FACE

Stable Isotope Laboratory

1st Year Report

Sampling of Nitrogen Compounds for Determination of Isotopic Values in Benthic Macroalgae, Sediment Organics, and Seawater

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Executive Summary

- This report summarizes work completed in 2007 in the Stable Isotope Laboratory at RSMAS as part of the FACE effort.

- Over 100 sites were chosen for the collection of surface sediment samples stretching from Miami to West Palm Beach. The sites were arranged in a grid pattern designed to capture any chemical signature associated with treated sewage outfalls. These samples were analyzed for the stable N and C isotopic composition of the organic material yielding a mean value for N of +3.82 ‰ and for C of -17.85 ‰.

- A total of 206 algal samples were collected from a variety of sites. These yielded a nitrogen isotopic composition of +4.21 ‰ and a carbon isotopic composition of -19.36 ‰.

- Water samples have been analyzed for $\delta^{15}N$ of NO_3^-, NH_4^+, and DON.

- These algal and sediment samples and the $\delta^{15}N$ of the NO_3^- show no evidence of isotopically positive $\delta^{15}N$ influencing the algal samples, although the most positive $\delta^{15}N$ values in the sedimentary organic material was found associated with some of the outfalls.

- The absence of such evidence emphasizes the need to further understand the nitrogen cycle in the coastal waters, before using isotopically positive $\delta^{15}N$ values to make conclusions regarding the sources of nitrogen in coastal waters.

Background

The purpose of this present study is to obtain samples from various locations around six South Florida treated waste water outfalls and analyze the stable nitrogen and carbon isotopic compositions.

This preliminary report is a documentation of the executed sampling plan and currently available data.

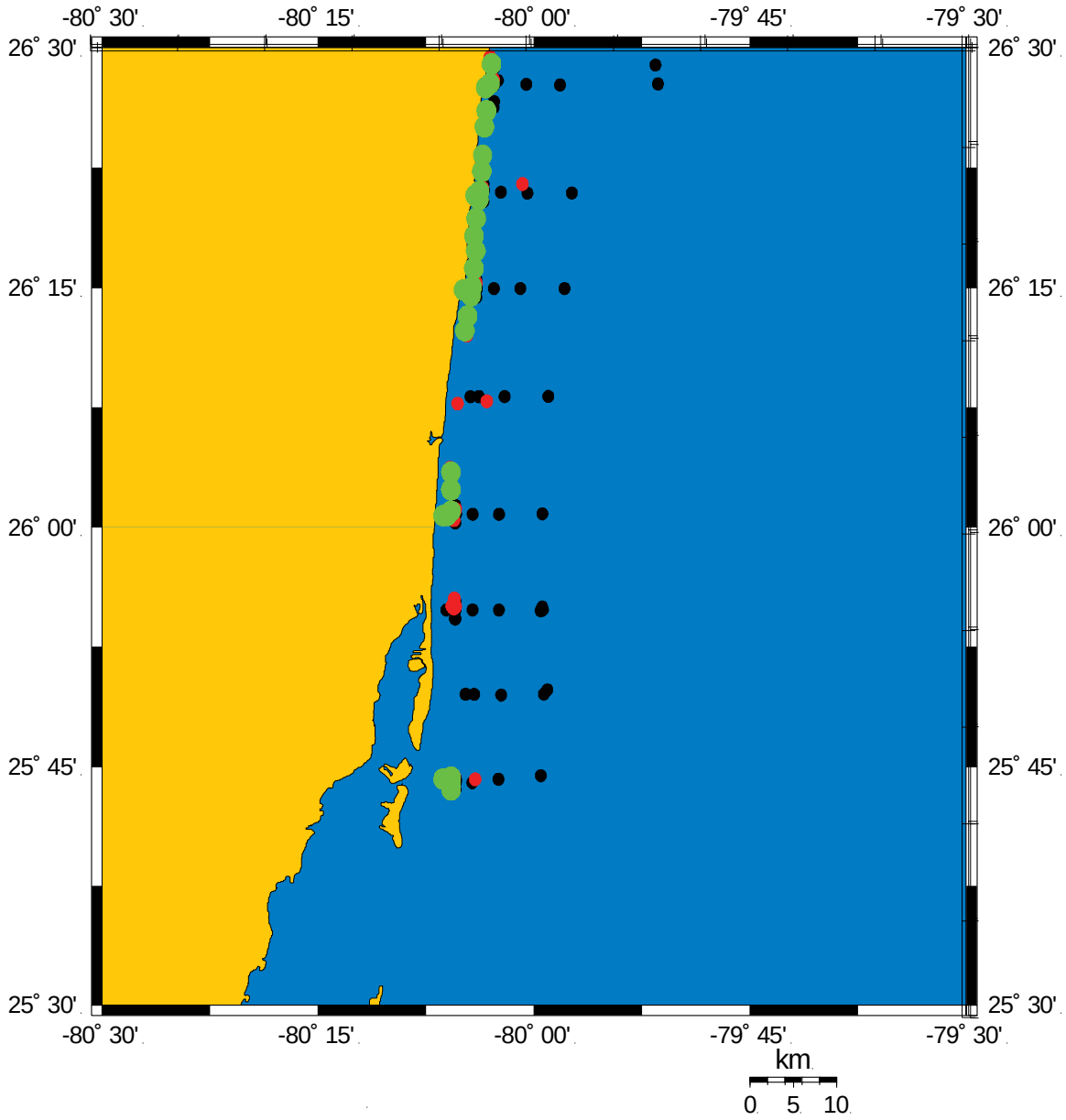


Figure 1: Location of sediment sample grabs (black dots), water column samples (red dots), and algal samples (green dots), collected during the October 2006 FACE

Sampling locations

A high spatial resolution sampling plan for collection of seawater and sediment was implemented in October 2006 during the Nancy Foster Cruise.

Sixty-four sampling sites were chosen for diver collection of benthic macroalgae and 110 sites for sedimentary organic material. These sites were conferred upon by PI Dr. Swart, research assistant Ms. Courtney Drayer, FACE director Dr. John Proni, Chief Scientist Dr. Tom Carsey, and Lt. Hector Cassanova in a meeting held at AOML August 2006. The location of these samples is shown in Figure 1.

Methods and Data obtained

Benthic Macroalgae

As samples were returned to RSMAS, they were sorted, identified, and separated by genus and/or species and dried in a low temperature (40 °C) drying oven for approximately 4-7 days. In total, 206 algae and sponge samples were collected and identified. Samples were then ground on a Wiley Mill through a 40 μm sieve. The samples were split and the second half was treated with 10% HCl followed by 2 rinses with ultra high purity deionized water and dried in a low temperature drying oven. Approximately 1-4 mg of sample was weighed out in at least duplicate in tin capsules for analysis of $\delta^{13}C$, $\delta^{15}N$, and C:N.

Sediment Organic Material

Sediments were collected both on board the Nancy Foster using a Shipek sampler and by diver operations. In total, 100 usable sediment samples were collected. These samples were freeze-dried upon return to RSMAS and the ground with a mortar and pistil. Approximately 500 mg of ground sample was treated with 10% HCl and then filtered on pre-rinsed and weighed 25mm GF/C filters. The material collected on the filters was split and placed in tin capsules for analysis of $\delta^{13}C$, $\delta^{15}N$, and C:N.

Instrumentation

Elemental and isotopic abundances of the sediment organic material and algal tissue samples have been determined using an Automated Nitrogen Carbon Analyzer (ANCA) interfaced to a stable isotope mass spectrometer (Europa Scientific Model 20-20). Isotopic abundances on the $\delta^{15}N$ and $\delta^{18}O$ of the NO_3^- have been determined using a trap and purge system interfaced with a GV IsoPrime Stable Isotope Ratio Mass Spectrometer. Data are reported relative to the conventional international standards, V-PDB for carbon, SMOW for oxygen and atmospheric nitrogen according to the following equations.

$$\delta^{15}N = (^{15}N/^{14}N \text{ sample} / {}^{15}N/^{14}N \text{ standard} -1)* 1000$$

$$\delta^{13}C = (^{13}C/^{12}C \text{ sample} / {}^{13}C/^{12}C \text{ standard} -1)* 1000$$

$$\delta^{18}O = (^{18}O/^{16}O \text{ sample} / {}^{18}O/^{16}O \text{ standard} -1)* 1000$$

Average values for each sample were determined and the standard deviation calculated. Samples that had standard deviations which were greater than two standard deviations

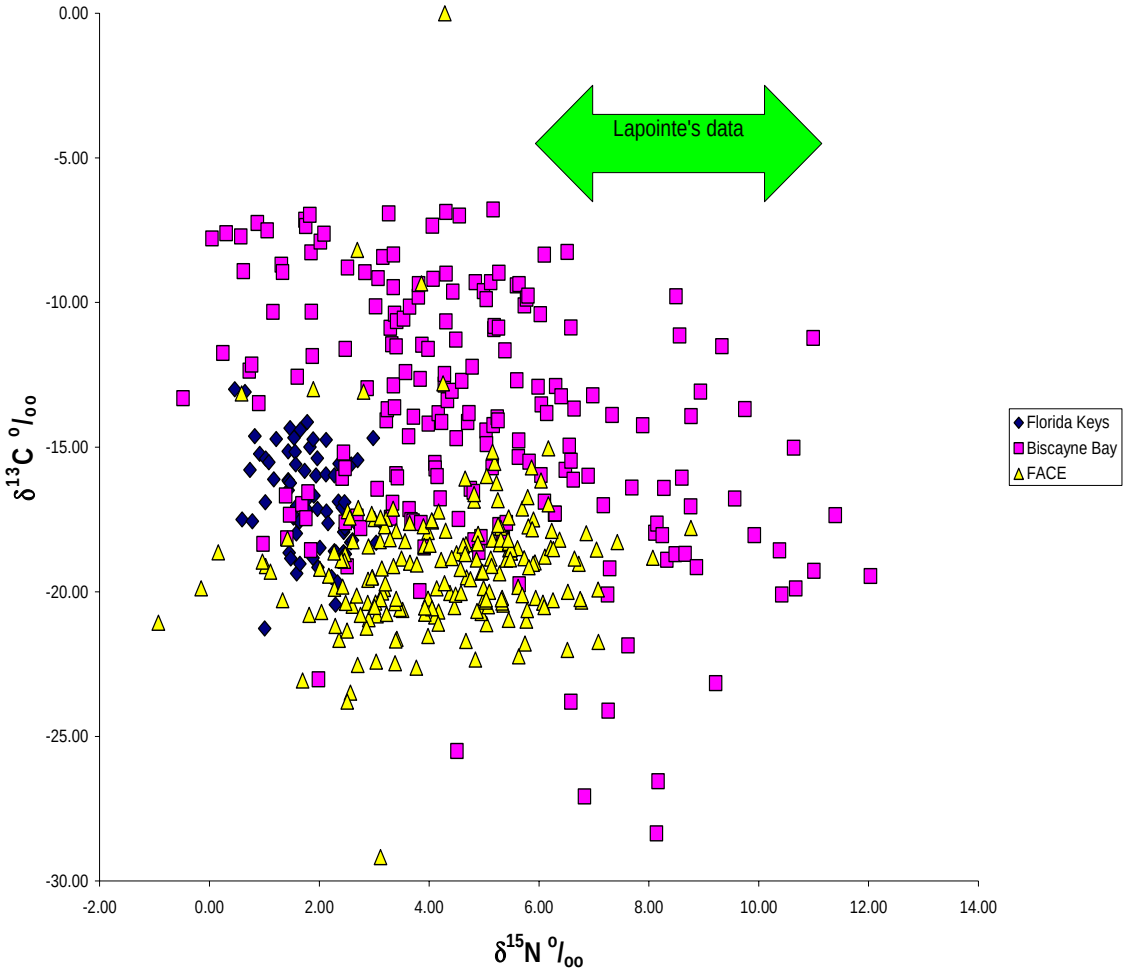


Figure 2: Comparison of C and N isotopic composition of algae from FACE (Figure 1), and samples collected from Biscayne Bay and the Florida Reef tract (unpublished data). The FACE data fall intermediate between Biscayne Bay (known to be contaminated with anthropogenic nitrogen and the Florida reef tract. Data from Lapointe (1997) are shown for comparison Lapointe did not published any carbon isotopic data.

from the mean were mark for reanalysis.

Seawater

Seawater samples were collected from surface and bottom water on board the Nancy Foster. The Stable Isotope Laboratory is the process of developing our facilities to process these data. Implementation of this method at Miami will await funding of new instrumentation. In the interim, Ms. Drayer traveled to New Bedford, Massachusetts in August 2007 and October 2007 to process these samples in a laboratory that is currently more equipped for such analyses. The procedure used for determining the $\delta^{15}N$ in

various dissolved nitrogen species is as follows; Nitrate is converted to nitrite through cadmium reduction and then to nitrous oxide with a 1:1 azide and acetic acid solution. The gas (N_2O) is then analyzed on a stable isotope ratio mass spectrometer (Mcllvin and Altabet, 2005). Additionally, an ammonium analysis can be completed by a step-wise hypobromite oxidation under basic conditions prior to the azide injection (Zhang et al., 2006). The nitrogen isotopic composition of dissolved organic nitrogen (DON) can also be determined by preceding the initial cadmium reduction with a persulfate digestion. Isotopic values for individual nitrogen species are then determined from a weighted average calculated from nitrite concentrations.

During visits to New Bedford in August and October 2007, all samples were analyzed for $\delta^{15}N$ of ammonia, nitrate and DON. Not all analyses were successful as a result of analytical and instrumental problems. Such problems will arise as the analyses are not carried out in house. In house capabilities will need new instrumentation.

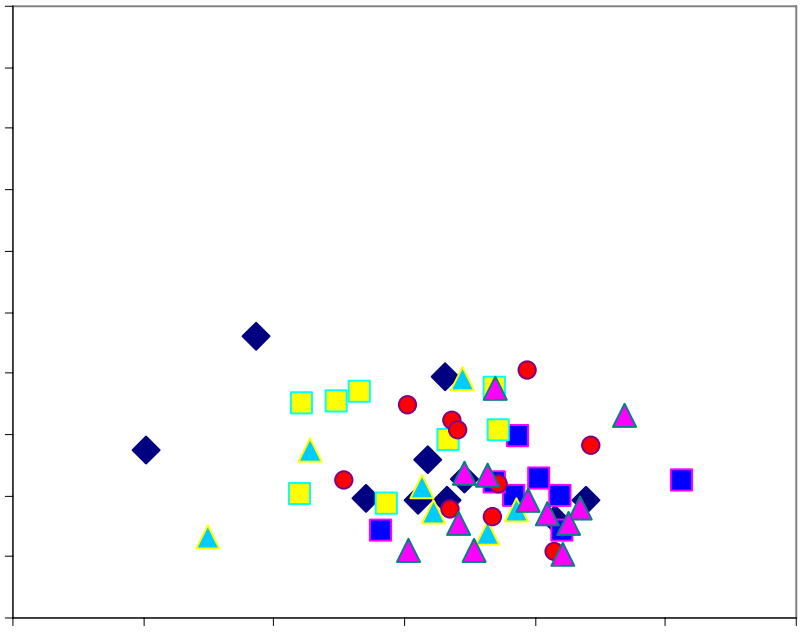


Figure 3: Carbon and nitrogen isotopic composition of organic material from sediment samples. There does not appear to be any statistically significant difference between the samples.

Results

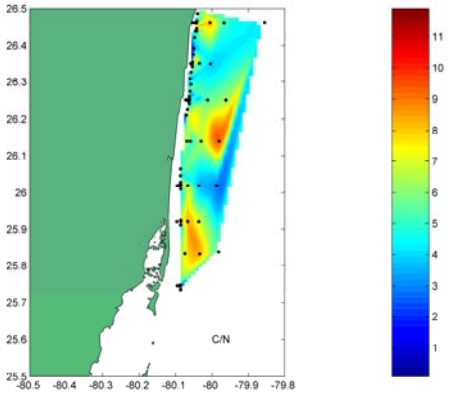


Figure 4a: Distribution of CN in organic material from sediment samples

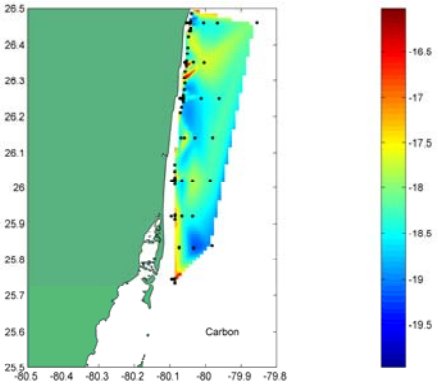


Figure 4b: Carbon isotopic composition of organic material in sediment samples

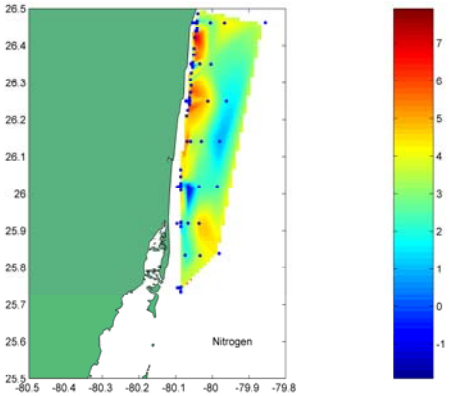


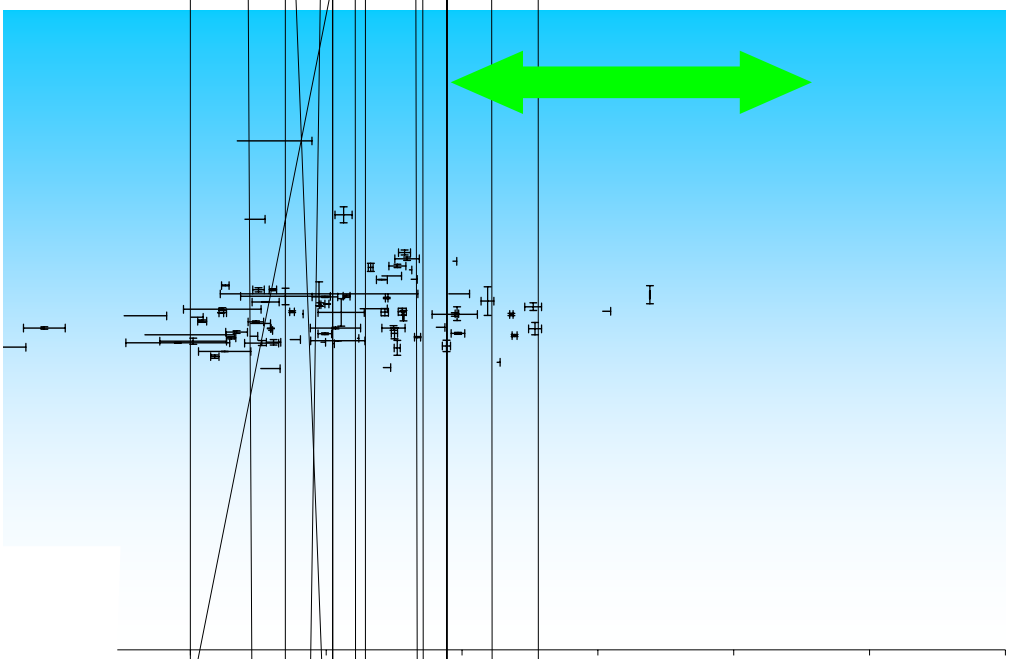
Figure 4c: Nitrogen isotopic composition of organic material in sediment samples

Algae: The mean $\delta^{15}N$ value for all algae samples is + 4.21 (+/- 1.62, n=206). The mean $\delta^{13}C$ value for all algae samples is - 19.36 (+/- 2.62, n=206). These data combined with data from Biscayne Bay and Florida Reef tract are shown in Figure 2.

2000; Thornton and McManus, 1994; Rogers, 2003). A more distinctive characteristic of this sewage derived POM is its depleted $\delta^{13}C$ value, ~ -22 to -27 ‰. Larger enrichments in $\delta^{15}N$ take place during the volatilization of NH_4^+ to NH_3 which occurs at high pH values, typical in animal waste (Heaton, 1986). As a result, ^{14}N is preferentially lost from the solution and the residual NH_4^+ becomes elevated in ^{15}N leading to $\delta^{15}N$ values of > +10 ‰. The NH_4^+ is utilized preferentially by algae and thus this isotopic signature may be reflected in the $\delta^{15}N$ of the algae tissues. If the NH_4^+ is not assimilated by organisms, it is eventually converted to nitrate (nitrification). The process of nitrification fractionates the NH_4^+ leaving it enriched in ^{15}N and the NO_3^-, enriched in ^{14}N (α= ~ 1.020 to 1.035). If all the NH_4^+ is converted to NO_3^- then the $\delta^{15}N$ of the NO_3^- will be the same as the original NH_4^+. Normally however, only a portion of the NH_4^+ is converted to NO_3^-. The NH_4^+ then becomes isotopically enriched in ^{15}N and the NO_3^- depleted. Similar mechanisms occur during denitrification, with the ^{14}N produced being isotopically light and the residual NO_3^- enriched. Enrichment through denitrification is believed to be cause of isotopic enrichments observed in deep waters. Such waters, which regularly upwell off the Florida Keys have reported $\delta^{15}N$ value of + 5.25‰ in contrast to + 4.24 ‰ in the surface waters (Leichter et al., 2007). Once on the shelf the nutrient-rich water mixes with water of lower nutrient concentration and lower $\delta^{15}N$ and the NO_3^- is rapidly assimilated by algae and other organisms. It is likely that as the concentration of nitrate decreases further that the $\delta^{15}N$ will increase further as a result of fractionation during assimilation. Such values have been suggested to be reflected in the $\delta^{15}N$ of various marine organisms. Benthic algae, which directly assimilate nitrate and ammonia, incorporate the $\delta^{15}N$ with only minimal amount of fractionation and are therefore believed to realistically record the ambient $\delta^{15}N$ of the DIN. In situations where elevated $\delta^{15}N$ values have been recorded in benthic macro algae, these have been interpreted as having been influenced by sewage derived nutrients (Lapointe, 1997; Costanzo et al., 2001). In contrast organisms feeding higher up the food chain become enriched in $\delta^{15}N$ by between 2-4 ‰ per trophic level (DeNiro and Epstein, 1981) by natural means. Utilization of the $\delta^{15}N$ in these organisms as indicators of the origin of nitrogen needs to take into consideration the organisms trophic position. For example, the $\delta^{15}N$ of coral tissues is enriched relative to the zooplankton on which the corals feed as well as the zooxanthellae which translocate organic compounds to the coral (Swart et al., 2005). The absolute $\delta^{15}N$ therefore is dependent upon the $\delta^{15}N$ of the base of the foodweb, the POM and the trophic level. In spite of uncertainty of trophic enrichment, various workers have attempted to use higher trophic level organisms, such as sponges, crustaceans, and corals as possible indicators of sewage input. In the case of the corals, Heikoop et al (2000) separated corals derived from reefs affected and unaffected, by sewage. The affected reefs had consistently higher $\delta^{15}N$ values (+8 ‰) compared to the unaffected or reference reefs (+ 5‰). In another example, the $\delta^{15}N$ of crustaceans was documented to decrease from + 12 to +8 ‰ with increasing distance away from a potential sewage source (Risk and Erdmann, 2000). Ward-Paige et al (2005b) compared the $\delta^{15}N$ of sponges growing at various locations along the Florida Keys with one sponge sample from Belize. They interpreted the higher mean values of the Florida sponges ~ +5 ‰ compared to the one example from Belize (+ 2.2 ‰) as reflecting a greater contribution from anthropogenic nutrients. In a related study (Ward-Paige et al. 2005a), the $\delta^{15}N$ and

rganic material from gorgonians was measured over a 20 year time period in
ch had consistently higher and lower nutrient values. These workers noted that
with higher nutrients possessed slightly higher $\delta^{15}N$ values (+3.9 vs. +2.9 ‰)
he values in the gorgonians with the heavier values had increased by ~ 1 ‰ over
0 years. In the most recent work on the Florida Reef tract (Lamb, 2007; Lamb
t, 2008), data was presented which shows that there is little anthropogenic
aterial influencing the reef communities. Since the particulate organic material
the major source of inorganic nutrients, it seems unlikely that human sewage
or impact on the reefs in this location at least.

ata: Considering that the stretch of the Florida coast between Miami and



is considered to have a heavy loading of anthropogenically derived
ng from numerous sewage outfalls, it is surprising that the algae and
rganic material in the sediments shows no indication of unusually
n. In addition there appears to be no indication of an isotopically

positive signature in the $\delta^{15}N$ of the nitrate. In comparison with algal data from Biscayne Bay and the Florida Reef tract, the algal FACE samples fall in an intermediary position, the $\delta^{15}N$ values being considerably more negative than those measured by Lapointe (1997), but slightly more positive than data from the Florida reef tract. The sediment data, which integrate the average input of organic carbon and nitrogen, have even more negative values (+3.86 ‰) (Figure 3 & 4). From the distribution maps shown in Figure 4, it appears that the heaviest $\delta^{15}N$ values in the sediments occur closest to the coast and perhaps associated with the sewage discharge areas. The sediment organic material is better at integrating the $\delta^{15}N$ rather than the $\delta^{15}N$ of algae which is dependent upon seasonal variations in environmental parameters. The relatively negative $\delta^{15}N$ of the nitrate can be explained by the positive $\delta^{15}N$ of the NH_4^+ (+9 to + 30 ‰). The difference between the NO_3^- and NH_4^+ is best explained by the process of nitrification which produces isotopically depleted NO_3^- while enriching the $\delta^{15}N$ of the residual NH_4^+. The range of the $\delta^{15}N$ of the nitrate reflects mixing between nitrate produced by nitrification and nitrate originating from upwelling.

Preliminary Conclusions

The data acquired to date suggests the following

1. Large differences occur between the $\delta^{15}N$ of nitrate and ammonia with the ammonia being significantly more positive (+20 to +30‰) compared to the nitrate (-4 to +4 ‰). These differences arise as a result of fractionation during the nitrification of the ammonia. The ammonium already has a positive $\delta^{15}N$ value as a result of the positive values of anthropogenic waste material and fractionation during the volatilization of ammonium at high pH values.
2. All algae and sediment samples possessed value between 0 and + 6 ‰. Such values are not unusually positive and do not suggest that the ammonia, which has very positive values is significantly influencing the nitrogen isotopic composition. Instead the algae are utilizing a mixture of ammonium and nitrate. Nitrate derived from nitrification has relative negative isotopic composition (- 4 to + 4 ‰), while upwelling nitrate has a more positive $\delta^{15}N$ (~ + 6 to +10 ‰).
3. The sediment $\delta^{15}N$ data, while not being unusually positive, do show slightly higher values associated with discharge sites. This pattern of distribution needs to be studied further in order to ascertain whether it is real or a result of a sampling artifact.

4. References

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