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Sources of dissolved inorganic nitrogen in a coastal lagoon adjacent to a major metropolitan area, Miami Florida (USA)

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ABSTRACT

Between 2006 and 2007, a study was carried out to determine the relative importance of natural and anthropogenic input of nitrogen into Biscayne Bay (South Florida, USA) using $\delta^{13}C$ and $\delta^{15}N$ values of algae, seagrasses, and particulate organic material, $\delta^{18}O$ and $\delta^{15}N$ of the NO_3^- and $\delta^{13}C$ of the dissolved inorganic carbon. The $\delta^{15}N$ values of all components showed a strong east to west gradient approaching more positive values (+7 to +10‰) close to the land-sea interface. The nitrogen could have emanated from the local waste water treatment plant, septic systems within the region, or nitrogen which had been affected by denitrification and leached from the local landfill, wastewater which had been injected into the Floridan aquifer and leaked back to the surface, and/or some other as yet unidentified source. The measured NO_3^- $\delta^{15}N$ and $\delta^{18}O$ values indicated that the dissolved nitrate originated from anthropogenic sources and was fractionated during assimilation.

1. Introduction

This paper has investigated the stable nitrogen and carbon isotope dynamics of algae, seagrasses, and particulate organic matter (POM) collected from Biscayne Bay, a semi-enclosed estuary in South Florida, USA. The results of this study have significant implications for the utilization of stable N and C isotopes as indicators of anthropogenic influence in any such body of water adjacent to a large metropolitan area.

1.1. Geographical setting of Biscayne Bay

Biscayne Bay is a large semi-enclosed body of water situated immediately to the east of the metropolitan area of Miami, home to a population of approximately 2 million people (Fig. 1). The natural shore line of the northern portion of Biscayne Bay has largely been replaced by artificial barriers and the center of the northern bay has been extensively dredged and is occupied by numerous artificial islands. The southern bay is generally preserved in its original state with some anthropogenic modifications and is part of Biscayne National Park (BNP), primarily a marine national park. Adjacent to BNP and located within the region's watershed are a wastewater treatment plant (South Dade Wastewater Treatment Plant (SDWWTP), large highly developed urban areas with septic systems, the major solid waste disposal site for Miami-Dade County (Black Point Landfill), and a nuclear power plant (Turkey Point) with its associated cooling canals. The treated wastewater from the SDWWTP is injected into the Floridan aquifer at a depth of ~1000 m. This aquifer is separated from the unconfined surficial aquifers by confining layers which limit exchange and are supposed to prevent the waste water from reaching the surface and influencing Biscayne Bay. This paper suggests that there is significant contamination of the bay by waters associated with one or more of these potential sources.

1.2. Hydrology of Biscayne Bay

The waters in Biscayne Bay are mixtures of seawater, freshwater derived from the mainland (through canals and groundwater discharge), and local precipitation. This mixture creates a gradient of salinity from near freshwater values immediately adjacent to the western coast to normal marine salinity towards the east (Fig. 2). As a result of barrier/artificial islands (Miami Beach, Fisher Island, Virginia Key, and Key Biscayne), a series of mudbanks (Safety Valves), and Pleistocene islands (Soldier Key, Elliott Key, and the northern Florida Keys Complex) to the east (Fig. 1), water in Biscayne Bay can become seasonally isolated leading to salinities slightly elevated above normal seawater values (Dole, 1914; Serafy et al., 1997; Smith, 1896). The present salinity balance in Biscayne Bay has been significantly altered compared to the 19th and early 20th centuries, when there were reports of ocean going ships entering the bay to replenish their freshwater supplies from

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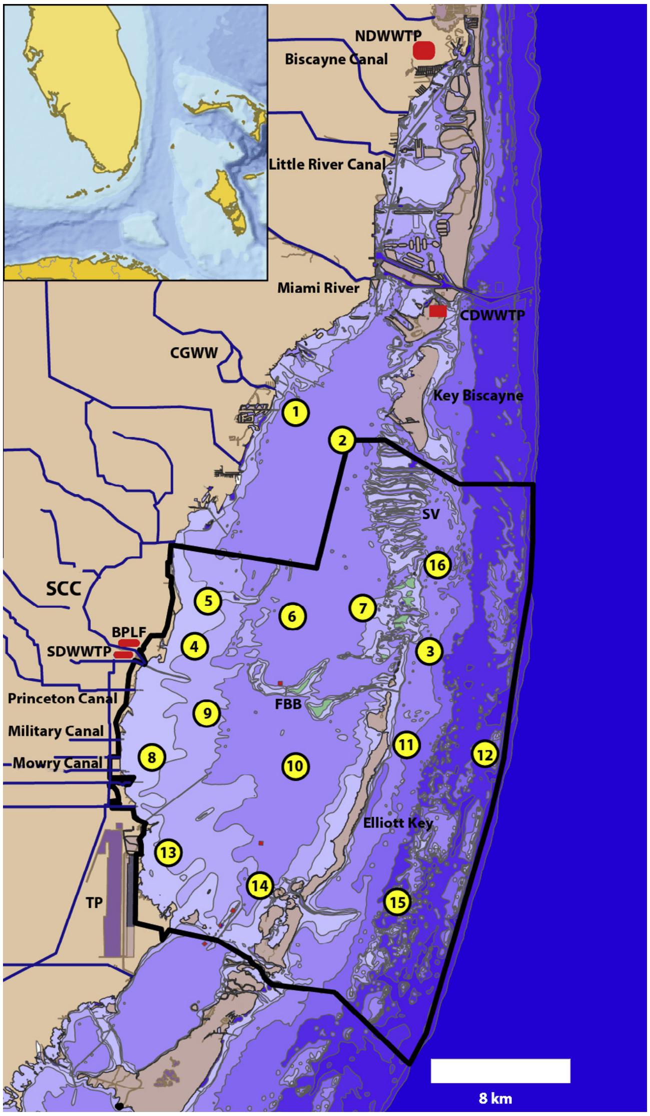

Fig. 1. Location map of Biscayne Bay and the 16 quarterly stations used in this study. Also shown are the various canals and features referred to in the text: North Dade Waste Water Treatment Plant (NDWWTP), Central Dade Waste Water Treatment Plant (CDWWTP), Rosenstiel School of Marine and Atmospheric Science (RSMAS), Coral Gables Water Way (GCGWW), Snapper Creek Canal (SCC), Safety Valves (SV), Feather Bed Bank (FBB), Black Point Land Fill (BPLF), South Dade Waste Treatment Plant (SDWWTP), and Turkey Point Nuclear Plant Cooling Canals (TPCC). Biscayne National Park is outlined by the solid black line. The bathymetric contour lines are drawn every 1 m. The dark blue color is deeper than 7 m. Areas of FBB and SV are semi-emergent at low tide (green color) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

springs that upwelled into the bay (Kohout, 1966; Munroe and Gilpin, 1990). These springs resulted from the high hydrological head of freshwater on the adjacent mainland. At the present, groundwater levels are considerably lower than in the past and this phenomenon has largely disappeared. Presently freshwater input into the Bay is largely derived from direct precipitation (53%), with lesser amounts from canals (37%) and groundwater (10%) (Stalker et al., 2009). All canals within the region have saltwater control structures in order to reduce the inland intrusion of saline water. The flow through these canals is managed by the South Florida Water Management District (SFWMD) which balances the dangers of saltwater intrusion and flood control, while at the same time providing sufficient water to meet the demands of local agriculture, industry, and the human population.

An extensive water drainage system (Fig. 1) is linked to Biscayne Bay through a number of canals (Mowry Canal, Military Canal, Princeton Canal, Snapper Creek Canal, and the Coral Gables Water Way) and the Miami River. In the northern portion of the Bay, the Biscayne Canal and the Little River Canal contribute freshwater. These waterways deliver, in addition to freshwater, a range of anthropogenic chemicals derived from agriculture, urban development, septic systems, and industry. Past efforts to study the temporal and spatial patterns of water quality were based on water samples collected monthly at stations throughout Biscayne Bay, between 1994 and 2008 by Florida International University (Caccia and Boyer, 2007). These data show high concentrations of dissolved nutrients such as phosphate and dissolved inorganic nitrogen (DIN) close to the coast line, particularly in the southern portion of the Bay (Fig. 2). For comparison, concentrations of DIN from the Florida Keys are approximately 0.1–0.5 μM (Szmant and Forrester, 1996), while adjacent to the coast in Biscayne Bay values average as high as 10 μM (Caccia and Boyer, 2007). These high concentrations have been reported as being derived mainly from the input of canal sourced nutrients derived from the agricultural runoff rather than other anthropogenic sources (Caccia and Boyer, 2007). Additional sources of nutrients might be derived from the Black Point Landfill (Meeder and Boyer, 2001) and supplied through groundwater input. This study employs stable nitrogen and carbon isotopes of biotic and biotic-derived components in Biscayne Bay together with nitrogen and oxygen isotope ratios of the nitrate in the water in order to help ascertain the origin of the nutrients being delivered to Biscayne Bay and in particular to ascertain whether there are any patterns which could be used to distinguish contributions from the SDWWTP, Black Point Landfill, or agricultural sources.

1.3. Stable N, C, and O isotopes as indicators of the source of nitrate in the marine environment

1.3.1. Nitrogen

There have been numerous studies which have used the $^{15}N/^{14}N$ ratios (reported as $\delta^{15}N$ values relative to atmospheric nitrogen) as a source indicator for nitrogen in aquatic ecosystems (McClelland et al., 1997; Schell and Michener, 1994; Valiela et al., 2000). Although there are multiple biogeochemical processes which can lead to enrichment of ^{15}N in the environment, elevated $\delta^{15}N$ has frequently been cited as evidence for significant anthropogenic N loading (Costanzo et al., 2001; Heaton, 1986; McClelland and Valiela, 1998; Sammarco et al., 1999). This enrichment arises both as a result of an estimated 3.5‰ increase in the $\delta^{15}N$ value per trophic level, a phenomenon which arises because organisms preferentially excrete ^{14}N enriched NH_4^+ (DeNiro and Epstein, 1981) in their waste products and subsequent sewage treatment which may cause further ^{15}N enrichments if there is substantial NH_4^+ volatilization or partial tertiary treatment (Kendall et al., 2008).

In marine waters off the Florida Keys, $\delta^{15}N$ values as low as +4.5‰ have been cited as being positive evidence for contamination by anthropogenic nitrogen (Lapointe et al., 2004). However, other studies have indicated that the situation in this area is not so clear (Lamb, 2007; Lamb et al., 2012; Swart et al., 2005a). For example, the $\delta^{15}N$ of coral tissues and zooxanthellae from the Upper Keys places them in a category unaffected by pollution according to the definition of Heikoop et al. (2000). In addition, studies of the $\delta^{15}N$ of particulate organic material and algae has shown no evidence for anthropogenic sources (Lamb and Swart, 2008) and values of the $\delta^{15}N$ of the nitrate, upwelled from deeper waters had values similar to algae (Lamb et al., 2012; Leichter et al., 2007). The variation observed in those studies was suggested to

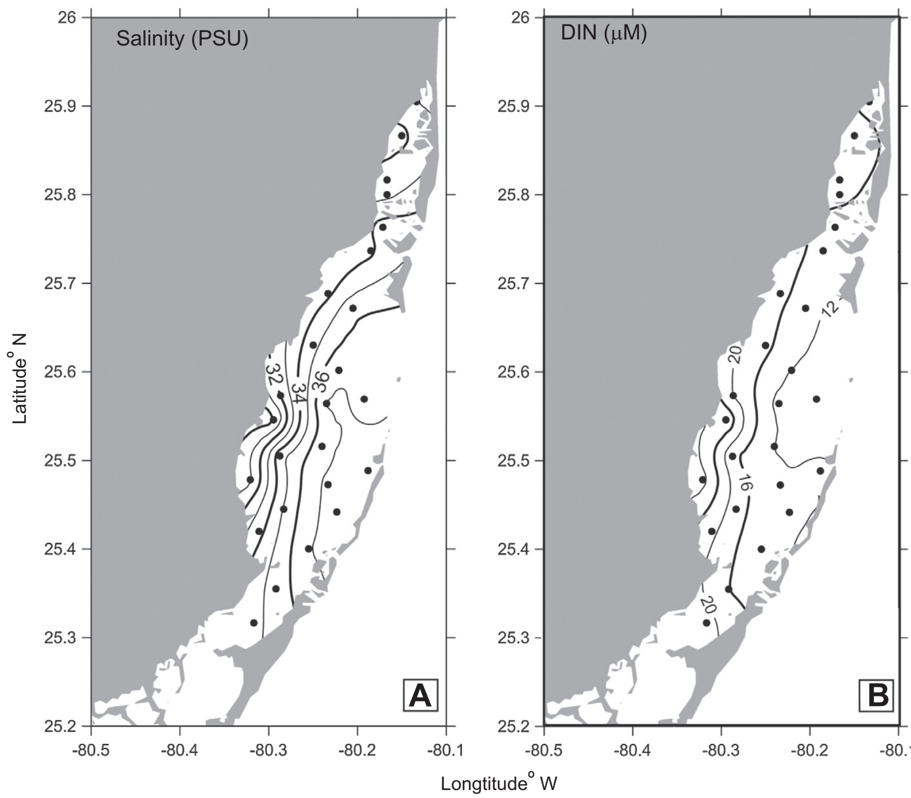

Fig. 2. Contour maps of the mean: (A) Salinity, and (B) Total nitrogen (μM) from monthly samples collected between 2006 and 2007 by Florida International University (Caccia and Boyer, 2005, 2007).

result from the preferential assimilation of ^{14}N by the algae, thereby leading to an enrichment in the heavier isotope compared to the ambient DIN (Lamb, 2007). The extent to which the lighter isotope preferentially accumulated is dictated by the fractionation factor (α) exerted during assimilation, which in turn varies according to species and physiological conditions. Although there is still considerable uncertainty about the fractionation during assimilation of NO_3^- or NH_4^+ into algae, the fractionation factor (α) is estimated to be between ~1.002 and 1.010 (Goericke et al., 1994; Wada and Hattori, 1978).

In addition to assimilation, the actual extent of enrichment in ^{15}N in DIN can be influenced by a range of factors specific to the environment such as fractionation during nitrification (Casciotti et al., 2003; Delwiche and Steyn, 1970) and denitrification, usually within the sediments (Barford et al., 1999; Cline and Kaplan, 1975; Granger et al., 2006; Miyake and Wada, 1971). Furthermore such environments can have inputs from 'natural' high end trophic feeders such as fish and birds, both of which have elevated δ^{15}N values (Hobson, 2011; Lamb et al., 2012). As a result there can be a considerable range in the δ^{15}N values of DIN which arise from natural processes and therefore it is not practical to assign a precise value for δ^{15}N values which would unequivocally indicate the presence of anthropogenic sewage.

1.3.2. Carbon

The δ^{13}C of organic material has been used in conjunction with δ^{15}N in order to identify anthropogenic and land-based influences within ecosystems. Generally the δ^{13}C of the dissolved inorganic carbon (DIC) and particulate organic carbon (POC) should decrease closer to the land as contributions from isotopically depleted terrestrial sources increase (Lamb and Swart, 2008; Rogers, 2003; Sammarco et al., 1999). Although changes can be reflected in the δ^{13}C of photosynthetic organisms (through the δ^{13}C of the local DIC), the δ^{13}C of these organisms is also influenced by the physiology as well as a large number of environmental factors like insolation, temperature, and salinity. While these additional factors can complicate the interpretation of changes in δ^{13}C, the measurement of this variable can provide insight into gradients between the land and marine ecosystems (Bouillon et al., 2002, 2003; Lin et al., 1991).

1.3.3. Oxygen

The δ^{18}O of nitrate in parallel with δ^{15}N has also been used to trace the origin and transformations of NO_3^- (Kendall, 1998). It has been shown that NO_3^- derived from precipitation can have quite positive δ^{18}O values (+20 to +70‰) while NO_3^- derived from the nitrification of NH_4^+ appears to have a constant offset from the δ^{18}O of water and dissolved O_2 of about 2–3‰ in most marine systems (Casciotti, 2009). In the oceans the nitrate δ^{18}O is balanced between this initial signal and subsequent enrichments produced by fractionation during either denitrification and/or assimilation. These removal processes can result in δ^{18}O values elevated up to +30‰ (Casciotti, 2009; Knapp et al., 2008; Sigman et al., 2009; Wankel et al., 2006, 2009). Generally if nitrate is derived or transformed by different sources or processes, different correlations arise between the δ^{15}N and the δ^{18}O of the nitrate. For example, during the assimilation of nitrate by algae or microbial denitrification, ^{15}N and ^{18}O are fractionated to the same extent leading to a 1:1 correlation between δ^{15}N and δ^{18}O. If a large proportion of the nitrate is resupplied through nitrification from an isotopically light source then the slope between these isotopes becomes significantly larger than unity, while if nitrate is derived from an isotopically heavy source, such as sewage, then the slope is significantly lower than unity (Wankel et al., 2009).

Removal of NO_3^- by denitrification under low O_2 conditions also discriminates against ^{15}N with α values of between 1.02 and 1.03

(Cline and Kaplan, 1975). This process may be expected to be operating in the narrow deep box canals, which have a high oxygen demand as a result of organic matter accumulation, resulting in low oxygen levels (Kruczynski and McManus, 2002), and thus contribute DIN to Biscayne Bay. Therefore if these processes were operating alone, as the concentration of DIN decreased, the $\delta^{15}N$ and $\delta^{18}O$ of the DIN would increase. Thus a positive correlation between $\delta^{15}N$ and $\delta^{18}O$ in the residual NO_3^- distinguishes between the 'source' and 'processing' explanations for elevated $\delta^{15}N$ in impacted systems. In contrast, low $\delta^{15}N$ values can be achieved where natural biological N_2 fixation (−2 to 0‰) is an important N source (Hoering and Ford, 1960). The $\delta^{15}N$ of industrially produced fertilizer can also be low because industrial N_2 fixation gives a $\delta^{15}N$ close to 0‰. The $\delta^{18}O$ of fertilizer derived NO_3^- is similar to atmospheric O_2 (~25‰), although this signal is lost upon assimilation. Atmospheric derived nitrate has low $\delta^{15}N$ values (Kendall, 1998), but enriched $\delta^{18}O$ values.

2. Methods

2.1. Field work

Algae and seagrasses were collected approximately quarterly over a two year period from 16 locations within Biscayne Bay (Table 1 and Fig. 1). This sampling frequency was selected in order to obtain samples from both the dry season in South Florida (November to May) and wet season (June to October). Sampling was usually carried out during two or more trips depending on weather conditions. The locations of the sites were designed to capture any onshore to offshore gradients within Biscayne National Park (Fig. 1) and to coincide with pre-existing water quality stations, where the concentrations of NH_4^+, NO_3^-, salinity and other nutrients were collected and measured on a monthly basis by Florida International University (Caccia and Boyer, 2005) (11 of the 16 stations corresponded with the FIU stations). Additional water samples were collected during June and July of 2006, including locations along the Coral Gables Water Way (CGWW) which were sampled for the analysis of the $\delta^{15}N$ and $\delta^{18}O$ of the nitrate. These water samples were filtered at the time of collection and remained frozen until analysis. At each of the locations snorkelers collected available benthic organisms. These typically consisted of multiple species of green algae and usually only one sample of *Thalassia* sp. The same species were not always collected or present at every site, either as a result of a true absence or due to site conditions (low visibility or poor weather conditions). The following genera were collected where present; *Avrainvillea* sp., *Caulerpa* sp., *Cladophora* sp., *Dasycladus* sp., *Dichtoyta* sp., *Jania* sp., *Laurencia* sp., *Penicillus* sp., *Thalassia* sp., Turf algae, *Udotea* sp., and *Ulva* sp. In total 214 samples of green algae, 101 sea grasses, and 54 red algae were collected from the quaterly sampling stations. Approximately 100 samples of green algae and surface sediments were collected throughout Biscayne Bay in June 2006. In all cases the entire specimen of algae was collected and analyzed, but for the seagrass samples (*Thalassia* sp.). only the blades were analyzed. In addition, various types of turf algae were collected, but these were not identified to the genus level. Aliquots of the water samples collected at monthly intervals in 2006 by FIU were analyzed for the $\delta^{13}C$ of the DIC and these data are reported in this paper.

2.2. Particulate organic material

Sampling of POM was conducted on a monthly basis at the same time and locations as the quarterly water column and sub-aquatic vegetation sampling. These samples were collected using a portable pump system (2000 gph bilge pump fitted with a 1.5 in. diameter flexible hose; 12 V deep cycle marine battery) and two stacked Nitex mesh sieves (150 μm, 20 μm) made from 6-in. PVC and marine hose clamps. The upper water column was sampled at a depth of 1.5 m for 30 min, allowing more than 3500L of water to pass through the sieve system. The >150 μm and 20–150 μm samples were rinsed with deionized water from Nitex screens into 30 mL bottles immediately following the sampling interval and were kept on ice for transport to the laboratory. These two size fractions were broadly characterized in terms of speciation using traditional light microscopy and the 20–150 μm sample was used for isotopic analyses. This phytoplankton size fraction was selected to limit the contribution of zooplankton to the POM samples and large particles after the approach of Evans et al. (2006). Additionally, a water sample (1 L) was collected after passing through the sieves (<20 μm size fraction), which was kept on ice until filtration on Whatman Anodisc membrane filters (0.2 μm pore size) in the laboratory. All size fractions and membrane filters were then dried at 70 °C, powdered, and analyzed for stable isotope ratios.

2.3. Seawater dissolved inorganic carbon

Samples collected for DIC were kept cool until filtration at the Rosenstiel School of Marine and Atmospheric Sciences (RSMAS) at the University of Miami through a 0.1 μM filter. Samples were

Table 1
Mean $\delta^{13}C$, $\delta^{15}N$ values and ±SE of all green algae, seagrass, red algae, and POM.

Site	Latitude °N	Longitude °W	Green algae		Seagrass		Red algae		POM	
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
1	25.6883	80.2333	−17.8 ± 0.8	6.1 ± 0.7	−11.3 ± 0.9	4.3 ± 0.7	−14.5 ± 1.1	8.0 ± 0.0	nm	6.6
2	25.6717	80.2050	−15.3 ± 0.3	5.1 ± 0.1	−9.5 ± 0.3	3.9 ± 0.3	np	np	nm	4.7
3	25.5428	80.1520	−15.8 ± 0.9	4.7 ± 0.9	−12.9 ± 0.8	5.7 ± 0.3	−18.6 ± 1.7	6.0 ± 0.3	nm	4.5
4	25.5458	80.2947	−16.5 ± 0.6	9.1 ± 0.2	−13.2 ± 1.0	9.6 ± 0.9	−18.5 ± 1.1	10.9 ± 0.5	nm	8.6
5	25.5733	80.2867	−15.6 ± 0.2	7.4 ± 0.3	−16.0 ± 1.7	6.8 ± 1.0	−17.8 ± 2.0	9.2 ± 0.4	nm	7.2
6	25.5642	80.2351	−15.6 ± 0.4	3.7 ± 0.2	−16.6 ± 2.4	4.9 ± 1.0	−18.3 ± 0.9	3.6 ± 0.1	nm	4.0
7	25.5692	80.1925	−14.3 ± 1.0	3.2 ± 0.3	−10.1 ± 0.8	3.2 ± 1.0	−15.8 ± 0.5	2.8 ± 0.3	nm	3.0
8	25.4783	80.3208	−16.0 ± 0.4	9.3 ± 0.2	−11.7 ± 2.5	6.8 ± 0.8	−19.2±	10.9 ± 0.3	nm	7.4
9	25.5050	80.2875	−14.3 ± 0.2	5.5 ± 0.2	−8.6 ± 0.6	5.3 ± 0.5	−17.4 ± 0.4	6.8 ± 0.9	nm	7.2
10	25.4725	80.2333	−13.9 ± 0.8	2.1 ± 0.2	−9.1 ± 1.3	5.3 ± 1.2	−18.1 ± 0.0	7.7 ± 0.0	nm	3.2
11	25.4858	80.1657	−17.1 ± 0.0	2.9 ± 0.0	−12.8 ± 2.7	3.9 ± 1.1	np	np	nm	3.7
12	25.4805	80.1181	−17.8 ± 0.5	2.5 ± 0.2	np	np	−16.1	6.1	nm	3.4
13	25.4202	80.3108	−15.4 ± 0.0	5.2 ± 0.1	−9.1 ± 0.8	4.9 ± 0.8	−16.3 ± 0.4	8.3 ± 0.9	nm	5.1
14	25.4000	80.2550	−14.1 ± 0.1	4.2 ± 0.4	−11.0 ± 0.8	2.3 ± 0.2	−16.8 ± 1.8	4.6 ± 0.8	nm	4.6
15	25.3903	80.1715	−14.2 ± 0.2	2.4 ± 0.4	−12.6 ± 2.8	2.1 ± 0.4	−14.8 ± 0.6	2.8 ± 0.1	nm	3.0
16	25.5955	80.1469	−8.9 ± 1.9	2.1 ± 0.1	−8.8 ± 0.0	1.8±0.0	np	np	nm	4.8

nm = Not measured, np = not present at site.

subsequently poisoned with $HgCl_2$. These samples were then stored in Wheaton bottles until analysis.

2.4. Laboratory analyses

2.4.1. Benthic samples

All collected samples 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. Entire samples were then ground on a Wiley Mill through a 40 μm sieve. These samples were then split with one portion being archived and the second treated with 10% HCl (overnight), followed by 2 rinses with ultra-high purity deionized water and dried in a low temperature drying oven. Approximately 1–4 mg of acid treated sample was weighed out for analysis of $\delta^{13}C$, $\delta^{15}N$, and C:N. Replicate analyses of organic samples which did not contain carbonate and which were treated with and without the acid revealed that the procedure had no statistically significant effect upon the $\delta^{13}C$ and $\delta^{15}N$ values.

2.4.2. Sediment organic material

Sediment samples were collected throughout Biscayne Bay by snorkelers. These samples were freeze-dried upon return to RSMAS and then ground with a mortar and pestle. Approximately 500 mg of ground sample was treated with 10% HCl and then filtered on pre-rinsed and weighed 25 mm GF/C filters. The material collected on the precombusted filters was split and each half analyzed for its $\delta^{15}N$, $\delta^{13}C$, and C:N values.

2.4.3. Seawater DIN $\delta^{15}N$ measurements

Nitrate was converted to nitrite through cadmium reduction and then to nitrous oxide for mass spectrometer analysis with an azide/acetic acid solution (Lamb et al., 2012; McIlvin and Altabet, 2005).

2.4.4. Water quality

Samples for salinity and a range of water quality measurements were collected on a monthly basis by FIU at 25 sites throughout Biscayne Bay (Fig. 2). In this paper we use the measurements of salinity, NO_3^-, and NH_4^+ made on samples collected from many of the same locations from which samples were taken for this study (11 out of the 16 sites had matching water quality data collected during the same month). These data have been reported previously (Caccia and Boyer, 2005, 2007) and the appropriate methods are contained within these papers.

2.4.5. Seawater dissolved inorganic carbon

The DIC in the sample was converted to CO_2 by acidification under vacuum, the CO_2 removed using a stream of He and passed to the mass spectrometer for isotopic analysis.

2.5. Instrumentation

Elemental and isotopic abundances for solid materials were determined using an Automated Nitrogen Carbon Analyzer (ANCA) interfaced to a stable isotope mass spectrometer (Europa Scientific Model 20-20) at the University of Miami. All analyses were performed in duplicate and the values presented represent the mean of the two analyses. Replicate analyses agreed to better than ±0.1‰ for C and ±0.2‰ for N. The CO_2 produced from the acidification of the DIC samples was also analyzed using the same mass spectrometer. The POM samples were analyzed using an elemental analyzer interfaced to a Finnigan-Delta C at Florida International University. Analytical reproducibility for solid materials is ±0.2‰ and ±0.1‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Isotopic abundances for dissolved N species $\delta^{15}N$ and $\delta^{18}O$ (NO_3^-) have been determined using a trap and purge system interfaced with a GV IsoPrime Stable Isotope Ratio Mass Spectrometer at University of Massachusetts, Dartmouth. For dissolved N species, the reproducibility is typically ±0.2‰ for $\delta^{15}N$ and ±0.4 for $\delta^{18}O$. Data are reported relative to the conventional international standards, V-PDB for carbon, SMOW for oxygen (in nitrate) and atmospheric nitrogen.

2.6. Statistics

Differences between sample stations were tested using a Mann–Whitney *U* test and reported statistically significant at the 95% confidence limits ($p < 0.05$), unless stated otherwise. Correlations between variables were calculated using a Spearman's rank correlation coefficient. Contour maps were constructed using a Kriging routine in Surfer 9.0. The Kriging method generates an interpolated grid based on estimates derived from a sampled dataset (Cressie, 1990; Isaaks and Srivastava, 1989).

3. Results

3.1. Biotic components

The data were combined into three different groups: (i) all green algae, (ii) red algae, and (iii) seagrass (exclusively *Thalassia*

Table 2
Mean values of the $\delta^{15}N$ (‰) of green algae, number of samples collected and ±SE of the analyses from each site and sampling visit.

Site	June–July 2006	*n*	August–September 2006	*n*	January–February 2007	*n*	April–May 2007	*n*	July–August 2007	*n*	November 2007	*n*	February 2008	*n*	Mean
1	4.9	1	nc		nc		4.6	2	9.0	1	nc		6.0	2	6.1 ± 0.7
2	4.8	5	5.3	3	nc		5.7	3	5.9	5	4.4	7	4.8	3	5.1 ± 0.1
3	nc		5.2	1	nc		nc		6.6	1	2.5	2	nc		4.7 ± 0.9
4	7.6	1	9.8	2	9.6	2	9.1	1	9.7	5	8.5	2	nc		9.1 ± 0.2
5	6.6	3	7.1	2	7.8	3	5.9	5	10.0	5	nc		7.0	2	7.4 ± 0.3
6	4.3	4	1.6	3	3.6	4	4.1	3	4.6	4	nc		4.3	1	3.7 ± 0.2
7	0.9	1	4.0	2	nc		3.4	2	3.8	2	3.7	4	3.4	1	3.2 ± 0.3
8	7.3	1	9.3	4	10.0	1	9.4	4	10.1	6	10.0	6	9.3	7	9.3 ± 0.2
9	4.2	2	7.0	3	nc		3.6	5	6.8	9	5.9	3	5.2	8	5.5 ± 0.2
10	nc		1.5	3	nc		2.8	1	1.1	3	2.8	1	2.6	4	2.1 ± 0.2
11	2.9	3	3.9	5	nc		nc		2.9	2	nc		nc		2.9 ± 0.0
12	nc		nc		nc		nc		2.9	1	nc		2.1	3	2.5 ± 0.2
13	nc		nc		nc		nc		4.9	6	5.6	1	nc		5.2 ± 0.1
14	nc		2.9	4	nc		4.2	2	2.5	6	6.9	1	4.7	1	4.2 ± 0.4
15	2.	1	nc		nc		1.2	2	3.9	1	nc		3.4	1	2.4 ± 0.4
16	nc		2.1	3	nc		nc		nc		nc		nc		2.1 ± 0.1
Mean	5.1 ± 0.5	22	6.1 ± 0.5	35	7.8 ± 0.9	10	5.9 ± 0.5	30	7.2 ± 0.3	57	5.8 ± 0.5	27	5.6 ± 0.4	33	

nc = Not collected. Samples were not collected at the site, usually as a result of poor weather conditions or low visibility during the collection date.

Table 3
Summary of sea grass (*Thalassia* sp.) sampled from 16 quarterly sites.

Site	June–July 2006	August–September 2006	January–February 2007	April–May 2007	July–August 2007	November 2007	February 2008	Mean
1	4.4	3.3	4.4	3.8	7.7	nc	2.4	4.3 ± 0.7
2	3.9	2.9	nc	2.9	nc	5.2	4.8	3.9 ± 0.5
3	6.1	6.4	nc	6.1	nc	nc	4.8	5.7 ± 0.4
4	8.8	8.6	7.5	4.3	8.8	11.8	12.3	9.6 ± 1.1
5	7.4	7.7	nc	3.2	9.2	8.4	9.7	6.8 ± 1.2
6	2.4	1.3	2.2	8.4	nc	6.7	5.6	4.9 ± 1.1
7	2.5	−0.4	nc	4.8	nc	5.9	3.4	3.2 ± 1.1
8	5.8	8.8	7.2	5.6	nc	nc	nc	6.8 ± 0.8
9	6.5	5.7	nc	nc	4.9	4.1	nc	5.3 ± 0.5
10	nc	3.6	nc	9.1	3.4	5.0	nc	5.3 ± 1.4
11	2.1	1.7	nc	3.1	nc	3.1	7.5	3.9 ± 1.1
12	nc	nc	nc	nc	nc	nc	nc	
13	nc	3.5	nc	nc	nc	6.0	5.3	4.9 ± 0.8
14	nc	2.2	nc	nc	2.5	nc	nc	2.3 ± 0.2
15	1.8	2.9	nc	nc	0.7	nc	1.1	2.1 ± 0.5
16	nc	1.8	nc	nc	nc	nc	nc	1.8
Mean	5.8 ± 0.8	4.5 ± 0.7	6.8 ± 1.8	5.0 ± 0.7	8.7 ± 1.3	7.0 ± 0.8	6.5 ± 1.0	

nc = Not collected. Samples were not collected at a site, usually as a result of poor weather conditions or low visibility. In some locations (BB12) seagrass was never present. The SE is shown for each of the sites and each of the sampling dates.

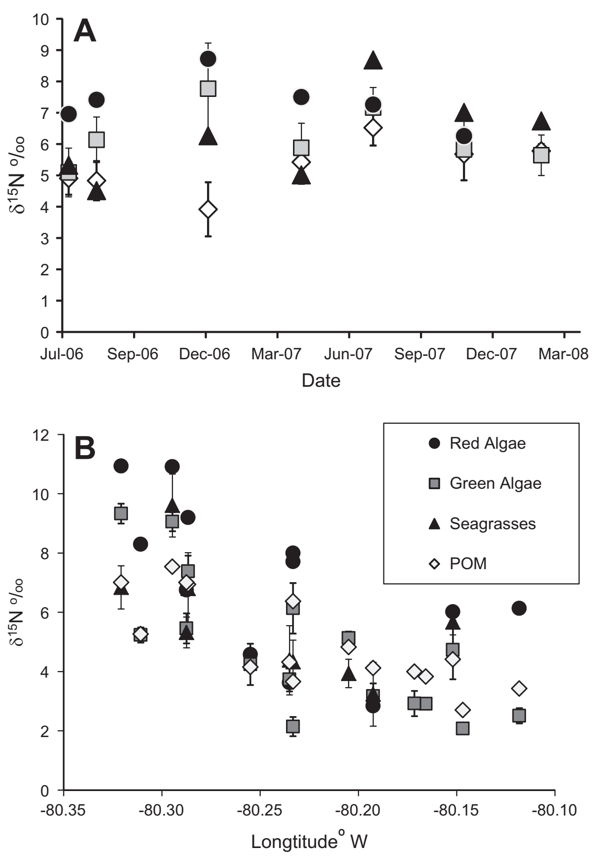

Fig. 3. (A) Mean variations in the δ^{15}N of green algae, red algae, sea grass, and POM with respect to time throughout the course of the study; error bars represent ±1 standard error. Errors are not shown for red algae and seagrass for purposes of clarity. (B) Data green algae, red algae, sea grass, and POM plotted against longitude. Error bars represent ±1 SE. Data have been omitted from the red algae and POM for purposes of clarity.

testudinum) (Tables 1–3). No statistically significant temporal changes in δ^{15}N were found in any of the groups ($p \ll 0.05$) throughout the study period as illustrated in Fig. 3a. Due to this absence of a temporal relationship, all data for individual specimens have been combined to provide mean values for the groups at each of the 15 sites. Analyses of these data show that all groups exhibited an increase in δ^{15}N towards the coast ($p < 0.05$) (Figs. 3b and 4).

Contour plots of the mean δ^{15}N values for each group (Fig. 4) show the geographic distribution patterns seen in Fig. 3. Values were relatively positive close to the shoreline, and became more depleted at stations further away from the mainland. This landward/seaward contrast in δ^{15}N was a general observation and is well illustrated by Fig. 5, which shows a comparison of all the species analyzed from two sites, one near the coast (BB8) and one of the outer sites (BB6). The δ^{15}N values for all species collected from the site near the coast were more positive than at the site further away. For the green algae, a wider geographic range of sites were sampled during the first sampling trip in 2006 (Fig. 6d). These data showed essentially the same pattern as seen from the 16 quarterly stations, but included higher (enriched) values associated with Key Biscayne, CGWW, and northern Biscayne Bay. No statistically significant relationships relative to distance from the coast were evident in the δ^{13}C data (Fig. 6a and c).

3.2. Non-biotic components

3.2.1. Sedimentary organic material

The δ^{15}N, δ^{13}C, and C:N values for the sedimentary organic material (SOM) average +3.9(±1.46), −15.5(±1.6), and 9.0(±2), respectively. The spatial patterns of the SOM δ^{15}N were similar to those observed in the biotic components (Fig. 6b). The SOM from areas close to the shoreline in southern Biscayne Bay were enriched in δ^{15}N. Patterns of elevated δ^{15}N were evident (i) adjacent to the Black Point Land Fill, (ii) south of Black Point to the Military Canal, (iii) and adjacent to the CGWW (Fig. 1). There was an inverse correlation between δ^{13}C and δ^{15}N ($r = -0.45$, $n = 55$, $p < 0.001$). There were no statistically significant correlations between C:N and either δ^{13}C or δ^{15}N.

3.2.2. Particulate organic material

The δ^{15}N of the POM mimics the magnitude and spatial distribution seen in the biological components (Table 1) and the SOM. Values were enriched close to the western margin and depleted seaward, towards the east (Fig. 4a).

3.3. Water quality data

3.3.1. Nitrate

The monthly concentration of NO_3^- varied over the study period (2006–2008) from as high as 27 μM close to the coast (BB8) in November 2007 to less than 0.05 μM at BB11 (Fig. 1). Typically at Site BB8 there was a strong seasonal cycle in the concentration

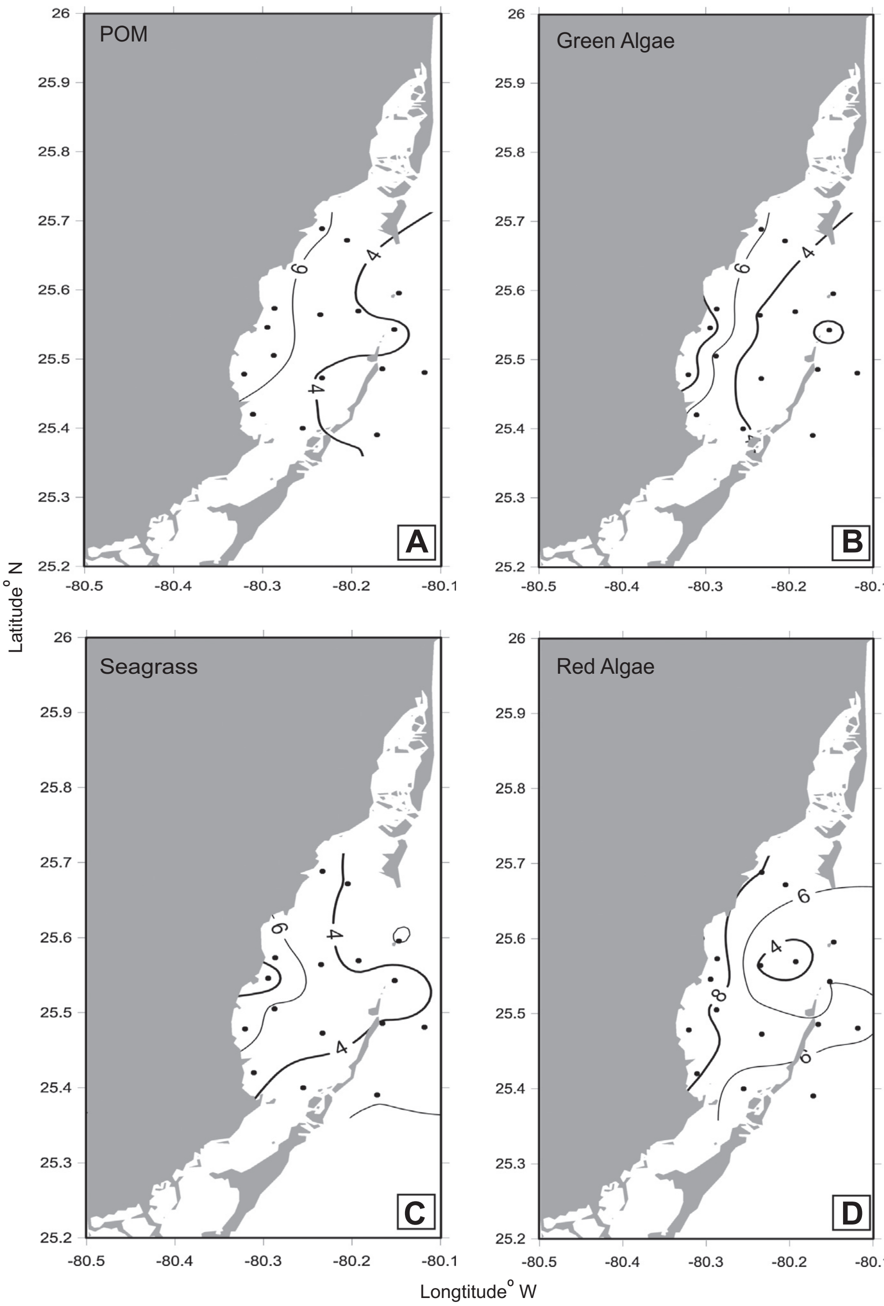

Fig. 4. The mean $\delta^{15}N$: of (A) POM, (B) green algae, (C) seagrasses, and (D) red algae collected from the 16 quarterly sampling stations over the two year study period.

of both NO_3^- and NH_4^+ with the highest values occurring during the late wet season (October–December). These patterns were present at all sites located close to the coast (BB1, BB4, BB5, BB8, BB9, and BB13). This seasonal cycle was observed at the other sites, but was less pronounced further away from the coast (BB6 and BB10), with wet season concentrations of NO_3^- reaching only ~1.0 μM.

3.3.2. Ammonium

The concentration of NH_4^+ also showed a seasonal cycle close to the coast, but with much lower maximum values (5–10 μM). At the central sites within the bay, no seasonal cycles were observed.

3.3.3. Salinity

Salinities varied seasonally between ~25 and ~36 PSU at the coastal stations, with the lowest values occurring near the end of the wet season (October–November). In the central areas (BB6) salinity varied between 36 and 39 PSU with no clear seasonal pattern. At the coastal sites (BB1, BB4, BB5, BB8, BB9, and BB13) the concentration of NH_4^+ and NO_3^- varied inversely with salinity. The correlation coefficient values varied from 0.8 at BB8 to 0.5 at BB1, but were statistically significant in all cases. In contrast sites further away from the coast (BB6) showed no statistically significant correlation between salinity and the concentration of NO_3^- or NH_4^+ (Fig. 7).

When averaged over the entire study period, the $\delta^{15}N$ of the biotic components showed statistically significant inverse correlations between $\delta^{15}N$ and salinity (see Table 4 and Fig. 8a). These inverse correlations also occurred when the data were compared from individual sampling periods, although the correlations are in some instances not statistically significant. The mean concentration of NO_3^- over the two year study from 11 sites is also positively correlated with the $\delta^{15}N$ of the POM, green algae, red algae, and

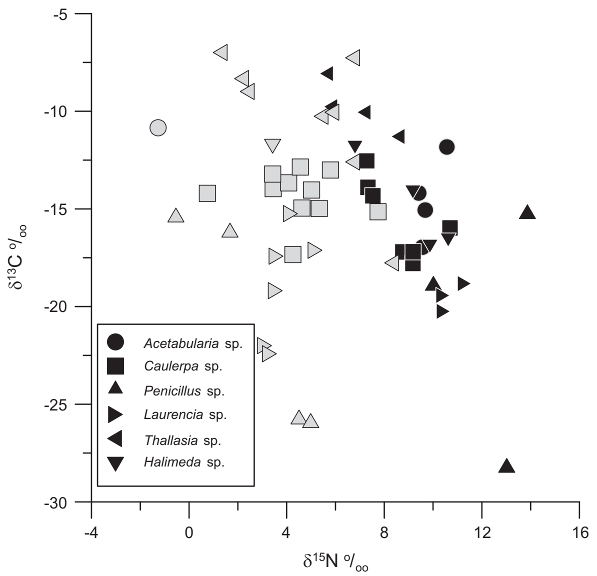

Fig. 5. The δ^{15}N and δ^{13}C of individual algae analyzed from two sites, BB8 and BB6. Site BB8 is close to the shore line and is represented by the darker symbols compared to BB6 (Fig. 1). There are no statistically significant differences in the δ^{13}C between the two sites.

seagrasses over the sampling time period (Fig. 8b and Table 4). In contrast the concentration of NH_4^+ is only statistically correlated with the δ^{15}N of the green algae (Table 4).

3.3.4. Dissolved inorganic nitrogen

The mean δ^{18}O and δ^{15}N values of the NO_3^- are shown in Fig. 9a and Table 5. In order to distinguish geographic patterns, the data have been separated into four spatial groupings: BNP, CGWW, north Biscayne Bay, and offshore Virginia Key. The δ^{15}N data from the CGWW displayed a wide range of values, from close to zero further inland, to much more enriched (δ^{15}N = +9‰) values further towards the mouth of the canal. The δ^{18}O of the CGWW samples exhibited a positive correlation with respect to δ^{15}N ($p < 0.01$), but this might be an artifact because the samples collected from the upper portion of the CGWW had much more depleted δ^{15}N values compared to those located near the canal mouth. The δ^{15}N of the other three groupings had similar mean values compared to the CGWW, but a much lower standard deviation. The most enriched δ^{15}N DIN values occurred in samples closest to the shoreline (Fig. 9b). The $\delta^{18}O_{nitrate}$ values of the bay sites, adjacent to the canals, were all more isotopically enriched than the canal samples, and showed a weak correlation ($p > 0.05$) with $\delta^{15}N_{nitrate}$. Overall, the range and spatial relationship in $\delta^{15}N_{nitrate}$ values match the biological samples and SOM δ^{15}N values. This relationship supports that NO_3^- is potentially the control on the observed spatial δ^{15}N patterns in Biscayne Bay.

3.3.5. Dissolved inorganic carbon

The δ^{13}C of the mean DIC varied seasonally with the most negative values (−6.4‰) occurring in May 2006 and the most positive during December 2006 (−1.5‰). Spatially the most negative δ^{13}C values occurred close to the coast line and decreased towards the open bay (Fig. 10).

4. Discussion

4.1. Algae, seagrasses, and particulate organic material

The δ^{15}N data presented in this study exhibit a clear relationship relative to the coastline. The ^{15}N enrichment was particularly elevated in all samples near the Black Point Land Fill and the adjacent sewage treatment facility (SDWWTP) (Fig. 3b). These gradients indicate that freshwater, derived from the mainland in the form of direct runoff or groundwater, is likely the source of δ^{15}N enriched DIN. This pattern was persistent throughout the two years of the study. While the quarterly sampling concentrated on BNP in the south, the more spatially extensive focused sampling effort of green algae during 2006 extended the survey to the northern portions of the bay (Figs. 1 and 6), areas which also have the potential to contribute nitrogen to BNP. These data (Fig. 6) had higher green algae δ^{15}N values adjacent to the CGWW, Key Biscayne, northern Biscayne Bay, and Fisher Island/Virginia Key. The CGWW is a man-made canal, constructed in the 1920s, lined with residential dwellings and allows for small boat access to Biscayne Bay. Along the length of the CGWW most of the houses have septic tanks which leak effluent through the porous Miami Limestone into the canal. The Miami limestone is known for being one of the most permeable carbonate aquifers (Halley et al., 1977; Neal et al., 2008) in the world and therefore waste water discharged into domestic septic systems quickly reaches the canal. During ebb tides the CGWW is therefore a point source for DIN with relatively enriched δ^{15}N values. Key Biscayne is a large residential community where septic systems are still widely used for single family dwellings. These systems can also contribute nutrients to the Bay through the groundwater. The effluent outfall from the Virginia Key waste water treatment plant discharges approximately 10 km off the east coast of Florida, into the Gulf Stream. Here the discharge plume rises to the surface, by virtue of its low density, and is frequently swept inshore by eddies from the Florida Current. In contrast to the biota and the sediments in Biscayne Bay and immediately surrounding Key Biscayne, these same components show relatively more depleted δ^{15}N values (+3 to +4‰) in the more open marine areas. The extensive spatially focused sampling of green algae, also provides additional data in Biscayne Bay south of Featherbed Bank where elevated ^{15}N is also observed. It appears that water carrying DIN with more positive δ^{15}N values is funneled south of this natural divide (Featherbed Bank) in Biscayne Bay producing a larger anthropogenic signal in this region. Within this area the city of Homestead has a permit for wastewater reuse to discharge onto the land surface adjacent to the C-103 (Mowry Canal). It is possible that this type of discharge may contribute to the observed values in this area (Figs. 1 and 6).

An interesting feature, documented by the data presented here, is the similar range of δ^{15}N values in seagrass, a rooted vascular plant, when compared to the various algae examined. This pattern is consistent with observations by McClelland et al. (1997) from Waquoit Bay and its associated watersheds. It was proposed in that study that in areas where there is an abundance of DIN, the seagrasses obtain most of their nutrients from the water column (Short and McRoy, 1984) rather than the sediments and therefore they have δ^{15}N values similar to macroalgae. In areas with low nutrients, it is suggested that the seagrasses have more negative δ^{15}N values as they obtained their DIN from the sediments where nitrification leads to more depleted δ^{15}N values. Such locations might also have higher concentrations of nutrients such as phosphorus. This hypothesis would tend suggest that there is an abundance of water column DIN in Biscayne Bay, which is in fact the case close to the coastline. It would also predict that there should be an increasing difference between seagrasses and other algae further from the coast, where the observed measurements of the concentration of DIN decreases (Fig. 8). However, there is no change in the relationship between seagrass and algae in Biscayne Bay as the ambient NO_3^- concentration decreases (Fig. 3b and Fig. 5). This may suggest that even though the measured concentrations of NO_3^- are low (<1 μM), that there is still sufficient NO_3^- to allow the seagrass to obtain the

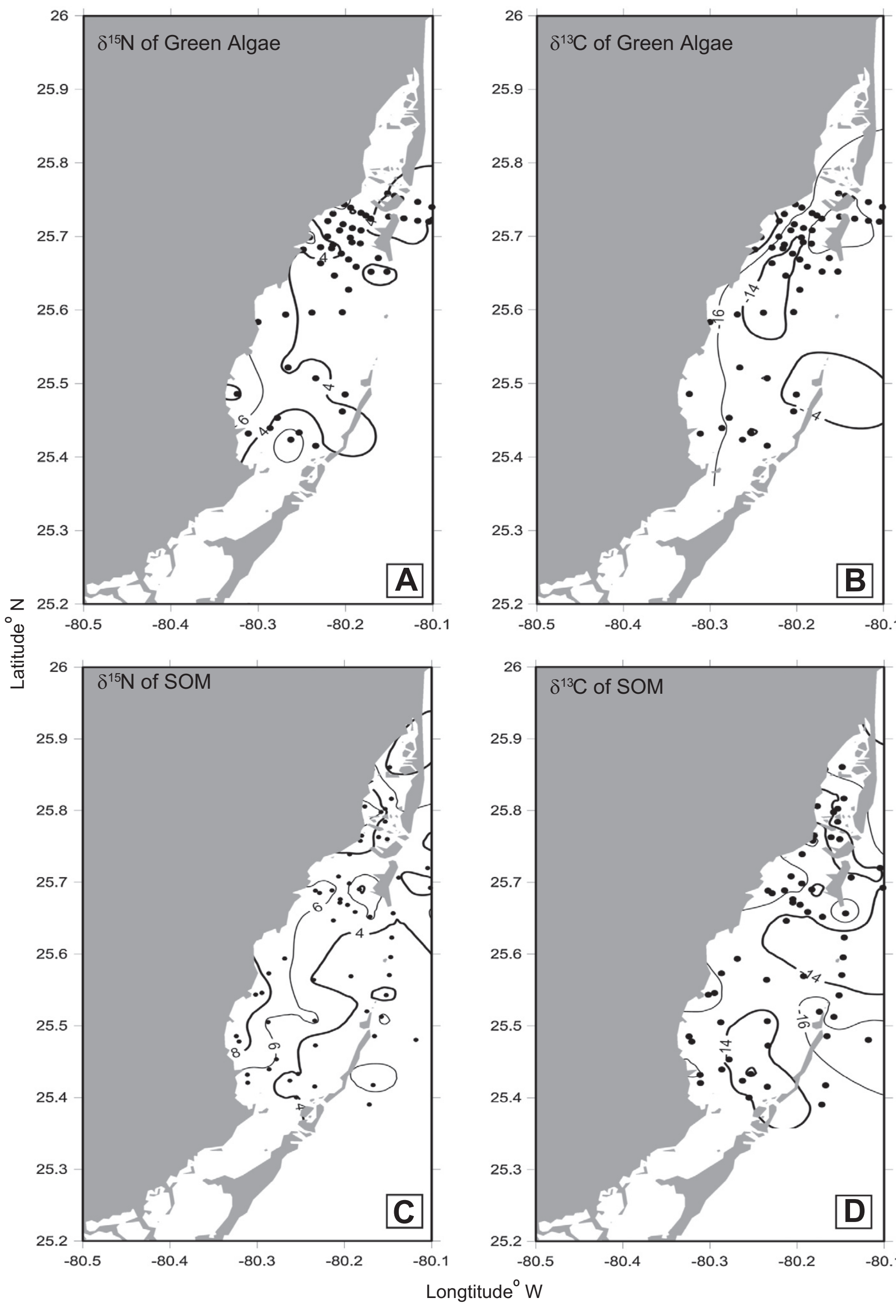

Fig. 6. The $\delta^{13}C$ and $\delta^{15}N$ of SOM (A and B) and green algae (C and D) collected during the sampling periods in June and July 2006.

majority of their DIN from the overlying water column rather than the interstitial pore water. However, in other studies in environments with low DIN, seagrasses had similar $\delta^{15}N$ values to the ambient DIN (Yamamuro et al., 2003) and therefore it may be that seagrasses always obtain their nutrients from the ambient water rather than the porewaters.

4.2. NO_3^- *isotopic composition*

4.2.1. Nitrogen

The concentration of NO_3^- in the water and its $\delta^{15}N$ and $\delta^{18}O$ values provide additional information regarding nitrogen sources for the biological materials and POM collected in this study. The more positive $\delta^{15}N_{nitrate}$ values in the CGWW are consistent with a waste water effluent source, probably derived from septic systems. The more negative $\delta^{15}N_{nitrate}$ values were found near the northern end of the navigable portion of the CGWW and potentially resulted from the leaching of fertilizers applied to lawns. In Biscayne Bay more enriched $\delta^{15}N_{nitrate}$ values reflect contributions of nitrate derived from sewage derived sources as well as natural fractionation during assimilation.

4.2.2. Oxygen

The depleted $\delta^{18}O$ of the nitrate in the CGWW is consistent with nitrification of terrestrial sources or rapid N turnover in CGWW (Fig. 9). The values of nitrate $\delta^{18}O$ in the bay are all more positive and reflect both contributions from atmospheric NO_3^- and fractionation during assimilation by organisms in the bay (Wankel et al., 2006, 2009).

The process of denitrification is unlikely to significantly alter the N isotopic signals within the bay, given a well oxygenated water column and lack of isotopic effect for sedimentary

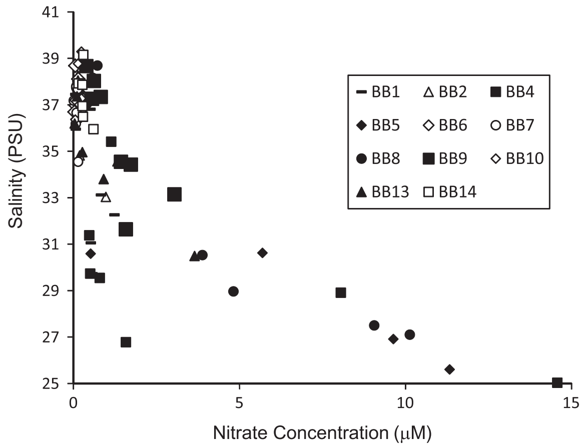

Fig. 7. Relationship between salinity and the concentration of NO_3^- at the various sites shown in Figure 1.

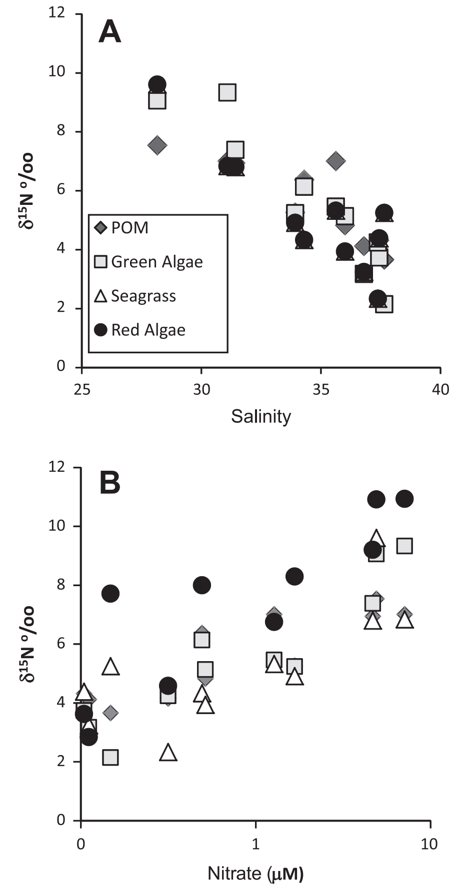

Fig. 8. (A) Variations between the $\delta^{15}N$ of the various components and the salinity during the month that the samples were collected, and (B) variations between $\delta^{15}N$ and the concentration of NO_3^-.

denitrification. The poor correlation between nitrate $\delta^{18}O$ and $\delta^{15}N$ values in Biscayne Bay indicates the potential effect of the N source's isotopic signature on the $\delta^{15}N$ values. There was a weak positive correlation observed in the Virginia Key data, but no trends existed between $\delta^{18}O$ and $\delta^{15}N$ in other areas. This observed relationship is opposite to data collected from the Florida Reef tract where the slope between $\delta^{18}O$ and $\delta^{15}N$ is close to unity (Lamb, 2007; Lamb et al., 2012). The absence of a correlation between $\delta^{18}O$ and $\delta^{15}N$ suggests that fractionation during assimilation of nitrate by algae is not the principal cause of enrichment in these isotopes. Alternatively, a reduced slope could be caused by recent nitrification of anthropogenic or other sources enriched in ^{15}N, such as upwelled water which is partially depleted in NO_3^-. As Biscayne Bay is semi-isolated from the Gulf Stream and there is a nutrient gradient away from the coast, a dominant anthropogenic source is the more probable explanation for the reduced slope between $\delta^{18}O$ and $\delta^{15}N$. Whereas atmospheric derived NO_3^- may still be a source, but it's highly enriched $\delta^{18}O$ is rapidly lost by mixing and therefore not discernible in the $\delta^{18}O$ of the nitrate.

4.3. Carbon isotopic composition of DIC

The $\delta^{13}C$ of the local DIC (Fig. 10) can be influenced by factors such as the nature of the organic material that is being oxidized and the extent to which the coastal system exchanges water with the open ocean. For example, Biscayne Bay has a fairly restricted circulation and limited exchange with the open ocean. Therefore this restricted nature might allow the oxidative products from the decomposition of organic material to linger, leading to a $\delta^{13}C$ in the DIC which is more isotopically negative when compared to water on the ocean side of Elliot Key. This difference is clearly displayed in the data from 2006, where the most enriched $\delta^{13}C$ values within the center of Biscayne Bay were ∼−3‰, while closer to shore values were as depleted as −10‰ (at the start of the 'wet' season). Values were not measured on the ocean side of Elliott Key during this study, but typically the $\delta^{13}C_{DIC}$ values of these waters have much more enriched values (−1 to +1‰) (Swart

Table 4
The Spearman Rank correlation coefficient between the $\delta^{15}N$ of POM, green algae, and seagrass and NO_3^-, NH_4^+ and salinity.

	June–July 2006	August–September 2006	January–February 2007	April–May 2007	July–August 2007	November 2007	February 2008	June–July 2006	Mean
POM									
NO_3^-	0.60	0.47	1.00	***0.78***	***0.65***	***0.90***	0.00	−0.31	***0.86***
NH_4^+	−0.20	0.33	0.65	0.47	0.36	0.00	0.00	−0.05	0.49
Salinity	0.20	−0.47	***−0.90***	***−0.70***	***−0.76***	***−0.90***	0.30	0.03	***−0.90***
Green algae									
NO_3^-		0.69	***0.92***	0.40	0.56	***0.83***	0.60	0.80	***0.90***
NH_4^+		***0.86***	***0.84***	0.40	−0.33	0.15	0.60	0.80	***0.62***
Salinity		−0.33	***−0.86***	−0.40	−0.36	−0.18	−0.70	−0.80	***−0.93***
Seagrass									
NO_3^-		0.66	0.54	0.80	−0.19	***0.88***	0.00	0.40	***0.67***
NH_4^+		***0.76***	***0.78***	0.80	0.09	0.03	0.00	0.80	0.47
Salinity		−0.28	−0.57	−0.80	−0.33	***−0.83***	−0.60	−0.40	***−0.61***

Values which are bold and in italics are statistically significant at the 95% confidence level.

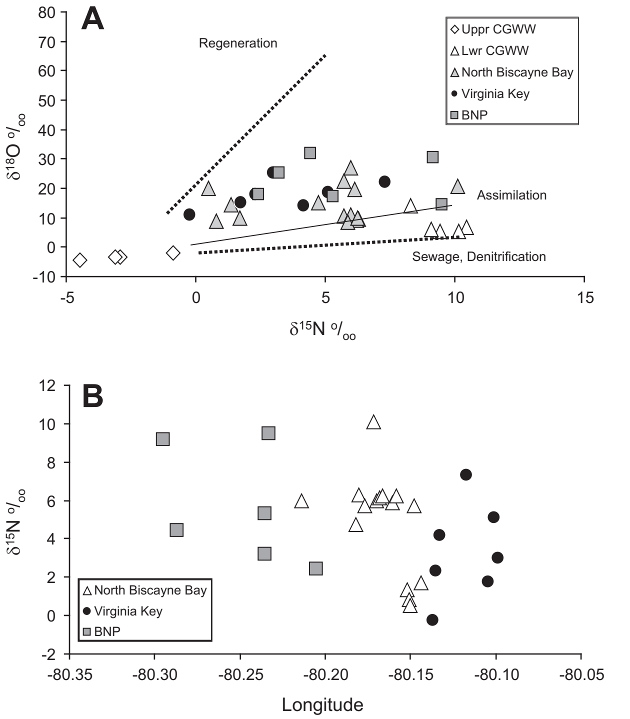

Fig. 9. (A) The δ^{15}N and δ^{18}O of nitrate samples collected from various sites in Biscayne Bay and surrounding environments. The solid line represents the 1:1 relationship between increasing δ^{15}N and δ^{18}O expected from assimilation or denitrification alone. (B) The relationship between the δ^{15}N of NO_3^- and longitude.

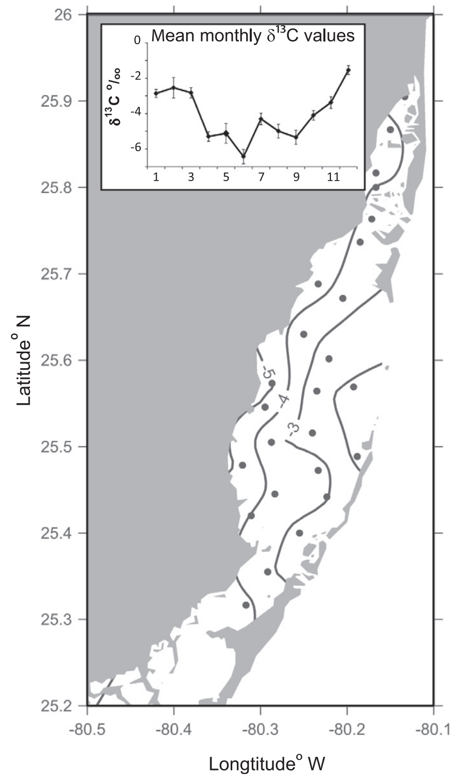

Fig. 10. The mean δ^{13}C of the DIC in monthly water samples collected at the locations shown. The inset shows the annual variation with δ^{13}C minimum values occurring in June 2006. The error bars represents ± one standard error.

Table 5
Summary and ±SE of δ^{15}N and δ^{18}O measured on nitrate.

	δ^{15}N ‰	δ^{18}O ‰
Coral Gables Water Way	4.0 ± 2.2	2.7 ± 2.1
North Biscayne Bay	4.9 ± 0.7	14.5 ± 1.1
Virginia Key (Ocean)	3.3 ± 0.9	17.7 ± 1.9
Biscayne National Park	5.7 ± 1.1	22.8 ± 2.8

et al., 2005b). This pattern, combined with the normal tendency for land based sources of organic carbon to have δ^{13}C values lower than −20‰, should lead to benthic organisms and SOM having more negative δ^{13}C values closer to the coast. In fact this pattern is only evident in the correlation between the negative δ^{13}C and δ^{15}N present in the SOM (Fig. 8). The green algae δ^{13}C values are relatively more depleted close to the coast, but this relationship was not statistically significant as seen in the δ^{15}N data. A range of physiological factors such as light availability, temperature, and salinity, in addition the ambient δ^{13}C of the DIC can affect the δ^{13}C value of algae. Therefore other environmental factors and conditions may be impacting these algae more than their primary carbon source.

4.4. Sedimentary organic material

The organic fraction in sediments (SOM) is composed of contributions from different sources of organic matter from within a specific area and therefore is an indicator of the integrated δ^{15}N at a particular location (Fig. 6). In the case of Biscayne Bay positive values are observed along the shoreline of the mainland, reflecting the contribution of organisms influenced by the isotopically positive nitrogen from the various sources discussed previously. Similar to the pattern observed in the green algae isotopic data, there appear to be more positive δ^{15}N values south of Featherbed Bank. This difference may result from the influence that Featherbed Bank has on separating the Bay into two distinct hydrological units. The influence of water from the CGWW can also be clearly seen extending into Biscayne Bay. In addition there is δ^{15}N enriched organic material around Key Biscayne reflecting contributions from septic systems in this community.

4.5. Origin of nitrate in Biscayne Bay

4.5.1. Agricultural sources

It has previously been calculated that 74% of the annual nitrate NO_3^- budget in Biscayne Bay is supplied from agricultural sources through the Mowry (C-103) and Princeton (C-102) canals (Fig. 1) (Caccia and Boyer, 2007). These canals enter Biscayne Bay in the approximate area where there is a focused concentration in total nitrogen (Fig. 2b). This elevated concentration of nitrogen is coincident with the enriched δ^{15}N values present in the algae and seagrasses (Fig. 3b). However, synthetic fertilizers do not have highly enriched δ^{15}N values (Freyer and Aly, 1974; Gormly and Spalding, 1979; Kendall et al., 2008), so therefore it seems likely that there must have been some input into these canals of sewage derived nitrogen or nitrogen which has undergone partial denitrification.

4.5.2. Waste water treatment plant

A potential source of nitrogen affecting Biscayne Bay is the effluent from the SDWWTP (Fig. 1). However, since all the treated water from the SDWWTP (367 million liters a day), is pumped into the Floridan aquifer to a depth of ~1000 m, it is unlikely that this source was responsible, unless there has been some significant leakage from the plant at a shallow depth into the surficial aquifers. Although, there has been some speculation that some of this waste water, after it is injected into the deep aquifer, may find its way back to the surface (Walsh and Price, 2010), this theory remains unproven. If leakage were the source of the heavy $\delta^{15}N$, then perhaps the DIN would have leaked over a broad area, and not show the characteristics of a source strongly associated with the immediate coastline as seen in the data presented in this study. Alternatively, the data presented here could provide the first evidence that these waters have reached the surface. Some of the waste water at the SDWWTP does get temporarily stored in surface pits during the wet season, which in South Florida is between May and October. Although, this water could have contaminated the canals entering Biscayne Bay, it would have introduced a seasonal pattern in the $\delta^{15}N$ data, a pattern which was not observed in these data (Fig. 3). In the northern portion of Biscayne Bay, the major source of DIN was in the form of NH_4^+ (Caccia and Boyer, 2005). Although, fewer samples were taken in this area, there was not the same enrichment of $\delta^{15}N$ in the algae and seagrass from this region.

4.5.3. Leakage from the landfill

Another source of anthropogenic DIN which may have contributed to Biscayne Bay is the Black Point Landfill. While the landfill probably does not contain significant animal waste, it does contain large quantities of organic material. This organic material will decompose producing NH_4^+ which is then transformed to NO_3^-. In previous studies it was found that high concentrations of NH_4^+ were present in shallow wells in Biscayne Bay and in canals adjacent to the landfill. These high concentrations implicated the landfill as a possible source. In anaerobic portions of the landfill, denitrification transforms this nitrate to N_2 gas and causes the residual nitrate to become isotopically positive as noted above (Cline and Kaplan, 1975). While this source is certainly capable of producing NO_3^- with an isotopically enriched signature, it probably can only supply a limited flux of $\delta^{15}N$ enriched NO_3^-.

An interesting comparison of the data presented in this paper can be made with the $\delta^{15}N$ of seagrasses in northeast Florida Bay. Florida Bay is an enclosed bay, located to the south of peninsular Florida, which receives input of water mainly from rainfall and by overland flow from the Everglades through Taylor Slough. Unlike Biscayne Bay, Florida Bay is relatively remote and not believed to be subjected to significant inputs of anthropogenic nitrogen. In a study of groundwater flow into this area (Corbett et al., 1999), it was determined that the seagrasses in the northeast portion of Florida Bay possessed fairly positive $\delta^{15}N$ values (∼+8‰). These workers concluded that positive $\delta^{15}N$ values were caused by fractionation during denitrification in the sediments, with the residual nitrate then diffusing into the overlying water column producing a positive $\delta^{15}N$ signal. Such a mechanism might potentially be operating in Biscayne Bay, but would be unlikely to have been responsible for the large flux of nitrogen previously measured.

5. Conclusions

The data presented in this paper shows a clear enrichment in $\delta^{15}N$ close to the shoreline of Biscayne Bay, and therefore indicate that a major input of nutrients in the near shore is derived from anthropogenic sources. Hence these data support the interpretation that high $\delta^{15}N$ values (∼+8 and higher) can be used as a tracer of anthropogenic input in certain geographical locations. Such locations might be semi-enclosed basins as Biscayne Bay in this study, Moreton Bay in Australia (Costanzo et al., 2001), or Waquoit Bay in Massachusetts, USA (McClelland et al., 1997). In more open oceanic settings, anthropogenic nutrients are quickly diluted by normal marine waters and any $\delta^{15}N$ signals originating from land-based sources of pollution are difficult to ascertain (Lamb, 2007; Lamb and Swart, 2008). Whether values lower than ∼+8‰ can be unequivocally interpreted as having been influenced by anthropogenic sources, as they have been in some instances (Lapointe et al., 2004), cannot be addressed by the data presented here, but it is likely that natural variability in $\delta^{15}N$ and isotopic fractionation by the mechanisms mentioned earlier in this paper, preclude this possibility. Although the precise origin of the ^{15}N enriched nitrogen in Biscayne Bay still needs to be determined, it is likely connected with the SDWWTP and/or the Black Point Landfill. These findings therefore represent a refinement of the findings of previous studies which investigated the supply of DIN to Biscayne Bay and concluded that the DIN originated from agricultural sources (Caccia and Boyer, 2005, 2007). Nutrients derived from agricultural sources, while having the potential to be delivered to Biscayne Bay in high quantities, do not possess elevated $\delta^{15}N$ values (Freyer and Aly, 1974) and therefore cannot be responsible for the large additions of DIN as previously suggested. The combined use of $\delta^{18}O$ and $\delta^{15}N$ allows the separation between the enrichment of $\delta^{15}N$ through assimilation processes, which would cause a positive covariance, the production of NO_3^- through regeneration, and the sewage derived nutrients (Wankel et al., 2006, 2009). In the case of Biscayne Bay it appears that the main source of ^{15}N enrichment close to the coast (<1 km) is from sewage influenced waste water rather than regeneration. However, the influence of the elevated $\delta^{15}N$ appears to diminish quickly with increasing distance from the coast and the $\delta^{15}N$ values for algae and other organisms approach those seen in the open marine sites at about 3–5 km offshore. The patterns observed in Biscayne Bay are opposite of that of the Florida reef tract, where sites as close as 1 km offshore were examined (Lamb et al., 2012), and no evidence was found for $\delta^{15}N$ enriched algae or POM. The difference between these two locations may be both the flux of DIN, which is probably much higher in Biscayne Bay, as well as the semi-enclosed nature of the bay when compared to the Florida reef tract.

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