



Macroalgal blooms on southeast Florida coral reefs II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen

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Abstract

Since 1990, coral reefs off southeast Florida have experienced an unprecedented succession of macroalgal blooms and invasions. To determine if anthropogenic land-based nitrogen (N) sources support these HABs, we collected macroalgal tissue for stable nitrogen isotope ($\delta^{15}\text{N}$) analysis at three spatially distinct depths ranging from the shallow subtidal to the shelf break (~ 43 m) along seven transects from Jupiter to Deerfield Beach, Florida, USA. This sampling was initiated during a historically significant drought in the spring of 2001 when rainfall, stormwater runoff, and upwelling were at a minimum. The sampling was repeated in the summer wet season following significant stormwater runoff and during a strong upwelling event.

Despite significant seasonal changes in N source availability, $\delta^{15}\text{N}$ values did not vary between samplings. Collectively, $\delta^{15}\text{N}$ values were significantly higher on inshore shallow reefs (+8.1‰) compared to mid (+6.1‰) and deep reefs (+6.7‰). Values were also elevated in the southern portion of the study area (e.g., Boca Raton, +8.5‰) where nearly 1.5 billion l/day of secondarily treated wastewater is discharged into the ocean via coastal outfalls. *Codium isthmocladum*, a macroalga that assimilates its nutrients entirely from the water column, was the dominant bloom species in the southern study area, where tissue $\delta^{15}\text{N}$ values matched source values of nearby sewage outfalls. The northern study area was dominated by species of the genus *Caulerpa*, particularly the invasive Pacific native *C. brachypus* var. *parvifolia*, which are capable of accessing benthic nutrient sources (e.g., submarine groundwater discharge, SGD) by means of root-like rhizoids. The northern area does not have sewage outfalls but features a highly transmissive geologic zone where SGD may be enriched with septic tank leachate and effluent from municipal deep injection wells.

Multiple lines of evidence supported the hypothesis that land-based sewage N was more important than upwelling as a N source to these HABs: (1) $\delta^{15}\text{N}$ values were highest on shallow reefs and decreased with increasing depth, indicating land-based sources of enrichment; (2) elevated $\delta^{15}\text{N}$ values occurred in these HABs during the dry season, prior to the onset of the summer upwelling; (3) elevated NH_4^+ concentrations occur on these reefs during both upwelling and non-upwelling periods and are kinetically preferred by macroalgae compared to upwelled NO_3^- . These findings provide a case study of a coupling between

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increasing anthropogenic activities and the development of macroalgal HABs, including invasive species that threaten economically important reef resources in southeast Florida.

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1. Introduction

The frequency, extent, and biomass of macroalgal blooms have increased in many tropical/subtropical coral reef communities in recent decades as a result of increasing land-based nutrient pollution (UNEP, 1994; ECOHAB, 1997; NRC, 2000). On coral reefs off southeast Florida, a succession of harmful macroalgal blooms (HABs) began in 1990 with extensive unattached forms of *Codium isthmocladum* that developed accumulations up to 2 m thick over the reef surface and on adjacent beaches (Fig. 1A and B). These HABs resulted in die-offs of sponges, hard corals, and soft corals due to hypoxia/anoxia in near-bottom waters and caused an emigration of reef fishes from the impacted areas (Lapointe, 1997; Lapointe and Hanisak, 1997). These initial *C. isthmocladum* blooms were followed by an extensive bloom of *Caulerpa verticillata* in 1997 (Lapointe, 1999; Fig. 1C). Previously, *C. verticillata* had not been observed on these reefs (Hanisak and Blair, 1988), and this invasive alga quickly spread from reefs off Riviera Beach near the Lake Worth Inlet northwards to reefs off Jupiter in northern Palm Beach County (Fig. 2).

Species of the tropical green algal genus *Caulerpa* are well known invaders of coastal waters, as demonstrated by the proliferation of *Caulerpa taxifolia* in the Mediterranean Sea (Meinesz, 1999; Verlaque et al., 2003). Evidence suggests that the rapid invasion of *C. taxifolia* in the Mediterranean (Meinesz and Hesse, 1991) was supported by land-based sources of nutrient pollution (Chisholm et al., 1997; Jaubert et al., 2003). Ironically, the Mediterranean native, *C. ollivieri*, has invaded a polluted harbor in the Bahamas where sewage has been implicated as a primary nitrogen (N) source supporting bloom formation (Lapointe et al., 2005a). In the Florida Keys, *C. verticillata* has likewise become abundant in sewage-polluted canal systems (Lapointe et al., 1994). Although the appearance of HABs involving *Codium isthmocladum* and *C. verticillata* has been considered

an indicator of escalating nutrient enrichment and eutrophication in southeast Florida (Lapointe and Hanisak, 1997), only limited attempts have been made to identify the specific N sources supporting these blooms (Lapointe, 1997). Most recently, while conducting the initial sampling (May 2001) for this study, we discovered a bloom of the invasive Pacific native *Caulerpa brachypus* var. *parvifolia* (henceforth referred to as *C. brachypus*, Fig. 1D and E) on reefs in Palm Beach County.

Both natural and anthropogenic sources may supply nutrients to HABs on reefs in southeast Florida. Episodic summertime upwelling has historically been important in this area (Green, 1944; Taylor and Stewart, 1958) but, because these HABs have developed only since 1990, we hypothesized that anthropogenic nutrient enrichment resulting from rapid population growth and associated land-based runoff from the watershed is the most significant nutrient source supporting these HABs. Urbanization of watersheds along the eastern coast of the U.S. has significantly increased nutrient loadings to coastal waters from a variety of sources including fertilizers, top soils, fossil fuel combustion, and municipal wastewaters (Howarth et al., 1996, 2000; NRC, 2000). Intensive agricultural activity in the Everglades Agricultural Area (EAA) in the inner region of the coastal plain enriches groundwaters and surface waters with N and phosphorus (P) from top soil and fertilizers. These are transported to coastal waters via either submarine groundwater discharges (SGD; Finkl and Charlier, 2003) or surface water discharges through the Port Everglades, Hillsboro, Boynton, Lake Worth, and Jupiter inlets (Fig. 2). Finkl and Charlier (2003) estimated that groundwaters deliver 5727 metric t/year of N and 414 metric t/year of P via SGD to the coastal reefs off Palm Beach County alone.

The watershed of southeast Florida between Palm Beach and Dade counties now supports nearly 7 million people. The domestic wastewater generated by this rapidly expanding population is disposed of either via

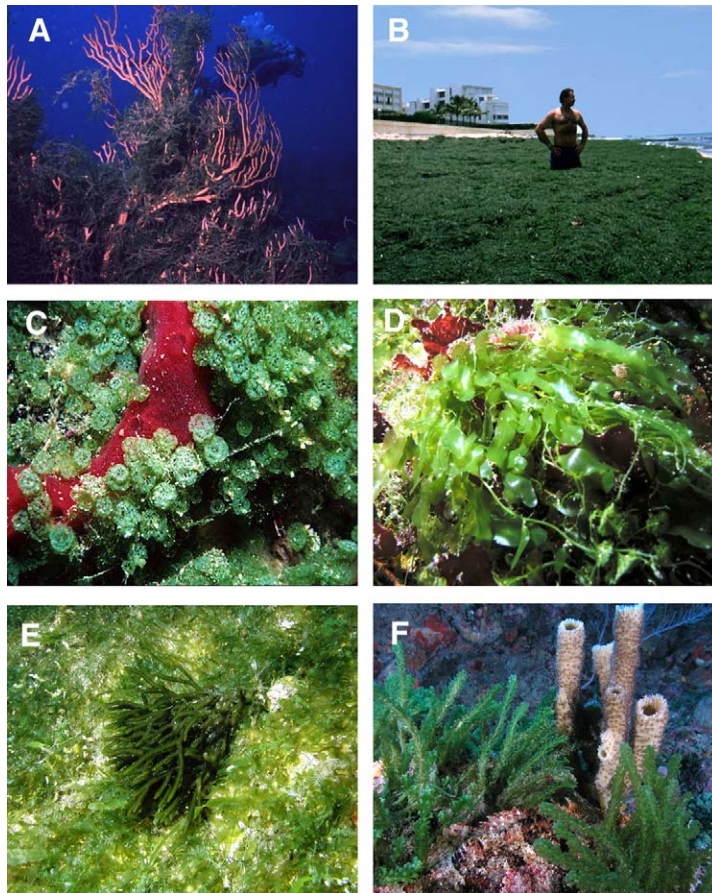


Fig. 1. Images of various green macroalgae that have formed harmful and invasive blooms on coral reefs off southeast Florida between 1990 and 2001: (A) *Codium isthmocladum* smothering octocorals at the “Football Field,” Lake Worth, FL., August 1990; (B) massive beach drift of *Codium isthmocladum* at Boynton Beach, FL., August 1992; (C) *Caulerpa verticillata* overgrowing sponge at North Colonel’s Ledge, off Juno Beach, FL.; (D) *Caulerpa brachypus* var. *parvifolia* near the Princess Anne off Riviera Beach, FL.; (E) *Codium isthmocladum* surrounded by the invasive *Caulerpa brachypus* var. *parvifolia* at North Colonel’s Ledge, Juno Beach, FL.; (F) *Caulerpa racemosa* at North Colonel’s Ledge, Juno Beach, FL.

septic tanks or by secondary treatment followed by discharge through ocean outfalls (1.5 billion l/day or ~6000 metric t of N/year; Hazen and Sawyer, 1994) or into Class 1 injection wells (1.9 billion l/day or ~7600 metric t of N/year; USEPA, 2003). Nearly 30 injection wells in Palm Beach and Broward counties pump nutrient-rich secondarily treated sewage effluent under pressure into the boulder zone at ~1100 m depth (Miller, 1997; USEPA, 2003). In Palm Beach County, at least one of these wells has been identified in a recent USEPA (2003) wastewater risk assessment for south Florida as having “a significant potential for vertical migration into overlying drinking water aquifers.”

Stable nitrogen isotope ratios ($\delta^{15}\text{N}$) can be used to effectively discriminate among natural and anthropogenic sources in marine food webs when the signatures of the various N sources are known (see reviews by Peterson and Fry, 1987; Owens, 1987; Lajtha and Michener, 1994). Atmospheric N and nitrogen fixation have baseline $\delta^{15}\text{N}$ values of 0‰ (Heaton, 1986; Owens, 1987). Enrichment of $\delta^{15}\text{N}$ in aquatic systems can result from N transformations that occur prior to, during, or following the treatment and discharge of wastewater. Volatilization of ammonia and isotopic fractionation by microbes during nitrification and denitrification produce residual DIN with

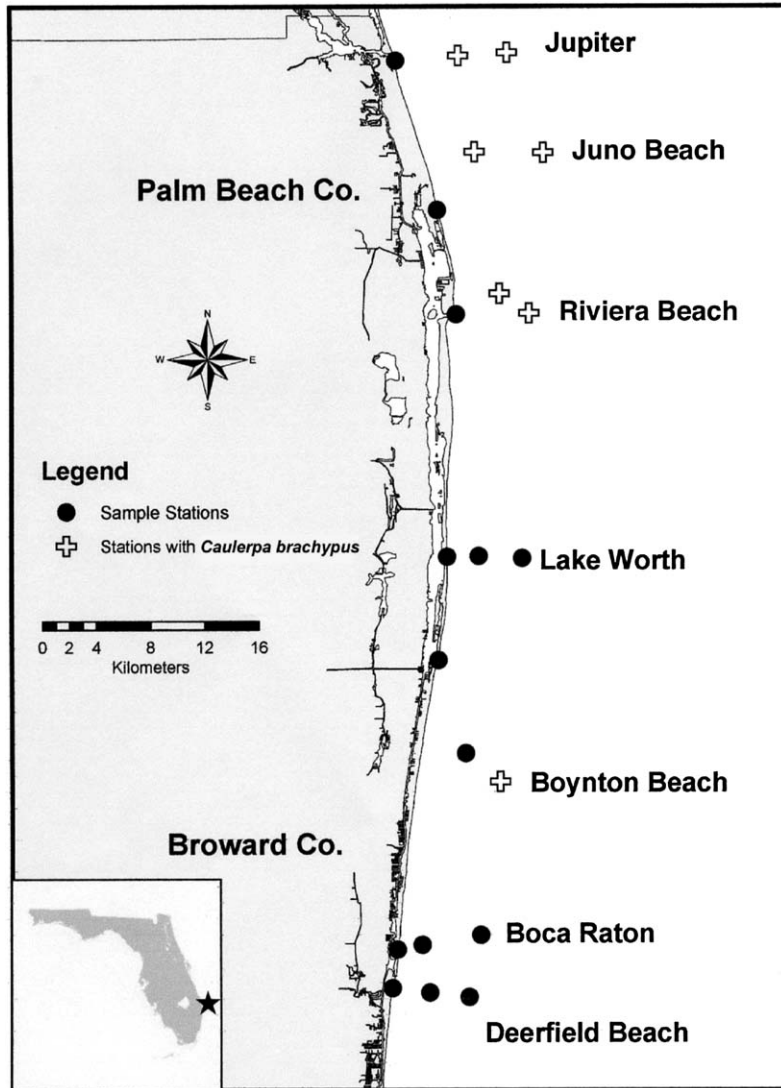


Fig. 2. Map of southeast Florida study area showing grid of sample stations. Sample stations where *Caulerpa brachypus* was discovered in May 2001 are denoted.

elevated $\delta^{15}\text{N}$ values of +6‰ to +22‰ (Heaton, 1986; Lindau et al., 1989). Globally, many case studies have used $\delta^{15}\text{N}$ as a tool to discriminate between natural and anthropogenic N sources supporting macroalgal growth (Hobbie et al., 1990; Lapointe, 1997; France et al., 1998; McClelland and Valiela, 1998; Rogers, 1999; Costanzo et al., 2001; Wayland and Hobson, 2001; Umezawa et al., 2002; Lapointe and Thacker, 2002; Gartner et al., 2002; Barile, 2004; Savage and Elmgren, 2004). Several studies have successfully

utilized $\delta^{15}\text{N}$ values in coral reef ecosystems to assess the extent of land-based N enrichment along gradients into the coastal ocean, on scales from several kilometers (Umezawa et al., 2002; Lapointe et al., 2004) to nearly 40 km across the Great Barrier Reef lagoon (Sammarco et al., 1999).

We hypothesized that, if sewage rather than upwelling was the primary DIN source supporting macroalgal HABs, then the highest $\delta^{15}\text{N}$ values would occur on the shallow reefs most influenced by

land-based wastewater discharges. Macroalgae that rely on N-fixation have low $\delta^{15}\text{N}$ values near the atmospheric signature of 0‰ (France et al., 1998; Table 1) in contrast to those using sewage N, which become increasingly enriched in $\delta^{15}\text{N}$ with increasing sewage N contributions over a range from +3‰ to +16‰ (Lapointe, 1997; Costanzo et al., 2001). This range includes secondary-treated wastewater discharges from sewage outfalls in the study area (Hoch et al., 1995; Table 1), as well as shallow (<10 m) groundwaters contaminated by septic tanks in the Palm Beach County watershed (Lapointe and Krupa, 1995; Table 1). In comparison, both fertilizers and organic peat from agricultural areas in the western region of the watershed have $\delta^{15}\text{N}$ values ranging from 0‰ to +3‰ (Heaton, 1986; Table 1) and can therefore be effectively discriminated from the wastewater N signature. The $\delta^{15}\text{N}$ value of upwelled NO_3^- from adjacent North Atlantic deep water is $\sim+4.8\%$ (Sigman et al., 2000; Montoya et al., 2002; Table 1), which coincides with the lower end of the sewage $\delta^{15}\text{N}$ range. Hence, additional evidence of the temporal and spatial presence of upwelling as a potential N and P source was needed. Accordingly, we collected seawater samples from a variety of reef sites during upwelling periods in this study for determination of DIN ($=\text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$) and soluble reactive phosphorus (SRP), and recorded sample depths and water temperatures.

Because the spring of 2001 was a period when climatological conditions were not favorable for upwelling and when a record drought occurred in south Florida (Abtew et al., 2002), we hypothesized

that N availability from upwelling and stormwater runoff was minimal compared to wastewater N loading in the study area prior to our initial May sampling. In contrast, the August sampling followed considerable wet season rainfall (60 cm between May and August, 2001; South Florida Water Management District), which coupled with strong upwelling during the August sampling, hypothetically reflected significant N contributions from both of these sources.

2. Materials and methods

2.1. Study sites and rationale for collection of macroalgae

To provide comprehensive spatial and temporal discrimination of N sources to macroalgal HAB communities in Palm Beach and northern Broward counties (Fig. 1A–F), tissue samples of abundant macroalgae were collected over a grid of 21 stations within seven transects extending offshore of Jupiter, Juno Beach, Riviera Beach, Lake Worth, Boynton Beach, Boca Raton, and Deerfield Beach in the dry (10–30 May) and wet (20–28 August) seasons of 2001 (Fig. 2). The sampling design involved depth stratification across the southeast Florida shelf in order to assess spatial variability among shallow subtidal (<5 m), mid-depth (25–30 m) and deep (~ 40 –43 m) reefs. This sampling network allowed us to quantify possible influences of anthropogenic versus natural N sources to the reef macroalgae. The HAB species *Codium isthmocladum* and *Caulerpa brachypus*, as well as other abundant macroalgae

Table 1
Source $\delta^{15}\text{N}$ values for the southeast Florida study area

Source and location	$\delta^{15}\text{N}$ (‰)	Reference
Ocean sewage outfall N. Broward County	+8.6	Hoch et al. (1995)
Septic tank effluent		
Jupiter Creek Monitor Well #4	+7.3	Lapointe and Krupa (1995a)
Jupiter Creek Monitor Well #5	+19.5	Lapointe and Krupa (1995a)
Tequesta Monitor Well #6	+4.6	Lapointe and Krupa (1995b)
Tequesta Monitor Well #10	+11.8	Lapointe and Krupa (1995b)
Upwelled Nitrate		
North Atlantic Ocean	+4.8	Sigman et al. (2000)
Inorganic fertilizer	0 to +3	Owens (1987)
Peat	0 to +3	Heaton (1986)
Atmospheric nitrogen	0	Owens (1987)

Table 2
Macroalgal species collected for $\delta^{15}\text{N}$ analysis in the wet and dry seasons, 2001

Species	Location													
	Jupiter		Juno Beach		Riviera Beach		Lake Worth		Boynton Beach		Boca Raton		Deerfield Beach	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Chlorophyta														
<i>Caulerpa brachypus</i>	D	M,D	M,D	M,D	M,D	D								
<i>Caulerpa mexicana</i>		S												
<i>Caulerpa racemosa</i>		D	S	S,D			S			D	S	S,D		D
<i>Caulerpa verticillata</i>	M	M		M	M	M								
<i>Caulerpa</i> spp.														D
<i>Codium isthmocladum</i>	M,D	M,D	S,M,D	M,D	M,D	M,D	D	D	S,D	M,D	D	M,D	D	M,D
Rhodophyta														
<i>Bryothamnion triquetrum</i>										S				S
<i>Ceramium</i> sp.							M	M						
<i>Galaxaura oblongata</i>								M	M					
<i>Halymenia elongata</i>									D		D			M
<i>Halymenia echinophysa</i>										D				
<i>Hypnea musciformis</i>														S
<i>Laurencia poiteaui</i>		S	S	S	S	S	S	S			S	S		
<i>Wrangelia</i> sp.						S								
Phaeophyta														
<i>Dictyota cronulata</i>	S							M,D		M		M		
<i>Dictyota menziesii</i>														S
<i>Lobophora variegata</i>											M	M	M	M
<i>Padina sanctae-crucis</i>			S						S					S
<i>Spatoglossum shroederi</i>	S													

S = shallow reefs (< 5 m), M = mid-depth reefs (25–30 m), D = deep reefs (40–43 m).

(particularly other *Caulerpa* spp.), were sampled and analyzed for tissue $\delta^{15}\text{N}$ (Table 2).

2.2. Collection and preparation of macroalgae

SCUBA was used to sample macroalgae from the 21 reef sites between 10 and 30 May 2001, and between 20 and 28 August 2001. We collected samples of *Codium isthmocladum*, *Caulerpa* spp., and other abundant macroalgae into nylon mesh bags. Water temperatures and depths at the reef sites were measured using Oceanic Datamax Pro PlusTM dive computers. Immediately following collection, macroalgae were identified (Littler and Littler, 2000), sorted to species, cleaned of debris, transferred to plastic ZiplocTM baggies, and held in a cooler for transport to the Marine Nutrient Dynamics Lab at HBOI. In the lab, the samples were rinsed briefly in deionized water to further remove debris and salt and then randomly sorted into five replicate composite samples per

species (~3–6 individual thalli per composite). The cleaned tissues were placed in plastic drying dishes and dried in a Fisher Scientific IsotempTM oven at 60 °C for 48 h. Dried macroalgal thalli were ground to a fine powder using a mortar and pestle and stored in plastic vials until analysis.

2.3. Analysis of macroalgal $\delta^{15}\text{N}$

Samples of dried, powdered macroalgae were analyzed for stable nitrogen isotope ratios with a Carlo-Erba N/A 1500 Elemental Analyzer and a VG Isomass mass spectrometer using Dumas combustion. The standard used for stable nitrogen isotope analysis was N_2 in air. $\delta^{15}\text{N}$ values (‰) were calculated as $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, with R equal to $^{15}\text{N}/^{14}\text{N}$. Values were statistically compared using one- and two-way ANOVA for main effects, after heterogeneity of variance of the data were tested using an F -test. Fisher's PLSD multiple comparisons

test was used to compare groups (e.g. sites, transects) within main treatment effects of ANOVA. The analyses facilitated comparison of discrete N sources assimilated by macroalgae among the study sites, transects, and between dry and wet season samplings. Results were considered significant when the probability (p) of the null hypothesis was less than 0.05 ($p < 0.05$).

2.4. Analysis of dissolved inorganic nutrients in upwelled water

One liter polyethylene HDPE bottles were used to collect samples ($n = 2$) of cold ($< 25^\circ\text{C}$), upwelled bottom water between 20 and 25 August 2001 at the following deep stations: Jupiter, Juno Beach, Riviera Beach, Lake Worth, and Deerfield Beach. Aliquots of the water samples were filtered through $0.45\ \mu\text{m}$ Whatman GF/F filters and analyzed for $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$ (SRP) by the Nutrient Analytical Services Laboratory, Chesapeake Biological Laboratory, Solomons, MD. NO_3^- and SRP were analyzed using a Technicon Auto Analyzer II whereas NH_4^+ and NO_2^- were analyzed using a

Technicon TRAACS 800. Detection limits for these analyses were $0.21\ \mu\text{M}$ for NH_4^+ , $0.01\ \mu\text{M}$ for $\text{NO}_3^- + \text{NO}_2^-$, $0.01\ \mu\text{M}$ for NO_2^- , and $0.02\ \mu\text{M}$ for SRP (see D'Elia et al., 1997).

3. Results

3.1. $\delta^{15}\text{N}$ of macroalgae

A total of 431 tissue samples of macroalgae were analyzed for $\delta^{15}\text{N}$ in this study. Results are presented below for the main effects of transect location and depth within the dry season, the wet season, and of interactions among seasons, locations, and genera.

3.2. Dry season sampling

A total of 209 samples of macroalgae (Table 2) were processed and analyzed for $\delta^{15}\text{N}$ from the May sampling of the 21 reef sites. Overall, there was a significant (ANOVA, $F = 11.12$, $p < 0.0001$) effect of location on the $\delta^{15}\text{N}$ values of macroalgae during the dry season (Fig. 3) – the lowest $\delta^{15}\text{N}$ values occurring

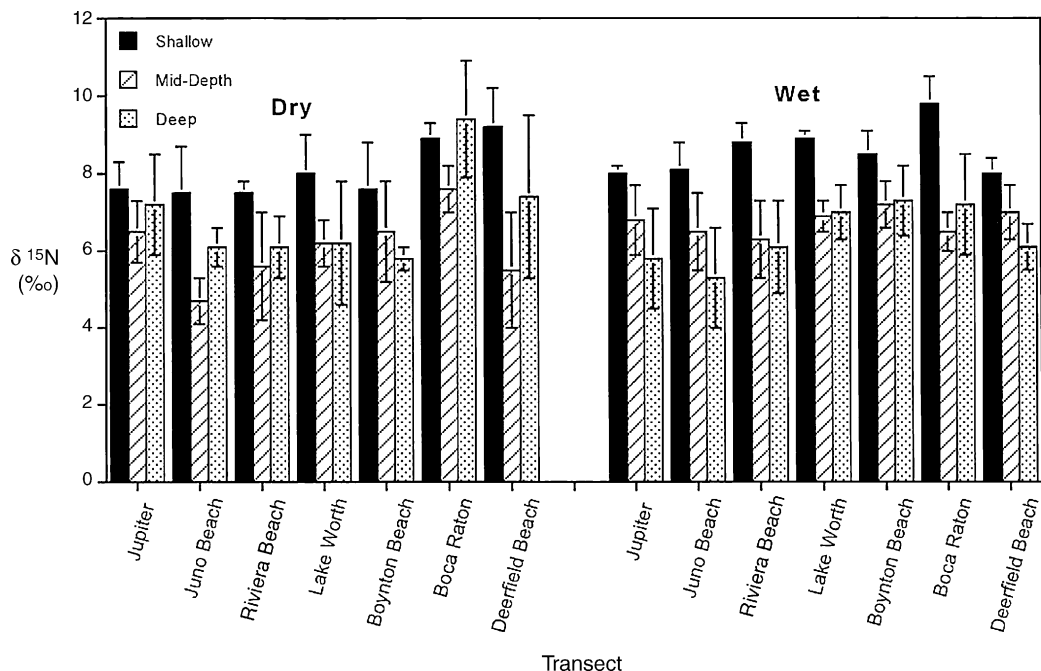


Fig. 3. $\delta^{15}\text{N}$ values for reef macroalgae at three depths along the seven transects in the dry (May) vs. wet (August) seasons, 2001. Values represent mean \pm S.E. ($n = 5\text{--}15$).

at Juno Beach ($+5.66 \pm 1.32\text{‰}$, $n = 32$) and the highest at Boca Raton ($+8.50 \pm 1.27\text{‰}$, $n = 24$) – reflecting a general trend of increasing $\delta^{15}\text{N}$ values from Juno Beach southward to Boca Raton (Fig. 3). The $\delta^{15}\text{N}$ values in macroalgae at Juno Beach were significantly lower (Fisher's post hoc test) than those at Jupiter ($p = 0.0005$), Riviera Beach ($p < 0.0001$), Lake Worth ($p = 0.0018$), Boynton Beach ($p = 0.0073$), Boca Raton ($p < 0.0001$), and Deerfield Beach ($p < 0.0001$). Conversely, the $\delta^{15}\text{N}$ values in macroalgae at Boca Raton were significantly higher than those at Jupiter ($p = 0.0003$), Juno Beach ($p = 0.0005$), Riviera Beach ($p < 0.0001$), Lake Worth ($p < 0.001$), Boynton Beach ($p < 0.001$), and Deerfield Beach ($p = 0.0045$).

The mean $\delta^{15}\text{N}$ values of reef macroalgae were significantly ($F = 47.54$, $p < 0.0001$) affected by depth during the dry season. At all locations, except Boca Raton (an outfall site), the highest $\delta^{15}\text{N}$ values occurred on shallow reef sites rather than deep reef sites (Fig. 3). Overall, the mean $\delta^{15}\text{N}$ of macroalgae on shallow reefs ($+8.06 \pm 1.10\text{‰}$, $n = 56$) was significantly ($p < 0.001$) higher than that of mid-depth ($+6.05 \pm 1.22\text{‰}$, $n = 80$) and deep reefs ($+6.65 \pm 1.76\text{‰}$, $n = 74$). The mean $\delta^{15}\text{N}$ of macroalgae on the mid-depth reefs was significantly ($p < 0.0008$) lower than that of the deep reef sites.

3.3. Wet season sampling

A total of 222 samples of macroalgae (Table 2) were processed and analyzed for $\delta^{15}\text{N}$ from the August sampling of the 21 stations. There was a significant (ANOVA, $F = 8.96$, $p < 0.0001$) effect of location on the $\delta^{15}\text{N}$ values of reef macroalgae. As in the dry season, the lowest $\delta^{15}\text{N}$ values occurred at Juno Beach ($+6.44 \pm 1.49\text{‰}$, $n = 40$) and the highest at Boca Raton ($+7.86 \pm 1.69\text{‰}$, $n = 30$) with a trend of increasing $\delta^{15}\text{N}$ values southward from Juno Beach to Boca Raton (Fig. 3). The $\delta^{15}\text{N}$ values of macroalgae at Juno Beach were significantly lower (Fisher's post hoc test) than those at Lake Worth ($p = 0.0045$), Boynton Beach ($p = 0.0013$), and Boca Raton ($p < 0.0001$). The highest $\delta^{15}\text{N}$ values of macroalgae were at Boca Raton, which were significantly higher than those at Jupiter ($p = 0.0004$), Juno Beach ($p = 0.0001$), Riviera Beach ($p < 0.0001$), and Deerfield Beach ($p = 0.011$).

There was a significant (ANOVA, $F = 87.23$, $p < 0.0001$) effect of reef depth on the $\delta^{15}\text{N}$ values of macroalgae in the wet season. At all locations, the highest $\delta^{15}\text{N}$ values occurred on the shallow reef sites rather than the mid-depth or deep reef sites (Fig. 3). Overall, the mean $\delta^{15}\text{N}$ of macroalgae on shallow reefs ($+8.58 \pm 0.87\text{‰}$) was significantly ($p < 0.0001$) higher than that of mid-depth reefs ($+6.7 \pm 0.89\text{‰}$), which was significantly ($p < 0.0004$) higher than that of the deep reef sites ($+6.3 \pm 1.26\text{‰}$).

3.4. Interactions among seasons, locations and genera

Two-way ANOVA indicated significant effects of location ($F = 52.36$, $p < 0.001$), genera ($F = 13.10$, $p < 0.001$), and the location \times genera interaction ($F = 2.45$, $p = 0.047$) on $\delta^{15}\text{N}$ values. The overall mean $\delta^{15}\text{N}$ value of *Codium isthmocladum* ($+6.95 \pm 0.97\text{‰}$, $n = 45$) was significantly higher than that of *Caulerpa* spp. ($+5.52 \pm 0.88\text{‰}$, $n = 55$) and the mean for all macroalgae ($+6.44 \pm 1.12\text{‰}$, $n = 169$, Fig. 4). For the shallow reefs, the mean $\delta^{15}\text{N}$ value of *C. isthmocladum* ($+8.37 \pm 1.0\text{‰}$, $n = 7$) was statistically similar to that of *Caulerpa* spp. ($+8.36 \pm 1.47\text{‰}$, $n = 15$) and to the overall mean for all macroalgae ($+8.31 \pm 1.03\text{‰}$, $n = 106$, Fig. 4). On the mid-depth reefs, however, the mean $\delta^{15}\text{N}$ value of *C. isthmocladum* ($+6.95 \pm 0.97\text{‰}$, $n = 45$) was significantly higher

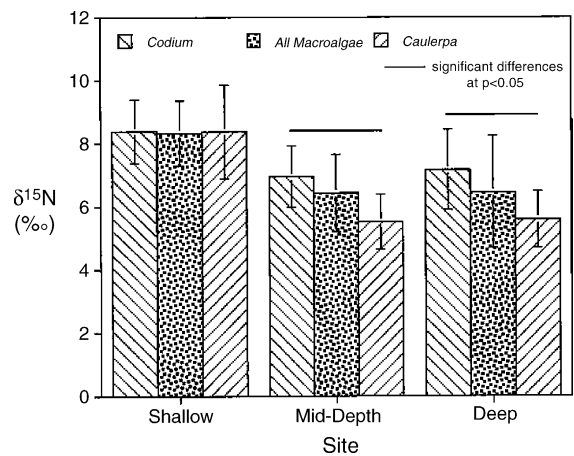


Fig. 4. $\delta^{15}\text{N}$ values for *Codium* spp., *Caulerpa* spp., and all macroalgae grouped by depth strata from all transects for the dry (May) and wet (August) season sampling in 2001. Values represent mean \pm S.E. ($n = 30\text{--}35$).

than that of *Caulerpa* spp. ($+5.52 \pm 0.88\%$, $n = 55$, $p < 0.001$) and higher than the overall mean for all macroalgae ($+6.44 \pm 1.12\%$, $n = 169$, $p < 0.047$; Fig. 4). Similarly, on the deep reefs the mean $\delta^{15}\text{N}$ value of *C. isthmocladum* ($+7.16 \pm 1.26\%$, $n = 65$) was significantly higher than that of *Caulerpa* spp. ($+5.59 \pm 0.89\%$, $n = 59$, $p < 0.001$) and higher than the mean for all macroalgae ($+6.47 \pm 1.53\%$, $n = 158$, $p < 0.047$, Fig. 4).

Although the overall effect of season was not significant among all transects and depths in the study ($p = 0.111$), significant differences in mean $\delta^{15}\text{N}$ values between the dry and wet seasons of 2001 were apparent in both *Codium isthmocladum* and *Caulerpa*

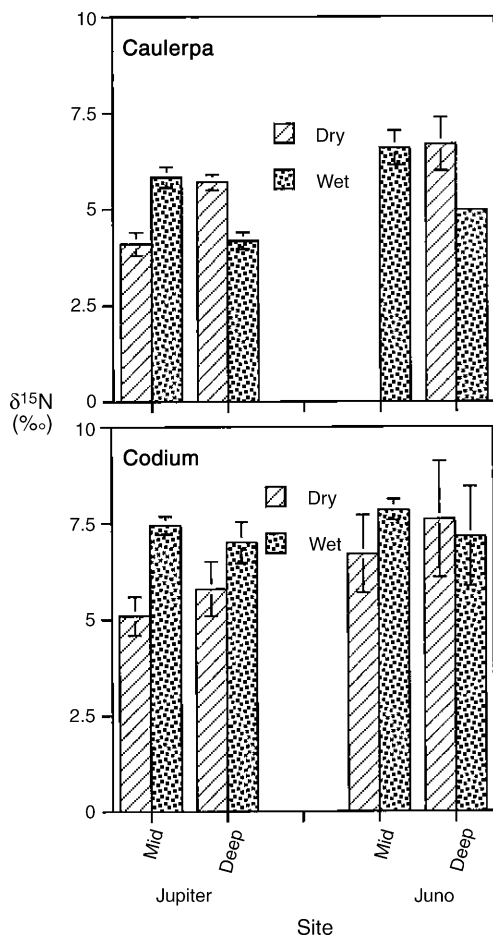


Fig. 5. $\delta^{15}\text{N}$ values of *Codium isthmocladum* and *Caulerpa brachypus* at mid and deep stations in the dry (May) and wet (August) seasons at Jupiter and Juno Beach. Values represent mean \pm S.E. ($n = 5-10$).

spp. from mid-depth and deep reefs in the northern study area off Juno Beach and Jupiter (Fig. 5). Two-way ANOVA of these data indicated significant effects of location ($F = 55.06$, $p < 0.001$), genera ($F = 99.38$, $p < 0.0001$), season ($F = 9.58$, $p = 0.003$), the season \times genera interaction ($F = 26.59$, $p < 0.0001$), the season \times location interaction ($F = 25.41$, $p < 0.0001$) and the season \times depth interaction ($F = 51.06$, $p < 0.0001$). $\delta^{15}\text{N}$ values were generally higher in *C. isthmocladum* than in *Caulerpa* spp. and increased from the dry season to wet season at both mid-depth and deep reefs off Jupiter, and at the mid-depth reef off Juno Beach (Fig. 5). The $\delta^{15}\text{N}$ values of *Caulerpa* spp. also increased from the dry to wet season on the mid-depth reef off Jupiter, but decreased at the deep reefs off both Jupiter and Juno Beach (Fig. 5).

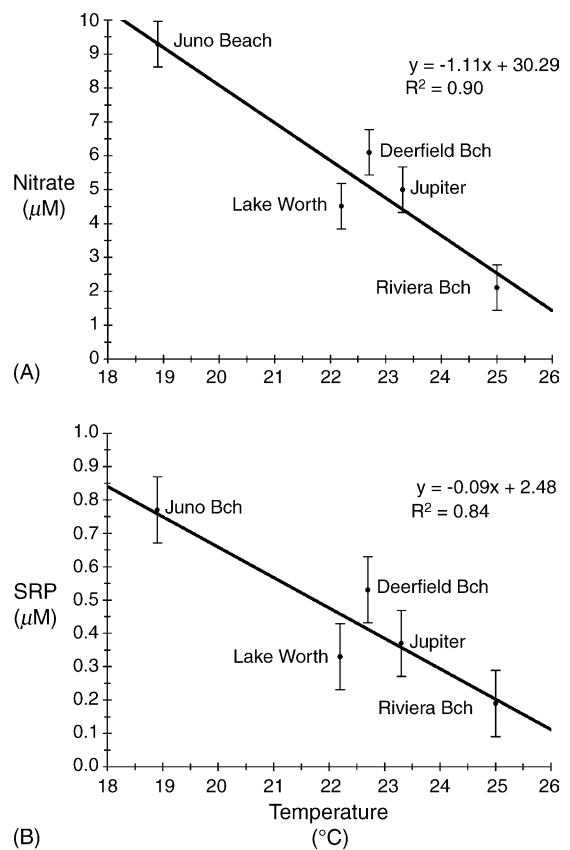


Fig. 6. Linear regressions for temperature vs. concentrations of nitrate (NO_3^- , A) and soluble reactive phosphorus (SRP, B) in the study area during the upwellings of August 2001. Values represent mean \pm S.D. ($n = 2$).

3.5. Nutrient concentrations of upwelled water

During the wet season sampling we observed elevated NO_3^- and SRP concentrations associated with a cold-water upwelling event. NO_3^- concentrations at the deep sites of five transects ranged from 2 to 9 μM during these upwellings, with the highest concentrations measured at the Juno Beach deep reef site. These elevated NO_3^- concentrations correlated significantly and negatively ($R^2 = 0.90$) with water temperature (Fig. 6A). SRP concentrations were elevated up to $\sim 0.75 \mu\text{M}$ in the upwelled waters and also correlated significantly and negatively ($R^2 = 0.84$) with water temperature (Fig. 6B).

4. Discussion

Results of this study support the hypothesis that anthropogenic N sources have contributed to the development of macroalgal HABs and the explosive invasion of *Caulerpa brachypus* on southeast Florida's coral reefs in recent years. This study also represents the first report on the distribution of *C. brachypus* var. *parvifolia* in the coastal waters of southeast Florida (Fig. 2). More broadly, these findings contribute to a growing recognition of the role of anthropogenic nutrient enrichment in relieving nutrient limitation while enhancing the productivity, biomass, and ultimate success of invasive HAB species, particularly chlorophytes in the genus *Caulerpa* (see also: Chisholm et al., 1997; Jaubert et al., 2003; Lapointe et al., 2005a).

4.1. The role of upwelling in nutrient enrichment of reefs

Although previous studies have described temperature anomalies associated with summer upwelling along Florida's southeast coast (Green, 1944; Taylor and Stewart, 1958; Smith, 1982), this study provides one of the first reports of NO_3^- and SRP concentrations associated with these phenomena in this study area. Coastal upwelling was not apparent during previous studies of the *Codium isthmocladum* blooms in the summer of 1994 when mean NO_3^- concentrations were relatively low ($0.66 \pm 0.33 \mu\text{M}$) compared to NH_4^+ ($0.96 \pm 0.76 \mu\text{M}$) and low temperatures

associated with upwelling did not occur (Lapointe, 1997). However, strong upwelling did occur during the present study in August 2001 when increasing NO_3^- concentrations up to 9 μM correlated significantly with decreasing temperature (to 19 °C) and reef depth (to 43 m). These upwelling events typically occur during summer months when southerly winds (parallel to shore) and onshore movement of the Florida Current produce conditions favorable for upwelling (Smith, 1982), which can persist for a period of 7–14 days (Lee and Mayer, 1977). At Conch Reef in the Upper Florida Keys, Leichter et al. (2003) reported NO_3^- concentrations of $\sim 4 \mu\text{M}$ associated with internal tidal bores (which they referred to as upwelling) but there was no significant correlation with temperature. Leichter et al. (2003) also reported relatively high concentrations of NH_4^+ (1.0–2.2 μM) associated with the elevated NO_3^- concentrations but did not consider the importance of SGD at Conch Reef (see Simmons, 1992) or NH_4^+ as a preferred N source to *C. isthmocladum*. At three stations along an 18 km transect extending from Big Pine Key offshore to the Looe Key Sanctuary Protection Area in the Lower Florida Keys, Lapointe et al. (2004) found that NH_4^+ concentrations increased significantly from the winter dry season to the summer wet season; this pattern correlated significantly with seasonal increases in rainfall, offshore advection of sewage-contaminated stormwater runoff, increased macroalgal biomass, and corresponding enrichment of macroalgal $\delta^{15}\text{N}$.

Previous nutrient kinetic studies with macroalgae provide mechanistic evidence as to why episodic, upwelled NO_3^- has not historically supported bloom formation and excessive biomass of *Codium isthmocladum* and *Caulerpa* spp. in southeast Florida. In controlled laboratory studies, uptake of DIN by *Codium fragile* subsp. *tomentosoides* was highly dependant upon light, temperature, and the source of DIN – i.e., NO_3^- versus NH_4^+ (Hanisak and Harlin, 1978). At temperatures of 20–25 °C, the uptake rate of NH_4^+ was ~ 7 -fold greater than that of NO_3^- and the presence of NH_4^+ inhibited uptake of NO_3^- . Similar preferences for NH_4^+ over NO_3^- have been reported for red macroalgae (D'Elia and DeBoer, 1978) as well as for natural phytoplankton communities (Conway, 1977). Because an average of 0.91 μM NH_4^+ was present in the near-bottom waters during the upwelling in August 2001, it is unlikely that NO_3^- was a major

DIN source to either *Codium isthmocladum* or *Caulerpa* spp. In a seasonal upwelling system similar to that of southeast Florida, Fujita et al. (1989) reported that relatively low concentrations of NH_4^+ (1.22 μM) supplied >100% of the N required for maximum growth of the chlorophyte *Ulva rigida*, even in the presence of much higher NO_3^- concentrations (10.8 μM).

The turbid, low light conditions and low temperatures associated with upwelling events could further limit the ability of *Codium isthmocladum* and *Caulerpa* spp. to assimilate upwelled NO_3^- . *Codium fragile* subsp. *tomentosoides* assimilated NH_4^+ more slowly in the dark than in the light, but the uptake rate in the dark (and under low irradiance) was still higher than the uptake rate for NO_3^- in the light (Hanisak and Harlin, 1978). Furthermore, N uptake was suppressed as temperatures decreased (Hanisak and Harlin, 1978). We observed these sub-optimal growth conditions during the summer 2001 upwelling event, which appeared to coincide with diminished bloom development. These observations support a conceptual model that predicts bloom formation to be highly dependant on the presence of $\sim 1 \mu\text{M}$ NO_4^+ , high downwelling irradiance, and temperatures of 25–29 °C (Lapointe and Hanisak, 1997), conditions that do not occur during cold, plankton-rich upwelling events in the southeastern United States (Atkinson et al., 1984; Atkinson, 1985).

4.2. Land-based nutrient enrichment in southeast Florida

Multiple lines of evidence from these studies support the hypothesis that N derived from land-based sources, rather than coastal upwelling, was the primary N source supporting the blooms of *Codium isthmocladum* and *Caulerpa* spp. on reefs in southeast Florida. First, the blooms of *C. isthmocladum* and *Caulerpa* spp. were well developed in mid-May 2001, prior to the onset of the summer upwelling period (Taylor and Stewart, 1958). Extensive blooms of *C. brachypus* were overgrowing sponges, hard corals, octocorals, and other macroalgal HAB species on reefs in northern Palm Beach County, whereas reef sites in southern Palm Beach County were primarily impacted by drift populations of *C. isthmocladum* (see Table 2). These observations in May 2001 support the conceptual model

proposed by Lapointe and Hanisak (1997) that drought periods may be an important environmental factor contributing to the formation of macroalgal blooms on deep reefs off southeast Florida. Like the initial blooms of *C. isthmocladum* that developed in southern Palm Beach County during the drought of 1989–1990, the blooms we observed in May 2001 occurred during one of the most severe droughts on record in South Florida (Abtew et al., 2002). Drought conditions reduce terrestrial stormwater runoff and increase water transparency in the coastal ocean, resulting in maximal downwelling irradiance, which we suspect is critical for bloom formation on these deep reefs (Lapointe and Hanisak, 1997). Water transparency during the May 2001 sampling was very high, most reefs having >33 m vertical visibility.

Secondly, the lack of a significant change in $\delta^{15}\text{N}$ values of macroalgae between the non-upwelling (temperatures >25 °C) May 2001 sampling and the August 2001 upwelling period supports the hypothesis that upwelled NO_3^- was not a major DIN source supporting the macroalgal blooms in the study area. The upwelling in August 2001 was most pronounced on the Juno Beach deep reefs where temperatures of 19 °C coincided with peak NO_3^- concentrations of 9.29 μM . The macroalgae from this deep site had the lowest $\delta^{15}\text{N}$ value of all sites during the August sampling and closely matched the $\delta^{15}\text{N}$ signature (+4.8‰) of North Atlantic deep-water NO_3^- (Sigman et al., 2000; Montoya et al., 2002). This pattern suggests that upwelled NO_3^- was reducing, not enriching, the higher background $\delta^{15}\text{N}$ values of macroalgae in the study area. This pattern can best be explained by the dominance of another N source with a higher $\delta^{15}\text{N}$ content than N associated with upwelling.

The significantly higher $\delta^{15}\text{N}$ values in macroalgae on the shallow reefs during this study clearly indicate a land-based source of N enrichment to the HABs. The magnitude of the $\delta^{15}\text{N}$ values obtained from the shallow reefs are in the range of human sewage and could be delivered to coastal waters of the study area via a variety of sources and pathways. An estimated 77,391 septic tanks occur in Palm Beach County with another 106,254 in Broward County (www.doh.state.fl.us). These on-site sewage disposal systems contaminate shallow groundwaters and downstream surface waters with elevated concentrations of NH_4^+ , NO_3^- , and SRP (Lapointe and Krupa, 1995).

The $\delta^{15}\text{N}$ values of these contaminated groundwaters range from +4‰ to +18‰, increasing with distance from the source as a result of fractionation associated with volatilization of ammonia and nitrification (Lapointe and Krupa, 1995a,b).

Other sources of sewage N to reefs off southeast Florida are ocean outfalls, which deliver NO_4^+ -enriched wastewaters directly into the water column in the southern part of our study area. Six ocean outfalls located off of southeast Florida include: Delray Beach (89 MLD), Boca Raton (65 MLD), North Broward (244 MLD), Hollywood (163 MLD), North Dade (370 MLD), and Central Dade (529 MLD), which collectively deliver ~ 1462 MLD of secondarily treated wastewater with high NH_4^+ concentrations ($\sim 900 \mu\text{M}$) into coastal waters (Hazen and Sawyer, 1994). Three of these outfalls, Delray Beach, Boca Raton, and North Broward, directly influence the water column in the southern part of the study area (Fig. 2).

Additional pathways for transport of sewage-derived NH_4^+ (and NO_3^-) to the mid-depth and deep reefs are via Classes I and V injection wells and SGD. Palm Beach and Broward counties have nearly 30 Class I injection well facilities that dispose of NH_4^+ -rich secondarily treated wastewater under pressure to ~ 1100 m depths (USEPA, 2003). One of these facilities along the coast in Palm Beach County has been identified in a recent EPA Wastewater Risk Assessment Report (USEPA, 2003) as a facility with upward vertical migration of effluent into a federally protected and regulated drinking water aquifer. Meyer (1989) described how upward vertical movement of groundwater from these depths occurs as a result of thermal convection, density gradients, and buoyant flow. Class V wells discharge into depths < 30 m and are used primarily for stormwater runoff rather than for sewage disposal. Swayze and Miller (1984) reported a highly permeable surficial aquifer unit between 20 and 33 m depths that could be enriched by the upward flow of wastewater and stormwater N from deeper zones. This geological unit extends offshore to the reef outcrops where the macroalgal HABs occur.

4.3. $\delta^{15}\text{N}$ source assimilation and fractionation in reef macroalgae

One of the challenges of using stable nitrogen isotopes in ecological studies is the “fractionation

effect” whereby assimilation of N at higher trophic levels leads to volatilization of the lighter ^{14}N , resulting in stepwise enrichment of ^{15}N through food webs (Minagawa and Wada, 1984). Macroalgae are useful for discriminating specific nutrient sources in marine ecosystems because they do not fractionate $\delta^{15}\text{N}$ values of their N sources in N-limited systems (France et al., 1998; Waser et al., 1999). Because the summer blooms of macroalgae on southeast Florida reefs are N limited (Lapointe, 1997; Lapointe et al., 2005b; water column N:P $< 16:1$, macroalgal tissue N:P < 35), fractionation of $\delta^{15}\text{N}$ source values by macroalgae would not be expected. Where fractionation has been documented between the N source (groundwater NO_3^-) and coral reef macroalgal tissue, enrichment in tissue $\delta^{15}\text{N}$ was slight (0.2–1.4‰; see Umezawa et al., 2002). For zooxanthellate reef corals, irradiance can confound the fractionation process when increased photosynthesis under high irradiance favors reduced fractionation of $\delta^{15}\text{N}$ (Muscatine and Kaplan, 1994; Heikoop et al., 1998). Fortunately, this is not the case with macroalgae. Low $\delta^{15}\text{N}$ values ($\sim +0.5\%$) are typical of macroalgae growing on shallow, oligotrophic reefs that experience high irradiance and natural N sources, such as nitrogen fixation (see France et al., 1998), whereas elevated $\delta^{15}\text{N}$ values of macroalgae occur in shallow sites receiving sewage N enrichment (Umezawa et al., 2002; Lapointe and Thacker, 2002).

High human population density and associated wastewater NH_4^+ loadings from sewage outfalls were associated with elevated $\delta^{15}\text{N}$ values of macroalgae in the southern study area where blooms of *C. isthmocladum* were centered. The $\delta^{15}\text{N}$ value of effluent from the North Broward outfall just south of Deerfield Beach was reported to be $\sim +8\%$ (Hoch et al., 1995), similar to the range of values of +8‰ to +12‰ widely reported for secondarily treated effluent (Lindau et al., 1989; Costanzo et al., 2001; Savage and Elmgren, 2004). The warm, buoyant fresh water effluents discharged from the sewage outfalls at 27 m depths quickly rise to the surface, potentially affecting shallow, mid, and deep reefs. The reported outfall $\delta^{15}\text{N}$ values closely matched the $\delta^{15}\text{N}$ signature of macroalgae on the mid and deep reefs near the Boca Raton outfall, as well as the shallow reefs throughout the study area.

In comparison, the northern study area had a lower population density, more diffuse anthropogenic N

enrichment (no outfalls), and the reefs were dominated by *Caulerpa* spp. The lowest $\delta^{15}\text{N}$ values ($\sim+5.7\text{‰}$) in macroalgae occurred at the most offshore station – Juno Beach in northern Palm Beach County – suggesting relatively low-level enrichment of sewage $\delta^{15}\text{N}$ at this site. In addition to effects of increased distance from the sewage outfalls in the southern portion of the study area, relatively lower $\delta^{15}\text{N}$ values in northern Palm Beach County may also reflect the contribution of more diffuse sewage N enrichment via SGD. These factors would tend to lower $\delta^{15}\text{N}$ values closer to that of Atlantic subsurface water ($\sim+4.8\text{‰}$, Sigman et al., 2000; Montoya et al., 2002). Umezawa et al. (2002) reported a similar dilution of $\delta^{15}\text{N}$ values of macroalgae associated with deep-water NO_3^- moving onto coral reef systems with high rates of nitrogen-fixation.

The pathway by which chronic anthropogenic nutrients enter coastal waters can confer competitive advantages upon *Codium* and/or *Caulerpa* and may contribute to their success in eutrophic coastal waters. The siphonaceous *Codium isthmocladum* relies entirely on the water column for nutrients, compared to the *Caulerpa* spp. that can access sediment and/or reef pore water nutrient pools with their root-like rhizoids. With these structures, *Caulerpa* spp. compete successfully with tropical seagrasses for sediment NH_4^+ in the Caribbean (Williams, 1984; Williams and Fisher, 1985). Different nutrient acquisition strategies may explain why *C. isthmocladum* blooms are best developed in southern Palm Beach County, where sewage from ocean outfalls is a primary source of N enrichment of the water column. In contrast, SGD is likely to be a more important N source to the reefs in northern Palm Beach County, which lacks sewage outfalls and where *C. brachypus*, *C. verticillata*, and *C. racemosa* accounted for extensive cover on the reefs (Fig. 1C–F; up to 80% cover, Lapointe et al., 2004). The ability of *Caulerpa* spp. to attach firmly to the reefs with their root-like rhizoids would also be an asset in these turbulent flow fields and would be a competitive advantage in accessing N pools in reef interstitial waters. Because thick mats of macroalgae may attenuate NH_4^+ flux from sediments to the overlying water column (McGlathery et al., 1997), dense *Caulerpa* blooms could intercept nutrient supplies which would otherwise be available to *C. isthmocladum* and other

macroalgae that depend on the water column for nutrients. This hypothesis is supported by the significantly higher $\delta^{15}\text{N}$ values of *C. isthmocladum*, compared to *Caulerpa* spp., on mid-depth and deep reefs, and may explain the prevalence of *C. isthmocladum* on reefs in southern Palm Beach County where the water column is chronically enriched by sewage effluent from ocean outfalls. Whereas *C. isthmocladum* was most prevalent in the southern portion of the study area, both *C. isthmocladum* and *Caulerpa* spp. were found throughout the study area (see Table 2), which facilitated this comparison.

The conclusion that sewage was a primary N source to these macroalgal blooms is supported by several other studies from the scientific literature reporting elevated $\delta^{15}\text{N}$ values in the range of those reported in this study. The $\delta^{15}\text{N}$ ratio of reef macroalgae ranged from $+5.7\text{‰}$ off Juno Beach to $+8.5\text{‰}$ off Boca Raton, values within the range reported for macroalgae growing on sewage N in both temperate and tropical coastal waters (Lapointe, 1997; McClelland and Valiela, 1998; France et al., 1998; Costanzo et al., 2001; Lapointe and Thacker, 2002; Umezawa et al., 2002; Barile, 2004; Savage and Elmgren, 2004; Table 1). The significant cross-shelf pattern of elevated $\delta^{15}\text{N}$ associated with land-based sources of pollution reported here parallels the findings of Sammarco et al. (1999) who reported that the influence of terrestrial N sources extended seaward across nearly 40 km of the Great Barrier Reef lagoon.

4.4. Cultural eutrophication of southeast Florida coral reefs

The SEFLOE II investigations (Hazen and Sawyer, 1994), funded by local wastewater utilities and performed in collaboration with the NOAA Atmospheric, Oceanographic and Meteorological Laboratory (AOML) in Miami, FL, assessed the environmental impacts of sewage outfalls in the southern portion of our study area. The SEFLOE II study reported that ambient concentrations of NH_4^+ on the reefs in north Broward County averaged $\sim 6\ \mu\text{M}$ and that maximum ambient concentrations were $\sim 36\ \mu\text{M}$. Those background NH_4^+ concentrations are some 6- to 36-fold higher than the mean NH_4^+ concentrations reported by Lapointe (1997) and Lapointe et al. (2005b) for this

area, which reflect not only the actual background concentration, but also the contribution of the wastewater loadings. Hazen and Sawyer (1994) used these erroneous NH_4^+ concentrations in a dilution model that supported their “finding of no significant impact” (FONSI) for the north Broward County outfall, which discharges ~ 1100 metric t/year of NH_4^+ into the water column over coral reefs in ~ 27 m depths. Section 403 of the USEPA (1994) Clean Water Act concluded: “Because of the relatively short term of the SEFLOE studies (several years), the long term or cumulative risks of nutrient loading and loading of other effluent constituents cannot be evaluated.”

The Ocean Regulatory Programs section of the USEPA (1994) Clean Water Act (CWA, Section 403) specifically addresses impacts on the marine environment from point source discharges. The section’s requirements are intended to ensure that no unreasonable degradation of the marine environment will occur as a result of the discharge and that sensitive ecological communities are protected. Unreasonable degradation is defined as “significant adverse changes in ecosystem diversity, productivity, and stability of the biological community within the area of discharge and surrounding biological community.” Sensitive ecological communities include unique species or communities, endangered or threatened species, species critical to the structure or function of the ecosystem, nursery/forage areas, and coral reefs. In our study, the $\delta^{15}\text{N}$ values in macroalgae ($\sim +8\%$) on coral reefs off Deerfield Beach closely matched the values reported by Hoch et al. (1995) for the NH_4^+ -rich effluent from the north Broward County outfall located “upstream” several kilometers to the south. While the SEFLOE II Report utilized empirical modeling estimates to suggest no impact of outfall sewage to the continental shelf biota, our study provides source-sink evidence of sewage N assimilation from outfalls into reef HABs. The southeast Florida reefs provide habitat to a number of endangered species (e.g. sea turtles) and finfish that have been affected by diseases associated with toxin production, possibly from epiphytes of these macroalgal HABs (Landsberg, 1995).

In summary, these results provide multiple lines of evidence that macroalgal assimilation of sewage N from ocean outfalls and other land-based sources (septic tanks, injection wells) supports HABs and their

degradation of coral reef habitats. If coral reef habitats are to be protected in the study area, we recommend that our results be considered in future National Pollution Discharge Elimination System (NPDES) permitting of the sewage outfalls, particularly as the NPDES process addresses the concept of “unreasonable degradation” of coral reef habitats. USEPA states that “if section 403 requirements for protection of the ecological health of marine waters are not met, a NPDES permit will not be issued.”

This evidence, that anthropogenic nutrient enrichment from land-based sewage discharges supports blooms of native *Codium isthmocladum* and the invasive *Caulerpa brachypus* var. *parvifolia* on coral reefs in southeast Florida, parallels recent reports that land-based sources of pollution, specifically human sewage, supported the invasion of *C. taxifolia* in the northwestern Mediterranean (Chisholm et al., 1997; Jaubert et al., 2003) as well as that of the Mediterranean native *Caulerpa ollivieri* in the Bahamas (Lapointe et al., 2005a). These findings suggest that current wastewater management practices in southeast Florida may be in conflict with federal and state initiatives to reduce the effects of land-based sources of pollution on sensitive ecosystems, such as coral reef habitats in southeast Florida. Implementation of advanced wastewater treatment (AWT) for the removal of N and P from sewage effluent, along with increased wastewater reuse for beneficial purposes, is critical to mitigate the impacts of these wastewater discharges on southeast Florida’s reefs. This study supports the conclusion of the Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP, see Windom, 1992) that nutrient pollution from sewage and other land-based sources poses the greatest present and future threat to the coastal marine environment (Windom, 1992).

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