under natural sunlight conditions; o) in the dark; and Δ) in the dark with NaN_3 added as a poisoned control.*

In order to further establish a link between the DON and the presence of proteins in the DOM pool of the Florida coastal Everglades, we have analyzed proteinaceous materials in several surface water samples of this system. This is a difficult task, since analytical methods for the analysis of dissolved proteins in natural water samples have not been previously developed. However, a combination of ultra-filtration with electrophoresis has allowed us to gain some insight into the protein levels and molecular distributions in the Everglades ecosystem. Water samples from C-111 canal, Taylor Slough, Florida Bay, Coot Bay, Blackwater River and the Shark River as well as leaf extracts from mangroves and sawgrass have been analyzed. Total protein concentrations were quite variable in these samples and showed highest concentrations in the Taylor Slough and one of the Florida Bay samples, while they were lowest in Coot Bay and the Blackwater River. The C-111 sample showed intermediate concentrations representing about 40% of the Taylor Slough sample. These preliminary results suggest that different regions within the Everglades system have different protein concentration levels as well as compositions. Different samples show some similarities and some variations in the protein bands that were detected in the gels. While three bands (74, 69 and 53 KDa) were present in all samples including the vegetation samples, the presence of lower molecular weight bands was more variable.

The general presence of the three bands indicated above may suggest the presence of recalcitrant proteins. It could however also suggest the presence of freshly leached proteins considering that they were also present in the leaf extracts. Similarly, the low levels of the lower molecular weight proteins and the larger qualitative variability between sites may be suggestive of either higher rates of biodegradation of these proteins resulting in low concentrations, or on

the contrary, they are recalcitrant remains of more highly degraded DOM. While the former explanation is more reasonable, more data is needed to better understand the biogeochemistry of protein materials and DON in general in this system.

In summary, our preliminary data suggest that different environments within the FCE will contribute DON to Florida Bay. While relative amount of protein-like materials in the freshwater marshes is lower than that observed for Florida Bay, the absolute concentrations of the DOM shows the opposite behavior. Therefore, both qualitative and quantitative aspects of this question need to be addressed, and the bioavailability of this material needs to be evaluated. This research project will focus on (1) a detailed molecular characterization and (2) an assessment of the bioavailability of the DON in Taylor Slough and Florida Bay (see Fig. 1).

METHODS

As mentioned above, the experimental approach of this project will be based on DON chemical characterizations and DON bioavailability assays (BDON).

Sampling Sites and Collection Protocol

Three sampling sites were distributed along a transect extending from the freshwater marsh of Taylor Slough through the mangrove fringe and into Florida Bay (see Fig. 1). The freshwater marsh site (TS/PH 2) and mangrove site (TS/PH 6) are sampled semi-continuously for TN and TP as part of the FCE LTER Program (see http://fcelter.fiu.edu). The Florida Bay site (TS/PH 9) is sampled monthly as part of the SERC Water Quality Monitoring Network (see http://serc.fiu.edu/wqmnetwork/). Water quality parameters such as total nitrogen (TN), dissolved organic nitrogen (DON), dissolved organic carbon (DOC) and other basic water quality parameters were determined in monthly grab samples taken from all sites. Four experiments were initiated to examine the physical, chemical, biological, and combined physical/biological effects on DON from the Everglades.

We collected water August 4, 2003 from 3 sites in the Everglades hydroscape. Our freshwater sampling point was located at 25.403 Lat, -80.607 Long and is the Florida Coastal Everglades Long Term Ecological Research (FCE LTER) site TS/PH 2. The water column was clear and depth was 0.75 m. The saltwater sampling sites were FCE LTER sites TS/PH 6 (Lat.25.214; Long –80.649) and 9 (Lat. 25.179; Long. –80.490). At TS/PH 6, the water column depth was 1.05 meters with a surface temperature of 29.9 ºC and DO 3.76 mg l^{-1}, pH of 7.82, and surface salinity 0.54 (practical salinity scale). The most marine sampling station, FCE LTER TS/PH 9 had a water column depth of 1.54 m, 30.78 ºC, DO 4.98, pH 8.33 and salinity 34.47.

Water samples were collected at 10 cm depth using acid washed, distilled water rinsed, and autoclaved distilled deionized water rinsed 8 L brown Nalgene bottles. Sample bottles were rinsed three times with sample water prior to collection. From the TS/PH 9 site we collected a 1 L brown Nalgene bottle for a bacteria inoculum.

Sediment samples were collected using clear acrylic coring tubes and sealed with butyl rubber stoppers. Sediment cores were brought to the surface and boat where we decanted the overlying water column with Tygon tubing. Sediments were sieved through a 500um sieve, while rinsing with ambient surface water and stored in sterile sampling bags in a dark cooler on ice.

Upon return to the laboratory, water samples were immediately passed through a Whatman GF/F filter, then through a sterile Gelman 0.2 µm cartridge filter, and stored in brown Nalgene

sample bottles. The 1 L water sample collected from TS/PH 9 was filtered through a sterile 0.8 μm filter and stored at 4 ºC to use as inoculum. Sediments were stored at 4 ºC in the dark.

Experimental Design

Experiment 1: Effect of increase in the salinity to the solubility of DOM. The first experiment was designed to test the effect of salinity on the flocculation of DOM derived from the Everglades. The idea was to quantify the amount of DOM from the Everglades that flocculates and/or precipitates out of solution upon transport to the higher salinity waters of Florida Bay. Low salinity water samples (200 ml) from TS/PH 2 and TS/PH 6 were placed in individual 250 ml Nalgene brown bottles to which varying amounts of artificial sea salt crystals (Instant Ocean) were added to effect treatment levels of 0, 5, 15, 25, and 35‰. The bottles were placed on a shaker table at 200 rpm for 1 h and then stood for 24h at room temperature. Dissolved organic carbon (DOC) concentration was measured after filtration through 0.2.μm membranes to estimate flocculation. Filtered samples were also analyzed for fluorescence properties, and molecular weight distribution using size exclusion chromatography (SEC). The experiment was conducted in duplicate.

Experiment 2: Photochemical decomposition of DOM. A 150 ml subsample of filtered (<0.2 μm) samples (TS/PH 2, 6, and 9) was irradiated using a solar simulator at 765 W m^2 (Suntest XLS+ solar simulator set; ATLAS Material Testing Technology LLC). After irradiation of designed period (0, 0.5, 1, 2, 4 and 7 days of continuous irradiation; 24 hour day periods of simulated sunlight), samples were analyzed for DOC concentration, fluorescence properties, and molecular size distribution analysis. The experiment was conducted in duplicate.

Experiment 3: DON Bioavailability (BDON) in Water Column. Water collected from TSPH 2, 6, and 9 was filtered through an 8 μm prefilter followed by a 0.2 μm cartridge prior to use. Salinity was adjusted to that of TS/PH 9 using synthetic seawater. DON bioavailability was quantified using 16 day incubations (Servais et al. 1989; Boyer and Groffman 1996) during which we measured loss of the DON fraction and any change in nutrients and bacterial N. Bottles were subsampled at 0, 2, 4, 8, and 16 days. On the first day of the experiment we poured a homogenized volume of 2 L of the 0.2 μm filtered water into each of twelve dark, sterile 2.5 L polycarbonate bottles that had been acid washed and DI rinsed. The salinity of each of the bottles was adjusted to that of TS/PH 9 (34.5) with NaCl with mixing on a stirring plate. We used NaCl instead of Instant Ocean to adjust salinity so as not to change DIN concentration of the sample.

We prepared duplicate bottles for both water column and sediment slurry incubations. The addition of 5 μM P as H_3PO_4 and 100 μM C as glucose assured that N limiting conditions were maintained in the bottles (Stephanauskas et al. 1999). A 10 ml bacterial inoculum of GF/F filtered water from TS/PH 9 was added at the initiation of the experiment. Duplicate bottles were incubated at 30 ºC, in the dark, in 2 L polycarbonate bottles covered with aluminum tape.

Bottles were sampled at 0, 2, 4, 8, and 16 days by collecting a 250 ml volume of water from each of the BDON assay bottles. Unfiltered samples were analyzed for TN, TP, alkaline phosphatase activity (APA, μM hr^{-1}), leucine aminopepdidase (LAP, μM hr^{-1}), bacterial numbers, and bacterial production. Subsamples were filtered through Whatman GF/F filters prior to analysis for NO_3^-, NO_2^-, NH_4^+, SRP, F_{max}, λ_{max}, FI, and Peak I.

Experiment 4: DON Bioavailability (BDON) with Sediment Addition. Sediments were collected at same sites as above using plexiglass cores, stoppered, and returned to lab in dark. Three cores per site were collected. In the lab, the 0.5-1.0 cm horizon of the replicate cores from each site were homogenized by stirring before being dispensed to duplicate bottles at 1 g wet weight l^{-1}. Ambient water and bacterial inoculum from each site were added as above. Incubations were same as above with the addition of gentle mixing of bottles on a daily basis. Sampling proceeded as described above, making sure to allow sediment to settle prior to filtration.

Analytical Methods

Fluorescence Analyses. Spectroscopic parameters such as total fluorescence intensity (F_{max}), maximum fluorescence emission wavelength (λ_{max}), synchronous fluorescence (Peak1), and fluorescence index (FI, f_{450}/f_{500}) were determined to assess seasonal variability in DOM characteristics. Fluorescence emission spectra were recorded at wavelength ranging from 300 to 700 nm, at an excitation wavelength of 370 nm in a 1 cm quartz fluorescence cells at room temperature (20^oC), using a Perkin Elmer LS50B spectrofluorometer equipped with a 150-W Xenon arc lamp as the light source. Synchronous excitation-emission fluorescence spectra of the water samples were obtained at constant offset value between excitation and emission wavelengths ($\delta\lambda=\lambda_{em}-\lambda_{ex}$). All spectra were recorded at an offset value of 30 nm with slit width of 10 nm. (see also Lu and Jaffe, 2001).

Chemical Analyses. Total neutral sugar content was analyzed colorimetrically using 2,4,6-ttripyridyl-s-triazine (TPTZ) Method (Myklestad et al. 1997). The principal of the analysis is as follows: hydrolyzed sugars were subjected to an oxidation reaction at alkaline pH, during which Fe_3^+ was reduced to Fe_2^+. The Fe_2^+ is then determined colorimetrically after complexation with the chromogen TPTZ to give violet color of the $Fe(TPTZ)_2^{+2}$.

One ml of hydrolysate of water samples was mixed in a test tube with 1ml of reagent A and kept for 10 min in boiling water. Then 1 ml of reagent B and 2 ml of reagent C were added immediately and mixed well on a Vortex-mixer. After 30 min the absorbance was read at 595 nm.

Reagent A: 0.7 mM Potassium ferricyanide in a mixed solution of 10 mM NaOH + 0.19 M Na_2CO_3

Reagent B: 2 mM Ferric chloride in a mixed solution of 2 M sodium acetate + 0.22 M citric acid + 5 M acetic acid

Reagent C: 2.5 mM of TPTZ in 3 M acetic acid

The molecular size distribution was determined by size exclusion chromatography (SEC) using 0.05 M Tris-buffer adjusted to pH at 7.0 with phosphoric acid as eluent. A 100 µl portion of water sample was injected into a YMC-Pack Diol-120G column (pore size 12 nm, I.D. 8.0 mm, length 500 mm; YMC Inc., Kyoto, Japan) and eluted at a flow rate of 0.7 mL min^{-1} at 23˚C. The elution curve was recorded by measuring the absorbance of the eluent at 254 nm. The column was calibrated with dextran of different molecular weights (Sigma-Aldrich, St. Louis, MO, USA). Weight average molecular weight (M_w) was calculated by: $M_w = \Sigma (A_i M_i) / \Sigma A_i$, where A_i and M_i are the absorbance in arbitrary units and the molecular weight estimated from

the calibration curve, respectively, at elution volume *i*. The total area of the chromatogram (ΣA_i) for all the samples was normalized. Polydispersity (d) was calculated as follows: $d = M_w / M_n$, where M_n (number average molecular weight) was calculated using the following equation: $M_n = \Sigma A_i / \Sigma (A_i / M_i)$. Because appropriate standard materials are not available, it is difficult to determine the precise molecular weight distribution of the FA fraction by SEC. Thus, the M_w values presented here do not necessarily equal the absolute Mw, and should instead be regarded as an index to compare the average size of the FA fractions measured under same conditions.

Water Quality/Nutrient Analyses. Water quality parameters were determined using standard techniques employed by the SERC Water Quality Monitoring Network (Boyer et al. 1997). Bulk samples were analyzed for total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC). TN was measured using an ANTEK 7000N Nitrogen Analyzer using O_2 as carrier gas (Frankovich and Jones, 1998). TP was determined using a dry ashing, acid hydrolysis technique (Solorzano and Sharp, 1980). TOC was measured by direct injection onto hot platinum catalyst in a Shimadzu TOC-5000 after first acidifying to pH<2 and purging with CO_2-free air.

Water samples were filtered through a sample rinsed 25mm Whatman GF/F into acetone-washed and sample rinsed 60ml high density polyethylene bottles for analysis of SRP, NO_x^-, NO_2^-, NH_4^+, and $Si(OH)_4$. These parameters were obtained by flow injection analysis (Alpkem model RFA 300). NO_3^- was calculated as $NO_x^- - NO_2^-$; DIN was calculated as $NO_x^- + NH_4^+$; and TON was defined as TN - DIN.

Enzyme Analyses. We used an alkaline phosphatase activity assay (APA) to determine the production of extracellular enzymes to mineralize phosphate from organic material (Hashimoto et al., 1985). Duplicate unfiltered 3 ml samples were incubated for 2 hours at 20º C after adding o-methylfluorescein phosphate. Initial and 2 hour readings were measured using a Gilford Fluoro IV Spectrophotometer (excitation = 430 nm, emission = 507 nm). The increase in fluorescence over the incubation period determined APA ($\mu M\ h^{-1}$).

We used a similar method to determine the leucine aminopeptidase activity (LAP) to estimate production of extracelluar enzymes which mineralize nitrogen from organic material (Hoppe, 1983). Duplicate unfiltered 3 ml samples were incubated for 2 hours after adding L-leucine-7-amido-4-methylcourmarine. Initial and 2 hour readings were measured using a Gilford Fluoro IV Spectrophotometer (excitation = 380 nm, emission = 440 nm) and used the change over the incubation period to determine LAP activity ($\mu M\ h^{-1}$).

Bacteriological Analyses. Bacterial counts were determined using epifluorescence microscopy with DAPI staining technique (Coleman, 1980, Porter and Feig, 1980). Samples were collected and preserved with Formalin buffered with phosphate solution to a final concentration of 2%. Aliquots were incubated at a final concentration of 25 $\mu g\ ml^{-1}$ DAPI in a filtration tower for 20 minutes prior to filtration onto a 0.2 µm black polycarbonate filter. The filter was then mounted onto a slide with low fluorescence immersion oil and examined under a 100W Hg bulb. Bacterial enumeration was performed by counting 10 sampling fields of a known size per slide, with a minimum of 300 cells per slide counted. The final value of cells ml^{-1} was obtained with a formula using the sample volume counted and the percentage of effective filter area counted.

Bacteria production was determined using ^{3}H-thymidine incorporation incubations (Bell, 1993) using triplicates of each sample and a 4% final concentration formalin blank for each. With each ^{3}H-thymidine incubation experiment we ran a blank sample for specific activity of the ^{3}H-thymidine. Filters were placed in scintillation vials to which 10 ml of Ultima Gold fluor was

added and counted in a Beckman liquid scintillation counter using external channels ratio correction. We converted activity to C using the following equation:

$$\mu g\ C\ l^{-1}\ h^{-1} = (moles\ l^{-1}\ h^{-1}) * (cells\ mole^{-1}) * (C\ cell^{-1})$$

where pico moles of thymidine incorporated were calculated through using the actual activity of the ^{3}H thymidine (dpm) versus the bacteria activity from live- killed (dpm). Cells $mole^{-1}$ was determined multiplying the thymidine conversion factor of 2×10^{18} cells $mole^{-1}$ by the moles of thymidine $l^{-1}\ h^{-1}$. We used a conversion factor of 20 fg C $cell^{-1}$, a value typically used in oceanic waters. Our observations of water from these Florida Bay sites over the past two years have shown that bacteria numbers are consistently lower and their size much smaller than those found in estuaries or other coastal systems. Thus we used the lowest conversion factor to multiply the number of cells by to estimate the change in C content.

The total dissolved nitrogen pool (TDN) in a sample is made up of dissolved inorganic N (DIN) and DON. The measurement of DON is confounded by the inclusion of bacteria in the DON pool. Loss of DON may be attributed to two processes: the degradation of DON to DIN and the conversion of DON to bacterial biomass. Since the bacterial biomass does not remain constant over the incubation period, both bacterial number and biovolume were also assessed. This allowed us to partition DON into living (DON_{Bact}) vs non-living (DON_{NL}) components.

At 0, 4, and 16 days, bacteria were classified as either spheres or rods and their diameters and lengths measured using a Whippled grid with smaller division of 2 nm. We assumed that half the bacteria were spheres, the other half rods. Cell volume (BV, μm^3) was calculated using standard equations. C content of the bacterial morphotypes (BC, fg C $cell^{-1}$) was calculated using a C:V ratio of 83 fg C μm^3, according to Vrede et al. (2002). The C:V used was for bacteria grown under N limitation and should therefore be applicable to this experiment. Bacterial N content (BN) was calculated using a C:N ratio of 7.5, also derived from N-limited growth (Vrede et al. 2002).

RESULTS

Experiment 1: Effect of increase in the salinity to the solubility of DOM

This experiment was conducted with the objective to assess the effects that salinity increments may have on the physical characteristics of DOM from two freshwater sites, namely TS/PH 2 and TS/PH 6. DOC concentration decreased slightly (0.4-1.3 mgC l^{-1} or 8-11% of original DOC) with increasing salinities up to of 35 (Fig. 8). However, since the decrease was very small, flocculation of DOM caused by the increase of salinity is considered to be a minor process in this environment.

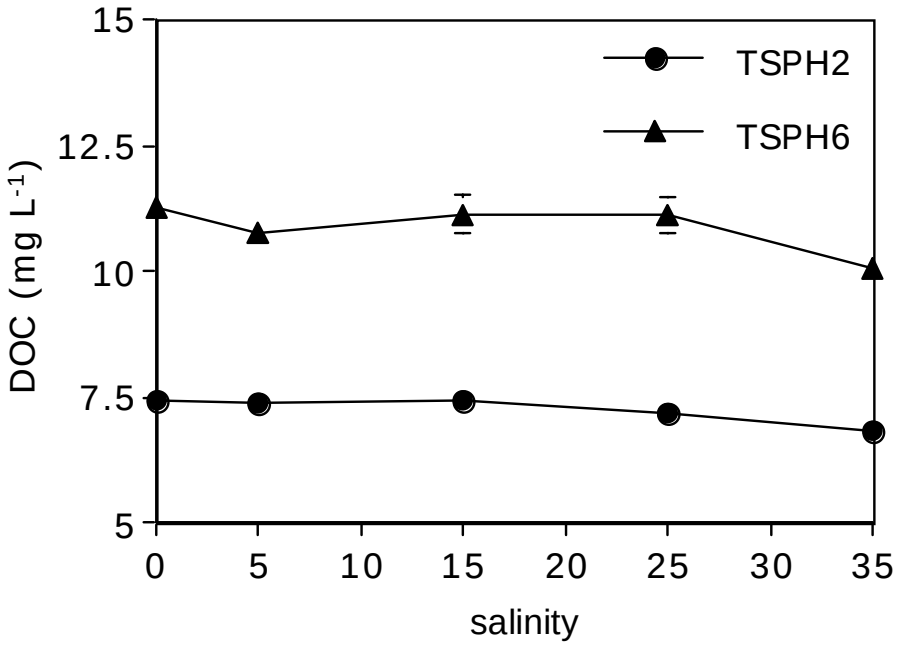


Fig. 8. *Plot of salinity vs. DOC concentration*

To investigate the compositional change of DOM, fluorescence properties were determined. These consisted of maximum fluorescence intensity (F_{max}), wavelength of maximum fluorescence intensity (λ_{max}), fluorescence index (FI), and the intensity of protein-like peak measured by synchronous fluorescence (Peak I). F_{max} was higher at TS/PH 6 than TS/PH 2 as a result of the higher DOC concentration in the mangrove site (Fig. 9). F_{max} did not change appreciably (less than 3% of the initial) with increments in the salinity, which suggested no appreciable removal of fluorophores caused by DOM flocculation processes. Maximum wavelength (λ_{max}) is often used as an index to estimate the source of DOM. For example, plant-derived DOM has a λ_{max} value around 420 nm, while microbial-derived DOM has a λ_{max} around 380 nm (De Souza Sierra et al. 1994). The longer λ_{max} for TS/PH 6 than TS/PH 2 indicated that plant (mangrove)-derived DOM contributed more to TS/PH 6, while TS/PH 2 was influenced by more microbial (periphyton)-derived DOM (Fig. 10). The λ_{max} did not change appreciably with changing salinity for either sample. However, it is important to understand, that because the emission spectrum (excitation at 313nm) shows a very broad hump, it is difficult to determine an accurate λ_{max} value.

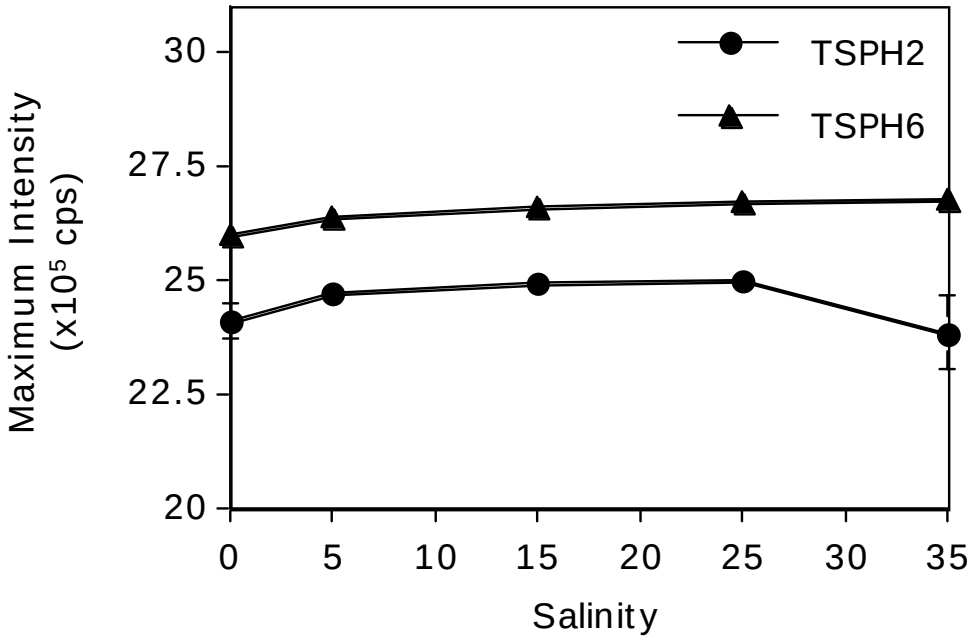


Fig. 9*. Plot of salinity vs. Maximum fluorescence intensity.*

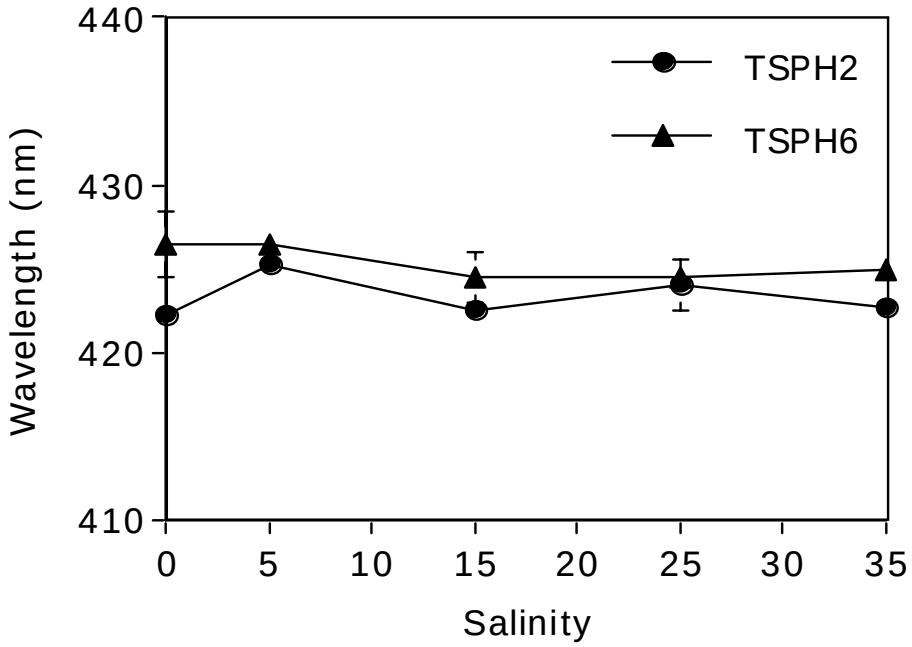


Fig. 10. *Plot of salinity vs. maximum wavelength*

The fluorescence index (FI) is more sensitive parameter of measurement as it is a ratio of 450:500 nm. FI was higher at TS/PH 2 than TS/PH 6, which is consistent with the larger influence of microbial-derived DOM at TS/PH 2 (Fig. 11). A slight increase in FI was observed for TS/PH 2 as salinity increased, probably as a result of change in ionic strength of samples, which may change fluorescence quenching processes (Lu and Jaffé, 2001). Peak I intensity decreased gradually after addition of artificial sea salt (Fig. 12). The salting out of proteinaceous

materials could be a plausible reason of the decrease, however, the decrease was only 7-9% of original peak intensity. This confirmed previous results that flocculation/precipitation of DOM caused by the increase of salinity is minor process in these regions of Florida Bay.

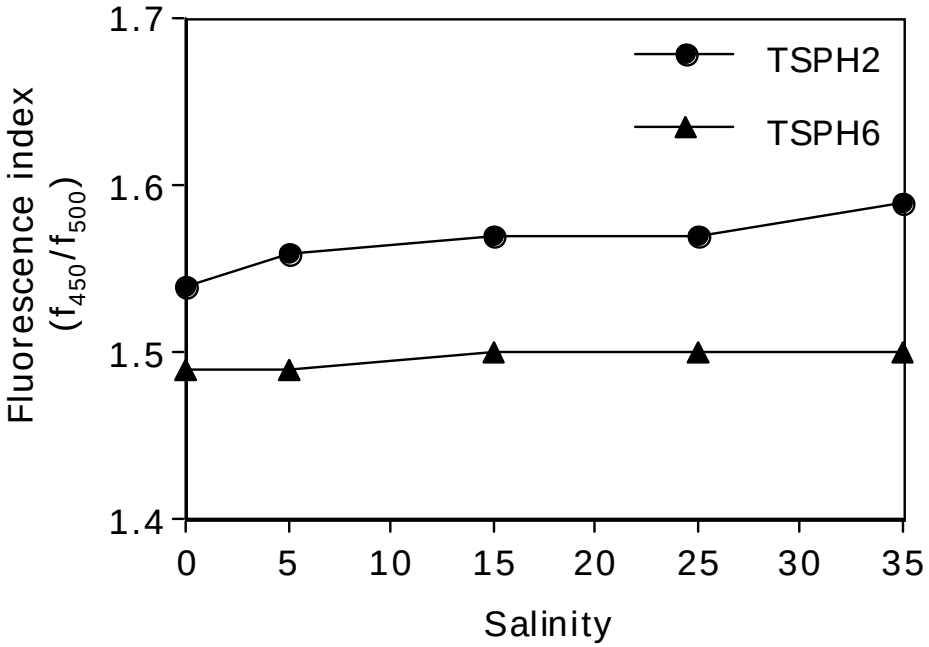


Fig. 11. *Plot of salinity vs. Fluorescence Index*

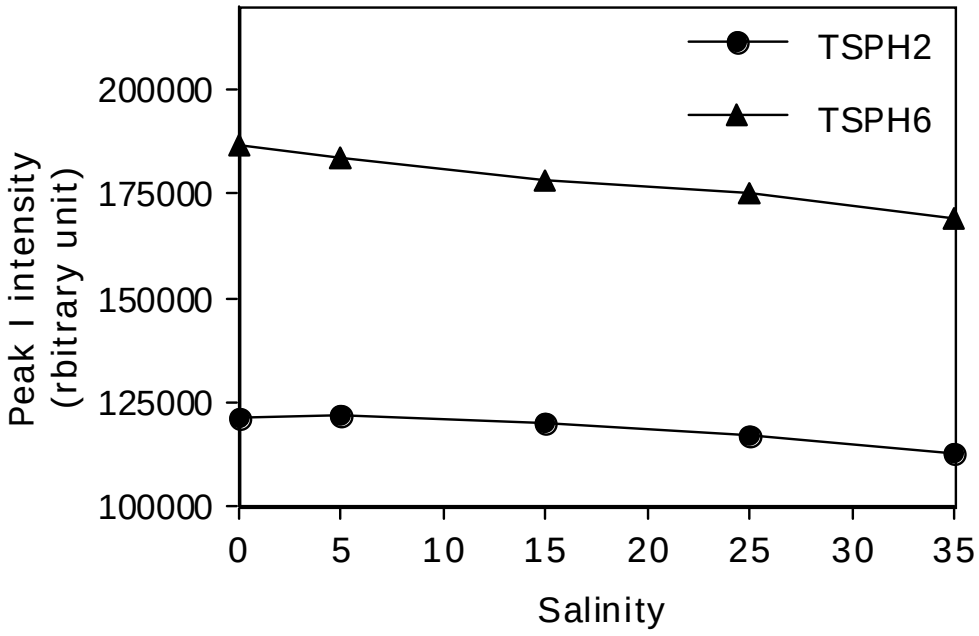


Fig. 12. *Plot of salinity vs. Peak I intensity*

In addition to optical properties, molecular weight based parameters were determined. The weight average molecular weight of DOM decreased significantly with salinity (Fig. 13a).

Polydispersity (an index of heterogeneity of molecular weight distribution) also increased as salinity increased (Fig. 13b). We interpret these results as follows: DOM in these areas is made up of loosely bound components held together by hydrogen and/or hydrophobic bonding. Increased salinity (and therefore ionic strength) brought about the breakdown of micelles into smaller components as well as physical contraction of DOM molecules. This interpretation seems contradictory to the traditional knowledge that suggests that molecular weight increases prior to flocculation/precipitation processes. However, these were not observed to any significant degree in this experiment. Further experiments are needed to examine the validity of these results.

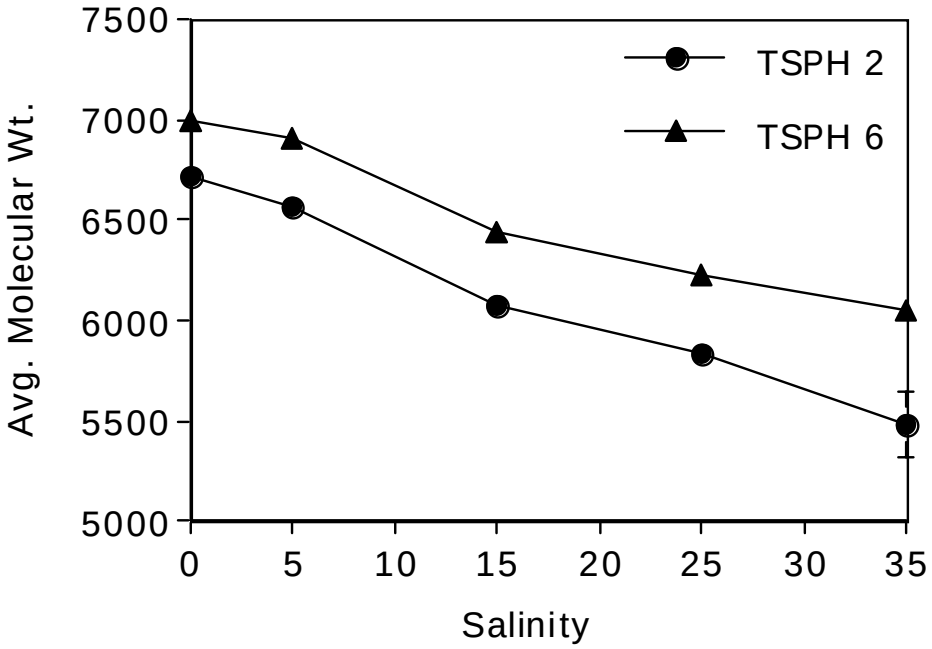


Fig. 13a. *Plot of salinity vs. weight average molecular weight*

Other researchers have noted the increased degradation of terrestrial DOM at elevated salinities (Stepanauskas et al. 1999; Wickner et al. 1999, Langenheder 2003). This effect was attributed to three possibilities: 1) the enhanced ability of marine bacteria to degrade macromolecules (González and Moran 1997; Langenheder et al. 2003), 2) increased ionic effect on exoenzyme activity (Chróst 1991, Wetzel 1992), and 3) flocculation and increased coiling of DOM with increased ionic strength (de Haan st al. 1987; Transvik and Sieburth 1989). We now add another factor to the list: disaggregation of macromolecular structure of DOM.

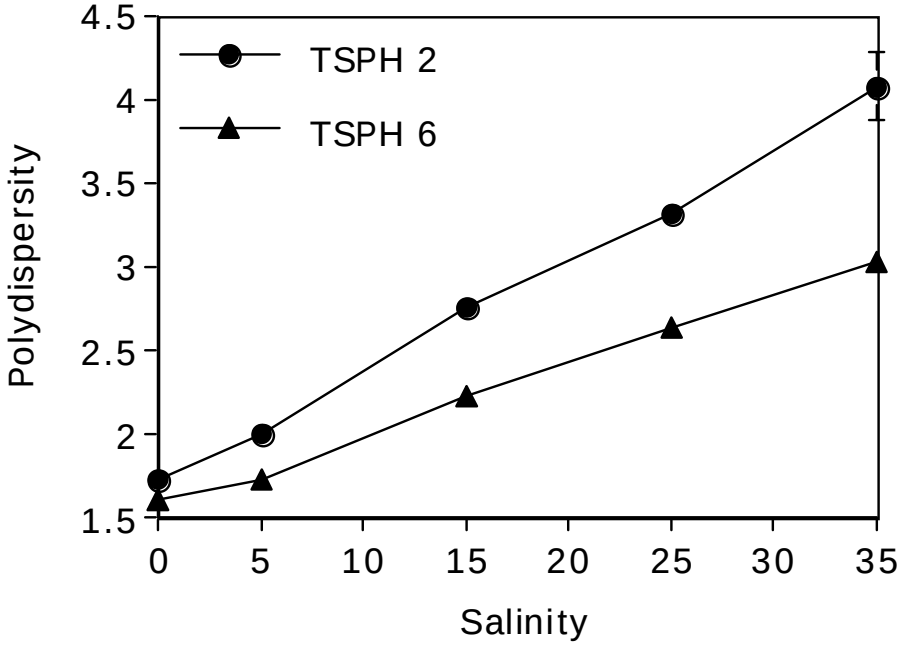


Fig. 13b. *Plot of salinity vs. polydispersity*

Experiment 2: Photochemical decomposition of DOM

In this experiment, the three surface water samples were exposed to solar radiation using a solar simulator system. DOC concentration did not change appreciably after exposure to simulated solar light (SSL) during a period of up to 7 days (Fig. 14). Thus complete degradation of DOM to CO and CO_2 was not occurring during this experiment. However, the UV absorption at 254nm decreased by 21%, 16%, and 5% for TSPH 2, 6, and 9, respectively after exposure to SSL for 1 day (Fig. 15). After a 7 day exposure, the absorption decreased by 46%, 43%, and 17% of the initial values. Thus, DOM at these sites, especially at TS/PH 2 and TS/PH 6, contained very photo-sensitive organic compounds. DOM from TS/PH 9 was less photosensitive, possibly because photosensitive compounds at this site had been already degraded under exposure to sun light in Florida Bay. UV absorption at 254nm normalized to C concentration (Specific UV absorption; SUVA; Fig. 16) is reported to have a positive correlation with aromatic C concentration (Chin et al., 1998). Thus humic substances that contain the most significant portion of aromatic C in DOM are likely to have been affected most severely by photochemical processes.

Another optical parameter, the S-value, $\ln(a_{254}/a_{436})/182$, is an index of the degree of development of electron transfer systems in DOM (in other words, they represent a proxy for the degree of poly-condensation of DOM). We observed that the S-value increased significantly after exposure to the SSL (Fig. 17). Since this value decreases as electron transfer system of chromophore develops, our result suggested that blue shift of absorption occurred by exposure to SSL.

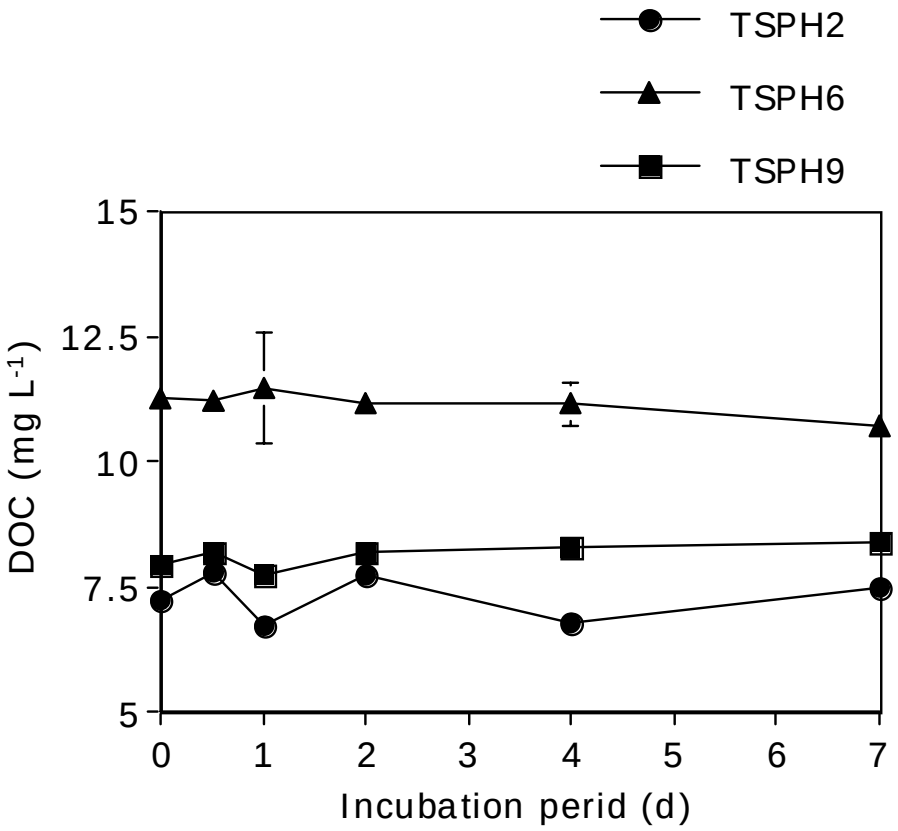


Fig. 14. *DOC concentration of water samples after exposing to solar simulated light.*

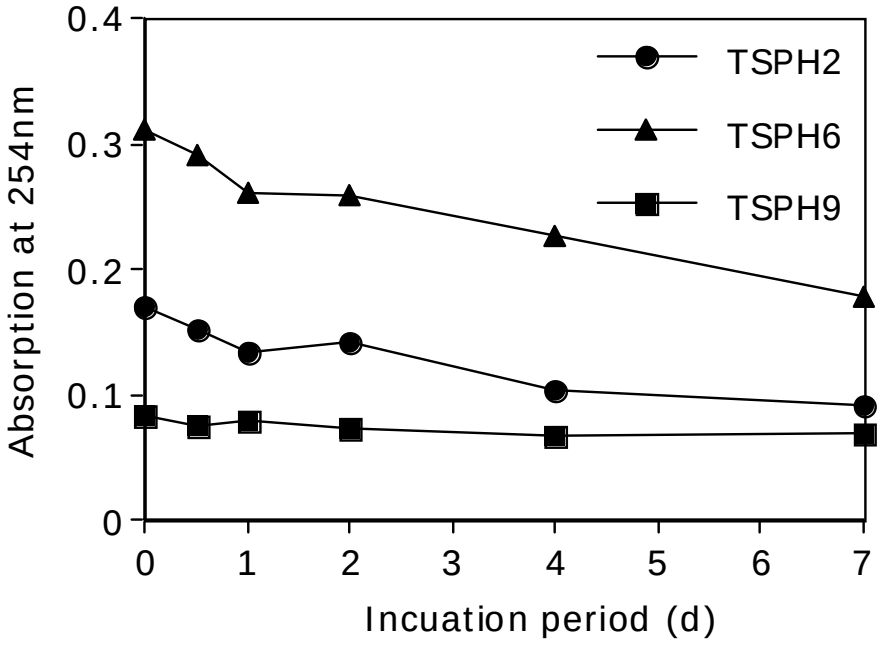


Fig. 15. *Change in the UV absorption (254nm) of water sample after exposing to solar simulated light*

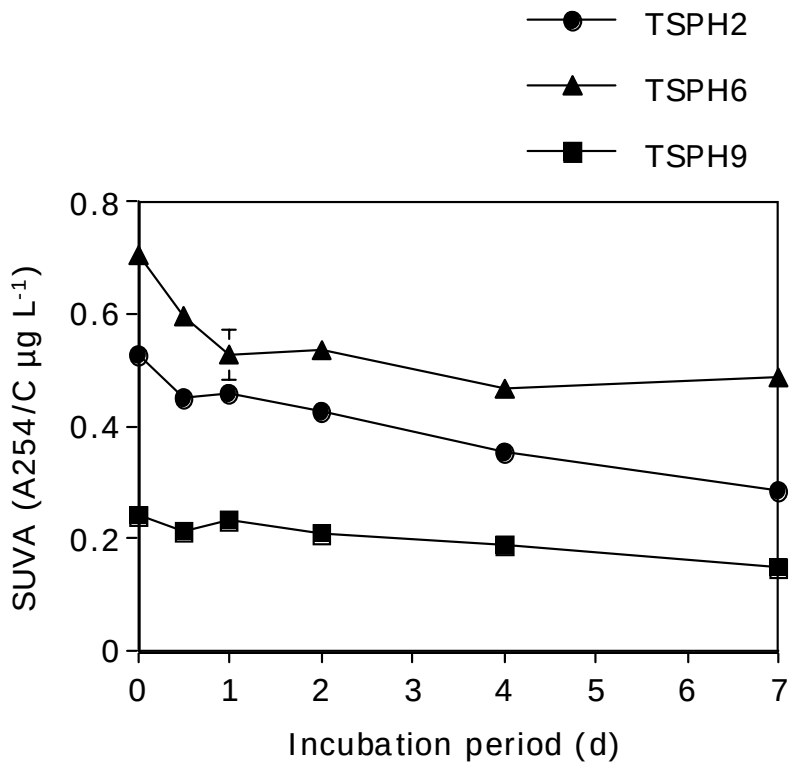


Fig. 16. *Change of specific UV absorption (SUVA) of water samples after exposing to solar simulated light*

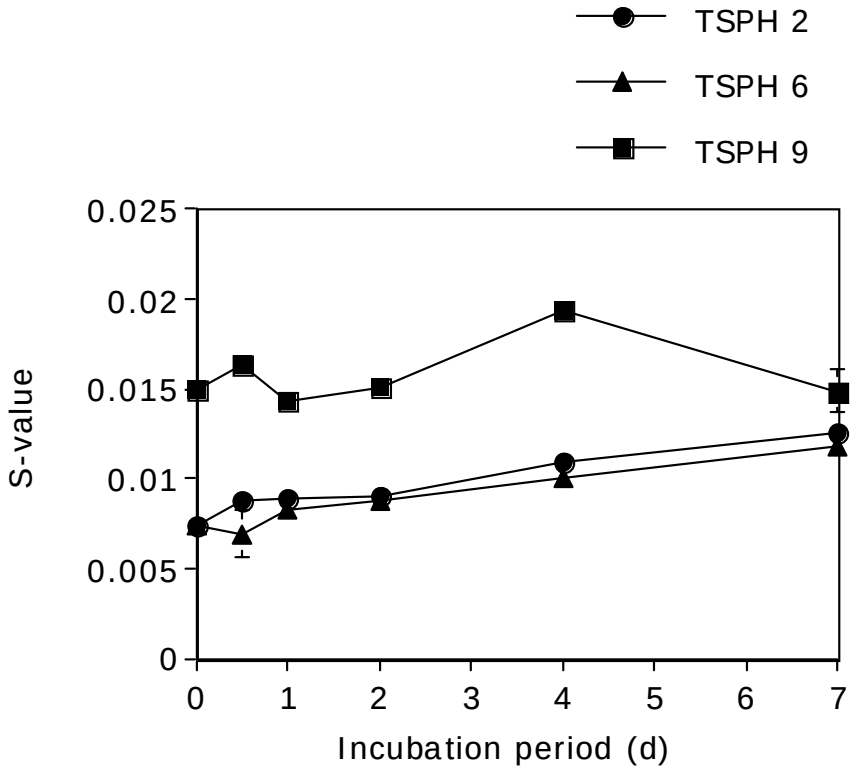


Fig. 17. *Change of S-value of water samples after exposing to solar simulated light*

Fluorescence intensity also decreased rapidly after exposure to SSL, suggesting the photodegradation of fluorophores (Fig. 18). Maximum wavelength increased slightly (Fig. 19), while the fluorescence index (FI) decreased significantly (Fig. 20). The decrease in FI. may be attributed to (1) the selective degradation of microbially-derived (or lowly polycondensed) DOM components (that have higher FI. values), and/or (2) polycondensation of fluorophore. The Peak I intensity also decreased for TS/PH 2 and TS/PH 6, but did not change appreciably for TS/PH 9 (Fig. 21).

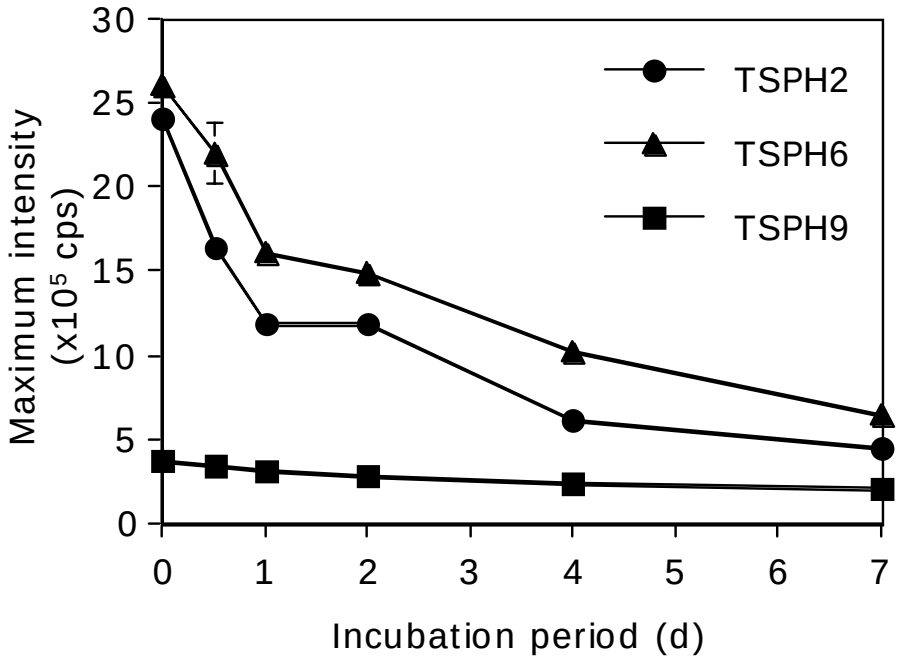


Fig. 18. *Change of maximum fluorescence intensity of water samples after exposing to solar simulated light*

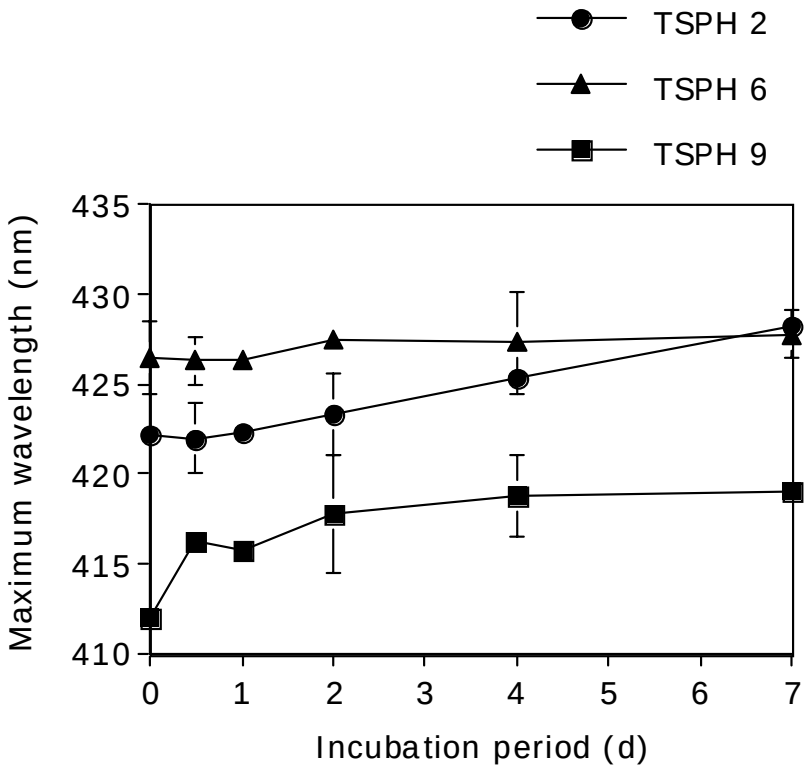


Fig. 19. *Change of the maximum wavelength of water samples after exposing to solar simulated light*

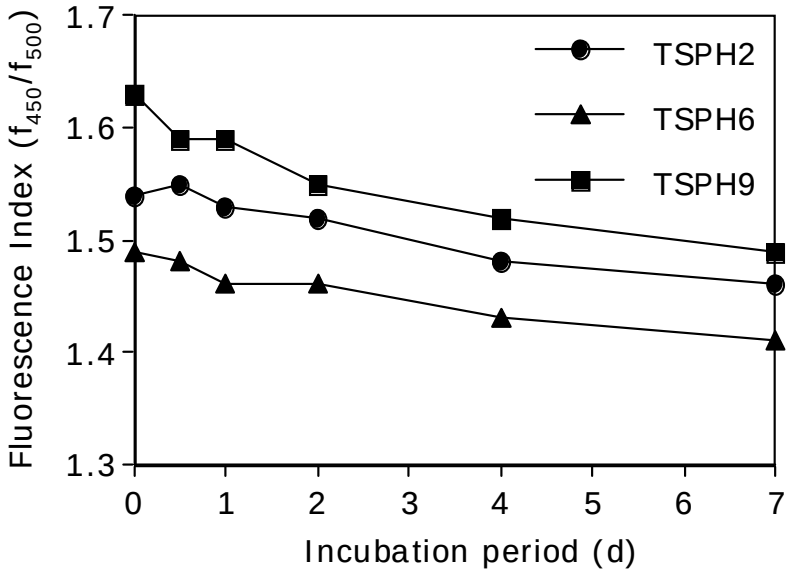


Fig. 20. *Change of the fluorescence index of water samples after exposing to solar simulated light*

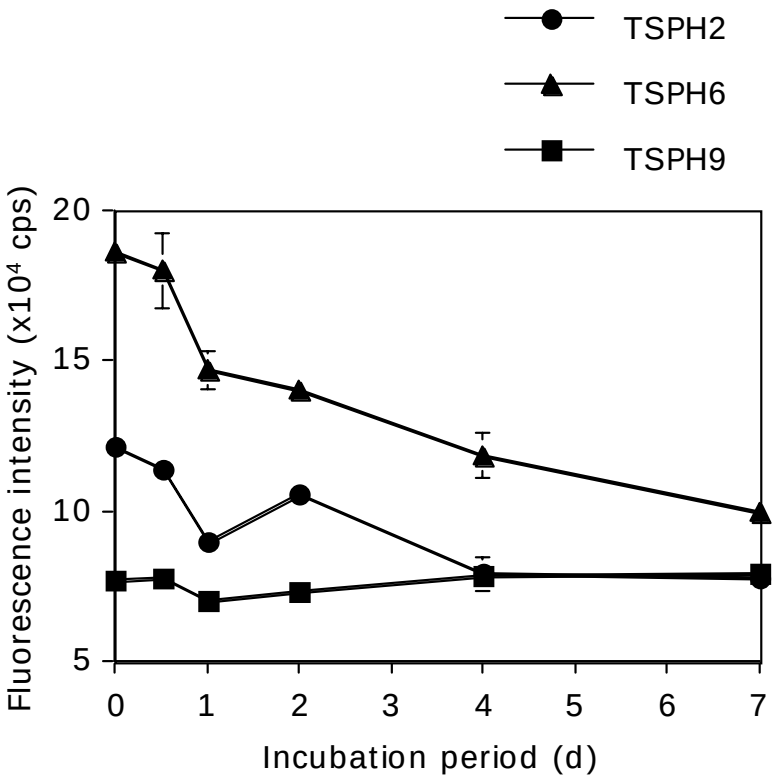


Fig. 21. *Change of the Peak I intensity of water samples after exposing to solar simulated light*

Phenolic substances as well as proteinaceous materials (tyrosine, tryptophan, and phenylalanine) are known to contribute to the Peak I signal. Since the Peak I intensity and UV absorption at 254 nm had a positive correlation (r^2=0.88; Fig. 22), the contribution to this signal by other aromatic compounds in addition to proteins is possible. DOM from TS/PH 2 and TS/PH 6 is known to contain remarkable amounts of aromatic C, while that from TS/PH 9 is quite low. Thus, the decrease in the Peak I intensity at TS/PH 2 and TS/PH 6 may be ascribed to the degradation of phenolic compounds.

Regarding the molecular distribution changes during light exposure, the weight average molecular weight decreased slightly after a 7 day exposure under SSL conditions (Fig. 23). The change detected was small, however, since the detection of elution curve is conducted using UV-absorption at 254nm, we might miss detecting those compounds that have lost UV-absorption characteristics after exposure to SLL.

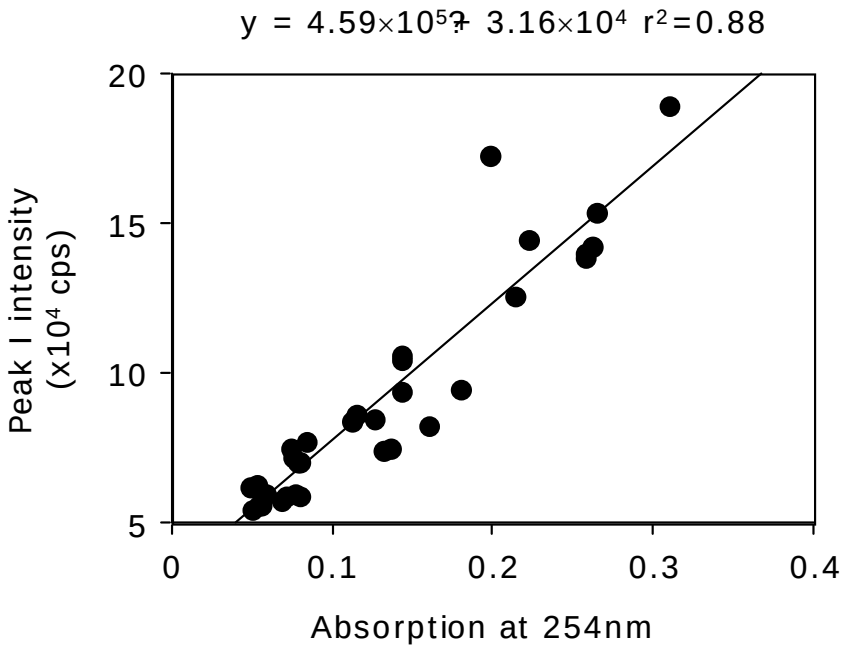


Fig. 22. *Correlation between UV absorption at 254nm and Peak I intensity*

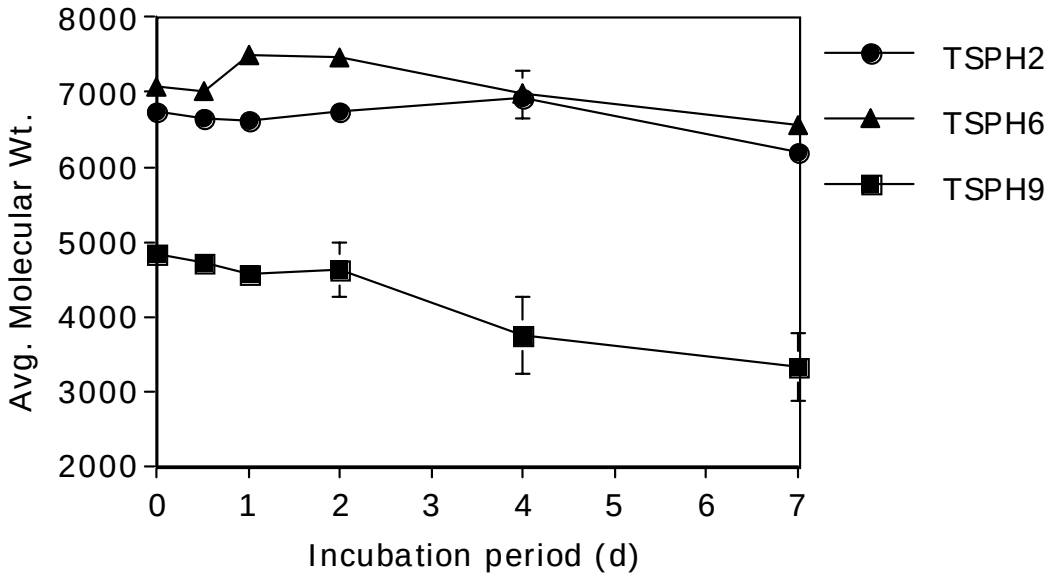


Fig. 23. *Weight average molecular weight of DOM after exposing to solar simulated light*

Overall, a significant degradation/modification was induced to a light-absorbing organic matter fraction in DOM (humic substances/fluorophore) by exposing water samples to sun light. The degradation was not equal for all the light-absorbing compounds, but lowly polycondensed materials were preferably disappeared. Light-absorbing compounds (like humic substances) are generally refractory against microbial degradation. Although complete degradation of DOM did not occurred in this experiment system, light-induced alternation will affect nutrient status and degradability of DOM by microorganisms. Thus sun light plays an important role for the dynamics of DOM in the Everglades.

Experiment 3: DON Bioavailability (BDON) in Water Column

Overall, only a minor amount of the DON from the three sites was bioavailable. This is consistent with our previous studies in the area (Boyer and Jaffé, unpublished results). Figures 24-26 show the dynamics of the DON components from the three water column samples. We experienced significant artifacts as a result of bottle effects after 8 days from sloughing of material from the bottle walls. Therefore, all BDON rate calculations were performed over 8 day incubation period. The most obvious aspect of these graphs is the small amount of total DON_{NL} loss. Langenheder et al. (2003) demonstrated that functional changes were associated with changes in community composition between freshwater and marine bacteria. They noted that even moderate changes in salinity had large effects bacterial community composition, which lead to altered growth characteristics. We got around this problem by adjusting salinity to 34.5 and using a bacterial inoculum from TS/PH 9 for all treatments. Therefore, our estimates are based on the premise that DON from all sites might be rapidly transported to the bay, so decomposition rates should reflect this possibility.

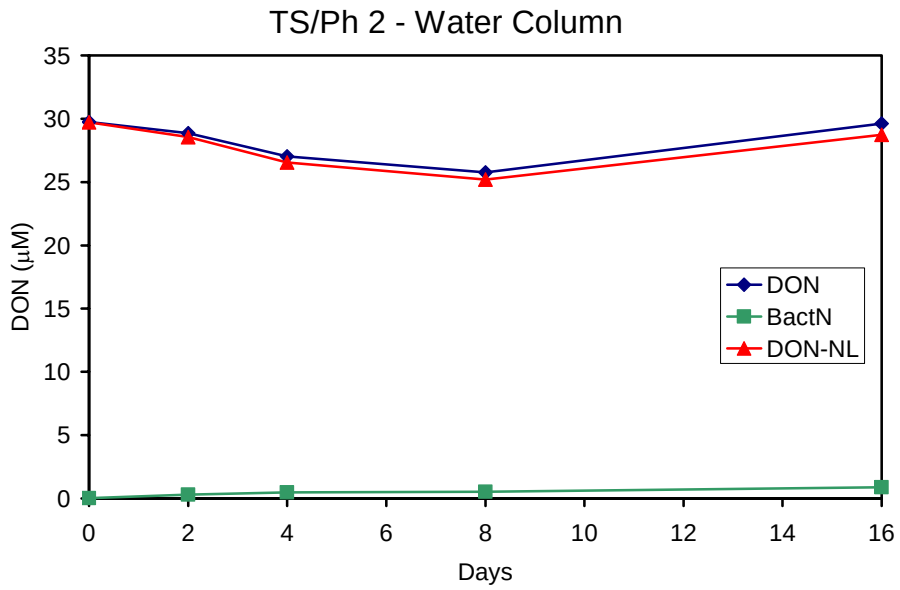


Fig. 24. *Change in DON at TS/PH 2 showing bacterial contribution to the DON pool (NL is non-living component).*

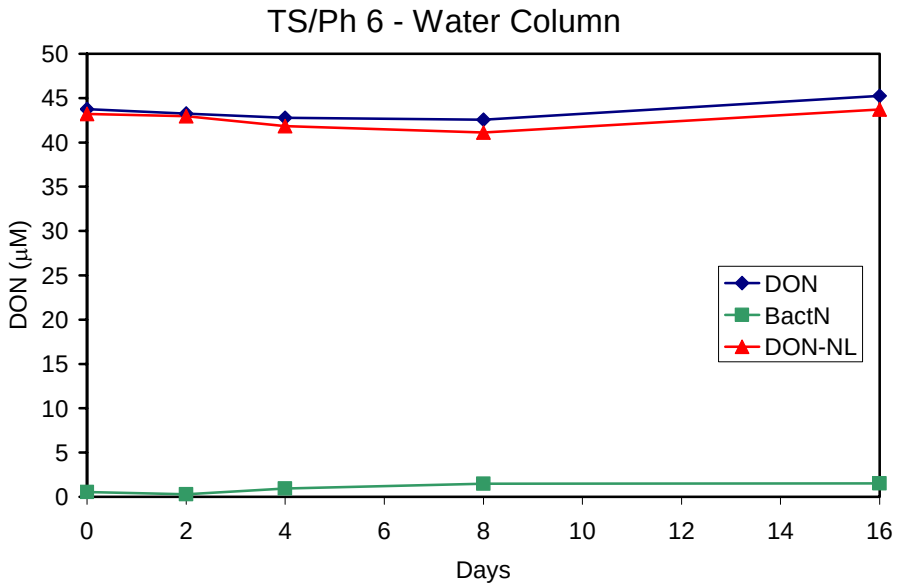


Fig. 25. *Change in DON at TS/PH 6 showing bacterial contribution to the DON pool.*

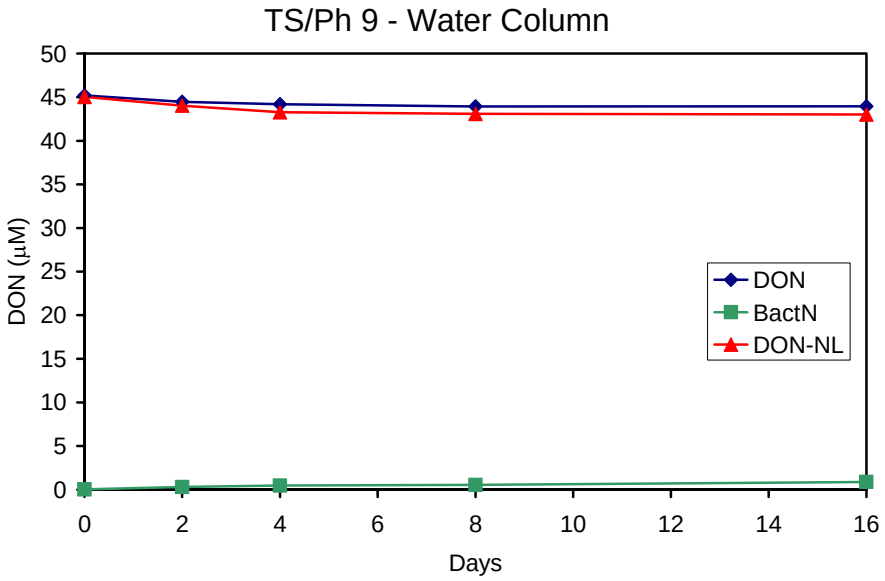


Fig. 26. *Change in DON at TS/PH 9 showing bacterial contribution to the DON pool.*

Data were fitted to a one phase exponential decay equation (Fig 27) where X is time, and Y is DON_{NL} concentration. Y starts out equal to Span+Plateau and decreases to Plateau with a rate constant K (d^{-1}). K values for TS/PH 2, 6, and 9 were 0.480, 0.403, and 0.254 d^{-1} (Table 1). Potentially bioavailable DON (BDON%) was calculated as Span/(Plateau+Span)*100.

$$Y = Span * e^{-K*t} + Plateau$$

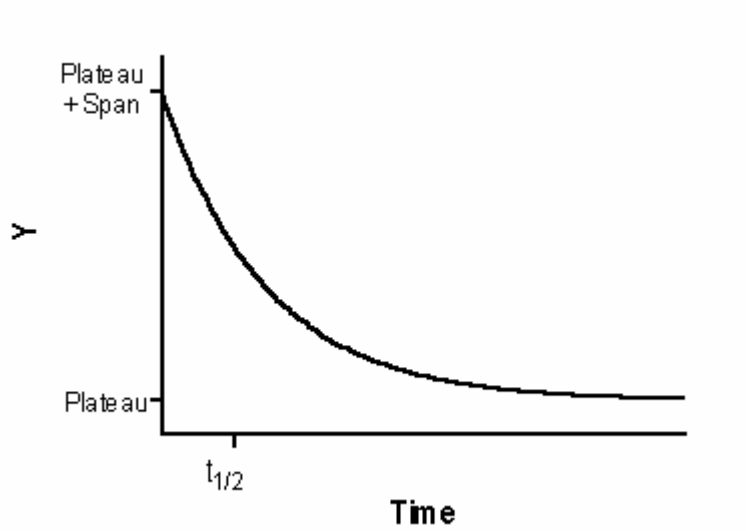


Fig. 27. *Example of one phase exponential decay equation.*

Water Column	TS/PH 2	TS/PH 6	TS/PH 9
SPAN (µM)	2.75	2.14	1.58
K (d^{-1})	**0.480**	**0.403**	**0.254**
PLATEAU (µM)	27.08	42.23	43.72
Half Life (d)	1.44	1.72	2.73
Bioavailability (%)	**9.21**	**4.82**	**3.49**
Degrees of Freedom	7	7	6
R^2	**0.378**	**0.303**	**0.285**

Table 1. One phase exponential decay fits for water column incubations.

In general, BDON was low; but DON bioavailability in the freshwater wetlands (9.2%) was higher than the mangrove (4.8%) and estuary site (3.5%). These values are comparable to other studies (Wetzel 1992; Stepanauskas et al. 1999), who observed 2 - 16% bioavailability for their marsh sites.

The rate constants tell only one part of the story; the plateaus for all sites were high relative to initial levels. This means that the rate of degradation was rapid in the beginning but that only a small portion of the total DON pool was degraded. It is important that any future modeling efforts incorporate this Plateau/Span information or else DON decomposition will be overestimated.

It is interesting that BDON is highest in the marsh, intermediate in the mangroves, and lowest in the bay itself. This suggests that the amount of bioavailable DON from the marsh and

mangrove that is transported to Florida Bay is low. The half lives among sources are different as well, meaning that the bioavailable fractions are probably of individual origin. This concept was also born out from the chemical characterization mentioned above (λ_{max}).

The bacterial contribution to the DON pool ranged from 0.12 – 3.4 % (0.04 – 1.5 µM N) and generally increased over time (Figs. 24-26). This increase was due to change in both bacterial numbers (Fig. 28) and total bacterial biovolume (BV_{Tot} in mm^3, Fig. 29). It is interesting to note that although bacterial abundance at TS/PH 6 and 9 were similar, BV_{Tot} was much greater at TS/PH 9. This may be the result of increased growth of bacteria at TS/PH 9 by the relaxation of C limitation there as well.

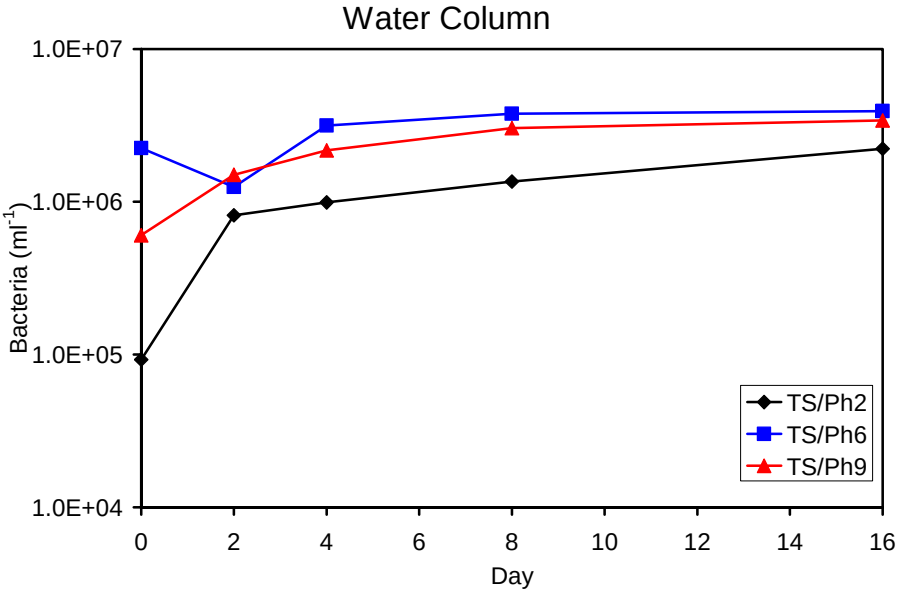


Fig. 28. *Change in total heterotrophic bacteria abundance at the three sites over time.*

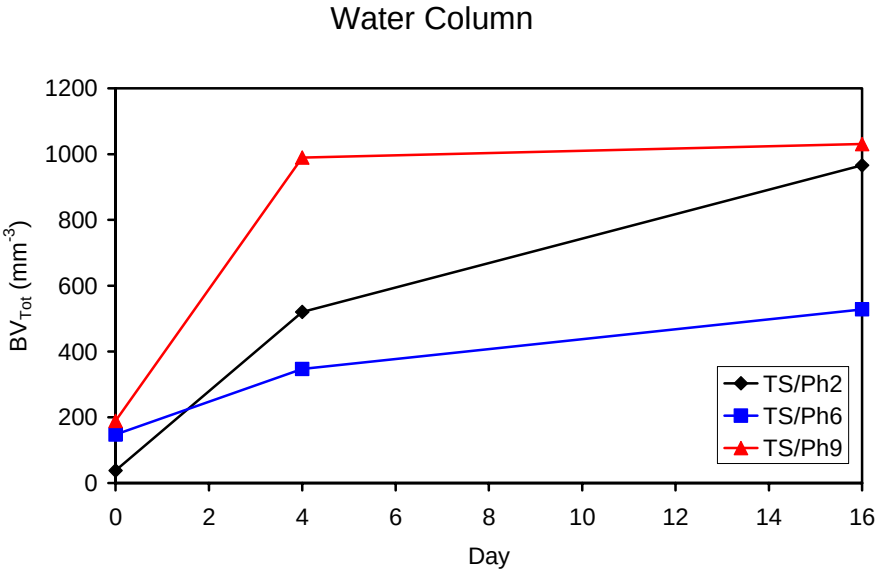


Fig. 29. *Change in total bacterial volume at the three sites over time.*

Bacterial production (BP, µgC l^{-1} h^{-1}) was stimulated by the addition of C and P in brackish and marine sites, relative to the freshwater marsh (Fig. 30). Under N-limiting conditions, this stimulation could only come from degradation of DON. However, C limitation has previously been suggested to occur in eastern Florida Bay (Boyer unpublished data).

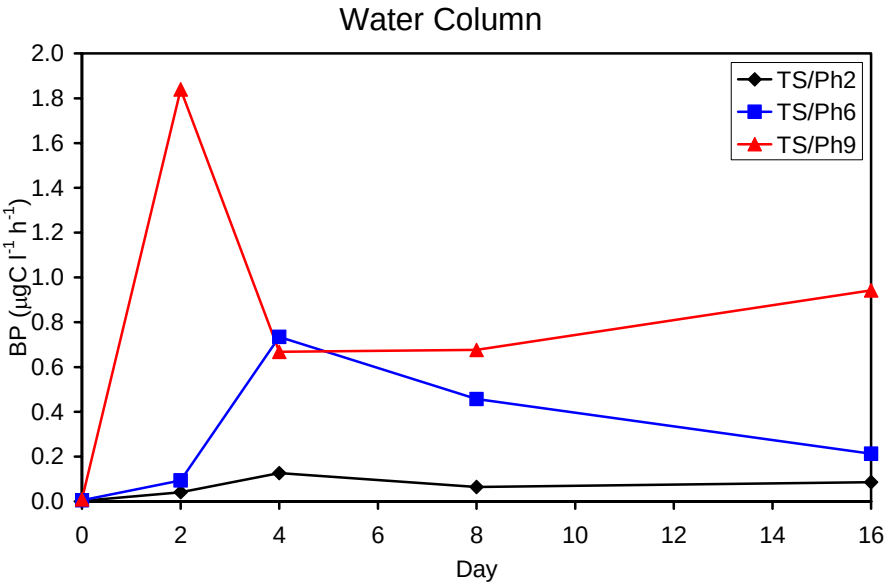


Fig. 30. *Change in bacterial production at the three sites over time.*

Concomitant with DON decay and bacterial growth and activity, we observed increases in leucine aminopepdidase activity (LAP, Fig. 31). LAP response was highest in the estuary and lowest in the marsh. Cell specific LAP activities (µM h^{-1} $cell^{-1}$) were also highest at TS/PH 9. Increase in LAP titer is a direct bacterial response to, and therefore a good indicator of, N limitation.

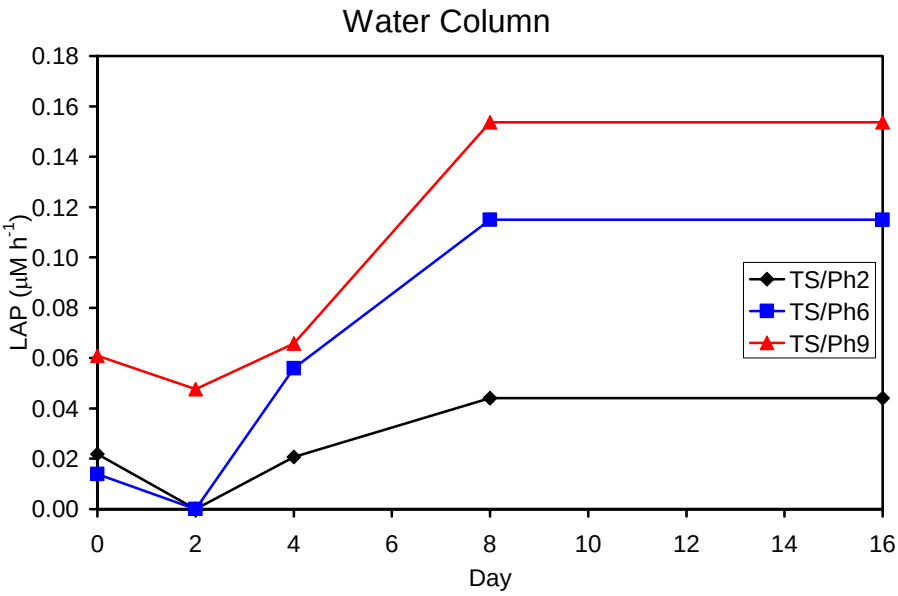


Fig. 31. *Increases in leucine aminopepdidase activity at the three sites over time.*

The actual concentrations of dissolved inorganic N (DIN) are shown in Figs. 32 & 33. As expected with N limitation, NO_x (NO_3+NO_2) was driven down or remained at very low levels (<0.5 μM) during the incubation.

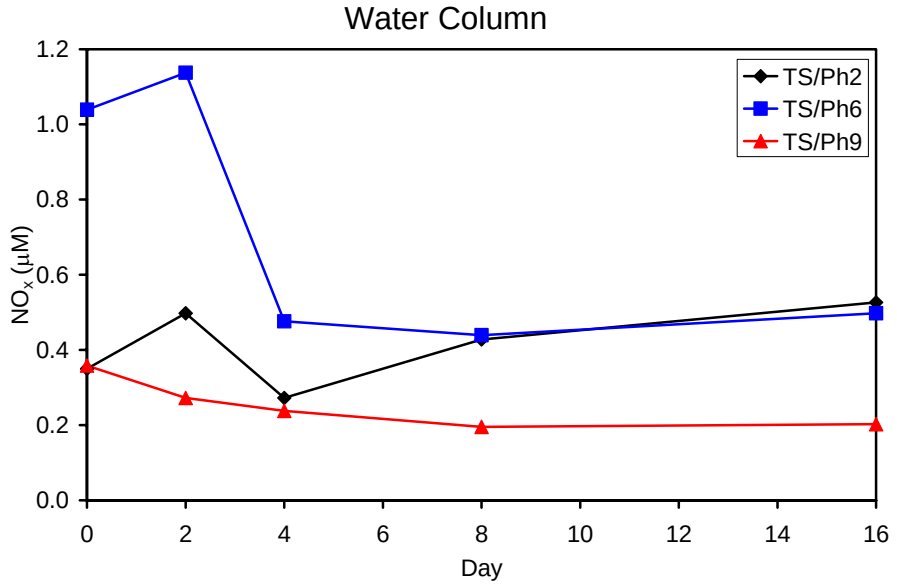


Fig. 32. *Concentrations of nitrate and nitrite at the three sites over time.*

Oddly, NH_4 levels in TS/PH 2 and 6 remained moderately high (3-5 μM) but did decline strongly towards the end of the incubation. We believe that this pattern was due to a sequential processing of DON by bacteria. Early on, the labile DON fraction was converted to biomass and the excess released as NH_4. After the bulk of the bioavailable DON was used up, the bacteria

began taking up the previously released NH_4. That little NH_4 was recycled at TS/PH 9 may have been because of the low initial bioavailability to begin with, but that is speculation.

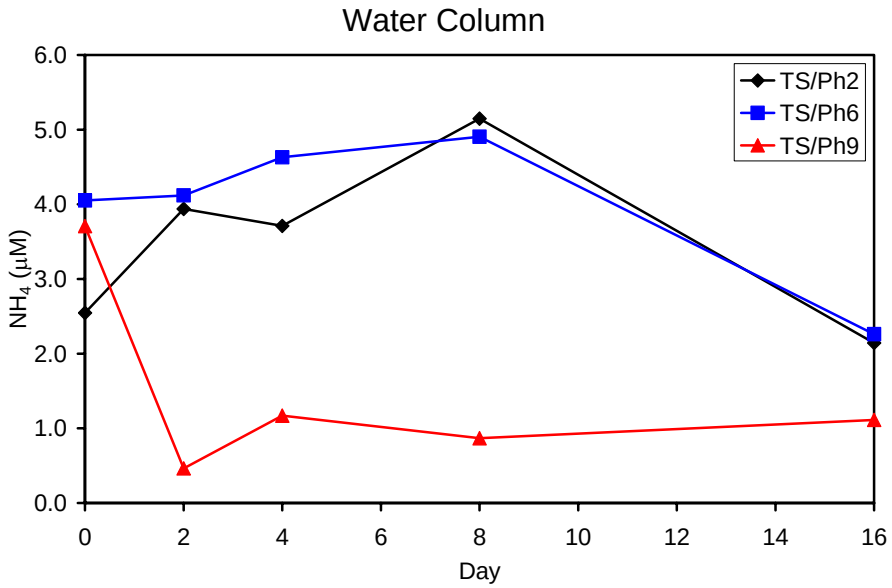


Fig. 33. *Concentrations of ammonium showing the difference among treatments.*

Phosphorus levels (total and soluble reactive) remained at or near the level of addition (Figs. 34 & 35) so no P limitation occurred, which might explain the high NH_4. As expected under P replete conditions, declines were observed in alkaline phosphatase activity (APA, Fig. 36).

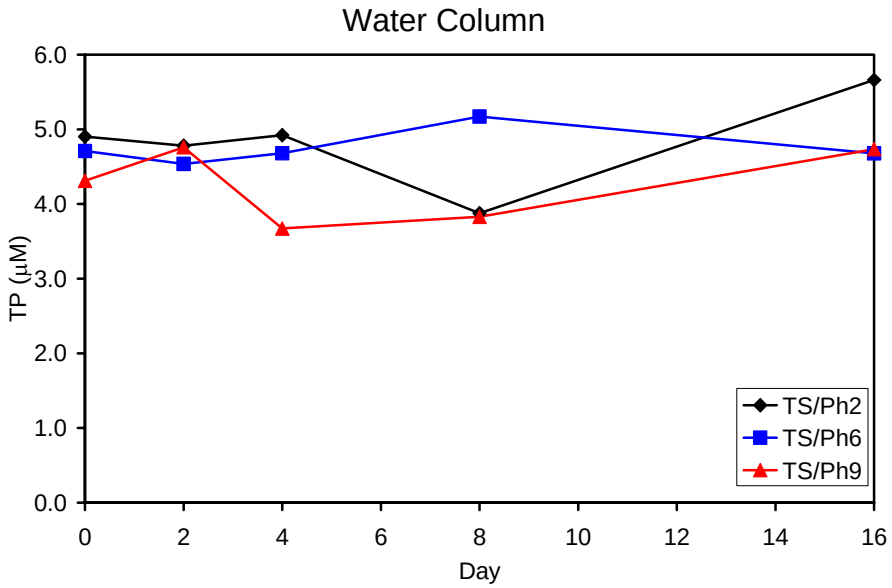


Fig. 34. *Concentrations of total phosphorus, all above P limitation threshold.*

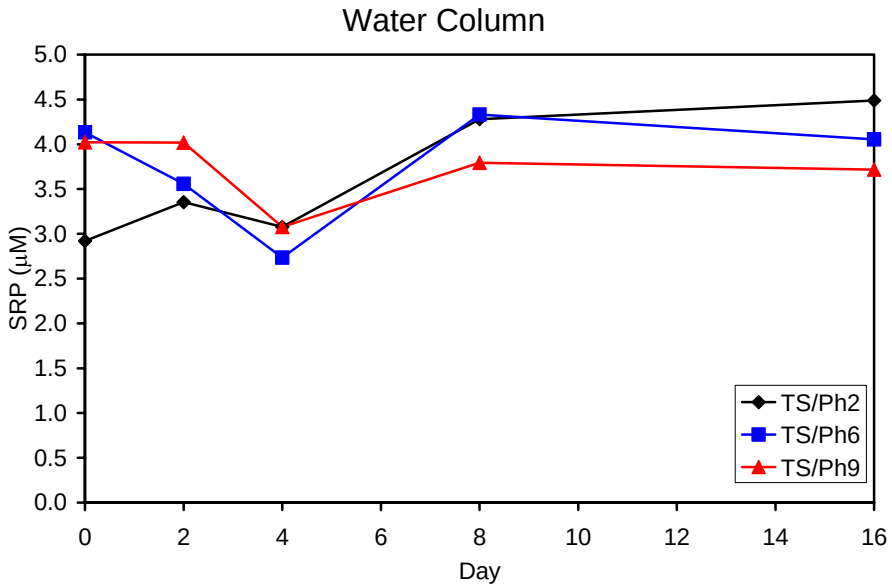


Fig. 35. *Concentrations of soluble reactive phosphorus, all above P limitation threshold.*

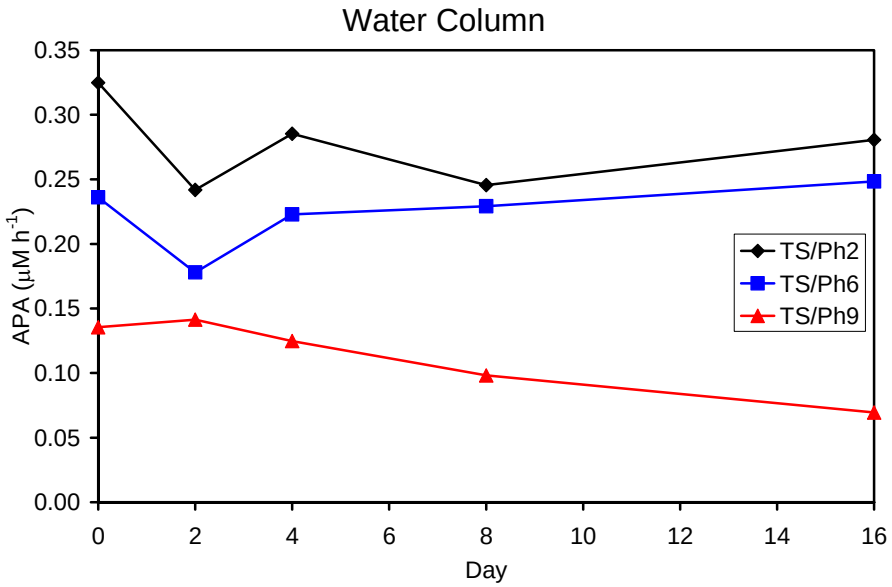


Fig. 36. *Alkaline phosphatase activity showing non-stimulation/decline over incubation.*

Optical fluorescence properties of the DOM were also measured in an effort to relate these to BDON. As mentioned previously, initial maximum wavelength of emission (λ_{max}) and fluorescence index (FI) were very different for the three sites, suggesting distinct sources of DOM at each site (Fig. 37 & 38). Plant-derived DOM has a λ_{max} value around 420 nm, while microbial-derived DOM has a λ_{max} around 380 nm. The longer λ_{max} for TS/PH 6 than TS/PH 2 indicated that plant (mangrove)-derived DOM contributed more to TS/PH 6, while TS/PH 2 was influenced by microbial (periphyton)-derived DOM. TS/PH 9 had the lowest λ_{max} of all, indicating almost non-existent terrestrial influence. Little change was observed in λ_{max} over the incubation period. However, it is important to understand, that because the emission spectrum

(excitation at 313nm) shows a very broad hump, it is difficult to determine an accurate λ_{max} value.

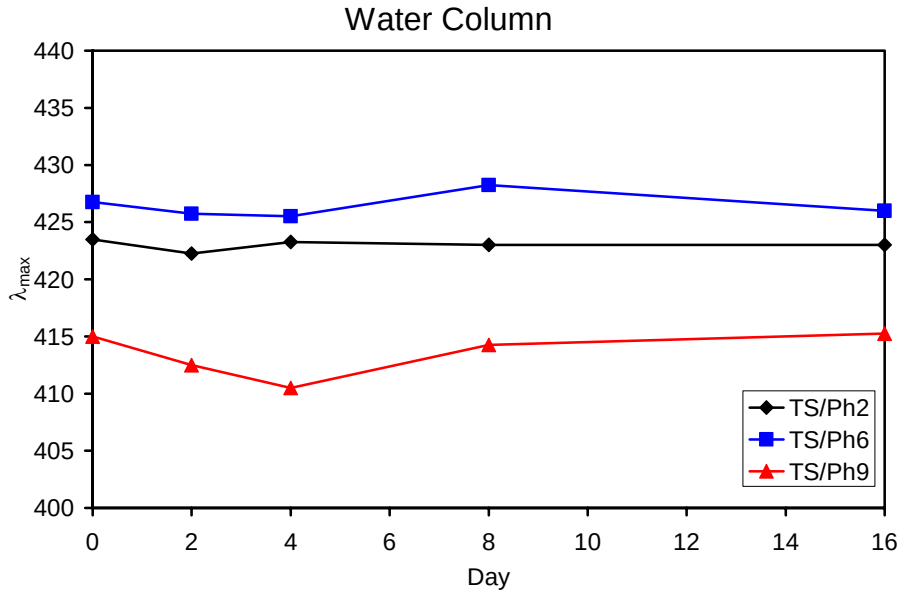


Fig. 37. *Maximum wavelength of emission at each site over the incubation.*

Fluorescence index (FI) was highest at TS/PH 9, indicating the marine origin of the DOM (Fig. 38). FI was higher at TS/PH 2 than TS/PH 6, which is consistent with the larger influence of microbial-derived DOM at TS/PH 2. A slight increase in FI was observed over the incubation as a result of selective decomposition.

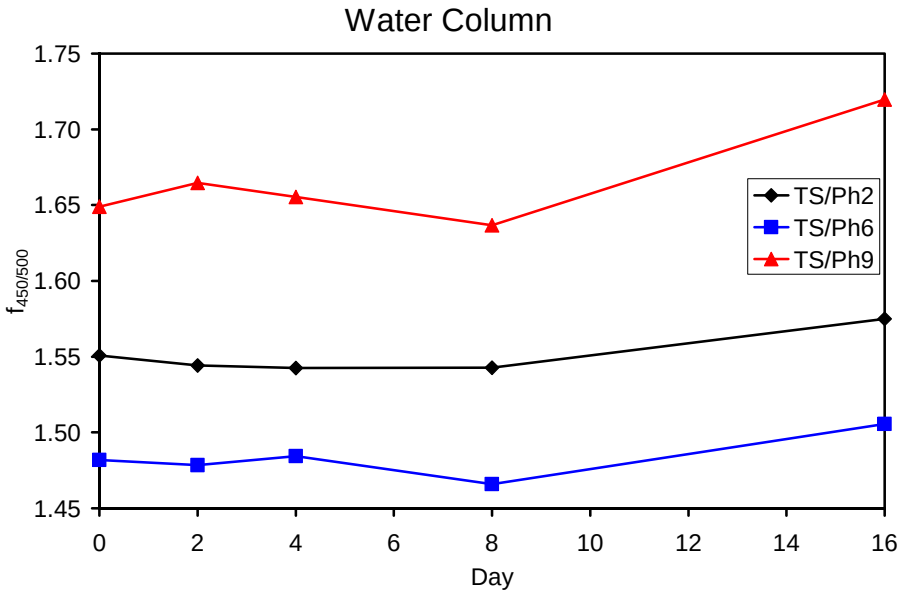


Fig. 38. *Fluorescence index at each site over the incubation.*

The protein-like peak of the synchronous fluorescence spectra (Peak I) was also measured to assess aspects of DOM quality (Fig. 39). TS/PH 9 had much higher Peak I value than the other sites. We expect this is due to presence of polyphenols released by seagrass in the estuarine site. Peak I intensity did not change over the incubation, indicating that these proteinaceous/polyphenolic materials were not degraded by microorganisms in the dark.

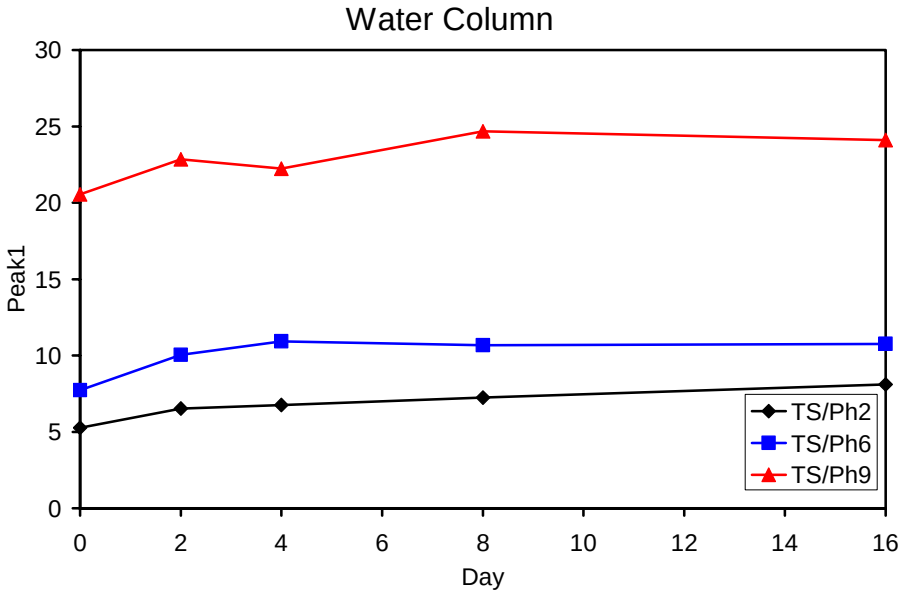


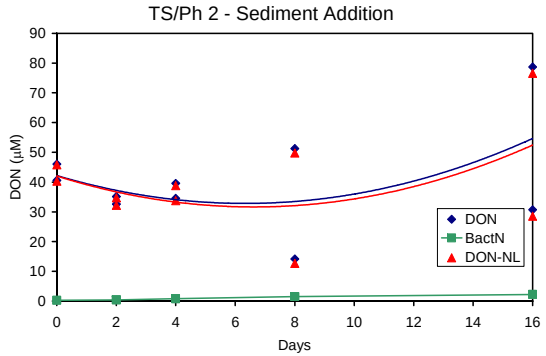
Fig. 39. *Fluorescence index at each site over the incubation.*

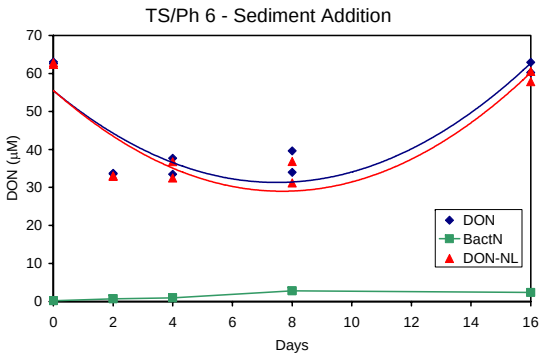
Experiment 4: DON Bioavailability (BDON) with Sediment Additions

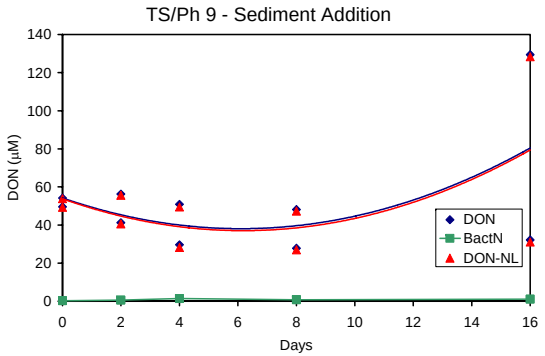
Results from the sediment addition experiment were obscured by artifacts of the general experimental design. A great amount of variability was introduced into the DON analysis because we did not filter samples from the bottles prior to measurement. This was not a problem in the water column experiment. The presence of sediment and associated floc turned out to cause large variability in measured values, even with replication. Results from TN analysis were too variable to fit a curve so no decay constants could be calculated. We hoped it would not be a problem if we let the bottles settle before withdrawing a sample, but it did not seem to help.

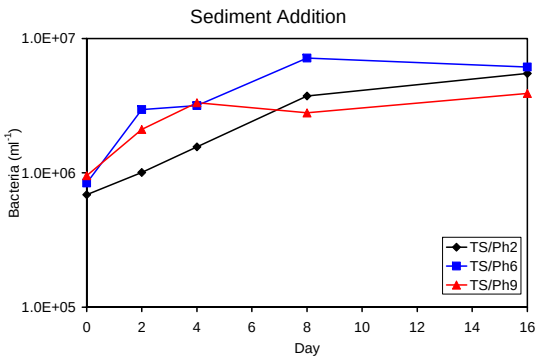
Overall, the trends in nutrient/biological variables were similar to the water column, but of greater magnitude (following figures). Initial bacterial counts, LAP, and BP, were 2-5X higher and increased over time. NO_x was low while NH_4 increased and then declined. TP, SRP, were comparable to the water column. Initial APA was higher but also declined over the incubation. No differences in the optical parameters were observed between treatments.

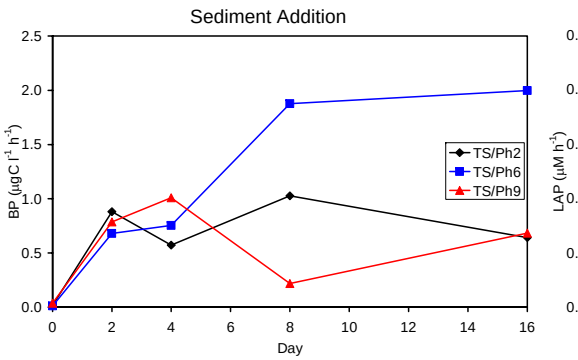
Because of the large variability inherent in the sediment addition experiment, we would not recommend performing this type of assay in the future.

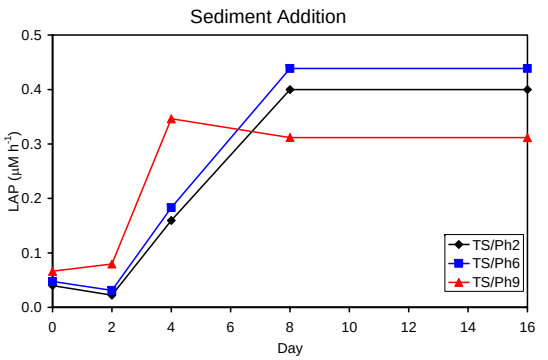

TS/Ph 2 - Sediment Addition
DON (µM)
Days
DON
BactN
DON-NL

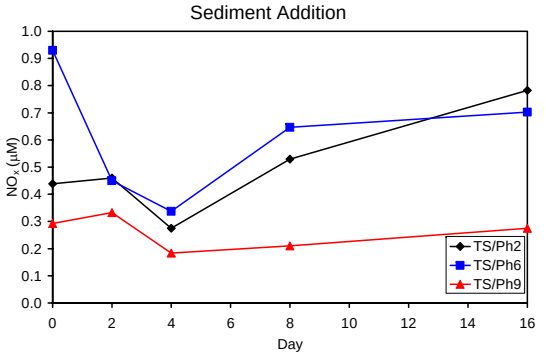

TS/Ph 6 - Sediment Addition
DON (µM)
Days
DON
BactN
DON-NL

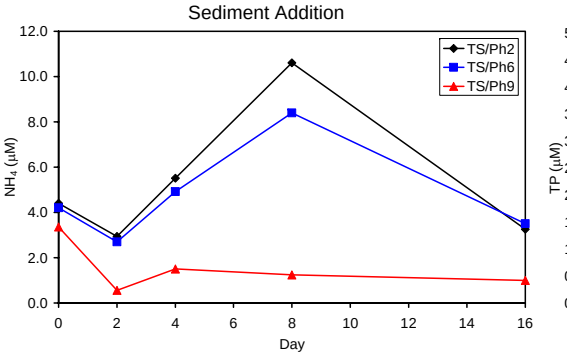

TS/Ph 9 - Sediment Addition
DON (µM)
Days
DON
BactN
DON-NL

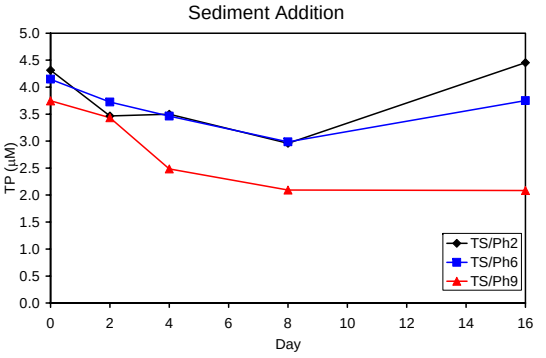

Sediment Addition
Bacteria (ml-1)
Day
TS/Ph2
TS/Ph6
TS/Ph9

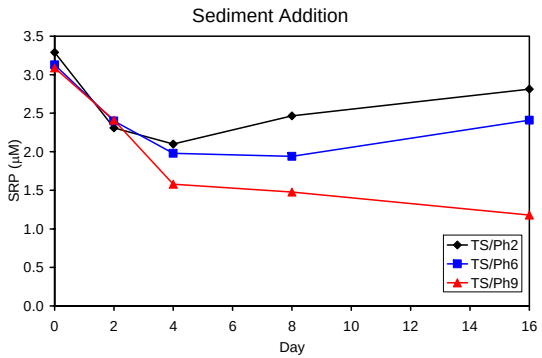

Sediment Addition
BP (µgC l-1 h-1)
Day
TS/Ph2
TS/Ph6
TS/Ph9

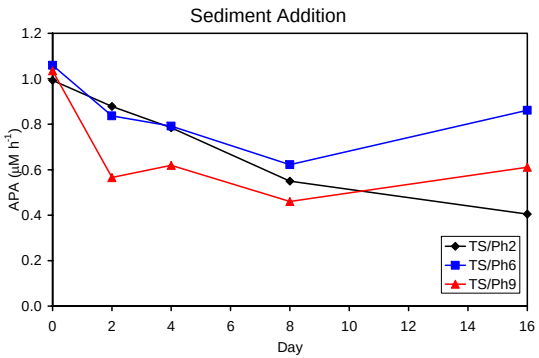

Sediment Addition
LAP (µM h-1)
Day
TS/Ph2
TS/Ph6
TS/Ph9

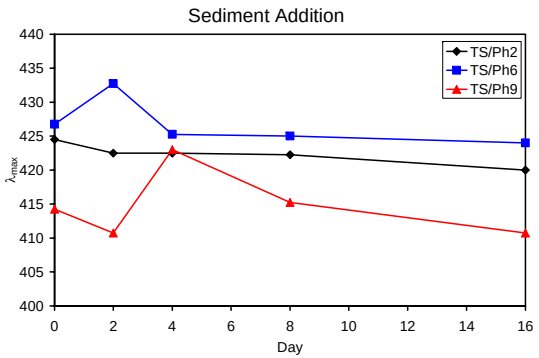

Sediment Addition
NOx (µM)
Day
TS/Ph2
TS/Ph6
TS/Ph9

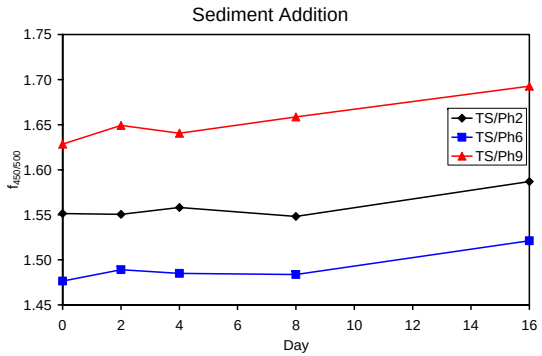

Sediment Addition
NH4 (µM)
Day
TS/Ph2
TS/Ph6
TS/Ph9

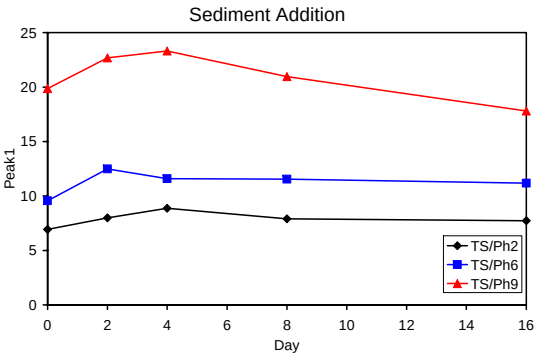

Sediment Addition
TP (µM)
Day
TS/Ph2
TS/Ph6
TS/Ph9


Sediment Addition
SRP (µM)
Day
TS/Ph2
TS/Ph6
TS/Ph9


Sediment Addition
APA (µM h-1)
Day
TS/Ph2
TS/Ph6
TS/Ph9


Sediment Addition
λmax
Day
TS/Ph2
TS/Ph6
TS/Ph9


Sediment Addition
f450:500
Day
TS/Ph2
TS/Ph6
TS/Ph9


Sediment Addition
Peak1
Day
TS/Ph2
TS/Ph6
TS/Ph9

CONCLUSIONS

Experiment 1. Overall, the first experiment performed showed that no significant flocculation or precipitation of DOM occurred with increase in salinity. This means that terrestrial DOM does not get trapped in the sediments but stays in the water column where it subjected to photolysis and advective transport. In other words, DON from the Taylor Slough or C-111 panhandle does not precipitate out at the entrance into the bay, but stays in the more mobile, soluble phase.

This experiment also showed that physical changes in the DOM occurred along a salinity gradient. The decrease in average molecular weight and increase in polydispersity may be explained by two different models: the disaggregation/dissociation of HMW complexes into many LMW components or the physical contraction of DOM structure. The exact nature of this physical process is not well understood at this time, but may affect photoreactivity and bioavailability of the DOM. Other researchers have noted the increased degradation of terrestrial DOM at elevated salinities. This effect was attributed to three possibilities: 1) the enhanced ability of marine bacteria to degrade macromolecules, 2) increased activity of exoenzymes with ionic strength, and 3) flocculation and/or contraction of DOM with increased ionic strength. We now add another factor to this list: disaggregation of macromolecular structure of DOM.

Experiment 2. Sunlight has a significant effect on the chemical characterization of DOM. While the DOC levels did not change significantly during photo-exposure, the optical characteristics of the DOM were modified. The environmental implications of this are conflicting: photo-induced polymerization may stabilize the DOM by reducing its bioavailability while photolysis may make the DOM more labile.

Experiment 3. In general, DON bioavailability was relatively low in this region. Bioavailable DON (BDON) in the freshwater wetlands (9.2% over 8 days) was higher than the mangrove (4.8%) and estuary sites (3.5%). The bacterial contribution to the DON pool was low but significant and was accounted for in the assays. Although bacterial abundances at TS/PH 6 and 9 were similar, the biovolume was much greater at TS/PH 9. This suggests that there is a potential for bioavailable DON from the marsh and mangrove to be transported to Florida Bay. However, we also showed that the composition of the DOM at each site is more distinct than we originally thought.

Elevated NH_4^+ levels observed were due to a sequential processing of DON by estuarine bacteria. First the labile DON fraction was converted to biomass and the excess released as NH_4^+. After the BDON was exhausted, the bacteria assimilated the previously released NH_4^+.

Experiment 4. A great amount of variability was introduced into the DON analysis because we did not filter samples from the bottles prior to measurement. We do not recommend performing this type of assay in the future. Overall, the trends in nutrient/biological variables were similar to the water column, but of greater magnitude.

Implications for the Nutrient Budget. These experiments show that, although the amount of DON loaded to the bay may be large, the fraction of DON available for microbial cycling is much smaller. However, since the median DIN concentration for TS/Ph 9 was 4.6 μM while BDON was 1.6 μM, the amount of recycled DON may indeed be a significant portion of the total DIN pool. We should add that more slowly decomposed DON that is not readily detected, and that might decompose over time periods exceeding that of the experiment, could also be a component of the BDON. Thus the 1.6 μM value for BDON would be a minimum estimate.

All this must be considered in context with the proposed CERP modifications to flows. As of the latest Initial CERP Update, the flows to Taylor Slough and C-111/Panhandle Basis are not

predicted to change very much from base conditions. Therefore we do not expect any great increases in TN loading in this region. In contrast, the proposed flow increases to the Shark River Slough are large and may have significant effects on transport of DOM to the Southwest Florida Shelf. We believe that future efforts in DON characterization and bioavailability should be concentrated in this area.

ACKNOWLEDGMENTS

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