The Impact of Anthropogenic Waste on the Florida Reef Tract

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THE IMPACT OF SEWAGE-DERIVED NUTRIENTS ON THE FLORIDA KEYS NATIONAL MARINE SANCTUARY

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Executive Summary

There is a growing perception by both scientific and recreational users of the Florida Keys National Marine Sanctuary (FKNMS) that coral reefs in the area are in a state of decline. Although there have been various hypotheses proposed to explain the perceived decline, some of the most plausible ideas are related to a combination of increased nutrient input into the reef system and their consequent promotion of algal growth combined with a pronounced reduction in the occurrence of the herbivorous sea urchin *Diadema anitllarum.* In addition to runoff, outfalls, and live-aboard boats, a major source of nutrients in the Florida Keys is the large number of septic systems. These have increased over the past decades as a result of the huge increase in tourism and population in the area. It is suggested that these septic systems leak wastewaters into the groundwater, eventually negatively impacting the reefs by various mechanisms. The work which has been carried out has attempted to trace components of this human waste in the reef environment along several transects from the Florida Keys toward the reef tract and therefore demonstrate unequivocally whether this hypothesis is correct. Specifically we have (i) attempted to use the organic compound coprostanol to detect the presence of septic tank sewage in the sediments, (ii) determined the origin (fixed atmospheric nitrogen, recycled municipal waste or upwelled nitrogen) of the nitrogen in sedimentary organic material as an indicator of the source of nitrogen in the corals and other biota based on the nitrogen isotopic composition, and (iii) determined the relative imporatnce of input of nitrogen compounds from Florida Bay.

Our investigations have revealed the following.

- No unusually high $\delta^{15}N$ values which could be ascribed to major input of anthropogenic waste into the reef environment
- Low coprostanol values throughout the study area with the exception of isolated sites in some canals in the keys.
- Occasional high $\delta^{15}N$ and Coprostanol, but generally no correlation between the two.
- No specific evidence of inputs of allochthonous nitrogen from Florida Bay

Based on our data we offer the following recommendations:

- The data produced by this study indicate that there is no discernable direct evidence for sewage contaminants from the Florida Keys reaching the reef tract. This result is in agreement with a smaller previous study conducted by the US EPA (Ferry, unpublished EPA study).

- The nitrogen cycle is not well understood in coral reef systems and attempts should be made to understand it thoroughly before a rush to judgement is made regarding $\delta^{15}N$. Prior to these studies we cannot recommend the use of $\delta^{15}N$ to indicate levels of pollution in coral reef environments.

- Sewage sterols (& other domestic pollution markers - eg. trialkylamines) are useful in assessing transport to open sea (issue of stability -microbial degradation rate- in warm, sub-tropical waters?). However, as a result of the instability of coprostonal in oxic environments it might not be useful to use this tracer in coral reef environments.

- There may be other anthropogenic activities which are adversely affecting the nutrient regime locally and may be impacting the health of corals and other biota.

- The nitrogen isotopic composition of sedimentary organic matter was unable to unequivocally assess the nutrient input to the Florida reef tract from Florida Bay.

- A comprehensive study of the nutrient dynamics in the Florida reef tract is required in order to definitively assess the impact of recent development in the Florida Keys on the health of the reefs in the area.

I. INTRODUCTION

Coral reefs are highly productive ecosystems which out compete other ecosystems in tropical oligotrophic waters. Although their ability to do this is not completely understood, various symbiotic relationships between nutrient fixing organisms and higher trophic levels are known to exist. These systems have been shown to be quite sensitive to eutrophic conditions, and nutrient levels as low as 0.1-0.2 μM dissolved inorganic phosphorous and 1μM dissolved inorganic nitrogen were found to have adverse effects on reefs (Bell, 1992). The impact of eutrophication on coral reefs can take many forms, some of which may not be immediately obvious. Initially, phytoplankton production will increase which can reduce light penetration and increase direct detrital sediment deposition, effectively smothering the coral (Endean, 1976). Increased organic sedimentation encourages filter feeders which compete with corals for space. Increases in water column nutrients also promote the growth of macroalgae. Numerous case studies have documented the adverse impact of macroalgal overgrowth due to nutrient enrichment from Hawaii (Banner, 1974), Bermuda (Lapointe and O'Connell, 1989), Barbados (Tomascik and Sander, 1985; 1987), the Seychelles (Littler *et al.,* 1991), the Red Sea (Merenger, 1981), and the Florida Keys (Lapointe and Clark, 1992, and Lapointe *et al.,* 1994) to name a few. Eutrophication may also inhibit recruitment and re-establishment of coral reefs which may not be immediately detected. Following storm damage to the reef, macroalgal communities may be able to take advantage of the enriched waters to establish themselves, thereby displacing the corals (Gabric and Bell, 1993).

Several recent studies have attempted to determine the extent to which sewage-derived contaminants reach offshore areas in the FKNMS; however, there is little consensus on this issue. One important mechanism for anthropogenic contaminant transport from the source area (Florida Keys development) to the potentially impacted areas (Sanctuary reef areas) is through groundwater flow. Lapointe *et al.* (1990) looked at the effect of on-site sewage disposal systems on groundwater in the Florida Keys, finding dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) levels in groundwater impacted by septic systems to be 4000 and 70 times greater, respectively, than groundwater in control areas. The level of SRP in groundwater is believed to be attenuated by adsorption in carbonate strata. The fate of sewage effluent injected into Class V wells in the Florida Keys has been studied by Shinn *et al.* (1994). Elevated levels of nitrogen (as ammonium) were found in groundwater as far as 4 miles offshore. Fecal coliform and streptococcal bacteria were also detected in several offshore wells. A ratio of fecal coliform to fecal streptococcal bacteria greater than 4 is considered indicative of human waste, and such ratios were measured as far as 4 miles offshore. The authors concede that definitive conclusions can not be drawn from these data because of uncertainties in coliform survival rates in marine environments; however, they state that this question warrants further investigation. They propose a tidal pumping mechanism driven by hydrostatic differences between Florida Bay and the Straits of Florida. The data suggest that areas of permeable Holocene reefs and limestone bedrock devoid of lime-mud sediment have the highest transmisivities, and therefore will be most impacted by groundwater nutrients. These areas correspond to a one-half mile wide nearshore belt of exposed Key Largo limestone, a narrow belt along the seaward side of the outermost reef bank, and the Holocene patch reefs which grow on topographic highs. These areas include most of the coral reef habitat within the Sanctuary, and indicate the existence of a significant pathway for sewage-derived contaminant impact on vital Sanctuary resources.

Several researchers have studied the levels of water column and sediment nutrients in the Florida Keys with contradictory results. Lapointe and Clark (1992) found elevated levels of dissolved and particulate nitrogen as far as 3 km from land, and concluded that anthropogenic nutrients were responsible for critically eutrophic conditions resulting in algal blooms, seagrass epiphytation and die-off, and declines in coral coverage in patch and bank reefs. More recently Szmant and Forrester (1996) reported slightly elevated DIN levels (~1μM NO_3^-) in nearshore areas which dropped off to oligotrophic levels (<0.25μM NO_3^-) within ~0.5 km of the shoreline. While they argue that essentially all water column nutrients are consumed by nearshore algal and seagrass communities, they admit that the potential for groundwater transport of nutrients has not been thoroughly addressed. Another recent study (Lapointe, 1997) measured DIN levels as high as 3.4 μM around algal blooms of the chlorophyte *Codium isthmocladium* on deep reefs 3 km offshore of Palm Beach county, Florida. In an attempt to determine the source of nutrients, Lapointe measured $\delta^{15}N$ values in *C. isthmocladium* tissues from one site over a five-month bloom period in 1995. He reported $\delta^{15}N$ values from +10.0 to +12.0 ‰ (vs. atmospheric N_2 std.), and from this concluded that the primary nutrient source was from wastewater-contaminated groundwater. Although the stable isotopic approach is quite valid for determination of organic source materials, for constituents with complex biogeochemical pathways and potentially multiple sources the results can be equivocal. The $\delta^{15}N$ values that Lapointe measured are too close to the expected values for nutrient inputs from upwelled waters from the Straits of Florida to independently substantiate his claim for a wastewater source.

The dramatic increase in human population and proportional increase of wastewater disposal in the Florida Keys is well documented. The potential for sewage-derived nutrients to impact the coral reef resources of the FKNMS is consistent with widely held views of the effects of eutrophication on coral reef communities, which prompted the US EPA to initiate this special study. A well-defined pathway and mechanism for groundwater transport of wastewater nutrients was postulated. Although there was a great deal of anecdotal evidence for increases in algal blooms, seagrass die-offs, and declines in coral reef health, the scientific evidence remained ambiguous. There was clearly a need for a definitive study to identify the magnitude of nutrient inputs from various sources to the coral reef communities within the FKNMS.

Detection of the presence of sewage contamination using coprostanol

Historically, sewage contamination in the marine environment has been established by counting fecal indicator (coliform) bacteria in water and sediment samples of affected sites. However, the extreme variability of coliform survival under varying environmental conditions (Rhodes and Kator, 1988) leads to considerable unreliability using this approach in coastal waters or in sediments (Doyle *et al.*, 1992). Environmental compartments such as marine sediments, suspended particulate matter, and water contain complex mixtures of sterols (Mackenzie *et al.*, 1982) which are often difficult to apportion to source because of commonality in different organisms and also due to subsequent diagenetic change in the environment. Coprostanol (5-beta-cholestan-3-beta-ol), formed in the gut of higher animals during the fecal metabolism of cholesterol, has been used extensively as a molecular marker for sewage contamination in the coastal environment, because of its relatively refractory nature in sediments (Hatcher and McGillivray, 1979; Venkatesan and Kaplan, 1990; Writer *et al.*, 1995; Nichols *et al.*, 1996). Sedimentary levels of this compound ranging from below detection limits (ng/g) to > 40 μg/g have been found in U.S. coastal waters, with levels greater than about 0.010 μg/g (a natural background level for marine sediments) being considered to be indicative of sewage pollution. Coprostanol and its related compounds cholesterol and cholestanol are themselves not contaminants of any concern, but merely indicators of both treated and untreated sewage sources. We will accordingly use these as proxies for the nutrient loads associated with these sources. It is the impact of the waste nutrients that has the most direct potential to disrupt the nutritive balance of offshore reef systems.

Sterols in general are poorly soluble in water thus coprostanol tends to associate with particulate matter and accumulate in sediments impacted by wastewater sources. Although resistant, coprostanol is known to be slowly degraded, especially in aerobic sediments. Other long lived sedimentary markers such as trialkylamines, which are present as trace components as the cationic surfactants of common chemical detergents, washing powders and fabric softeners, have also been used (Fernandez *et al.*, 1991; Chalaux *et al.*, 1992) along with the fecal sterols as indicators of the extent of sewage and waste water nutrient input.

Determination of the origin of nitrogen species in the FKNMS

The nitrogen isotopic composition ($\delta^{15}N$) of an organism's tissues approximates the $\delta^{15}N$ of its diet. In addition there is in most cases a slight enrichment as $\delta^{15}N$ depleted nitrogen in the form of urea and ammonia is lost. Field studies show that there is an average of 3.2 ‰ enrichment in $\delta^{15}N$ per trophic level. Nitrogen in excreted waste is mainly in the form of urea which is hydrolyzed to ammonia and converted to nitrate. During hydrolysis $\delta^{15}N$ depleted ammonia is lost by volatilization, causing the residual ammonium to be enriched in $\delta^{15}N$. This ammonium is converted to $\delta^{15}N$-enriched nitrate with $\delta^{15}N$ values from +10 to +20‰ (Heaton ,1986). The extent of enrichment in $\delta^{15}N$ is influenced by a range of factors and it is therefore not practical to assign a precise value for $\delta^{15}N$ in sewage. However, values are invariably higher than +10‰ (Heaton, 1986). The nitrogen isotopic method has been used to distinguish nitrogen derived from fertilizers (0‰) (Shearer et al. 1974; Kreitler, 1979; Heaton, 1986), nitrates produced from the oxidation of nitrogen waste (+10 to +22‰) (Kreitler ,1979, Lindau and Spalding 1984), and nitrates produced from the oxidation of organic nitrogen in the soil (+4 to +9‰) (Gormley and Spalding, 1979, Mariotti ,1974)

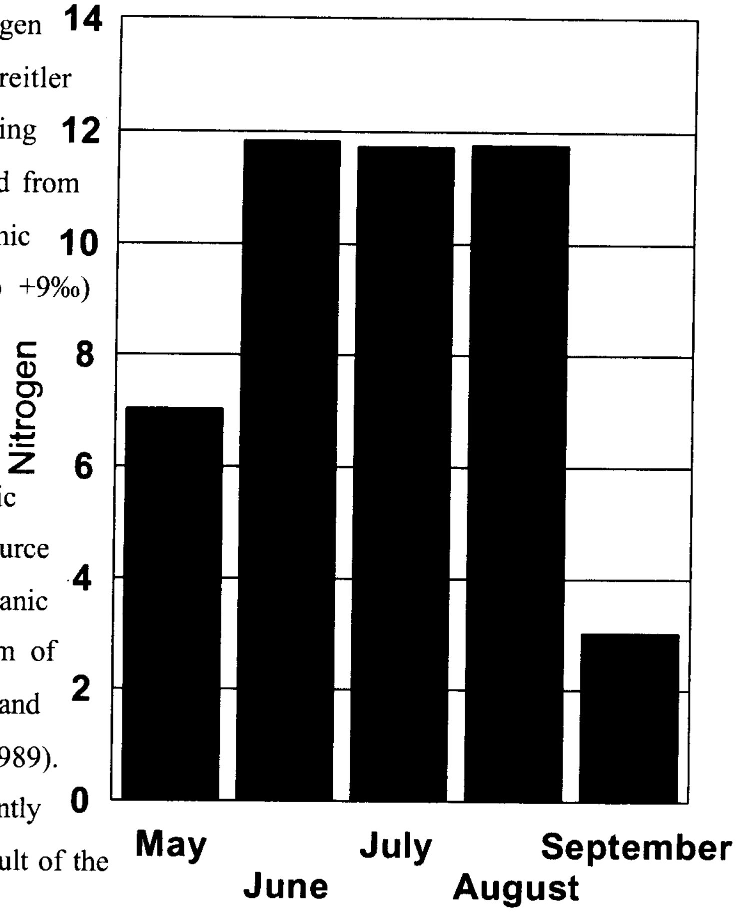

Figure 1: Data from Lapointe et al (1997) showing relatively positive N-isotopes which were suggested resulted from anthropogenic influences.

In the absence of significant anthropogenic input, the most important source of nitrogen to the oceanic euphotic zone is in the form of upwelled nitrate (Altabet and McCarthy 1985, Altabet 1989). The nitrogen is frequently isotopically enriched as a result of the process of denitrification. Hence the $\delta^{15}N$ is often quite high in oxygen depleted waters (Cline and Kaplan 1975, Liu and Kaplan 1989) and it is likely that high $\delta^{15}N$ will be found in organisms from these areas. Although the nitrogen isotopic composition has not been measured in waters from the Florida Keys, tropical North Atlantic water has nitrogen isotopic compositions between +7 and +10 ‰ (Liu and Kaplan, 1989). Hence we suggest that the two $\delta^{15}N$ end-members likely to influence the nitrate dynamics of the Florida Keys are isotopically distinct, (i) relatively positive nitrogen isotopic values derived from anthropogenic waste (+10 to +20‰), and (ii) slightly lighter nitrogen stemming from the upwelling of nitrate rich waters (+5 to +10‰). Stable nitrogen isotope analysis of organic matter has been successfully applied to determine the extent of sewage contamination in lacustrine (Coakley *et al.*, 1992, and Bachtiar *et al.*, 1996) and coastal marine environments (Sweeney *et al.*, 1980, and Van Dover *et al.*, 1992). The proposed study follows an approach similar to those taken by these authors.

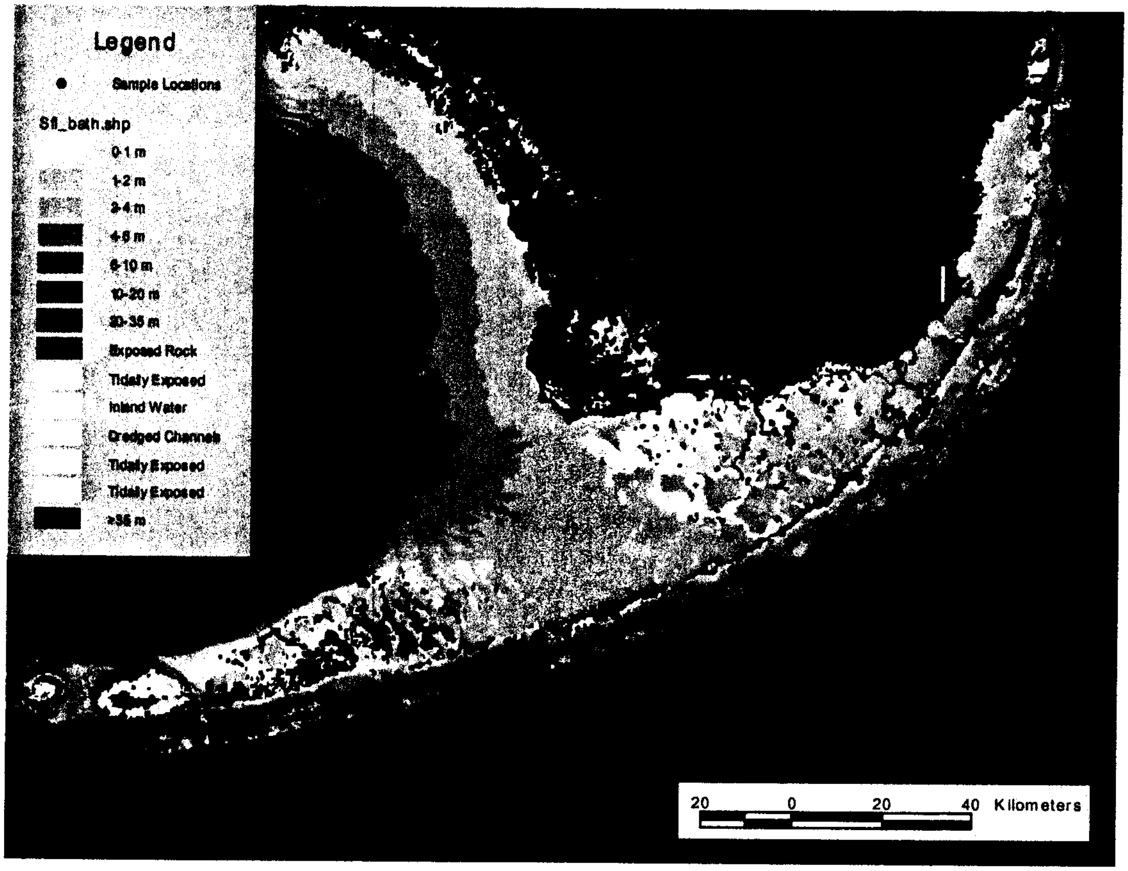

Figure 2: Representative sampling locations for suspended particulate matter and bottom sediments.

Although an attempt has been made to determine the source of nitrogen at one coral reef site in South Florida (Lapointe, 1997) (Fig. 1), we believe that the conclusion that wastewater nutrients are reaching the reef is not supported by the data presented in the work of Lapointe (1997). In order to resolve the uncertainties regarding these data a more thorough survey is needed, such as that carried out in this study. In particular it is important to examine changes in the $\delta^{15}N$ of the particulate organic nitrogen (PON) and organic material in the sediment as one moves away from the nitrogen sources in the Keys towards the potential source of nitrogen associated with upwelling. Without this information it would not be possible to unequivocally state whether values of +10 ‰ resulted from an input of nitrogen associated with sewage or from upwelling. Using both the $\delta^{13}C$ and $\delta^{15}N$ of the POM will enable us to distinguish between carbon and nitrogen derived from anthropogenic and mangrove sources (important for assessing the relative influence of Florida Bay), and also nitrogen added by fixation and upwelling. For example, the $\delta^{15}N$ of POM derived from mangrove sources can be expected to be close to 0 ‰, while its $\delta^{13}C$ is highly depleted (-25 to -30 ‰). These values will distinguish the organic material from Florida Bay (predominately mangrove detritus) from both anthropogenic and marine sources.

Determination of the relative importance of Florida Bay water in the FKNMS

Florida Bay outflow in the middle Keys (Long Key and nearby passes), and West Florida Shelf waters through the lower Keys passages (Mossier Channel, Bahia Honda) are potential sources of nutrients and turbidity to reef areas, and result in elevated nutrient and turbidity levels further offshore in the middle Keys (Szmant and Forrester 1996). Recent Florida Bay seagrass die-offs and algal blooms (Fourqurean et al 1992, Roblee *et al* 1992) have raised concerns that degraded Florida Bay waters may be reaching reef areas and promoting harmful algal blooms as far away as Looe Key Reef. The field sampling design of this study specifically targets sites which are representative of Florida Bay discharge into the FKNMS. Based on nutrient levels and isotopic signatures along this gradient (as well as all other survey sites) the potential for Florida Bay nutrient contribution will be assessed.

II. METHODS AND APPROACH

Fieldwork

We collected bottom sediment and suspended particulate samples from 50 sites located throughout the FKNMS. Because sediments tend to integrate short-term variations, little seasonal variation in sediment chemistry is expected, and therefore bottom sediments were collected from each site at one time throughout the study period. Particulate organic matter, which does vary seasonally, was collected at two times each year; once during each wet and dry season. These samples are being collected from approximately the same locations as the sediment samples. A representative set of sample locations is shown in Figure 2.

Sample collection was carried out from our 22' Boston Whaler and from the 57' R/V *Calanus* (University of Miami) and from selected roadside locations. Surficial sediment samples were collected by a Halltech dredge sampler and a subsample of the homogenized material was retained to yield a representative sample. POM samples were collected by filtering up to 20L of water (depending on POM concentration) through 0.45 μm precombusted glass-fiber filters in the field. As a result of the large amount of water necessary to obtain sufficient sample to analyze the particulate matter, we filtered the water samples in the field using a small portable pump.

Sample preparation

Surface sediment and particulate matter samples were prepared for isotopic analysis following a widely published method for organic materials (see for example Cifuentes *et al.*, 1988). This method involves the removal of carbonate carbon in 0.2 N HCl, followed by rinsing with distilled water and oven drying at low temperature (~60°C).

Surface sediment and particulate matter samples are subjected to an extraction and clean-up procedure prior to the identification and quantitation of the sterols by GC and GC-MS. Sterols are being extracted using slight modifications of established procedures (Writer *et al.*, 1995; Nichols *et al.*, 1996). Briefly, this involves saponification/extraction (0.5N KOH in MeOH), the extract then being filtered to remove sediment, subject to neutralization with HCl then partitioning into methylene chloride. The methylene chloride extract, taken to dryness under a N_2 stream, then is applied as a hexane solution to a packed column of neutral silica over neutral alumina. The sterol fraction, isolated by methanol elution, is converted to their corresponding OTMSi (trimethylsilyl) ethers by treatment with N,O-bis(trimethyl silyl) -trifluoroacetamide (BTSFA).

Sample analysis

The organic carbon and nitrogen contents as well as the isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of sediment and particulate matter is determined using a modified CN analyzer interfaced with a continuous-flow isotope-ratio mass spectrometer (CFIRMS) (Europa Scientific). This provides the C/N ratio of the samples in addition to the $\delta^{13}C$ content of the organic carbon (measured as CO_2) and the $\delta^{15}N$ content of the organic nitrogen (measured as N_2). The $\delta^{13}C$ may not be diagnostic of the nitrogen source, but provides information on the source of organic carbon.

Sterols are analyzed by manual, splitless, injection onto a Hewlett Packard 5890 GC coupled to a HP5971 MSD quadropole mass spectrometer using a 30 or 60m DB-5 column. The sterols are quantified against a gravimetric perdueuterated cholesterol-d7 standard, and against a benzo[b]fluoranthene internal standard. An external sterol standard mixture of coprostanol, epicoprostanol, cholesterol, and cholestanol is used as an extraction and procedural recovery check. Selected ion monitoring (SIM) response factors have been established for copropstanol and epicosprostanol. Quantification of other expected sterols will be made using the response factor of coprostanol for the purposes of enabling sterol ratios in the samples to be examined.

The closest equivalent EPA method for this analysis is U.S. EPA Method 3550A, which employs sonication extraction, prior to TMS derivitization.

A selection of the sediment samples were investigated for the occurrence of trialkylamine surfactants. These compounds were extracted by the same procedure as for the sterols, but eluting from the silica-alumina columns by methanol-ether (Chalaux et al., 1992). Their identification was confirmed using GC-MS analysis (derivitization not necessary).

Data processing and interpretation

The stable isotope data have been interpreted using a simple mixing model to determine the relative contributions of various nutrient sources throughout the FKNMS. This multiple tracer mixing model approach has been applied in the Santa Monica Basin (Williams *et al.*, 1992) to successfully discriminate between sewage and marine nutrient sources in dissolved and particulate organic matter.

The results of the sterol analyses provide an independent means for the estimation of relative amounts of these fecal sterol markers in the sediments over the spatial area examined rather than their precise quantitation. The use of sterol ratios (Hatcher and

McGillivary, 1979) has been suggested as a way of removing any bias induced by the preferential association of coprostanol with sediments of varying particle size or organic carbon content.

III. SAMPLES

Field sampling trips for bottom sediment and suspended particulate matter have been successfully completed on the dates shown in Table 1. The samples collected in June 2000 were collected as part of an EPA funded study, which supported the formation of the National Center for Caribbean Coral Reef research. All samples collected to date have been analyzed for organic carbon and nitrogen content and isotopic composition. Sterol extraction methods have been optimized and standard quantitation for sterol analyses has been performed. Selected samples have been extracted for sterol analysis. Spatial data have been entered into a Geographical Information System (GIS) database (Arcview 3.1).

	Date	POM	Sediments
Summer 1998	7/20/1998- 7/29/1998	X	
Fall 1998	10/20/1998- 10/22/1998	X	
Winter 1998	12/15/1998 2/9/1999 2/23/1999- 2/26/1999	X	
Spring 1999	4/16/1999 4/27/1999- 4/28/1999	X	
Summer 1999	6/8/1999 6/10/1999 8/3/1999 9/27/1999	X	X
Summer 2000	6/9/2000- 6/12/2000	x	

Table 1: Sampling scheme

IV. RESULTS

Results of analyses on the $\delta^{13}C$ and $\delta^{15}N$ analysis of the POM and the sediment as well as the coprostanol analysis of the POM are listed in Appendix I. Plots of the data showing the spatial distribution of these parameters are shown in Appendix II. A summary of the mean value, standard deviations, and ranges of the data are shown in Table 2, 3, and 4.

	N (mg/l)	N s.d.	Range	$\delta^{15}N$	$\delta^{15}N$ s.d.	Range
Summer 98	0.03	0.25	0.001-0.142	2.6	1.6	0.6-9.4
Fall 98	0.06	0.10	0.001-0.109	1.4	0.9	-0.44-2.9
Winter 98	0.05	0.04	0.011-0.203	2.9	1.8	-0.46-6.8
Spring 99	0.01			4.4	2.6	-3.6-9.2
Summer 99	0.13	0.03	0.106-0.190	2.7	0.8	1.22-4.0
Summer 00				2.9	1.1	1.17-5.0

Table 2

	C (mg/l)	C s.d.	Range	$\delta^{13}C$	$\delta^{13}C$ s.d.	Range
Summer 98	0.15	0.18	0.05-1.03	-18.18	3.4	-12.6 - -30.9
Fall 98	0.26	0.43	0.00-2.03	-26.20	4.0	-19.2 - -34.7
Winter 98	0.24	0.28	0.05-1.51	-26.65	6.4	-12.6 - -35.2
Spring 99	0.50			-23.64	4.3	-18.4 - -35.6
Summer 99	0.47	0.30	0.23-1.01	-18.90	1.6	-14.0 - -20.9
Summer 00				-19.55	2.6	-15.8-- 24.12

Table 3

	Coprostanol ppm	s.d.	Range
Summer 98	0.086	0.162	0.00-0.740
Fall 98	0.587	2.56	0.00-11.15
Winter 98	0.060	0.202	0.00- 1.18
Spring 99	0.009	0.020	0.00-0.08
Summer 99	0.009	0.018	0.00-0.06

Table 4

V. DISCUSSION

In spite of the suggestion that the $\delta^{15}N$ of organic material might be used as an indicator of anthropogenic pollution in the marine environment, the values of $\delta^{15}N$ measured in this study fell within a comparative narrow range and showed no excessively high values which suggested direct influence of sewage.

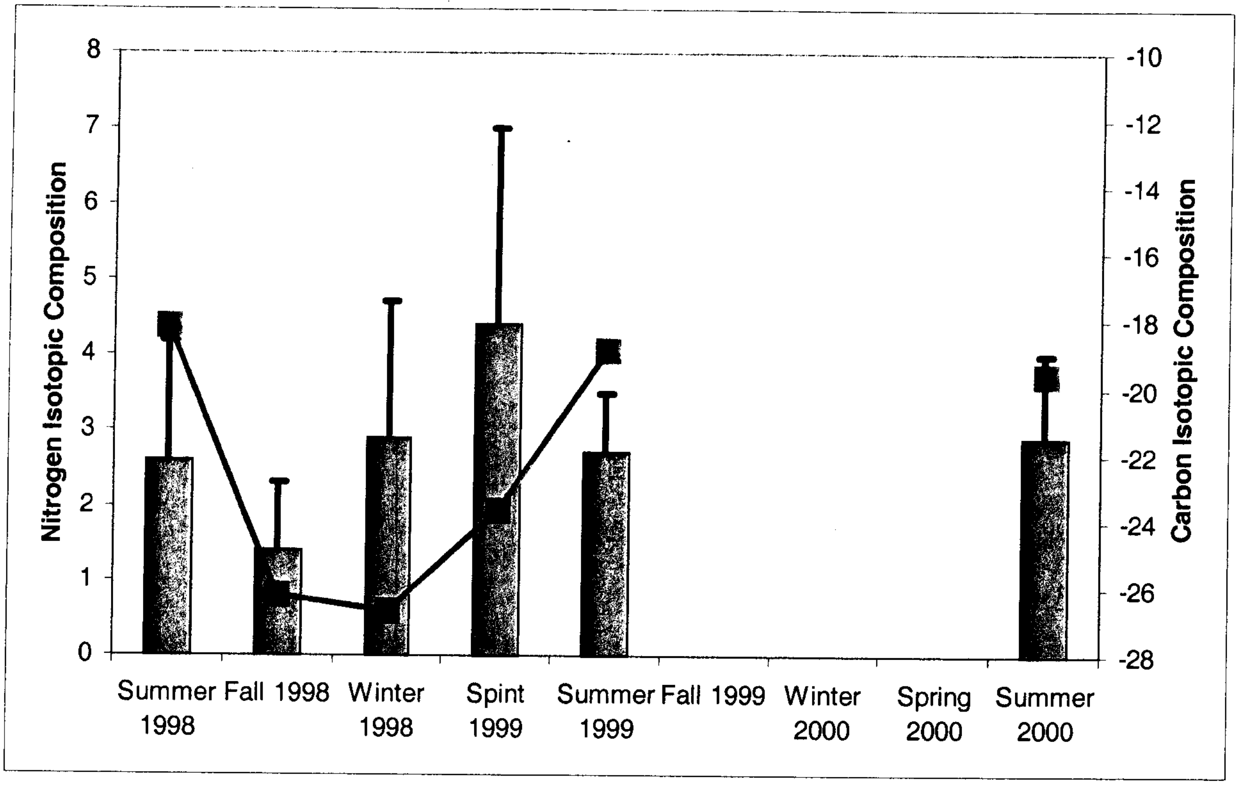

Figure 3: Ranges and means of $\delta^{13}C$ and $\delta^{15}N$ values measured on POM from the Florida Keys between 1998 and 2000. Bars =nitrogen, symbols = carbon. Error bars= 1 standard deviation.

CARBON AND NITROGEN ISOTOPIC COMPOSITION

The highest $\delta^{15}N$ values of +9.2 occurred in POM sampled during the spring of 1999 near Cudjoe Key (Appendix 1). Other higher values also occurred during this sampling period giving the spring of 1999 the highest $\delta^{15}N$ values of any period sampled (Figure 3). Despite the differences between the various seasons of the year there is no statistically significant difference between adjacent

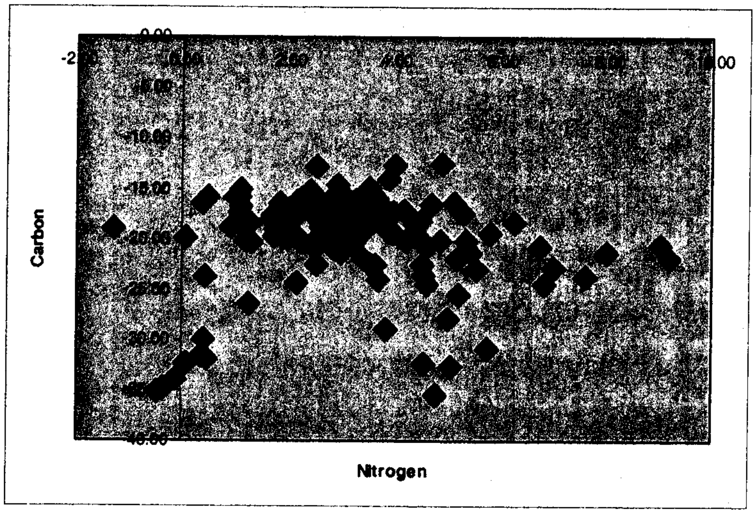

Figure 4: Cross plot of the of $\delta^{13}C$ and $\delta^{15}N$ compositions of POM measured during the study period.

seasons. Based on the short time interval of the study it appears that perhaps the Fall and Winter may have lower $\delta^{15}N$ values than the Spring and Summer time periods (Figure 3). Although the maximum $\delta^{15}N$ values of approximately +9 are similar to the maximum values mesured by other workers (Heikopp *et al.*, 1998). These values have interpreted as being affected by sewage. However the large range of variability and the inconstancy of the $\delta^{15}N$ values relative to potential sources of sewage suggests that further work is necessary. The $\delta^{13}C$ of the POM exhibited a range between -26 and -18 per mille. The more positive $\delta^{13}C$ values and therefore less terrestrially influenced samples appear to coincide during the summer (Figure 3). There appeared to be no correlation between $\delta^{13}C$ and $\delta^{15}N$ values (Figure 4). A positive correlation might have linked inputs from terrestrial sources with isotopically negative $\delta^{13}C$ values with more positive $\delta^{15}N$ values producing an inverse correlation between $\delta^{13}C$ and $\delta^{15}N$.

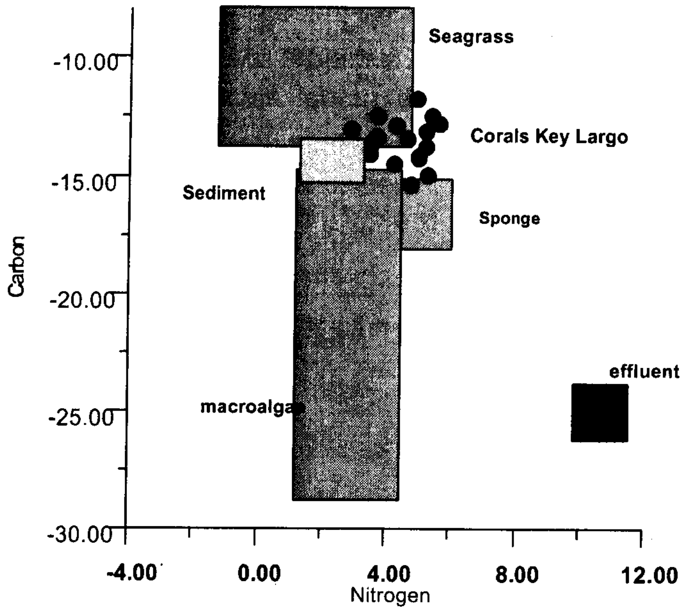

Figure 5: Ranges of C and N isotopic compositions measured in the study by Ferry, (unpublished EPA report).

A previous EPA study (Ferry, unpublished EPA study) measured the level of $\delta^{13}C$ and $\delta^{15}N$ in algae and other marine organisms in the lower Keys, including material growing adjacent to the sewage outflow in Key West. In this 73% of the dissolved inorganic nitrogen (DIN) was reported as being in the form of NH_4^+ with an isotopic composition of +11 per mille. Carbon and nitrogen isotopic compositions were not related to the distance from the sewage outfall and showed no relationship to the sedimentary $\delta^{15}N$. A summary of these data are shown in Figure 5 together with unpublished data from Swart on the $\delta^{13}C$ and $\delta^{15}N$ composition of the organic tissues of corals growing off Key Largo. These data indicate that the range of $\delta^{15}N$ of the sewage effluent is between +10 and +12 per mille while the range of $\delta^{15}N$ in other organisms is between -2 and +6 per mille. The range of data from the POM measured in this study generally fall in the same range as the data presented by Ferry (unpublished data) trending between the field defined by the sewage effluent and the macroalgae (Figure 6). Whether this means that there is actually some influence from sewage on the reef trace is still a matter of speculation. It is more likely that we are insufficiently knowledgeable about the nitrogen isotopic systematics on the reef tract.

STEROLS

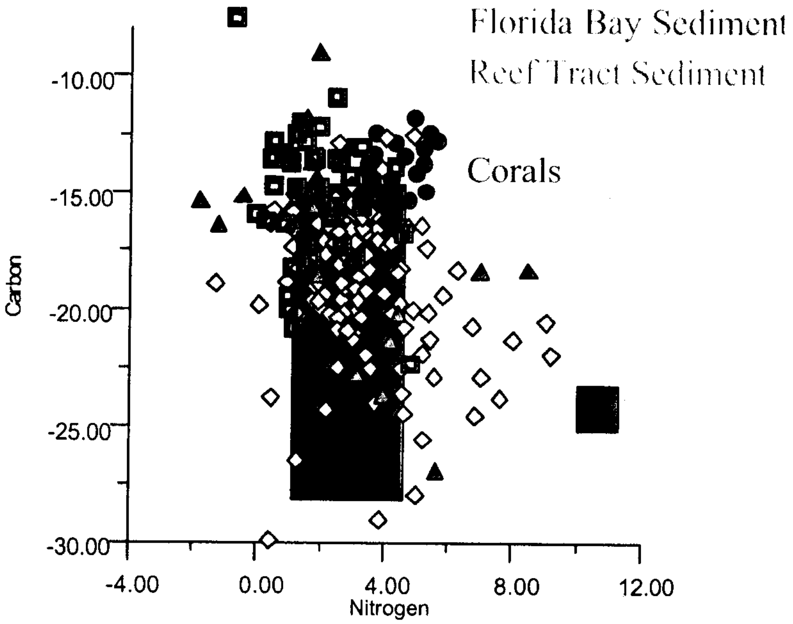

Figure 6: Comparison of data measured by Ferry (unpublished) with data measured in this study. Note that the data lie between the macroalgae and the effluent of the sewage outflow.

Coprostanol (5-beta-cholestan-3-beta-ol), the dominant human fecal sterol, is only formed in the gut of selected animals. Those studied so far include (besides humans) monkeys and seals. Herbivores such as cows, sheep, kangaroos, etc. do also have the microbial gut flora capable of converting delta 5 sterols to 5B-stanols. However since herbivores eat mostly plant matter, they convert far more of the dominant plant sterols, e.g. sitosterol to the C29 homologue, 24-ethylcoprostanol. As a first approximation, humans can be distinguished from herbivores by the ratio of C27 to C29 stanols - the overall sterol profiles are distinct (Leeming et al., 1996; Leeming and Nichols, 1996) enough for this purpose. Pigs being omnivores have a profile intermediate between the

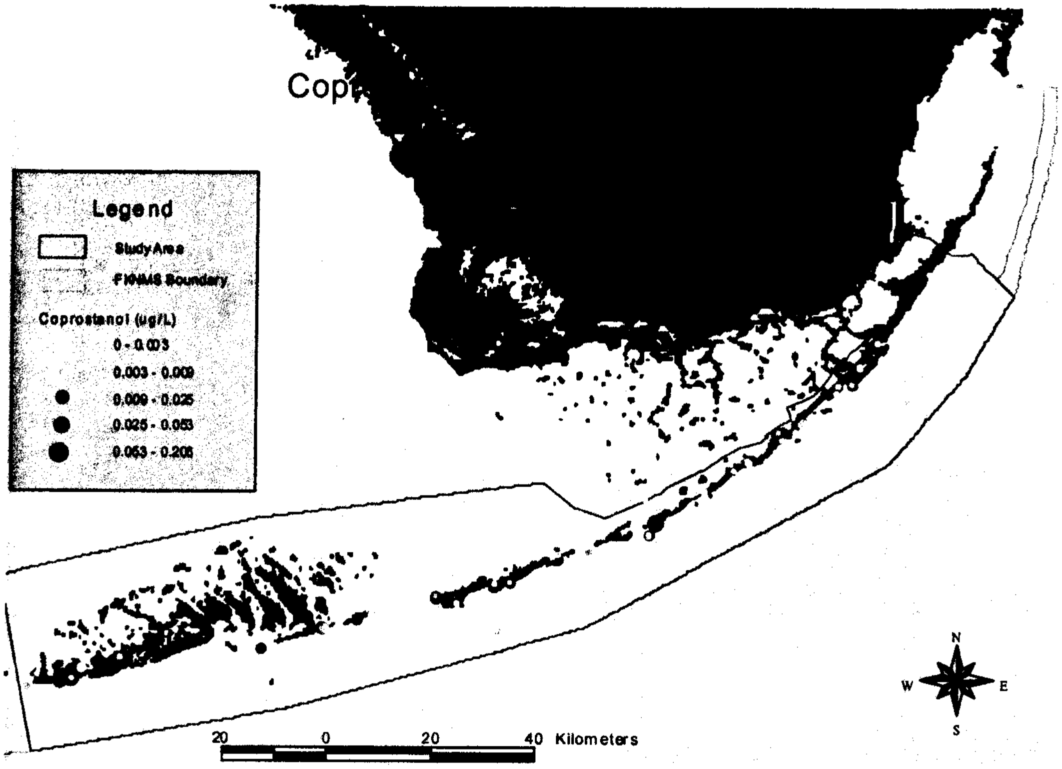

Figure 7: Distribution of coprostanol in samples measured from the Florida Keys. The highest concentrations were measured close to the Florida Keys, but there was no evidence of high values on the Florida reef tract.

two. In order to unambiguously distinguish pigs from humans and herbivores would probably require the use of additional biomarkers; bile acids are one such a possibility (Leeming, pers. communication). Birds on the other hand, as well as dogs, reptiles and invertebrates do not appear to have the gut flora capable of converting delta 5 sterols to 5B-stanols at all. So within some rational limits (i.e. taking into account such environmental factors such as plausible sources, residence times, water velocities etc.) the presence of indicator bacteria like thermotolerant or halotolerant coliforms, in the absence of 5B-stanols clearly indicates that fecal contamination is most likely not from humans or herbivores.

Concentrations of coprostanol were detected at a very wide range of concentrations (0 to 11 ppm), but in general were very low (Table 4). The mean concentrations were similar to values measured by Ferry (unpublished, EPA study). These data are also similar to other values measured in the region quoted by Ferry (unpublished EPA study).

Unfortunately a key reference mentioned by Ferry is missing from his list of reverences. Despite the generally low concentrations measured throughout the study area, higher values were detected close to the Florida Keys in areas most likely to be influenced by sewage effluent (Figure 7).The absence of higher values at distances further from the Florida Keys suggests that either the sewage is not making to the reefs or that the coprostanol is being degraded before it reaches sites further off shore. Microbial degradation of coprostanol from discharged raw or incompletely treated sewage wastes is most likely to take place within the water column or in the oxic sediment water interface. Sedimentary incorporation rates will be determined by the balance between the microbial and depositional environmental rates at the specific sites. In truly anoxic sedimentary environments, the sterol biomarkers would be expected to be stable for years. Estimates of burial incorporation rates would be in the 1% or less range, i.e. most of the input coprostanol would not survive microbial degradation time scales of more than a few months . Defintive biodegradation rates, and also any abiotic (e.g photochemical) consumption rates are not well established, especially for subtropical waters such as the Florida Keys.

The absence of a correlation between coprostanol and the $\delta^{15}N$ in the POM data suggests that the processes controlling the concentration of these parameters are decoupled (Figure 8) . For example if the absence of concentrations of coprostanol in the outer reefs is a result of degradation, then these process do not influence the $\delta^{15}N$ values or they influence the $\delta^{15}N$ in a different direction. One

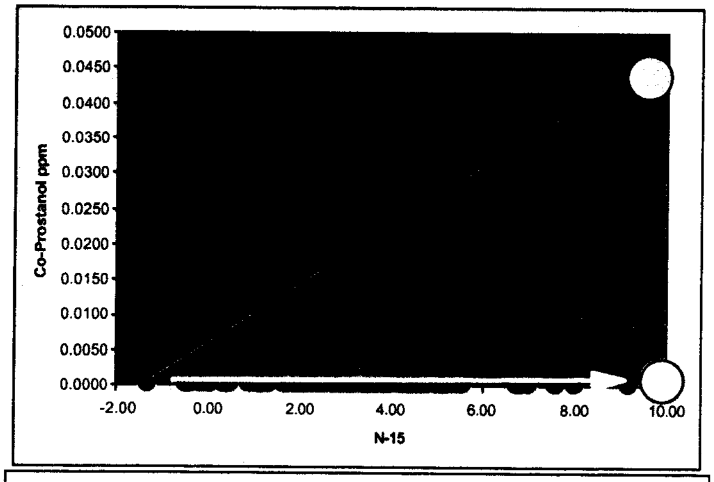

Figure 8: Correlation between the $\delta^{15}N$ of POM and the concentration of coprostanol. A large proportion of the samples analyzed show a wide range in $\delta^{15}N$ values, but no relation with the concentration of coprostanol.

possible explanation for these trends is that the trend of an invariant low concentration in the concentration of coprostanol with a wide range of $\delta^{15}N$ values is a result of the upwelling of nitrate which possess an isotopic enriched $\delta^{15}N$ value but essentially no coprostanol. In contrast the trend of increasing concentration of coprostanol with increasing $\delta^{15}N$ is a result of contribution from anthropogenic sewage sources (Figure 8). This hypothesis might be tested by combining the $\delta^{15}N$ data with the coprostanol and the $\delta^{13}C$ data (Figure 9). For example it might be expected that anthropogenic pollution would possess a high concentration of coprostanol, and more negative $\delta^{13}C$ value and a more positive $\delta^{15}N$ values. In contrast the upwelling nitrogen will possess a low concentraiton of coprostanol, a positive $\delta^{15}N$, and a relatively positive $\delta^{13}C$ value. Such trend however is not evident in the data (Figure 9).

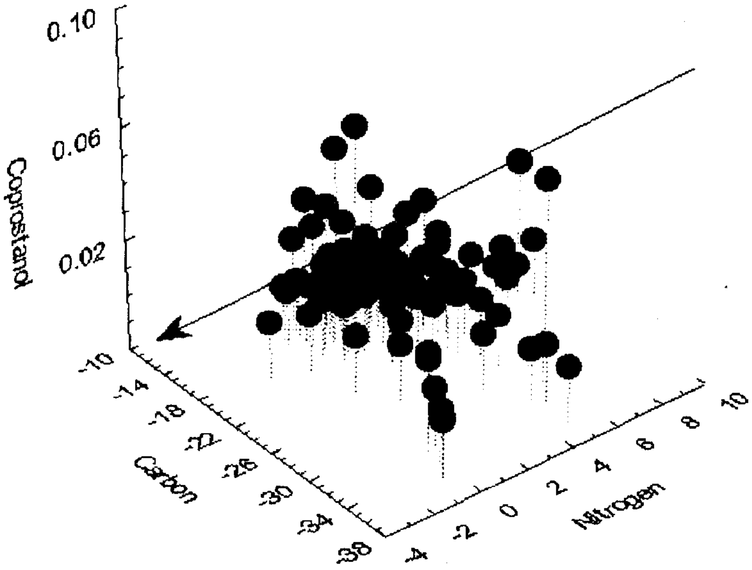

Figure 9: Combined $\delta^{15}N$, $\delta^{13}C$, and coprostanol data with arrow indicating the direction of the trend which should be present if the range in the data were a result of the mixing of nitrogen derived both from upwelling and pollution.

FLORIDA BAY

There has been widespread discussion as the effect that the outflow from Florida Bay might have upon the Florida reef tract. A comparison of the data collected in this study can be made with other data collected from Florida Bay during previous studies (Ellis and Swart, unpublished). These data show that the organic material in

Florida Bay have no unusual $\delta^{15}N$ compositions and range in value from 0 to +6 per mille. Organic material in the eastern portion of Florida Bay tends to be slightly isotopically more positive than material in the western portion of Florida Bay (Figure 10). The origin of these differences is not known at present, but is likely a result of natural processes rather than the input of anthropogenic nitrogen. The ranges of values is insufficiently diagnostic to allow the nitrogen isotopic composition to be used as a diagnostic indicator as to the input of allochthonous nitrogen emanating from Florida Bay to the reef tract.

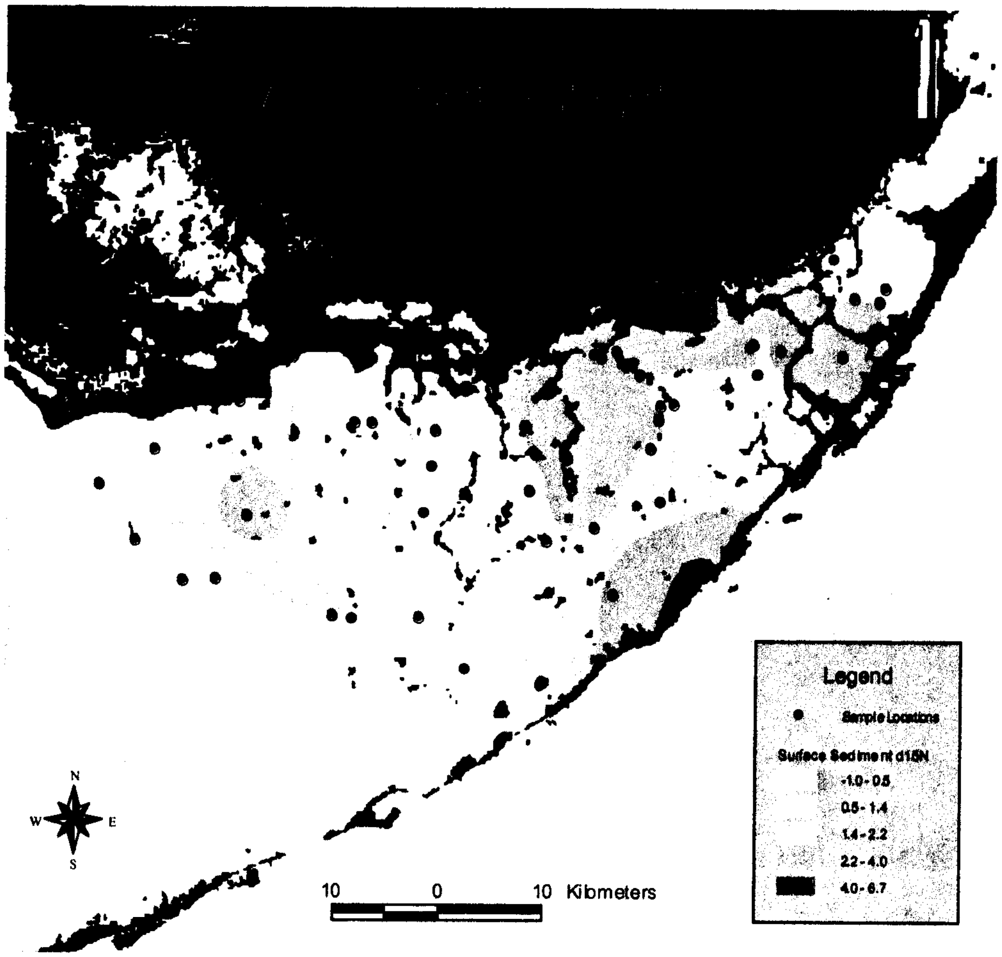

Figure 10: Contour map of the $\delta^{15}N$ of sedimentary organic material in Florida Bay (Ellis and Swart, unpublished data).

VI. CONCLUSIONS

- ☐ While the $\delta^{15}N$ of sediments and particulate material show a range of values, they do not show excessively heavy values which can be interpreted as reflecting input of anthropogenic waste. Hence the $\delta^{15}N$ of waste may not be sufficiently diagnostic to allow it to be used for the identification of anthropogenic derived sewage
- ☐ Coprostanol does not show a strong correlation with $\delta^{15}N$ values, but does show some high values particularly close to the Florida Keys.
- ☐ A model is proposed for two component mixing between the nitrogen originating from the upwelling of nitrate and nitrogen derived from anthropogenic sources.

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EPA Florida Keys Project
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Summer 1998 Sample Results

Name	Site Name	Date	Latitude	Longitude	Volume	N mg/l	δ^{15}N ppt	C mg/l	δ^{13}C ppt	C/N	Coprost ppm	Epicop ppm	Cholest ppm
EPA 7981	Key Biscayne Inshore (site 1)	07/29/1998	25.6450	-80.1283	20.0	0.0143	1.13	0.0587	-16.64	4.1	0.00	0.00	0.00
EPA 7984	Key Largo Inshore (site 4)	07/29/1998	25.0650	-80.3133	25.0	0.0112	1.74	0.0455	-17.59	4.0	0.07	0.03	4.1
EPA 7985	Key Largo Midshore (site 5)	07/29/1998	25.0933	-80.3567	15.5	0.0201	1.29	0.0898	-18.29	4.5	0.00	0.00	0.00
EPA 7986	Key Largo Offshore (site 6)	07/29/1998	25.1100	-80.3800	23.5	0.0130	1.74	0.0514	-19.63	3.9	0.00	0.00	0.00
EPA 79810	Long Key Inshore (site 10)	07/30/1998	24.7900	-80.8600	15.0	0.0167	0.50	0.0565	-15.77	3.4	0.00	0.00	0.00
EPA 79811	Long Key Midshore (site 11)	07/30/1998	24.7533	-80.8450	21.0	0.0155	2.09	0.0656	-17.29	4.2	0.00	0.00	0.00
EPA 79812	Long Key Offshore (site 12)	07/30/1998	24.7133	-80.8333	23.0	0.0135	1.33	0.0546	-20.31	4.0	0.00	0.00	0.00
EPA 79813*	Marathon Inshore (site 13)	07/30/1998	24.7000	-81.0317	21.0	0.0134	1.79	0.0492	-19.02	3.6	0.00	0.00	0.00
EPA 79814S	Marathon Midshore (site 14) Surface	07/30/1998	24.6733	-81.0217	21.0	0.0151	2.58	0.0656	-18.13	4.3	0.00	0.00	0.00
EPA 79815S	Marathon Offshore (site 15) Surface	07/30/1998	24.6417	-81.0133	25.0	0.0140	0.06	0.0644	-19.85	4.6	0.00	0.00	0.00
EPA 79817A	7 Mile Bridge Midshore (site 17)	07/30/1998	24.6367	-81.1917	22.0	0.0141	2.01	0.0644	-20.02	4.6			
EPA 79818*	7 Mile Bridge Offshore (site 18)	07/30/1998	24.5983	-81.1833	28.5	0.0113	1.96	0.0503	-18.84	4.3			
EPA 79822S	Key West Offshore (site 22) Surface	07/31/1998	24.4550	-81.8283	21.0	0.0162	2.27	0.0724	-20.24	4.5			
EPA 79823S	Key West Midshore (site 23) Surface	07/31/1998	24.4867	-81.8283	18.0	0.0168	2.69	0.0602	-19.72	3.6			
EPA 79824D	Key West Inshore (site 24) Bottom	07/31/1998	24.5367	-81.8283	16.5	0.0223	3.08	0.1027	-20.12	4.6			
EPA 79852*	Bahia Honda	07/20/1998	24.6653	-81.2579	8.2	0.0342	2.90	0.1288	-16.22	3.8	0.12	0.07	10.98
EPA 79853	Key West Harbor	07/20/1998	24.5623	-81.8055	13.0	0.0217	2.94	0.0885	-16.62	4.1			
EPA 79854	Garrison Bight Ramp	07/20/1998	24.5607	-81.7843	14.0	0.0264	4.49	0.1353	-19.78	5.1	0.00	0.00	0.00
EPA 79855	Smathers Beach	07/20/1998	24.5522	-81.7669	6.0	0.0777	4.03	0.3788	-12.62	4.9	0.17	0.08	10.90
EPA 79856*	Stock Island Ramp	07/20/1998	24.5741	-81.7327	14.2	0.0184	2.08	0.0699	-17.11	3.8	0.11	0.05	10.13
EPA 79857	Cow Key Channel	07/20/1998	24.5718	-81.7471	12.0	0.0402	4.62	0.2809	-20.82	7.0	0.30	0.15	19.36
EPA 79859	South Stock Island	07/21/1998	24.5669	-81.7299	12.0	0.0442	3.54	0.2103	-14.88	4.8			
EPA 79860	Sugarloaf Key	07/21/1998	24.6421	-81.5708	13.4	0.0189	0.39	0.0728	-16.40	3.9			
EPA 79861	Cudjoe Key	07/21/1998	24.6630	-81.4694	10.5	0.0310	3.74	0.1479	-16.64	4.8	0.08	0.05	15.59
EPA 79862	Big Pine Key	07/21/1998	24.6686	-81.3746	13.0	0.0223	2.54	0.1078	-15.92	4.8	0.00	0.00	0.00
EPA 79863	Doctor's Arm Canal	07/21/1998	24.7018	-81.3525	16.3	0.0316	1.11	0.1821	-17.37	5.8	0.34	0.18	11.37
EPA 79864	Pine Point	07/21/1998	24.7243	-81.3904	7.7	0.1424	5.76	1.0331	-30.85	7.3	0.37	0.03	50.17
EPA 79865	Spanish Harbor Keys	07/21/1998	24.6499	-81.3171	15.2	0.0274	2.94	0.1405	-14.69	5.1	0.00	0.00	0.00

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Copro Epicopr Cholest
ppm ppm ppm

Name	**Site Name**	**Date**	**Latitude**	**Longitude**	**Volume**	**N**	**$\delta^{15}N$**	**C**	**$\delta^{13}C$**	**C/N**	**Copro**	**Epicop**	**Choles**
						mg/l	**ppt**	**mg/l**	**ppt**		ppm	ppm	ppm
EPA 10983	Key Biscayne Offshore (site 3)	10/22/1998	25.6433	-80.0817	19.0	0.0178	2.90	0.1044	-25.75	5.9	0	0	0
EPA 10984	Key Largo Inshore (site 4)	10/21/1998	25.1067	-80.3783	13.2	0.0007	1.20	-0.0050	-25.92	-7.0	0	0	0
EPA 10986*	Key Largo Offshore (site 6)*	10/21/1998	25.0667	-80.3150	19.0	0.0123	1.06	0.0465	-22.79	4.0	0	0	0
EPA 10987*	Channel 5 Inshore (site 7)*	10/21/1998	24.8183	-80.7600	9.4	0.0491	1.42	0.2304	-25.14	4.4	0	0	0
EPA 10988	Channel 5 Midshore (site 8)	10/21/1998	24.8000	-80.7467	16.5	0.0230	1.37	0.1317	-27.20	5.7	0	0	0
EPA 109810	Long Key Inshore (site 10)	10/21/1998	24.7883	-80.8617	7.2	0.0386	0.24	0.2565	-29.88	6.6	0	0	0
EPA 109811	Long Key Midshore (site 11)	10/21/1998	24.7550	-80.8450	12.5	0.0189	2.65	0.0883	-19.17	5.0	0	0	0
EPA 109812	Long Key Offshore (site 12)	10/21/1998	24.7133	-80.8350	18.3	0.0051	2.14	0.0349	-26.26	6.9	0	0	0
EPA 109813	Marathon Inshore (site 13)	10/20/1998	24.6983	-81.0317	8.6	0.0617	0.81	0.2519	-24.59	3.6	11.15	0.31	33.26
EPA 109814	Marathon Midshore (site 14)	10/20/1998	24.6767	-81.0233	18.1	0.0234	1.74	0.0916	-25.25	3.7	0	0	0
EPA 109815	Marathon Offshore (site 15)	10/20/1998	24.6433	-81.0150	19.3	0.0355	0.48	0.1516	-33.47	4.3	0	0	0
EPA 109816	7 Mile Bridge Inshore (site 16)	10/20/1998	24.6733	-81.2050	8.2	0.0601	0.19	0.3892	-32.48	6.5			
EPA 109817	7 Mile Bridge Midshore (site 17)	10/20/1998	24.6383	-81.1933	18.3	0.0671	1.07	0.2803	-27.43	3.4	0	0	0
EPA 109818	7 Mile Bridge Offshore (site 18)	10/20/1998	24.6017	-81.1817	18.6	0.0423	-0.44	0.1828	-34.71	4.3	0	0	0
EPA 109819	Looe Key Inshore (site 19)	10/20/1998	24.6083	-81.4233	6.2	0.5102	0.84	2.0272	-28.52	3.7	0	0	0
EPA 109820	Looe Key Midshore (site 20)	10/20/1998	24.5767	-81.4183	9.0	0.0227	2.49	0.1494	-23.15	6.6	0	0	0
EPA 109821	Looe Key Offshore (site 21)	10/20/1998	24.5367	-81.4133	18.4	0.0050	2.23	0.0363	-23.58	7.2	0	0	0
EPA 109822	Key West Offshore (site 22)	10/20/1998	24.4550	-81.8283	10.0	0.0785	0.63	0.3537	-24.86	3.8	0	0	0
EPA 109823	Key West Midshore (site 23)	10/20/1998	24.4883	-81.8283	5.9	0.0218	2.04	0.1597	-22.95	7.3	0	0	0
EPA 109824	Key West Inshore (site 24)	10/20/1998	24.3700	-81.8283	4.9	0.0296	2.04	0.2213	-20.84	7.5	0	0	0

EPA 79866	Boot Key Harbor	07/21/1998	24.7055	-81.1227	6.0	0.0508	2.54	0.2462	-12.89	4.9	0.10	0.05	3.86
EPA 79867	Sombrero Beach	07/21/1998	24.6924	-81.0869	8.0	0.0398	2.42	0.1651	-17.19	4.1	0.00	0.00	0.00
EPA 79868	Key Colony	07/21/1998	24.7290	-81.0198	8.5	0.0374	4.51	0.1454	-18.27	3.9	0.00	0.00	0.00
EPA 79869	Coco Plum Beach	07/21/1998	24.7328	-80.9936	4.5	0.0616	2.19	0.2231	-16.20	3.6	0.10	0.05	2.34
EPA 79870	Grassy Key	07/21/1998	24.7814	-80.9114	17.5	0.0175	1.70	0.0574	-18.05	3.3	0.00	0.00	0.00
EPA 79871	Largo Sound	07/22/1998	25.1290	-80.3967	11.0	0.0306	3.26	0.1739	-20.37	5.7			
EPA 79872	Pennekamp Cut	07/22/1998	25.0986	-80.4088	4.0	0.0712	1.14	0.3374	-15.12	4.7	0.05	0.03	8.80
EPA 79873	Ocean Reef Harbor	07/22/1998	25.2989	-80.2758	12.0	0.0247	3.05	0.1113	-21.03	4.5	0.00	0.00	0.00
EPA 79876	Lake Largo	07/22/1998	25.0801	-80.4351	16.0	0.0178	3.44	0.0722	-17.69	4.1	0.00	0.00	0.00
EPA 79877	Tavernier Key	07/22/1998	25.0076	-80.5051	14.7	0.0180	2.26	0.0680	-16.00	3.8	0.00	0.00	0.00
EPA 79878	Basin 31	07/22/1998	24.8852	-80.7649	10.5	0.0264	1.02	0.1019	-15.80	3.9	0.00	0.00	0.00
EPA 79879	Fla. Bay Trans. Offshore	07/22/1998	24.8256	-80.7447	16.0	0.0157	1.11	0.0521	-15.83	3.3	0.00	0.00	0.00
EPA 79880	Long Key	07/22/1998	24.8396	-80.7831	11.0	0.0349	4.01	0.1980	-17.24	5.7	0.00	0.00	0.00
EPA 79881	Anne's Beach	07/22/1998	24.8466	-80.7447	4.0	0.0900	0.42	0.6913	-29.94	7.7	0.74	0.31	16.06
EPA 79883	Snake Creek	07/23/1998	24.9534	-80.5877	18.0	0.0014	4.72	0.0600	-16.56	42.8			
EPA 79885	Tavernier Canal	07/23/1998	25.0086	-80.5347	16.0	0.0091	9.04	0.0462	-20.57	5.1	0.38	0.13	15.68

EPA Florida Keys Project
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Winter 1998 Summary Results

Name	Site Name	Date	Latitude	Longitude	Volume	N mg/l	$\delta^{15}N$ ppt	C mg/l	$\delta^{13}C$ ppt	C/N	Coprost ppm	Epicop ppm	Cholest ppm
EPA 129805	Key Largo Midshore (site 5)	12/15/1998	25.0950	-80.3583	16.5	0.083	-0.13	0.376	-34.10	4.5	0.000	0.000	0.000
EPA 129806	Key Largo Offshore (site 6)*	12/15/1999	25.0683	-80.3167	17.5	0.066	-0.35	0.498	-34.86	7.5	0.000	0.000	0.000
EPA 129807A	Channel 5 Inshore (site 7)*	12/15/1998	24.8217	-80.7567	11.0	0.097	0.08	0.439	-32.38	4.5	0.130	0.070	7.730
EPA 129809	Channel 5 Offshore (site 9) Surface	12/15/1998	24.7667	-80.7250	14.0	0.032	0.49	0.171	-31.97	5.8	0.000	0.000	0.000
EPA 129816A	7 Mile Bridge Inshore (site 16)	12/14/1998	24.6717	-81.2067	9.5	0.203	-0.46	1.505	-35.17	7.4	0.000	0.000	0.000
EPA 129819A	Looe Key Inshore (site 19)	12/14/1998	24.6100	-81.4217	8.0	0.105	0.37	0.473	-31.63	4.5	0.090	0.030	6.440
EPA 29910	Long Key Inshore (site 10)	02/10/1999	24.7883	-80.8617	16.0	0.022	0.44	0.138	-23.79	5.6	0.000	0.000	0.000
EPA 29913	Marathon Inshore (site 13)	02/09/1999	24.6983	-81.0300	16.5	0.078	1.27	0.573	-26.47	5.7	0.000	0.000	0.000
EPA 29915	Marathon Offshore (site 15)	02/09/1999	24.6400	-81.0167	5.0	0.052	4.88	0.170	-20.08	3.3	0.020	0.010	8.270
EPA 29924S	Key West Inshore (site 24)Surface	02/09/1999	24.5400	-81.8267	12.0	0.014	3.43	0.067	-21.96	5	0.040	0.010	5.360
EPA 29925	Knight Key	02/23/1999	24.7069	-81.1235	9.0	0.069	5.30	0.179	-17.43	2.6	0.110	0.030	5.490
EPA 29926	Litle Duck Key	02/23/1999	24.6821	-81.2290	11.0	0.056	5.15	0.144	-16.46	2.6	0.040	0.030	3.650
EPA 29927	Spanish Harbor Keys	02/23/1999	24.6494	-81.3173	5.5	0.148	4.90	0.455	-12.56	3.1	0.180	0.070	5.220
EPA 29928	Doctor's Arm	02/23/1999	24.6958	-81.3560	8.0	0.056	3.77	0.193	-17.09	3.4	0.200	0.100	8.710
EPA 29929	Key West Airport	02/23/1999	24.5566	-81.7475	10.5	0.061	4.20	0.170	-17.23	2.8	1.180	0.090	3.450
EPA 29930	Garrison Bight	02/23/1999	24.5603	-81.7846	12.0	0.062	6.76	0.182	-20.76	2.9	0.000	0.000	0.000
EPA 29931	Stock Island	02/24/1999	24.5753	-81.7321	10.1	0.063	3.41	0.192	-16.82	3.1	0.000	0.000	0.000
EPA 29932	Big Coppitt Key	02/24/1999	24.5943	-81.6638	5.8	0.110	3.18	0.302	-18.63	2.8	0.000	0.000	0.000
EPA 29933	Saddle Bunch Keys	02/24/1999	24.6416	-81.5708	12.0	0.050	3.69	0.126	-15.98	2.5	0.000	0.000	0.000
EPA 29935	Sombrero Beach	02/24/1999	24.6924	-81.0861	8.2	0.020	1.89	0.084	-17.79	4.1	0.000	0.000	0.000
EPA 29936	Coco Plum Beach	02/24/1999	24.7334	-80.9936	11.0	0.018	2.43	0.088	-15.29	4.8	0.000	0.000	0.000
EPA 29937	Duck Key	02/24/1999	24.7770	-80.9237	13.0	0.014	2.31	0.062	-16.60	4.4	0.000	0.000	0.000
EPA 29938	Fiesta Key	02/24/1999	24.8398	-80.7814	14.0	0.015	3.30	0.073	-16.61	4.9	0.030	0.010	4.420
EPA 29939	Bud'n'Mary's	02/24/1999	24.8975	-80.6601	17.3	0.012	3.02	0.064	-15.99	5.4	0.000	0.000	0.000
EPA 29940	Tiki Bar	02/24/1999	24.9384	-80.6118	11.0	0.017	3.09	0.088	-15.82	5.0	0.000	0.000	0.000
EPA 29941	Snake Creek	02/25/1999	24.9529	-80.5871	11.5	0.016	2.55	0.085	-16.74	5.3	0.000	0.000	0.000
EPA 29942	Harris Park	02/25/1999	25.0257	-80.4940	18.5	0.011	3.61	0.057	-18.43	5.3	0.090	0.040	8.730

EPA 29943	NURC	02/25/1999	25.0965	-80.4321	17.6	0.011	5.44	0.049	-21.31	4.4	0.000	0.000	0.000
EPA 29944	Garden Cove	02/25/1999	25.1714	-80.3674	3.0	0.090	2.64	0.729	-19.61	8.1	0.000	0.000	0.000
EPA 29945	Basin 31	02/25/1999	24.8851	-80.7648	13.0	0.033	3.07	0.196	-17.18	6.0	0.000	0.000	0.000
EPA 29946	Snake Creek Canal	02/25/1999	24.9580	-80.5902	13.5	0.014	3.29	0.069	-16.09	5.0	0.000	0.000	0.000
EPA 29947	Tavernier Canal	02/25/1999	25.0085	-80.5330	13.0	0.015	3.51	0.076	-15.86	5.1	0.000	0.000	0.000
EPA 29948	Tavernier Canal Mouth	02/25/1999	24.9954	-80.5281	14.1	0.013	2.93	0.062	-15.49	4.8	0.000	0.000	0.000
EPA 29949	Largo Sound	02/26/1999	25.1316	-80.3962	18.0	0.013	3.90	0.076	-19.30	5.8	0.000	0.000	0.000
EPA 29950	Pennekamp Cut Mouth	02/26/1999	25.0993	-80.4110	18.3	0.011	2.76	0.063	-19.12	5.6	0.000	0.000	0.000

EPA Florida Keys Project
The Impact of Sewage Contaminants on the Florida Reef Tract
Spring 1999 Sample Results

Name	Site Name	Date	Latitude	Longitude	Volume	N mg/l	$\delta^{15}N$ ppt	C mg/l	$\delta^{13}C$ ppt	C/N	Coprost ppm	Epicop ppm	Cholest ppm
FKEPA-49901	Key Biscayne Inshore (site 1)	04/17/1999	25.6450	-80.1233	16.0		4.80		-35.57		0.000	0.000	0.000
FKEPA-49904	Key Largo Inshore (site 4)	04/16/1999	25.1067	-80.3783	16.0		5.09		-32.72		0.000	0.000	0.000
FKEPA-49905	Key Largo Midshore (site 5)	04/16/1999	25.0933	-80.3517	13.4		8.02		-21.33		0.000	0.000	0.000
FKEPA-49906	Key Largo Offshore (site 6)	04/16/1999	25.0600	-80.3150	20.0		7.61		-23.80		0.000	0.000	2.070
FKEPA-49907	Channel 5 Inshore (site 7)	04/16/1999	24.8200	-80.7567	12.0		2.18		-24.30		0.000	0.000	0.000
FKEPA-49908	Channel 5 Midshore (site 8)	04/16/1999	24.7950	-80.7433	16.0		7.02		-22.90		0.000	0.000	0.000
FKEPA-49909	Channel 5 Offshore (site 9)	04/16/1999	24.7617	-80.7217	16.0		4.56		-32.39		0.000	0.010	3.860
FKEPA-49910	Long Key Inshore (site 10)	04/16/1999	24.7867	-80.8633	10.1		5.02		-27.93		0.000	0.000	3.870
FKEPA-49911	Long Key Midshore (site 11)	04/16/1999	24.7550	-80.8483	8.0		3.86		-29.01		0.000	0.000	0.000
FKEPA-49912	Long Key Offshore (site 12)	04/16/1999	24.7117	-80.8317	16.0		6.84		-24.54		0.000	0.000	0.000
FKEPA-49914	Marathon Midshore (site 14)	04/16/1999	24.6767	-81.0233	14.0	0.0120	0.94	0.0497	-18.87	4			
FKEPA-49925	Knight Key	04/27/1999	24.7076	-81.1249	11.1		4.23		-20.21		0.000	0.000	0.000
FKEPA-49926	Litle Duck Key	04/27/1999	24.6824	-81.2282	9.0		3.66		-22.48		0.020	0.010	2.480
FKEPA-49927	Spanish Harbor Keys	04/27/1999	24.6498	-81.3171	14.5		6.28		-18.37		0.070	0.030	4.360
FKEPA-49928	Doctor's Arm	04/27/1999	24.6982	-81.3409	11.6		5.83		-19.44		0.050	0.000	2.220
FKEPA-49929	Key West Airport	04/28/1999	24.5563	-81.7477	7.6		5.37		-20.19		0.000	0.000	0.000
FKEPA-49931	Stock Island	04/27/1999	24.5752	-81.7320	20.0		4.00		-19.37		0.080	0.010	1.840
FKEPA-49932	Big Coppitt Key	04/27/1999	24.5946	-81.6635	11.6		5.18		-21.90		0.000	0.000	0.000
FKEPA-49933	Saddle Bunch Keys	04/27/1999	24.6431	-81.5703	15.0		5.22		-25.57		0.000	0.000	0.000
FKEPA-49934	Cudjoe Key	04/27/1999	24.6635	-81.4679	13.0		9.19		-21.97		0.000	0.000	0.000
FKEPA-49935	Sombrero Beach	04/27/1999	24.6918	-81.0874	8.5		4.63		-24.49		0.010	0.000	2.200
FKEPA-49936	Coco Plum Beach	04/27/1999	24.7330	-80.9935	3.8		3.00		-21.30		0.000	0.000	0.000
FKEPA-49938	Fiesta Key	04/28/1999	24.8402	-80.7810	12.0		4.54		-22.52		0.000	0.000	0.000
FKEPA-49939	Bud'n'Mary's	04/28/1999	24.8950	-80.6597	8.5		3.57		-22.54		0.000	0.000	0.000
FKEPA-49940	Tiki Bar	04/28/1999	24.9387	-80.6111	20.0		-1.30		-18.93		0.000	0.000	0.000
FKEPA-49941	Snake Creek	04/28/1999	24.9526	-80.5864	8.0		4.58		-23.60		0.010	0.000	0.450
FKEPA-49942	Harris Park	04/28/1999	25.0248	-80.4937	5.5		2.55		-22.49				
FKEPA-49943	NURC	04/28/1999	25.0965	-80.4323	6.0		5.57		-22.92				
FKEPA-49944	Garden Cove	04/28/1999	25.1713	-80.3673	18.7		3.71		-24.01		0.000	0.000	0.000
FKEPA-499FB	Field Blank						-3.55		-73.73				

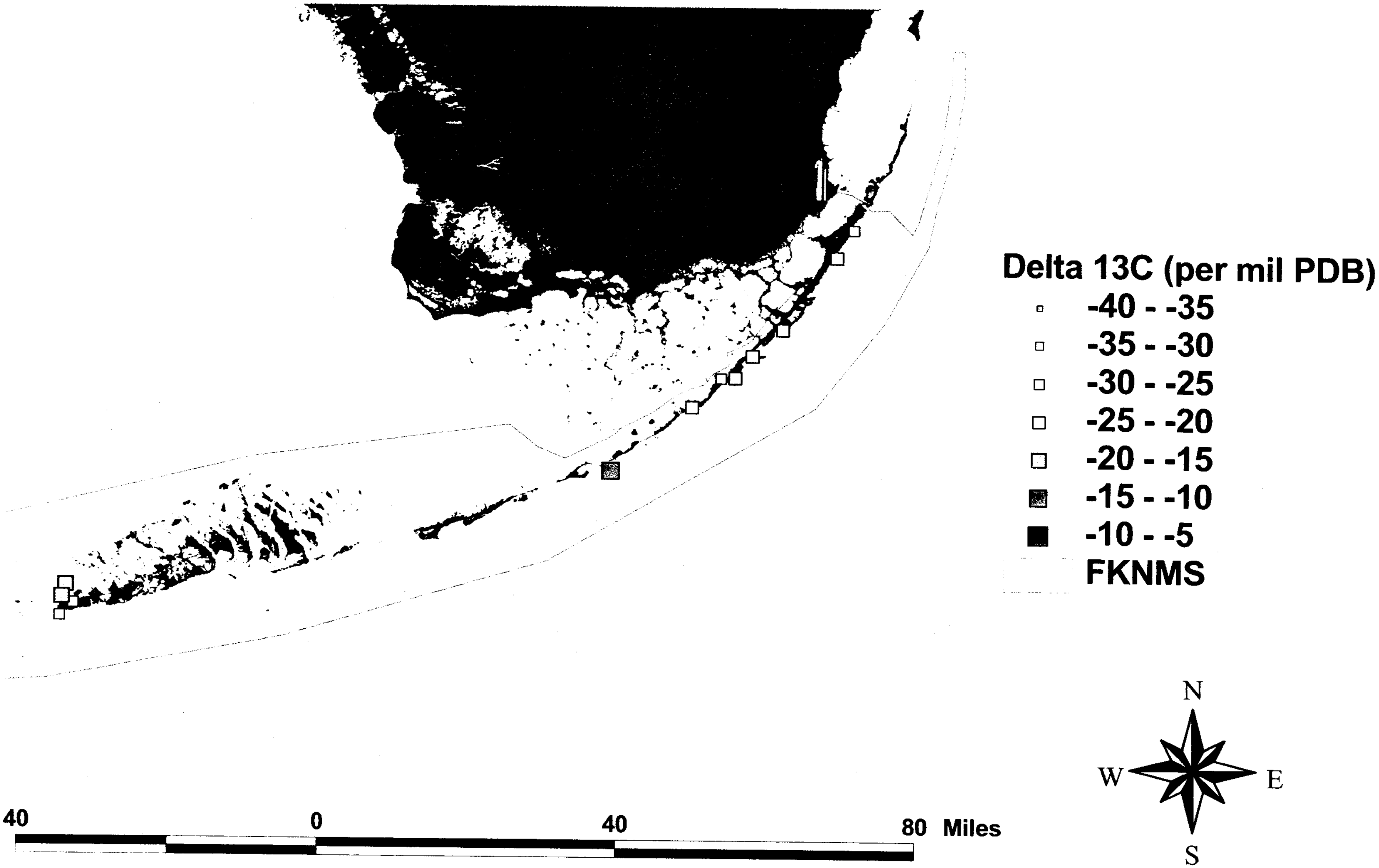
EPA Florida Keys Project
The Impact of Sewage Contaminants on the Florida Reef Tract
Summer 1999 Sample Results

Name	Site Name	Date	Latitude	Longitude	Volume	N mg/l	$\delta^{15}N$ ppt	C mg/l	$\delta^{13}C$ ppt	C/N	Coprost ppm	Epicop ppm	Cholest ppm
FKEPA-69901	Key Biscayne Inshore (site 1)	36319.00	25.65	-80.13	15.00	0.19	2.79	1.01	-17.73	5.36	0.06	0.03	7.22
FKEPA-69903*	Key Biscayne Offshore (site 3)	36319.00	25.64	-80.09		0.13	3.07	0.58	-19.15	4.20	0.00	0.00	0.00
FKEPA-69904*	Key Largo Inshore (site 4)	36319.00	25.11	-80.38			3.91		-13.98		0.00	0.00	0.00
FKEPA-69905*	Key Largo Midshore (site 5)	36319.00	25.10	-80.36			2.64		-18.76		0.00	0.00	0.00
FKEPA-89906	Key Largo Offshore (site 6)	36375.00	25.07	-80.32	17.85		2.13		-19.42		0.00	0.00	0.00
FKEPA-89907*	Channel 5 Inshore (site 7)	36375.00	24.82	-80.76			4.40		-18.47		0.00	0.00	0.00
FKEPA-89908	Channel 5 Midshore (site 8)	36375.00	24.79	-80.74	7.50		3.17		-19.64		0.00	0.00	0.00
FKEPA-89910	Long Key Inshore (site 10)	36375.00	24.79	-80.86	9.30		1.88		-16.37		0.04	0.03	5.35
FKEPA-89911	Long Key Midshore (site 11)	36375.00	24.76	-80.85	10.50		2.73		-20.19		0.01	0.05	7.79
FKEPA-89912	Long Key Offshore (site 12)	36375.00	24.72	-80.83	14.00		3.44		-19.25		0.00	0.00	0.00
FKEPA-89913*	Marathon Inshore (site 13)	36375.00	24.70	-81.03			1.91		-18.90		0.00	0.00	0.00
FKEPA-89914	Marathon Midshore (site 14)	36375.00	24.68	-81.02	17.50		2.65		-18.91		0.00	0.00	0.00
FKEPA-89915	Marathon Offshore (site 15)	36375.00	24.65	-81.02	14.50		2.54		-20.40		0.00	0.00	0.00
FKEPA-69916*	7 Mile Bridge Inshore (site 16)	36430.00	24.67	-81.20			2.14		-17.70		0.00	0.00	0.00
FKEPA-89917	7 Mile Bridge Midshore (site 17)	36375.00	24.64	-81.19	12.00		3.52		-18.33		0.02	0.01	12.85
FKEPA-69918	7 Mile Bridge Offshore (site 18)	36320.00	24.60	-81.18	12.00	0.11	1.91	0.23	-19.71	2.20			
FKEPA-69922	Key West Offshore (site 22)	36321.00	24.45	-81.83	12.00	0.11	1.22	0.24	-20.19	2.19			
FKEPA-69923	Key West Midshore (site 23)	36321.00	24.49	-81.83	12.00	0.11	2.51	0.30	-20.87	2.60			
FKEPA-69924*	Key West Inshore (site 24)	36321.00	24.53	-81.83		0.13	2.86	0.48	-20.94	3.62			

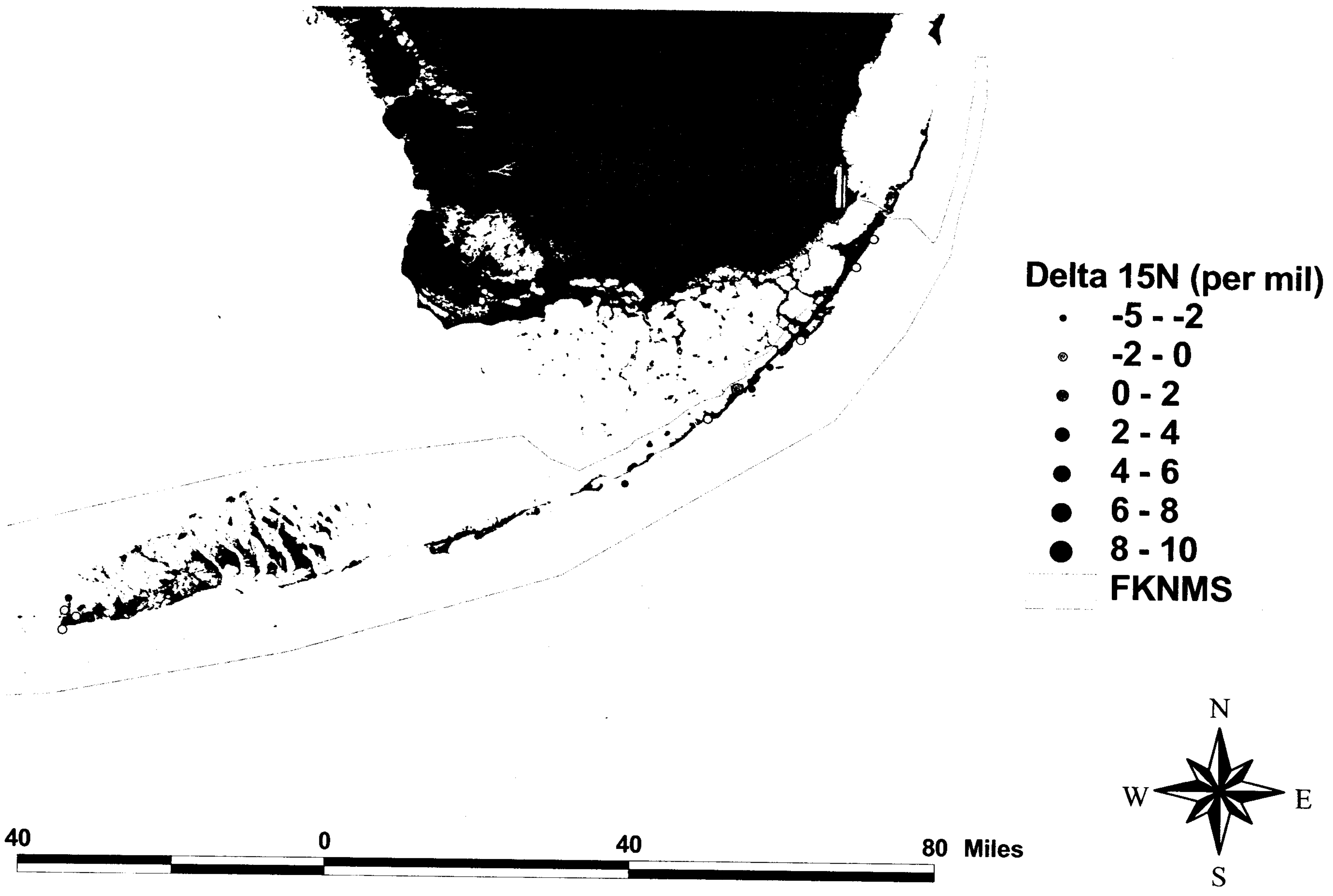
EPA Florida Keys Project
The Impact of Sewage Contaminants on the Florida Reef Tract
Summer 1999 Sediment Samples

Lab ID	Name	Date	Latitude	Longitude	N	δ^{15}N	C	δ^{13}C	C/N	C/N
					mg/l	ppt	mg/l	ppt		
18556	89901	08/03/1999	25.6450	-80.1300		1.11	1.41E-07		-16.28	
18284	89902	08/03/1999	25.6450	-80.1083	1.76%	1.92	2.92E-07	6.71%	-8.99	4
18557	89903	08/03/1999	25.6433	-80.0850		4.17	2.47E-07		-21.36	
18558	89904A	08/03/1999	25.1100	-80.3783		-1.79	8.30E-08		-15.33	
18289	89905	08/03/1999	25.0950	-80.3583	1.06%	1.52	4.75E-07	8.99%	-11.75	9
18560	89908A	08/03/1999	24.7917	-80.7400		2.17	2.37E-07		-15.15	
18559	89908B	08/03/1999	24.7917	-80.7400		-1.21	1.29E-07		-16.43	
18292	89910	08/03/1999	24.7867	-80.8600	1.89%	2.30	4.23E-07	10.88%	-15.90	6
18561	89911	08/03/1999	24.7550	-80.8483		-0.45	1.12E-07		-15.14	
18294	89913	08/03/1999	24.7017	-81.0283	1.30%	1.72	2.83E-07	6.90%	-15.62	5
18295	89914	08/03/1999	24.6767	-81.0233	1.35%	1.68	2.72E-07	7.83%	-13.71	6
18296	89917	08/03/1999	24.6350	-81.1933	1.20%	1.84	2.57E-07	7.85%	-14.29	7
18297	89924	08/03/1999	24.5333	-81.8283	1.48%	2.92	3.49E-07	7.83%	-17.82	5
18281	FBM29817	02/21/1998	25.0070	-80.5344	1.41%	4.43	5.10E-07	14.28%	-20.21	10
18282	FBM29818	02/21/1998	25.0853	-80.4573	3.17%	3.15	1.00E-06	32.71%	-22.81	10
18555	113099RWC	11/30/1999				3.97	4.81E-07		-23.67	
18554	113099WPPC	11/30/1999				1.91	2.30E-07		-18.54	
18551	12699KWC-1C	12/06/1999				8.48	3.62E-07		-18.35	
18553	12699KWC-1FDC	12/06/1999				6.99	2.61E-07		-18.38	
18552	12699KWC-2C	12/06/1999				5.62	5.70E-07		-26.88	

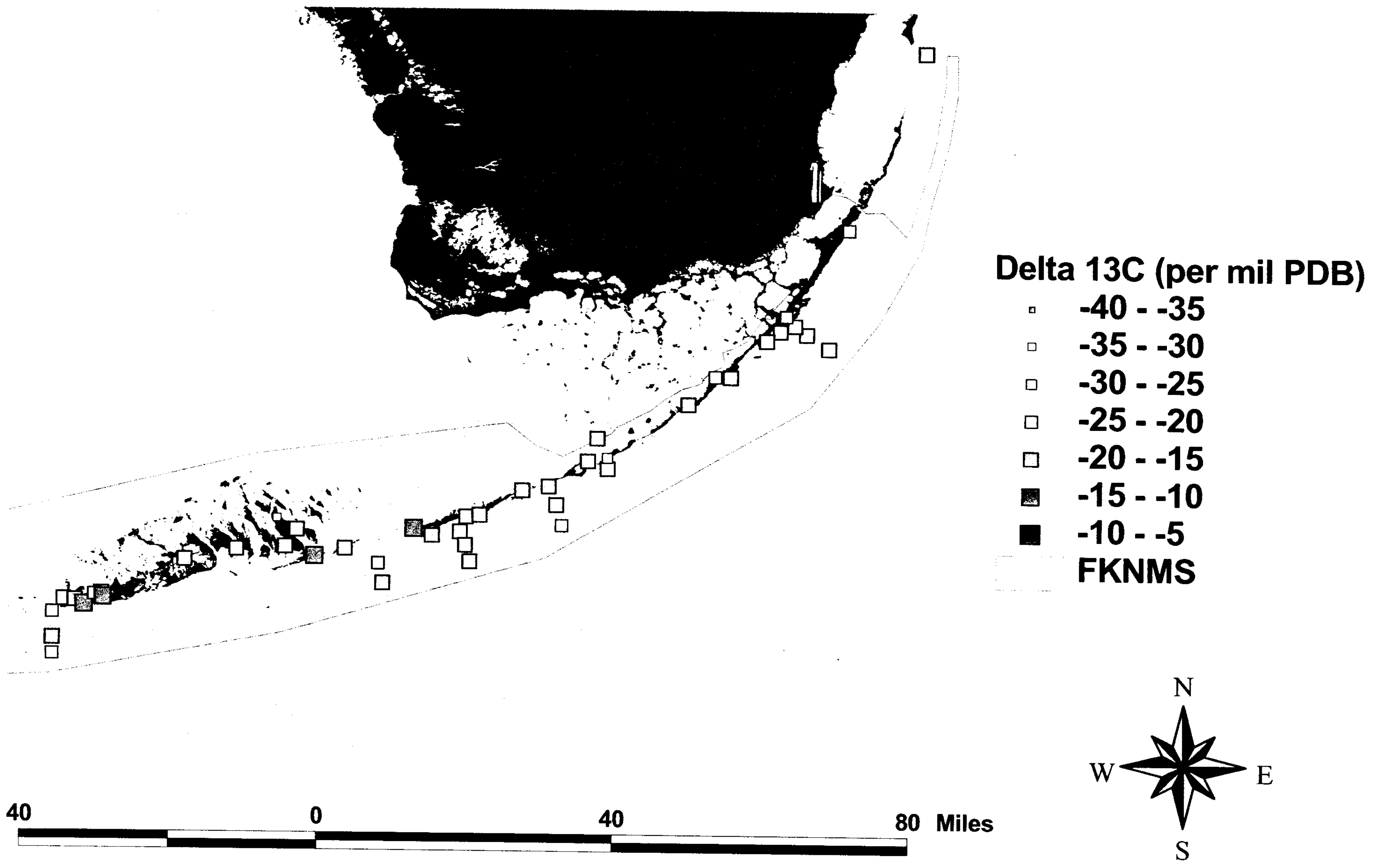
Spring 98 POM Carbon



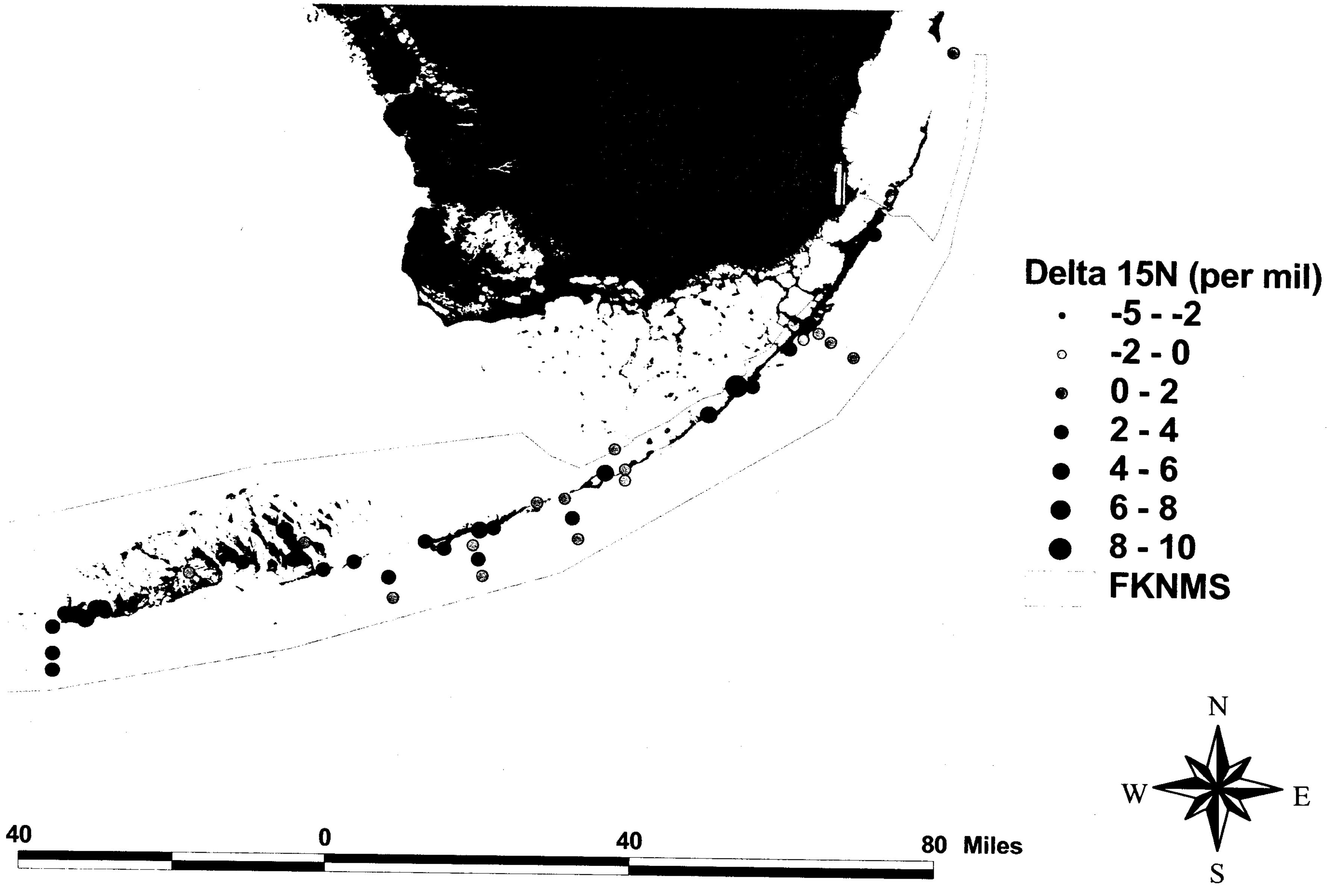
Spring 98 POM Nitrogen



Summer 98 POM Carbon



Summer 98 POM Nitrogen



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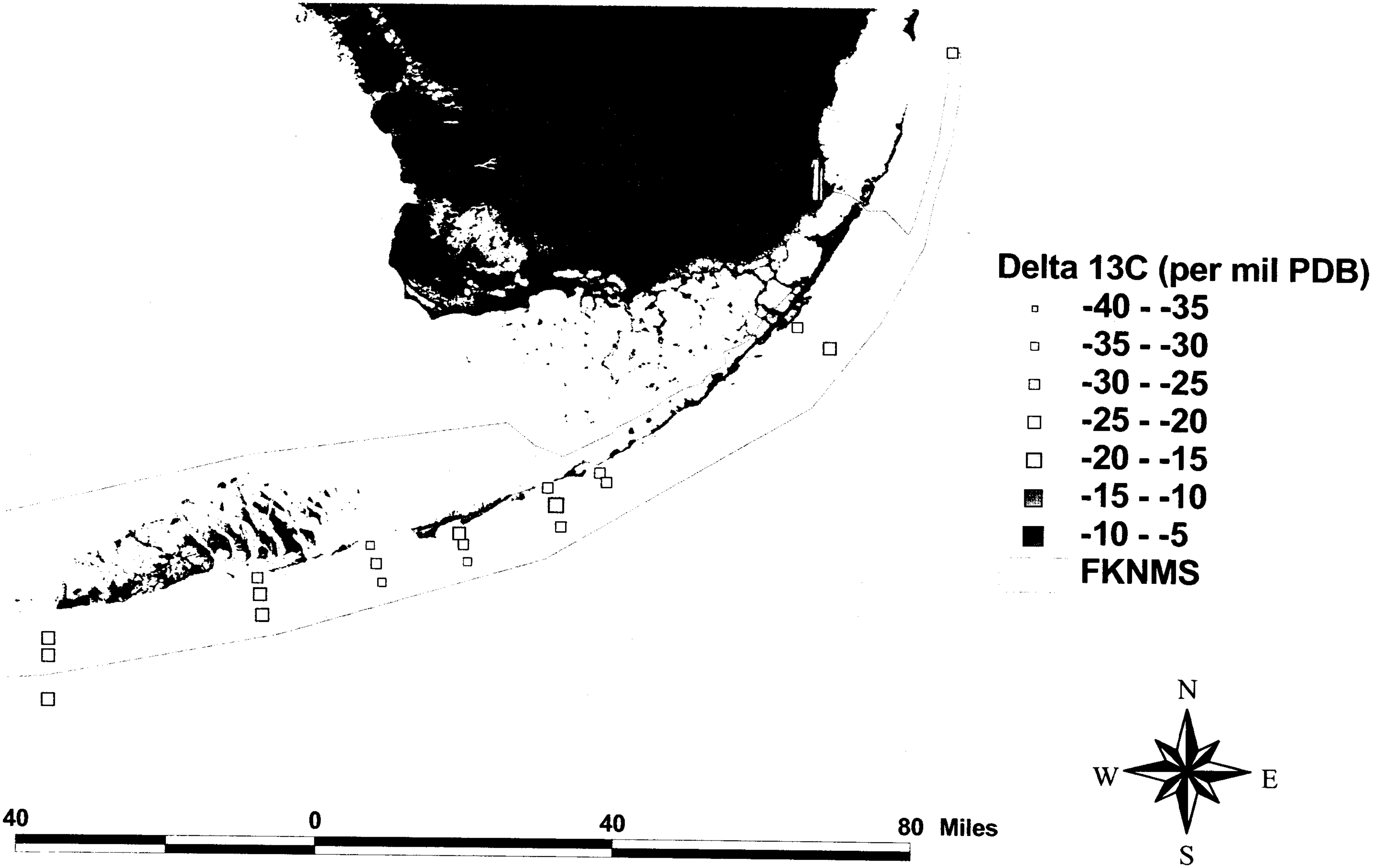
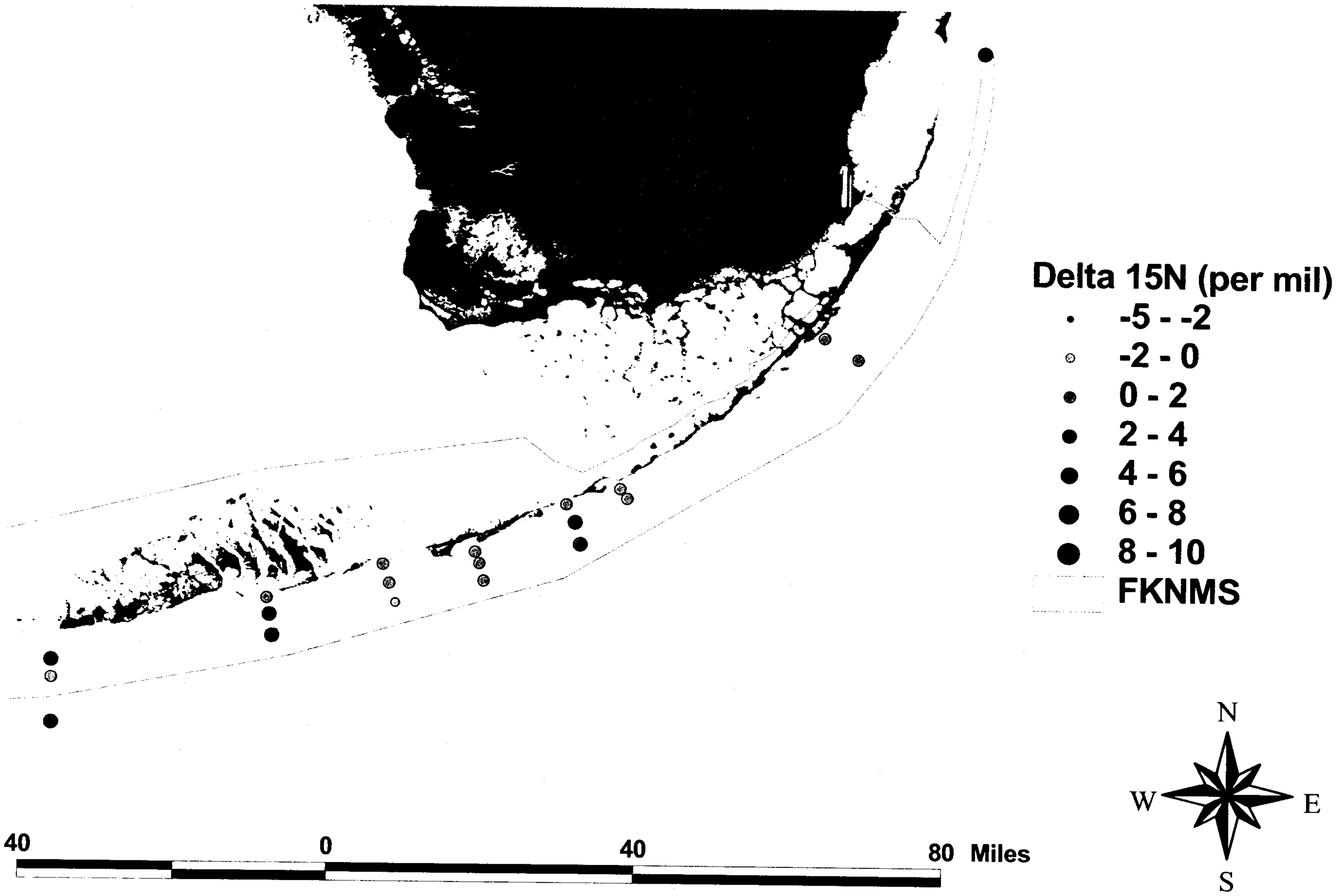
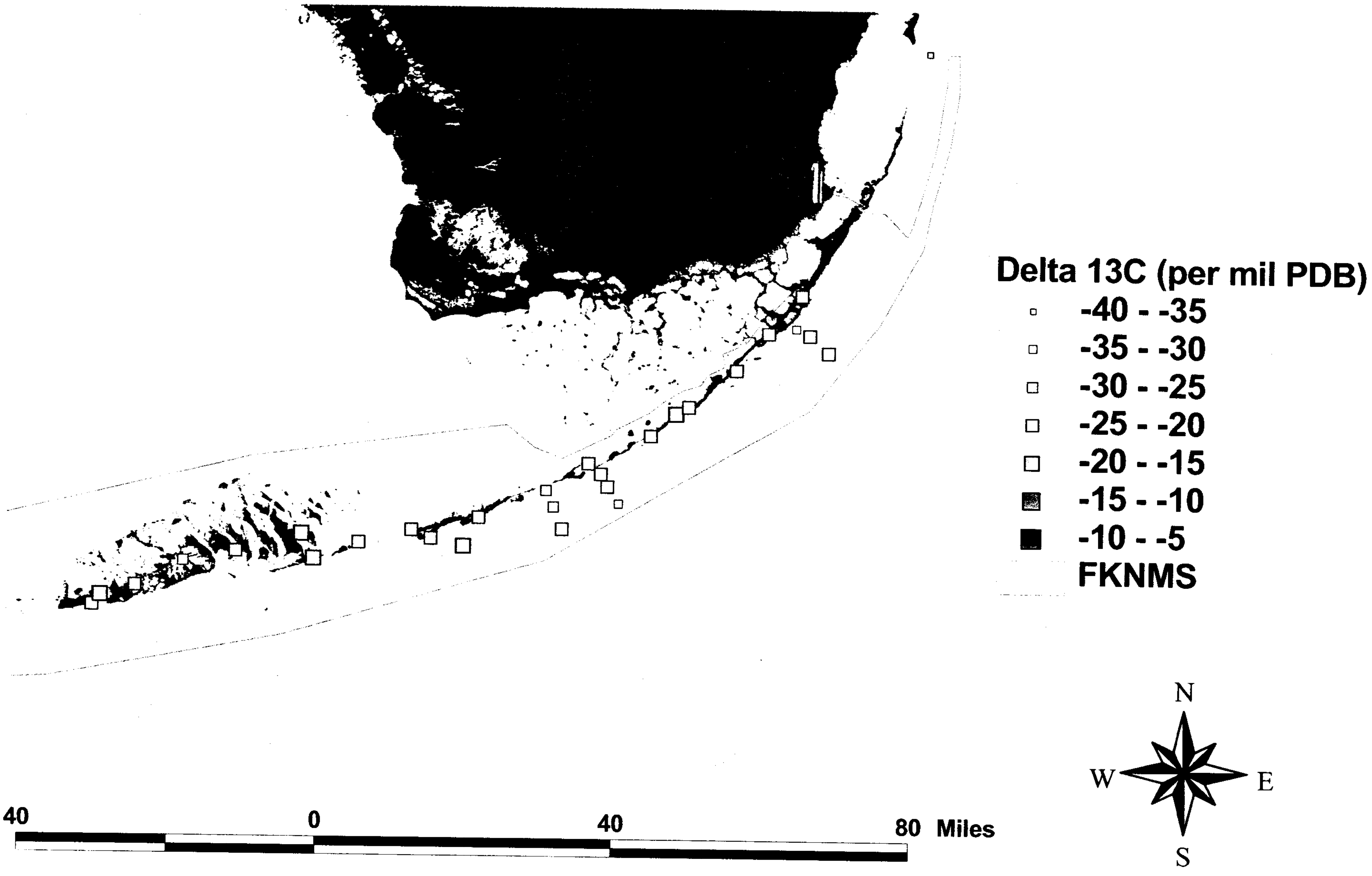


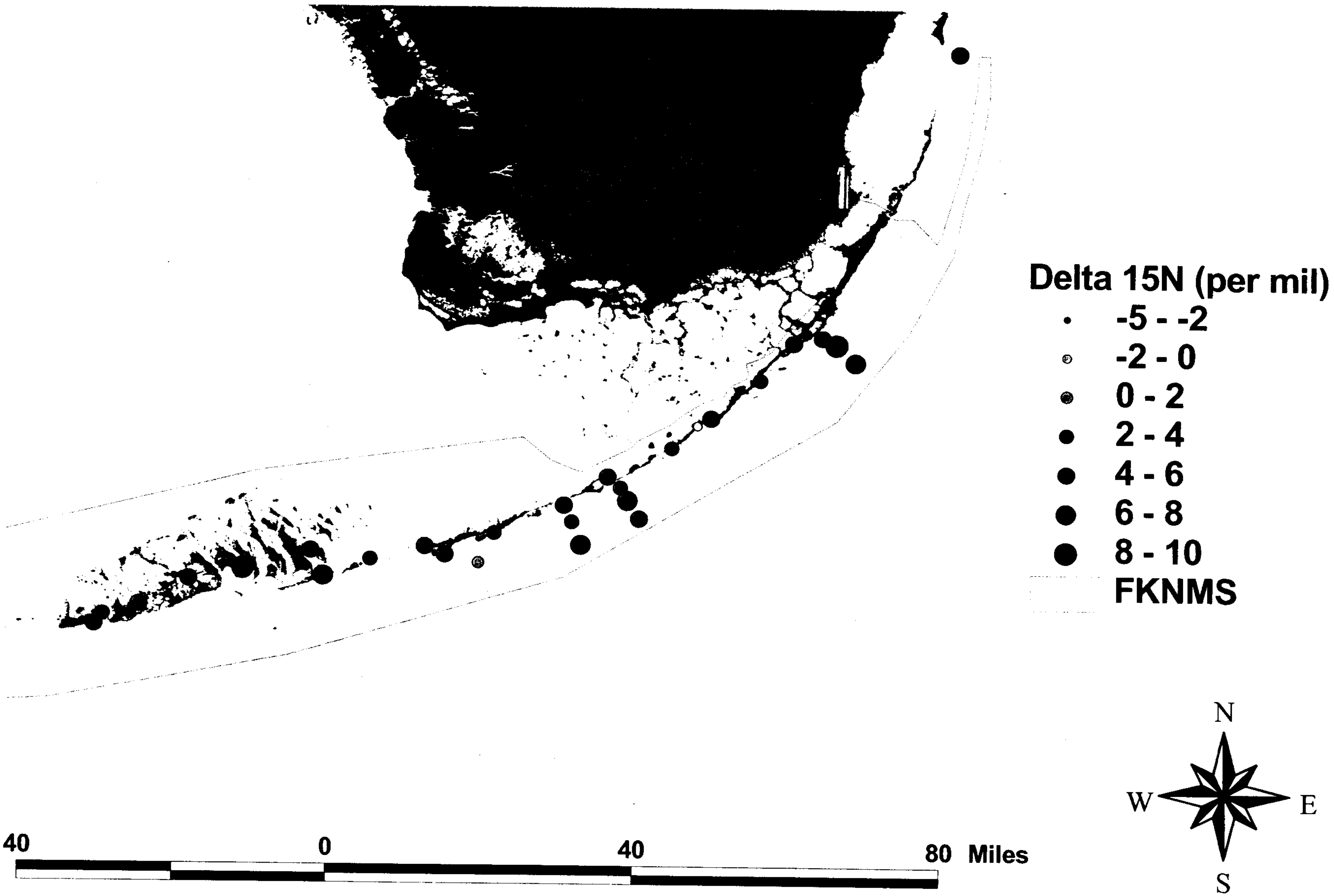
Fall 98 POM Nitrogen



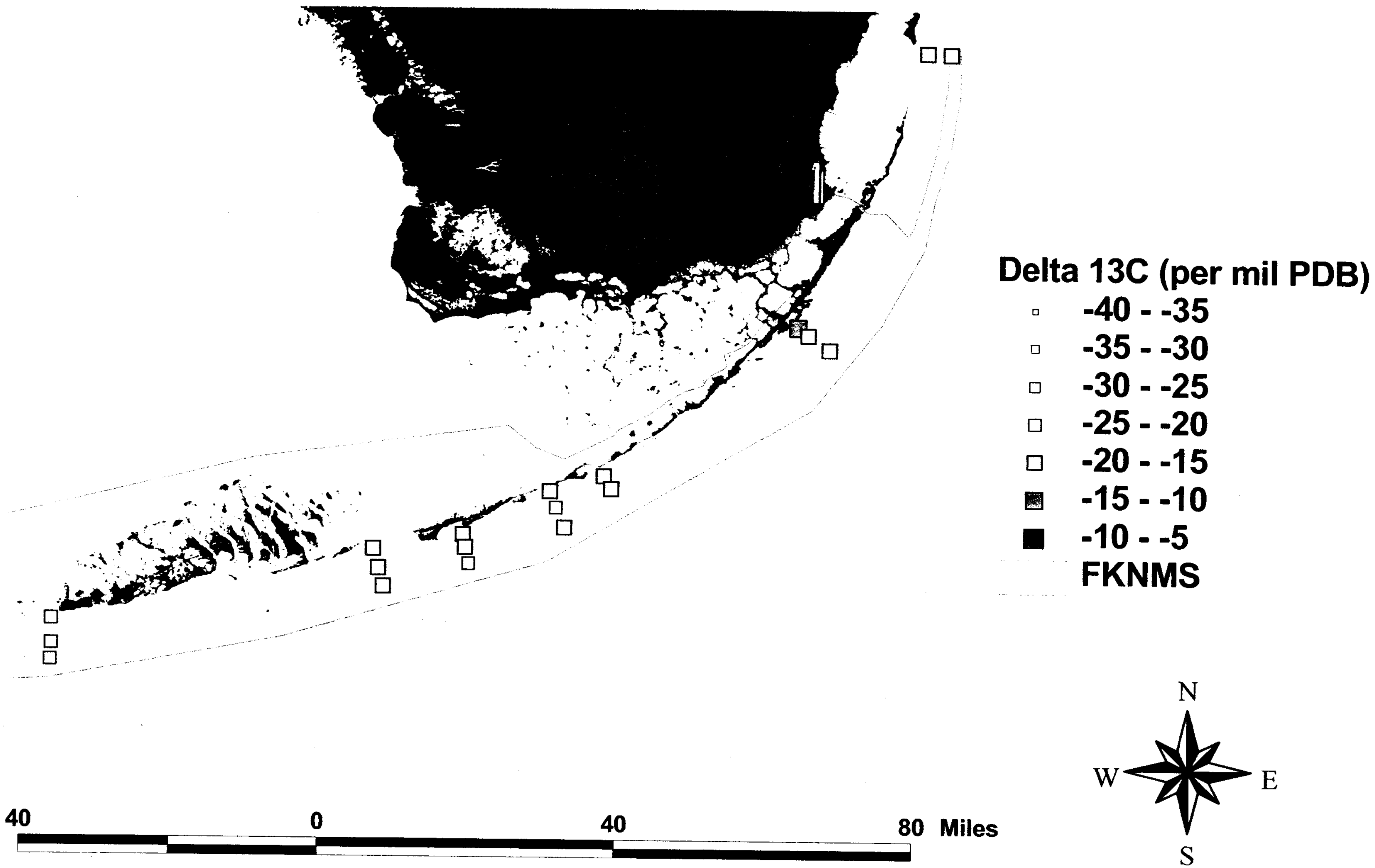
Spring 99 POM Carbon



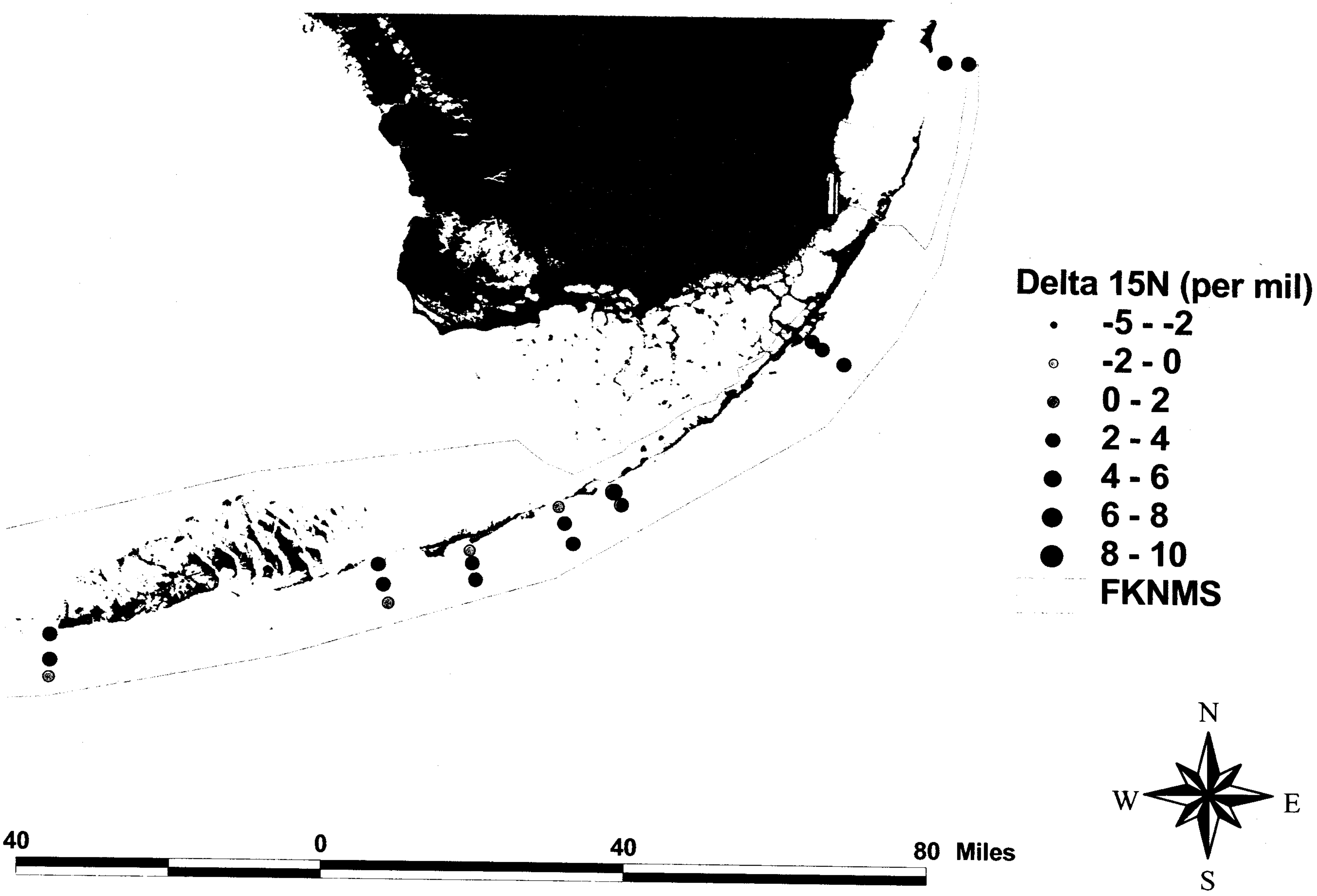
Spring 99 POM Nitrogen



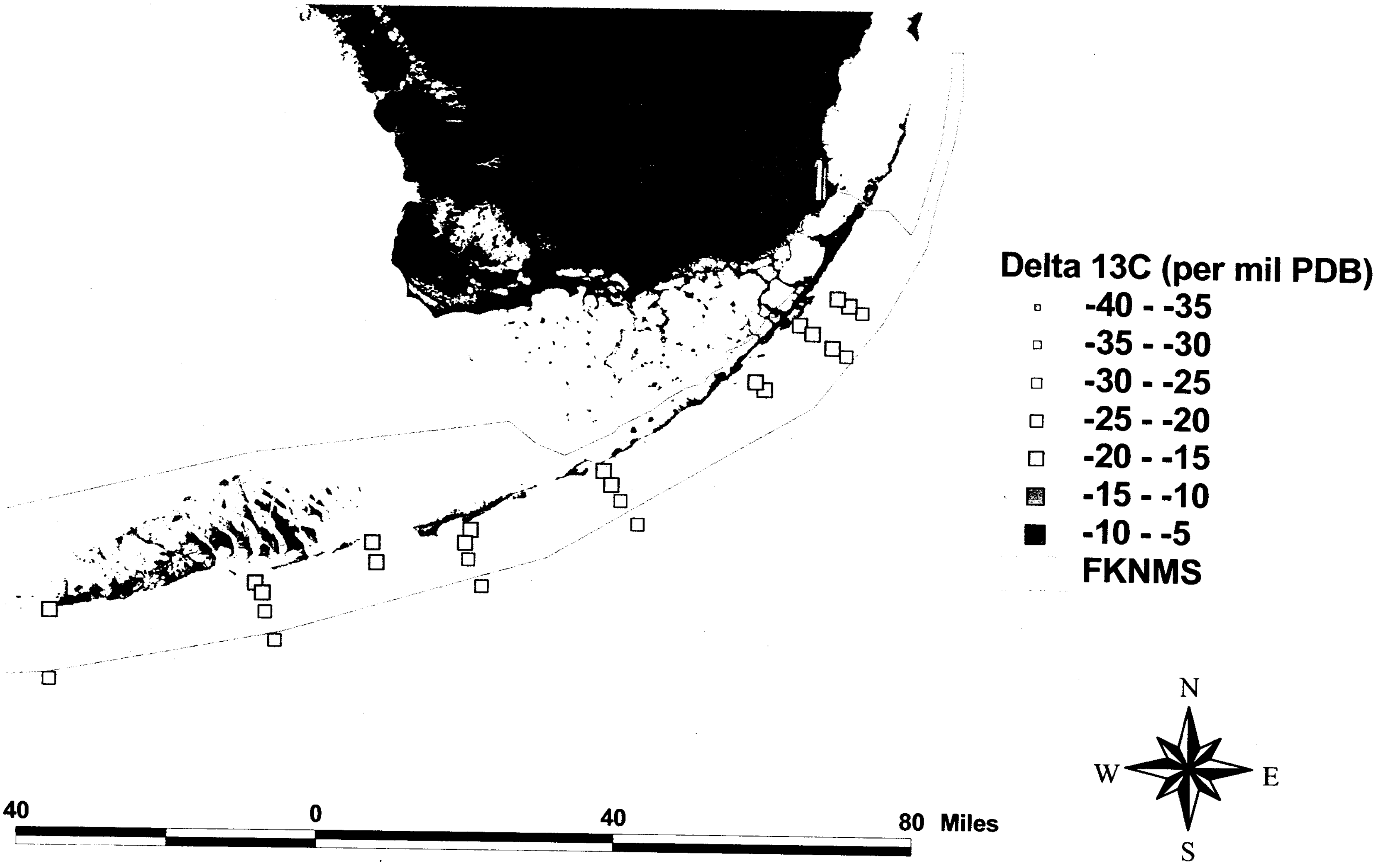
Summer 99 POM Carbon



Summer 99 POM Nitrogen



Summer 00 POM Carbon



Summer 00 POM Nitrogen