

## Nitrogen and oxygen isotopic signatures of subsurface nitrate seaward of the Florida Keys reef tract

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### *Abstract*

Hydrographic and nutrient structure and the nitrogen and oxygen isotopic compositions ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) of the nitrate pool seaward of the Florida Keys reef tract were measured in Summer 2003. Nitrate and soluble reactive phosphate concentrations were tightly associated with depth and temperature and were close to 0 in surface waters, increased to 2–10  $\mu\text{mol L}^{-1}$  and 0.1–1.0  $\mu\text{mol L}^{-1}$ , respectively, at intermediate depths associated with the thermocline and 15–25  $\mu\text{mol L}^{-1}$  and 1.0–1.5  $\mu\text{mol L}^{-1}$ , respectively, in deeper water. Ammonium concentrations were low (0–0.6  $\mu\text{mol L}^{-1}$ ) at all depths. Dissolved organic carbon concentrations ranged from 70–120  $\mu\text{mol L}^{-1}$  near the surface to 40–60  $\mu\text{mol L}^{-1}$  below the thermocline. Mean nitrate  $\delta^{15}\text{N}$  was 4.78‰ ( $\pm 0.05$ ) overall and varied from 4.24‰ ( $\pm 0.07$ ) within the chlorophyll maximum layer at 50–70 m depth to 5.26‰ ( $\pm 0.05$ ) below the thermocline at 150–242 m depth. Mean  $\delta^{18}\text{O}$  mean was 3.26‰ ( $\pm 0.08$ ) and varied from 4.89‰ ( $\pm 0.14$ ) within the chlorophyll maximum layer to 3.02‰ ( $\pm 0.07$ ) below the thermocline. These observations suggest an oceanic, deep-water nitrate source below the thermocline, a mix of sources from upwelling and  $\text{N}_2$  fixation, and the effects of fractionation associated with uptake by primary producers within the thermocline. The water column nitrate isotopic data support the general hypothesis that primary producers on the Florida Keys reef tract directly utilize the transiently available subsurface nitrate pool.

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Analysis of stable isotopes can be a powerful tool for identifying nutrient sources and transformation pathways in aquatic and marine ecosystems. In general,  $\delta^{15}\text{N}$  values (the ratio of  $^{14}\text{N}$  to  $^{15}\text{N}$  relative to an atmospheric reference standard) of organic and inorganic compounds within nitrogen cycles reflect both the isotopic signatures of nitrogen source pools and fractionation associated with uptake and biogeochemical transformation (Altabet 2005). Where nutrient sources have distinct isotopic compositions, it is possible to trace their uptake by primary producers and effect on consumer food webs (e.g., Fourqurean et al. 1997; McClelland and Valiela 1998; Cole et al. 2005). Analysis of  $\delta^{15}\text{N}$  values of organic matter in marine ecosystems has provided insight into decomposition processes (Saino and Hattori 1980), trophic-level dynamics (Hansson et al. 1997), and nitrogen sources to primary producers (Umezawa et al. 2002; Cole et al. 2005). Nitrate use and the nutrient status of oceanic systems in the present and past have been studied with the use of  $\delta^{15}\text{N}$  (Altabet 2005).

Denitrification in water column suboxic zones (Altabet et al. 1995) and estimates of terrestrial contribution of nitrogen to coastal marine sediments (Sweeney and Kaplan 1980) have also been assessed. Nitrogen isotopes of particulate organic material (POM), dissolved inorganic nitrogen (DIN), and dissolved organic nitrogen (DON) can also provide information about nitrogen sources such as nitrogen fixation (e.g., Karl et al. 2002; McClelland et al. 2003) and anthropogenic inputs (Wankel et al. 2006). Recently, tools have also been developed for measuring the oxygen isotopic composition of marine nitrate (Casciotti et al. 2002), offering a second dimension along which information relating to nutrient sources and transformation processes can be inferred (Wankel et al. 2006).

The input of anthropogenic nutrients poses a serious threat to a variety of nearshore ecosystems, including coastal embayments, estuaries, and coral reefs. With the use of discernable differences between  $\delta^{15}\text{N}$  values for natural versus anthropogenic sources of nitrogen, a number of studies have investigated nitrogen pollution in coral reef environments via measurement of tissue  $\delta^{15}\text{N}$  in benthic organisms (e.g., Sammarco et al. 1999; Heikoop et al. 2000; Swart et al. 2005). Recognition of an isotopic signal from anthropogenic nitrogen in benthic organisms depends on clear differentiation of  $\delta^{15}\text{N}$  in potential sources. For example, Umezawa et al. (2002) showed macroalgal  $\delta^{15}\text{N}$  decreased from +8‰ to +2‰ between 500 and 800 m from shore to fringing reef crests as evidence of a nearshore source of anthropogenic nitrate with a high isotopic signature. Coral  $\delta^{15}\text{N}$  along a nearshore–offshore gradient in the Great Barrier Reef, Australia (Sammarco et al. 1999), have also suggested both terrestrial nitrogen loading nearshore as well as input of oceanic nitrogen offshore. Studies in other ecosystems have used  $\delta^{15}\text{N}$  measurements in seagrasses (Fourqurean et al. 1997) and estuarine algae (McClelland and Valiela 1998) to infer nutrient loading from sewage and other terrestrial sources.

Extensive effort in recent years has been focused on attempts to measure nutrient sources and dynamics in the waters adjoining the Florida Keys (Kruczynski and McManus 2002). Human activities on land and in coastal waters can significantly increase the availability of both nitrogen and phosphorous in reef waters, especially for nearshore reefs (Garrison et al. 2003; Paytan et al. 2006). Although large increases in human population in the Florida Keys over the last several decades have increased the potential for anthropogenic nutrient loading nearshore, available data have not detected a signal of anthropogenic nutrients reaching the outer reefs of the Florida Keys reef tract, located 5–8 km from shore (Szmant and Forrester 1996; Kruczynski and McManus 2002). Although the possibility of nutrient transport via submarine groundwater movement through the carbonate platform of the Florida Keys reef tract has been investigated, neither tracer experiments (Corbett et al. 2000; Dillon et al. 2003), near-bottom salinity and temperature data (Leichter et al. 1996, 2003), or radium isotopic measurements have shown evidence of groundwater delivery to the reef tract (Paytan et al. 2006). Attempts to infer nutrient pollution sources from  $\delta^{15}\text{N}$  measurements in tissues of macroalgae and

gorgonians have also been made on reefs both nearshore and several kilometers from shore in the Florida Keys (Lapointe et al. 2004; Ward-Paige et al. 2005). These studies did not include determination of  $\delta^{15}\text{N}$  values for the purported nutrient sources, relying instead on the hypothesis that tissue  $\delta^{15}\text{N}$  values  $> 3\text{--}4\text{‰}$  indicate land-based nutrient pollution (Lapointe et al. 2004). In the same region, however Swart et al. (2005) found no clear signals in coral  $\delta^{15}\text{N}$  from offshore to nearshore reefs but did find significant temporal variation, suggesting seasonal changes in the relative importance of coral autotrophy and heterotrophy.

Independent of potential anthropogenic sources, the Florida Keys reef tract is also exposed to episodic input of nutrients from upwelling-associated uplift of isotherms by frontal eddies of the Florida Current and internal waves acting at tidal and faster frequencies (Lee et al. 1992; Leichter et al. 1996, 2003). The thermocline seaward of the Florida Keys is often relatively shallow (e.g., 50 m depth), especially in summer, and upward incursions of cool (22–26°C) water onto reef slopes occur frequently throughout the Keys (Leichter et al. 2003). Previous water column sampling has shown relatively high concentrations of subsurface nitrate (5–20  $\mu\text{mol L}^{-1}$ ) and soluble reactive phosphate (0.1–2.0  $\mu\text{mol L}^{-1}$ ) below the thermocline (Lee et al. 1992; Leichter et al. 2003) and clearly points to the potential for the offshore subsurface nutrient pool to be an important source of nutrients reaching reef slopes, especially at depths of  $\geq 10$  m. In addition to being attenuated with depth, incident internal waves interact with reef topography, producing significant heterogeneity in cool water exposure and residence times within, as well as across depths (Leichter et al. 2005).

A growing body of evidence supports the general hypothesis that exposure to the offshore nutrient pools has important consequences for benthic primary producers in this system. Both Vroom et al. (2003) and Smith et al. (2004) found increased growth rates of the green alga *Halimeda tuna* with increasing depth on the slope of Conch Reef. Smith et al. (2004) further found that *H. tuna* at 7 m depth showed a significant response in nutrient addition experiments, whereas individuals at 21 m depth showed no response, suggesting that these deeper individuals existed in nutrient-replete conditions. Isotopic evidence also suggests utilization of the pulses of subthermocline nitrate by a common benthic macroalga, *Codium isthmocladum*. Leichter et al. (2003) found that  $\delta^{15}\text{N}$  values of *C. isthmocladum* increased from approximately +2.0‰ at 9 m depth to +5.0‰ at 35 m, corresponding to increasing exposure to pulses of subthermocline water and thus a gradient in availability of offshore nitrate. This interpretation assumed that the isotopic signature of the offshore subthermocline  $\text{NO}_3^-$  pool was close to the average of  $\sim 5\text{‰}$  measured for marine  $\text{NO}_3^-$  in other settings (Sigman et al. 2000) and that isotopic discrimination by the algae during uptake and assimilation would likely be small because overall nitrogen content and C:N ratios suggest these algae were relatively nitrogen limited.

The study reported here was motivated by the lack of direct measurements of the isotopic composition of the

subsurface nitrate pool seaward of the Florida Keys reef tract. Detailed sampling of the water column hydrographic and nutrient structure seaward of the reef tract was combined with direct determination of the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of subsurface  $\text{NO}_3^-$ . Such measurements are useful for distinguishing likely sources of nutrients in the offshore pool and the possible utilization of those nutrients by reef-associated benthic primary producers. These data represent, to our knowledge, the first measurements of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values for subthermocline nitrate in this region.

## Methods

Field sampling was carried out aboard the R/V *Walton Smith* 11–19 Sep 03 within a region spanning a linear distance of  $\sim 200$  km seaward of the Florida Keys. Sampling of hydrographic structure and nutrient chemistry was conducted along onshore–offshore transects at six sites from Sand Key (SK;  $24^\circ 26.3' \text{N}$ ,  $81^\circ 53.1' \text{W}$ ) in the southwest to Carysfort Reef (CF;  $25^\circ 13.19' \text{N}$ ,  $80^\circ 12.1' \text{W}$ ) in the northeast (Fig. 1). At each of the six sites, a transect of 10 hydrographic sampling stations was established from near the seaward margin of the reef directly offshore at distances of 0, 0.9, 1.8, 3.7, 5.6, 7.4, 9.3, 11.1, 14.8, and 18.5 km from the reef. A single hydrocast was made from the ship at each station with a Seabird 911+ conductivity–temperature–depth (CTD) meter with a fluorometer and a water sampling rosette. Hydrographic data were averaged in 1-m vertical bins using data only from the downward-traveling portion of each cast, and oceanographic properties (salinity, density, vertical stratification statistics) were calculated from the 1-m averaged data. Chlorophyll *a* (Chl *a*) concentration was calculated from in situ fluorescence measured by a Wetstar fluorometer that was calibrated shortly before the cruise. Contour plots across casts within each transect were produced by kriging the data onto a uniform grid.

On three of the transects (Sand Key, Tennessee Reef, and Elbow) 12-liter Niskin bottles were used to collect water samples from four to six depths at each sampling station. Water samples were taken at the maximum depth, at regularly spaced intervals in the water column, and near the surface (3 m depth). Real-time output from the CTD and fluorometer was monitored during the casts, and at least one water sample was taken in the subsurface fluorescence maximum layer. Water samples were filtered immediately after the casts through GF/F filters ( $0.7 \mu\text{m}$  nominal pore size). Two 60-mL filtered samples from each Niskin bottle were frozen for postcruise determination of inorganic nutrient concentrations on an Alpkem autoanalyzer. A separate 1-liter filtered sample from each Niskin bottle was acidified with HCl to a pH of  $< 2.0$  and stored at room temperature for subsequent nitrate isotopic determination. Two 10-mL aliquots from each sample were acidified with trace metal–grade HCl ( $10 \mu\text{L}$  acid per sample), sealed in 20-mL glass ampoules that had been previously combusted at  $450^\circ\text{C}$  for 8 h, and stored for subsequent determination of dissolved organic carbon (DOC) concentration. Samples were subsequently sparged with inert gas to remove any dissolved inorganic carbon

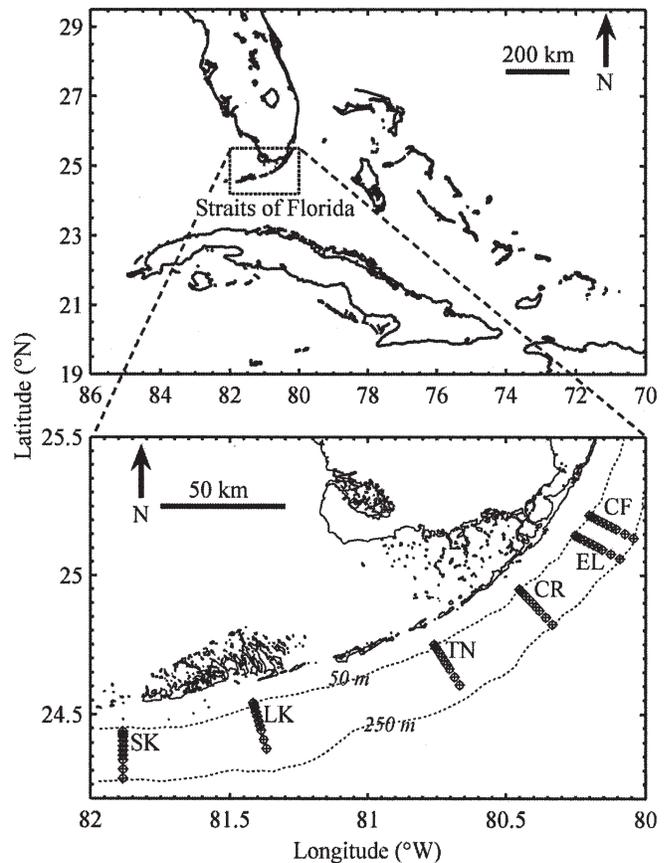


Fig. 1. Study area seaward of the Florida Keys at regional scale (top panel) with expanded view (lower panel) showing sampling transects seaward of Sand Key (SK), Looe Key (LK), Tennessee Reef (TN), Conch Reef (CR), Elbow Reef (EL), and Carysfort Reef (CF). Points show locations of sampling stations along each transect. Dashed lines indicate approximate location of the 50- and 250-m isobaths.

(DIC) and were injected into the  $680^\circ\text{C}$  combustion column to convert all organic carbon to  $\text{CO}_2$ . The  $\text{CO}_2$  gas was passed through a series of traps to remove water, through an elemental copper column scrubber, and through a membrane filter before passing through the nondispersive infrared analyzer to determine DOC. A mean value from three to five subsample injections from each DOC sample was calculated with a maximum coefficient of variation of 2%. DOC standards made from dried potassium hydrogen phthalate were measured daily at the beginning and end of each run to obtain standard curves.

Determination of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  was performed following the method of McIlvin and Altabet (2005) by reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  by cadmium and further reduction to  $\text{N}_2\text{O}$  by sodium azide in an acetic acid buffer. All samples were calibrated and adjusted for exchange and fractionation and blanked against the international nitrate isotopic standards USGS 34 and USGS 35 (Casciotti et al. 2002; Böhlke et al. 2003). The reference scale for N and O isotopic composition were atmospheric  $\text{N}_2$  and SMOW (standard mean ocean water), respectively. Standard materials were run before, after, and

at regular intervals between samples. Analytical precision measured from multiple determinations on standards was  $\sim 0.2\text{‰}$  for  $\delta^{15}\text{N}$  and  $0.7\text{‰}$  for  $\delta^{18}\text{O}$ . The  $\text{NO}_3^-$  concentration limit for successful isotopic determination was  $\sim 0.5 \mu\text{mol L}^{-1}$ . Samples taken near the surface all had  $\text{NO}_3^-$  concentrations substantially below this level and therefore were not included in the isotopic analysis. All samples from 50 m depth and deeper on the SK transect had nitrate concentrations  $> 2.0 \mu\text{mol L}^{-1}$ . By contrast, samples from the Tennessee Reef (TN) and Elbow Reef (EL) transects contained a significant number of samples with relatively low nitrate concentration in which  $\text{N}_2\text{O}$  yield was insufficient for reliable isotopic determination. To minimize potential bias associated with isotopic determination of only the small number of samples with sufficient nitrate concentrations at these sites, the entire runs of the TN and EL samples were excluded from further analysis. Isotopic determinations for the 16 samples from the SK transect were considered a representative and unbiased sampling of nitrate across a range of concentrations from 2.0 to  $25.0 \mu\text{mol L}^{-1}$ . Overall mean  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values across depths on the SK transect were calculated, and means by depth ranges associated with specific features in the hydrographic and fluorescence data were also calculated. These depth-specific features were the chlorophyll maximum layer at 50–70 m depth, the thermocline region at 90–120 m, and the cool subthermocline region at 150–242 m. The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  means (and variance) were weighted by sample nitrate concentration. Weighted means differed from nonweighted means by only 0.1–0.5%. Kruskal–Wallis nonparametric analysis of variance was used to test for differences in mean  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values among the three depth ranges.

## Results

Water column stratification was strong, reflecting late summer conditions with surface temperatures at or near their annual maximum (28–30°C). The relationship between temperature and salinity across all sampling stations points to clear differentiation of water masses with depth (Fig. 2). Surface waters with temperature  $\geq 28.5^\circ\text{C}$  showed the widest range of salinities from  $\sim 35.0$  to 36.5. At intermediate temperatures between  $28.5^\circ\text{C}$  and  $16^\circ\text{C}$ , salinities varied from  $\sim 36.0$  to 36.7. This temperature range was associated with the thermocline at middepths, with highest salinities at temperatures of 22–24°C. The temperature–salinity (TS) relationship at these middepths was more variable than at the surface or at greater depths, suggesting mixing of water masses within the thermocline. The TS relationship in subthermocline water cooler than  $\sim 16^\circ\text{C}$  was relatively tight, with salinities only varying between  $\sim 35.5$  and 36.0.

The vertical hydrographic structure varied among sites and sampling days. Although it is not possible to distinguish between temporal and spatial variability with the nonsynoptic sampling scheme among sites, the patterns suggest that the dynamics of the Florida Current influence the distribution of water masses. Figure 3 shows cross-shore sections of the hydrographic data seaward of SK,

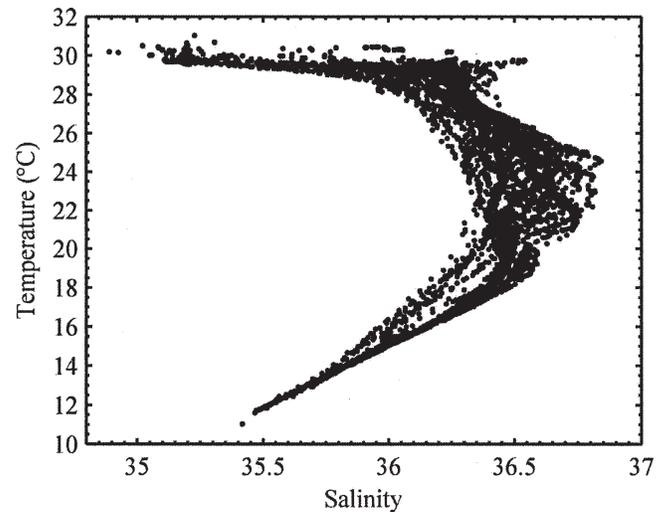


Fig. 2. Temperature–salinity relationship for 1-m binned CTD data from all sampling stations.  $n = 6,106$ .

TN, and EL on 12, 14, and 16 Sep, respectively. Four vertical regions within the water column are evident in the hydrographic and fluorescence sections: (1) a surface layer that appears nearly isothermal, (2) a relatively thin zone just below this isothermal layer characterized by a sharply defined subsurface chlorophyll maximum layer associated with the 20–22°C isotherms at the top of (3) a zone of strong thermal and density gradients (the thermocline/pycnocline) at intermediate depths, and (4) a relatively cool isothermal subthermocline region. At SK, the surface isothermal layer extended from the surface to  $\sim 25$ –40 m depth, the chlorophyll maximum layer comprised a band at  $\sim 50$ –70 m depth at the top of the thermocline/pycnocline that extended to  $\sim 120$ –150 m depth, and the relatively isothermal subthermocline region extended from 150 m to the bottom (260 m at the furthest offshore station). Isopycnal surfaces were close to horizontal at SK, and the 26.0 ( $\sigma_t$ ) isopycnal, corresponding roughly to the bottom of the chlorophyll maximum layer was at  $\sim 60$ –75 m depth. By contrast, seaward of TN and EL, on 14 and 16 Sep, the surface layers were thicker, extending to  $\sim 40$  m at TN and 60 m at EL. The thermocline/pycnocline region was between  $\sim 40$ –125 m depth at TN and 60–200 m depth at EL. The coolest, subthermocline layer existed only as a relatively thin wedge close to the bottom.

Corresponding to the variation in isotherm depth among transects, the depth of the chlorophyll maximum layer also varied. Specifically, the depth of the chlorophyll maximum layer midway along each onshore–offshore transect (at the 9–11-km stations) was  $\sim 52$  m at SK, 60 m at TN and 100 m at EL. In addition to being deeper off of TN and EL than SK, the subsurface chlorophyll maximum layer was more vertically diffuse and less continuous at EL than at SK. The variation in depth of the chlorophyll maximum layer appears to be driven by the physical variation in isotherm depth among sampling days and sites; however, alongshore variation in chlorophyll production cannot be ruled out.

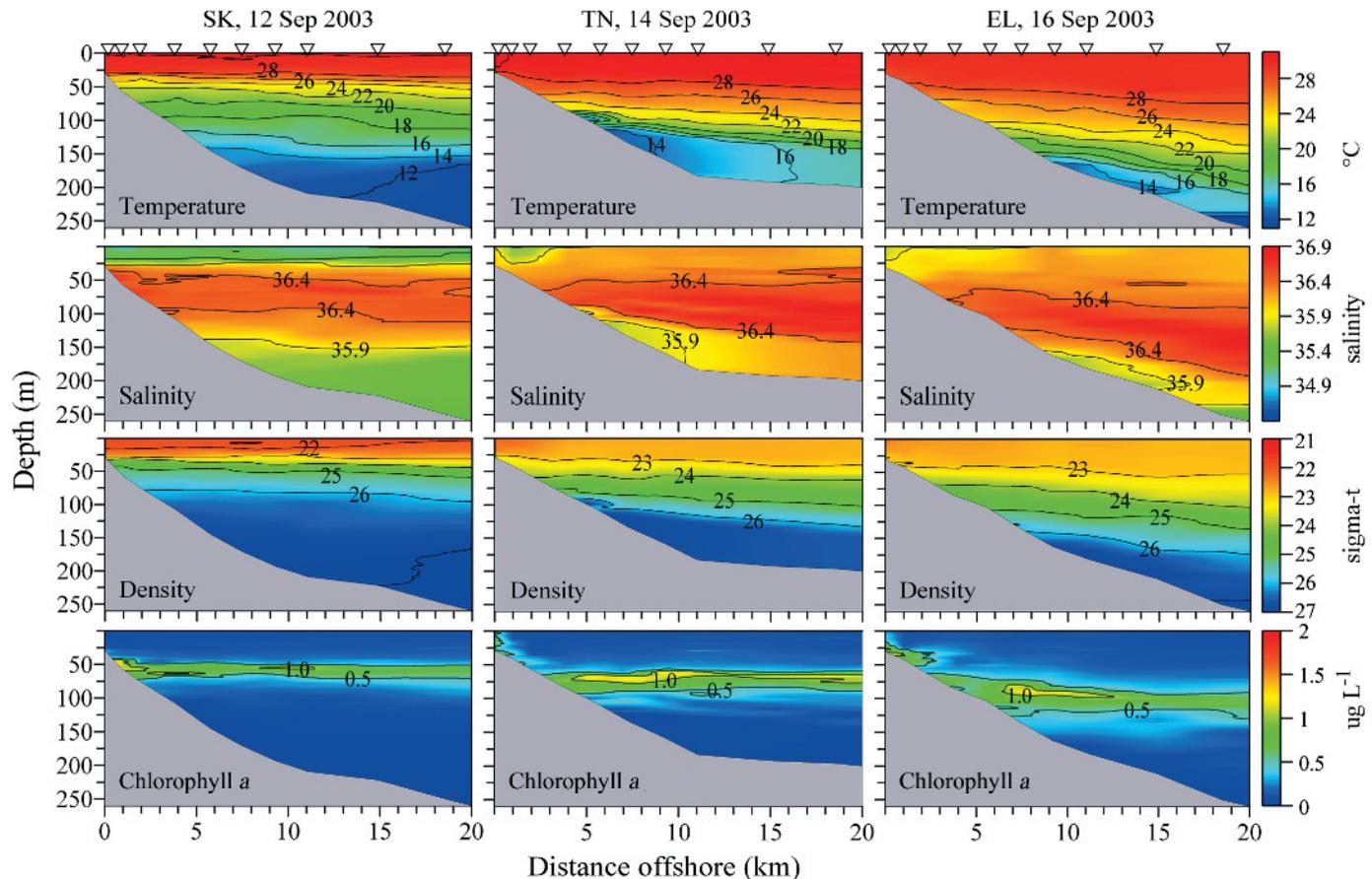


Fig. 3. Hydrographic sections of temperature ( $^{\circ}\text{C}$ ), salinity, density ( $\sigma\text{-t}$ ), and Chl *a* concentration ( $\mu\text{g L}^{-1}$ , from fluorescence) along transects seaward of Sand Key, Tennessee Reef, and Elbow Reef on 12, 14, and 16 Sep 03, respectively. Arrows along top axes indicate locations of individual CTD casts. Grey region at lower left of each plot represents bottom profile extrapolated from depth soundings at each sampling station.

At TN and EL, the 26.0 isopycnal was at 100–130 m and 150–170 m depth and isopycnal surfaces were distinctly tilted upward toward shore. The vertical tilt of isopycnals upward toward shore implies horizontal pressure gradients and corresponding alongshore flow to the northeast in the Florida Current. Surface current speeds of 0.5–1.5  $\text{m s}^{-1}$  to the northeast on 14 and 16 Sep offshore of TN and EL were estimated from the ship's downward-looking acoustic current meter while holding position at CTD stations and from the set of the ship's course while traveling between stations.

Nitrate ( $\text{NO}_3^-$ ) and soluble reactive phosphate (SRP) were very low (measured values of 0–0.2  $\mu\text{mol L}^{-1}$  with a detection limit of  $\sim 0.05 \mu\text{mol L}^{-1}$ ) in the isothermal surface waters in the top 25–50 m of the water column at all of the sampling stations. However, a distinct nutricline existed at depth with relatively high concentrations of  $\text{NO}_3^-$  and SRP below the stratified layer. Temperature appears to be a useful indicator of water masses independent of the vertical variation in hydrographic structure evident among sites and sampling days. The relationships of inorganic nutrients and DOC to temperature (and thus water masses) are shown in Fig. 4. Cross-correlation coefficients of these relationships are presented

in Table 1. Concentrations of  $\text{NO}_3^-$ , SRP, and DOC were strongly correlated with temperature (and with depth).  $\text{NO}_3^-$  and SRP concentrations ranged from 2 to 15  $\mu\text{mol L}^{-1}$  and 0.1 to 1.0  $\mu\text{mol L}^{-1}$ , respectively, at intermediate depths, and from 15 to 25  $\mu\text{mol L}^{-1}$  and 1.0 to 1.5, respectively, in the deep, subthermocline waters. Ammonium ( $\text{NH}_4^+$ ) concentrations were low (0–0.6  $\mu\text{mol L}^{-1}$ ) at all depths and showed no correlation with depth, temperature, or concentrations of the other nutrients. DOC concentrations ranged from 70–120  $\mu\text{mol L}^{-1}$  near the surface to 40–60  $\mu\text{mol L}^{-1}$  below the thermocline.

The nitrate  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values mapped onto cross-shore contours of nitrate concentrations for the SK transect are shown in Fig. 5. The relationship between depth and nitrate concentration,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  for all of the samples across the SK transect are shown in Fig. 6. Nitrate concentration varied significantly among these samples, ranging from  $\sim 2.0$  to nearly 25.0  $\mu\text{mol L}^{-1}$  between 50 and 250 m depth. Overall nitrate  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values varied from 3.55‰ to 5.52‰ (mean 4.78‰  $\pm$  0.05 SE) and 2.73‰ to 6.06‰ (mean 3.26‰  $\pm$  0.08), respectively, for samples taken between 50 and 242 m depth along the SK transect. Weighted mean isotopic values grouped by the depth-

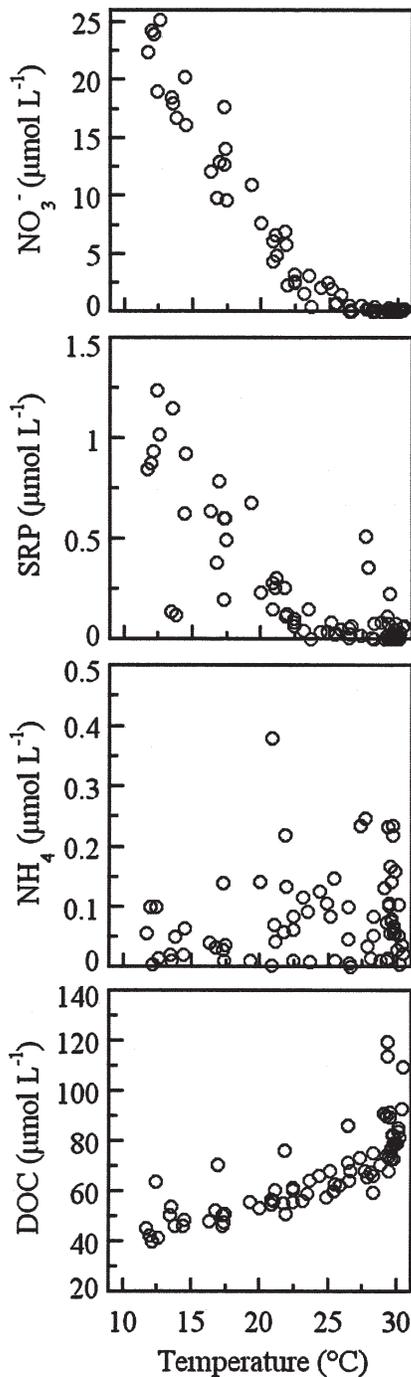


Fig. 4. Concentrations of nitrate ( $\text{NO}_3^-$ ), soluble reactive phosphate (SRP), ammonium ( $\text{NH}_4^+$ ), and dissolved organic carbon (DOC) plotted in relationship to temperature ( $^\circ\text{C}$ ) for all water samples at SK, TN, and EL. Correlation coefficients are shown in Table 1.

specific zones evident in the hydrographic profile for SK are shown in Table 2. Nitrate was low to absent in the surface isothermal layer and isotopic determinations were not possible. Mean  $\delta^{15}\text{N}$  varied from 4.24‰ ( $\pm 0.07$  SE) within the chlorophyll maximum layer at 50–70 m depth to 5.26‰ ( $\pm 0.05$  SE) below the thermocline at 150–242 m depth. Mean  $\delta^{18}\text{O}$  varied from 4.89‰ ( $\pm 0.14$  SE) within

Table 1. Cross-correlation coefficients between depth, temperature, and dissolved nutrient and dissolved organic carbon concentrations. Significant correlations at  $p < 0.05$ , for the samples ( $n = 73$ ) are shown in bold.

|                 | Depth        | Temperature  | $\text{NO}_3^-$ | SPR          | DOC  |
|-----------------|--------------|--------------|-----------------|--------------|------|
| $\text{NO}_3^-$ | <b>0.88</b>  | <b>-0.94</b> |                 |              |      |
| SPR             | <b>0.83</b>  | <b>-0.81</b> | <b>0.86</b>     |              |      |
| DOC             | <b>-0.68</b> | <b>0.79</b>  | <b>-0.68</b>    | <b>-0.56</b> |      |
| $\text{NH}_4^+$ | -0.05        | 0.12         | -0.12           | -0.12        | 0.07 |

the chlorophyll maximum layer to 3.02‰ ( $\pm 0.07$  SE) below the thermocline. The Kruskal–Wallis nonparametric analysis of variance indicated significant differences among depth groups ( $\chi^2 = 9.42$ ,  $p < 0.01$  for  $\delta^{15}\text{N}$  means;  $\chi^2 = 10.7$ ,  $p < 0.01$ , for  $\delta^{18}\text{O}$  means), with multiple comparisons indicating significant differences between the surface and both deeper depths for  $\delta^{15}\text{N}$  and between the subthermocline zone and both shallower depths for  $\delta^{18}\text{O}$ . The differences among the means were larger than the machine precision (0.2‰ for  $\delta^{15}\text{N}$ , 0.7‰ for  $\delta^{18}\text{O}$ ).

The relationships between the natural log of nitrate concentration and nitrate  $\delta^{18}\text{O}$  (Fig. 7A) and  $\delta^{15}\text{N}$  (Fig. 7B) values across the range of measured concentrations along the SK transect are shown in Fig. 7.  $\delta^{18}\text{O}$  was significantly correlated with  $\ln[\text{NO}_3^-]$  ( $r^2 = 0.78$ ,  $p < 0.05$ ). The linear regression of  $\delta^{18}\text{O}$  as a function of  $\ln[\text{NO}_3^-]$  (dashed line, Fig. 7A) had a slope of  $-1.1$ . Linear

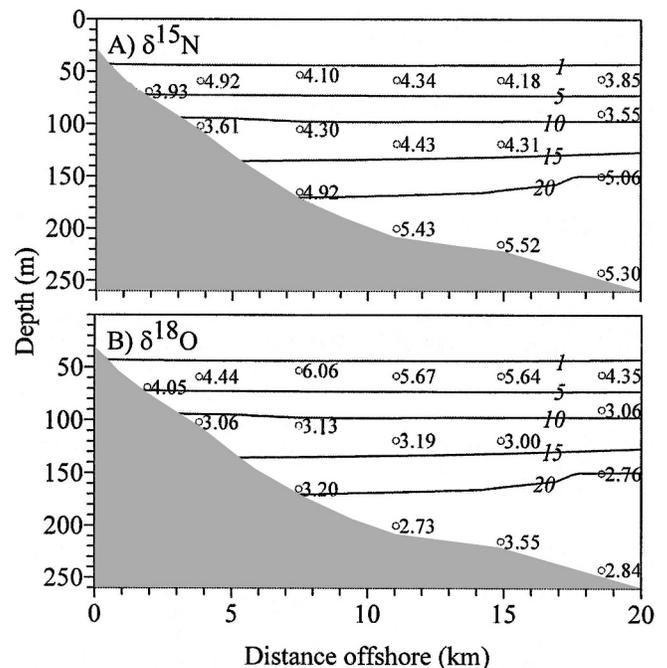


Fig. 5. Contoured section plots of nitrate concentration ( $\text{NO}_3^-$ ,  $\mu\text{mol L}^{-1}$ ), and point values of (A)  $\delta^{15}\text{N}$  (‰) and (B)  $\delta^{18}\text{O}$  (‰) at the SK transect on 12 Sep 03. Open circles indicate depth and location of each water sample, with adjacent numbers indicating isotopic values determined for each  $\text{NO}_3^-$  sample. Italicized numbers label contours of  $\text{NO}_3^-$  concentration.

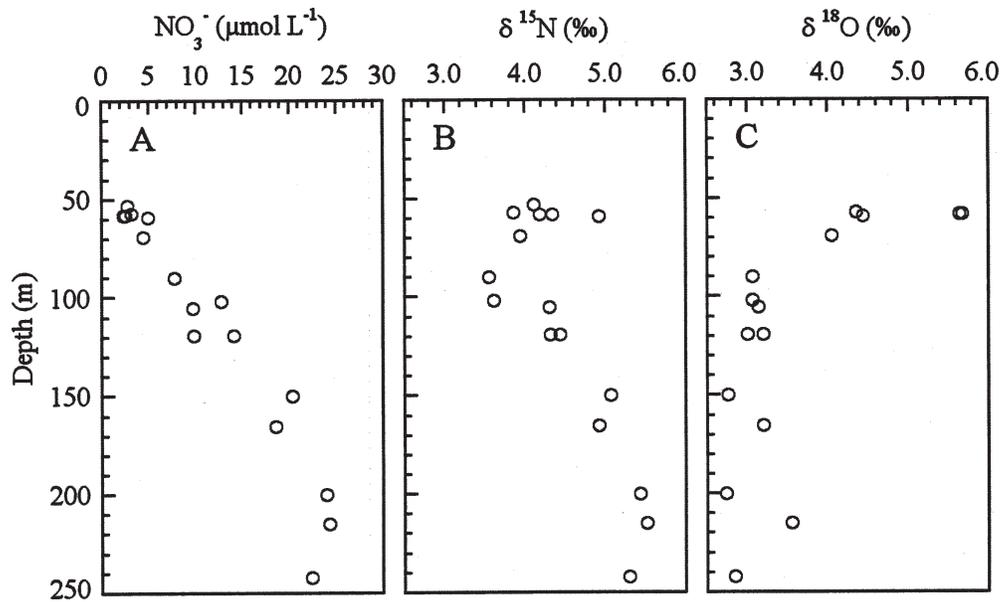


Fig. 6. Relationships between depth and (A) nitrate ( $\text{NO}_3^-$ ) concentration, (B) nitrate  $\delta^{15}\text{N}$  (‰), and (C) nitrate  $\delta^{18}\text{O}$  (‰) for water samples from the SK transect on 12 Sep 03. Isotopic determinations were possible for samples with  $\geq 2.0 \mu\text{mol L}^{-1}$  nitrate, corresponding to samples collected at  $\geq 50$  m depth.

regression of  $\delta^{15}\text{N}$  as a function of  $\ln[\text{NO}_3^-]$  (not plotted) was not significantly different from zero.

## Discussion

The hydrographic and nutrient data present a picture of the nutrient pool seaward of the Florida Keys representative of summer conditions when the water column is strongly stratified and surface temperatures are at or near their annual maximum. The water column was characterized by (1) a warm and relatively isothermal surface layer down  $\sim 50$  m depth, (2) a chlorophyll maximum layer at the top of (3) an intermediate zone with a TS relationships suggestive of mixing, and (4) significantly cooler deep water with relatively high concentrations of inorganic nutrients. This general hydrographic structure, including the presence of the large pool of available inorganic nutrients below the thermocline, is consistent with prior observations (Lee et al. 1992; Leichter et al. 2003) and provides a context in which to interpret the sources and dynamics of nutrients in this system.

Table 2. Nitrate  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  (mean  $\pm$  SE) weighted by nitrate concentration in three depth ranges corresponding to patterns in the hydrographic and fluorescence data offshore of SK 12 Sep 03 (Fig. 3). Within columns, an asterisk indicates a mean that differs significantly at  $p < 0.05$ .  $n$  = sample size in each depth range.

| Depth range (m) | $\delta^{15}\text{N}$ (‰) | $\delta^{18}\text{O}$ (‰) | $n$ |
|-----------------|---------------------------|---------------------------|-----|
| 50–70           | $4.24 \pm 0.07$           | $4.89 \pm 0.14^*$         | 6   |
| 90–120          | $4.07 \pm 0.09$           | $3.08 \pm 0.01$           | 5   |
| 150–242         | $5.26 \pm 0.05^*$         | $3.02 \pm 0.07$           | 5   |
| Overall         | $4.78 \pm 0.05$           | $3.26 \pm 0.08$           | 16  |

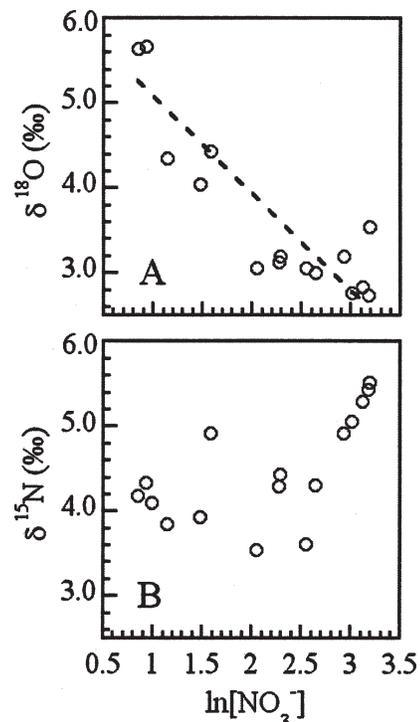


Fig. 7. Relationships between the natural log of nitrate concentration and (A) nitrate  $\delta^{18}\text{O}$ , and (B) nitrate  $\delta^{15}\text{N}$  for water samples from the SK transect on 12 Sep 03. Dashed line in (A) shows linear regression between natural log of nitrate concentration and  $\delta^{18}\text{O}$ . Regression equation:  $\delta^{18}\text{O} = -1.145(\ln[\text{NO}_3^-]) + 6.24$ ,  $r^2 = 0.78$ ,  $n = 16$ ,  $p < 0.05$ . Regression between natural log of nitrate concentration and  $\delta^{15}\text{N}$  (not shown) was not significantly different from zero.

For nitrate in the subthermocline water masses  $\geq 150$  m depth, mean  $\delta^{15}\text{N}$  of 5.26‰ and mean  $\delta^{18}\text{O}$  of 3.02‰ are consistent with values for oceanic nitrate most likely produced by remineralization of dissolved and particulate organic nitrogen in deep water. Specifically, Sigman et al. (2000) showed relatively invariant nitrate  $\delta^{15}\text{N}$  values of  $4.8 \pm 0.2\%$  for a range of deep ocean basins including the North Atlantic, and Casciotti et al. (2002) reported  $\delta^{15}\text{N}$  values of  $\sim 5.1$ – $5.5$  for subthermocline nitrate in the North Pacific.  $\delta^{18}\text{O}$  values close to 3.0‰ are similarly consistent with deep oceanic nitrate pools after adjusting for the most recent determination of  $\delta^{18}\text{O}$  relative to SMOW for reference materials (Casciotti et al. 2002; Böhlke et al. 2003; Sigman et al. 2005).

For samples taken at 50–70 m depth within the chlorophyll maximum layer, we observe a clear increase in  $\delta^{18}\text{O}$  values with decreasing nitrate concentration and an increase in the variance of both nitrate  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  relative to deeper water samples. Mean  $\delta^{15}\text{N}$  values appear to be slightly lower within the thermocline than in deep water; however, the main pattern for  $\delta^{15}\text{N}$  appears to be increased variance within the thermocline rather than clear differences in mean values across the thermocline. These combined isotopic patterns likely reflect both physical mixing processes within the thermocline and isotopic effects associated with biological utilization of nitrate in the subsurface chlorophyll maximum layer. Additionally, remineralization of nitrate could mask the apparent effects of fractionation. Within the thermocline, mixing of warm surface water containing low nutrient concentrations with cooler subthermocline water containing much higher nutrient concentrations could produce the observed water masses with intermediate temperature and nutrient concentrations. Such mixing within the thermocline would be consistent with the observed variance in the TS relationships at intermediate depths. Additionally, the marked chlorophyll maximum layers closely associated with the thermocline are indicative of high concentrations of phytoplankton cells, and the reduced concentrations of  $\text{NO}_3^-$  relative to deeper water likely reflect a drawdown of nutrients associated with uptake by phytoplankton (Sigman et al. 2000). Mixing (of water with different nitrate sources and isotopic signatures), nutrient uptake, and remineralization would be expected to affect the resulting isotopic composition of nitrate within the thermocline; however, their relative roles are difficult to assess without knowledge of residence times for water masses within the thermocline and nutrient uptake and remineralization rates.

One source of the somewhat depleted (lighter) nitrate  $\delta^{15}\text{N}$  values within the thermocline relative to deeper water could be an input of nitrate from biological  $\text{N}_2$  fixation whose products are ultimately nitrified to  $\text{NO}_3^-$  below the euphotic zone. The  $\delta^{15}\text{N}$  of the  $\text{NO}_3^-$  produced via  $\text{N}_2$  fixation should be between  $-2\%$  and  $0\%$ , reflecting the atmospheric source of  $\text{N}_2$  ( $0\%$  by definition) and possibly including some fractionation (Karl et al. 2002).  $\delta^{18}\text{O}$  for  $\text{NO}_3^-$  derived ultimately from  $\text{N}_2$  fixation should be set by nitrification processes and thus also in the range of  $0\%$  to  $+3\%$  (Casciotti et al. 2002; Sigman et al. 2005). The

influence of  $\text{N}_2$  fixation on thermocline  $\text{NO}_3^-$  would be consistent with prior observations of *Trichodesmium* blooms in the region (McClelland et al. 2003) and lowered  $\delta^{15}\text{N}$  for  $\text{NO}_3^-$  in the subtropical mode water of the Sargasso Sea (Knapp et al. 2005). It is also possible that nitrate with lower  $\delta^{15}\text{N}$  relative to deep water could enter the system via atmospheric deposition of  $\text{NO}_3^-$ . Typical isotopic values for atmospherically derived nitrate are  $-10\%$  to  $+2\%$   $\delta^{15}\text{N}$  and  $+60\%$  to  $+85\%$   $\delta^{18}\text{O}$  (Hastings et al. 2003; Wankel et al. 2006); thus, atmospheric deposition could result in both apparent  $\delta^{15}\text{N}$  depletion and  $\delta^{18}\text{O}$  enrichment. Measurements of  $\delta^{17}\text{O}$  of nitrate could clarify inferences with respect to atmospheric deposition. Although analysis of the present measurements cannot rule out the possibility of an effect of atmospheric deposition, it seems unlikely that  $\text{NO}_3^-$  potentially deposited via rainfall near the surface would be detected only at intermediate depths of  $\sim 50$  m and not closer to the surface. Also, it is not necessary to invoke atmospheric deposition to explain the patterns observed here. Specifically, the data can be explained by mixing of deep-water  $\text{NO}_3^-$  with  $\text{NO}_3^-$  from within the thermocline and above. The latter is likely to have isotopic values influenced by  $\text{N}_2$  fixation, isotopic fractionation associated with nitrate uptake within the thermocline, or both.

It has been noted in a number of studies that as nitrate is taken up and assimilated by phytoplankton cells, discrimination against  $^{15}\text{N}$  relative to  $^{14}\text{N}$  and against  $^{18}\text{O}$  relative to  $^{16}\text{O}$  results in a remaining pool of  $\text{NO}_3^-$  that is heavier (enriched) in both isotopes relative to the source (Needoba et al. 2004). In upwelling systems, the magnitude of isotopic fractionation can be estimated by the linear fit between isotopic values and the natural logarithm of nitrate concentration where the negative slope of the linear fit estimates the fractionation factor (Altabet 2001). Although deep water might not be the sole source of the nitrate sampled within the thermocline, the relationship between  $\delta^{18}\text{O}$  and  $\ln[\text{NO}_3^-]$  is significant, as would be expected from uptake. The slope of the linear regression suggests a fractionation factor of  $\sim 1$ – $1.5\%$ . A much larger  $\delta^{15}\text{N}$  fractionation factor of 5.2 has been estimated for  $\text{NO}_3^-$  concentrations of  $\sim 3.5$ – $10 \mu\text{mol L}^{-1}$  in high-nutrient–low-chlorophyll regions of the equatorial Pacific (Altabet 2001). In contrast, no significant relationship between  $\delta^{15}\text{N}$  and  $\ln[\text{NO}_3^-]$  is discernable. This is likely because of multiple sources of N isotopic signatures, specifically deep  $\text{NO}_3^-$  and  $\text{N}_2$  fixation, whereas the source effect on  $\text{NO}_3^-$   $\delta^{18}\text{O}$  via nitrification is uniform in this region given only small variations in the  $\delta^{18}\text{O}$  of seawater. Further investigation could aid in understanding the apparent differences between oxygen and nitrogen fractionation in the Florida system.

Potential sources of nutrients in this system in addition to deep water, atmospheric deposition and  $\text{N}_2$  fixation are terrestrial runoff and groundwater, both of which are potentially strongly influenced by anthropogenic activities. However, terrestrial runoff, groundwater, or both do not appear to be likely sources of the observed high concentrations of subsurface nitrate measured offshore. The Florida Keys reef tract lies  $\sim 5$ – $8$  km seaward of the islands of the

Florida Keys, and the offshore distance to our sampling stations ranged up to an additional 18.5 km seaward of the reef tract, at which bottom depths range from 150 to 250 m. The salinity data in the water column and near the bottom show no indication of freshwater influence, and the measured dissolved organic carbon concentrations were consistent with oceanic rather than inshore water masses (Del Castillo et al. 2000). Although significant anthropogenic nutrient loading has been detected close to shore in the Florida Keys (Kruczynski and McManus 2002; Paytan et al. 2006), the nutrients in potential groundwater and runoff sources are likely to enter nearshore waters and be taken up by benthic or water column primary producers, or both, relatively close (e.g., hundreds of meters) to shore. For example, the signal shown by Umezawa et al. (2002) for a terrestrially derived nitrogen source to reef macroalgae in the Ryukyu Islands, Japan, was found only within hundreds of meters of the shore. Heikoop et al. (2000) found no clear signal of sewage nitrogen on coral reefs >1 km from the city of Zanzibar, on the coastline of East Africa, likely because of rapid dilution in surface waters. Additionally, although the measured values of nitrate  $\delta^{15}\text{N}$  between 4.0‰ and 5.5‰ are entirely consistent with oceanic nitrate, they differ from values for nitrate in groundwater and runoff measured elsewhere. Umezawa et al. (2002) measured  $\delta^{15}\text{N}$  values of 5.2–7.8‰ for nitrate in groundwater and runoff and suggested true values could be somewhat higher because of incomplete nitrate conversion in their analytical methods. The  $\delta^{15}\text{N}$  values of nitrate and ammonium in agricultural runoff derived from nitrogenous fertilizers are likely to be isotopically depleted, –2‰ to +4.7‰ and –12.5‰ to +3.6‰, respectively (Paerl and Fogel 1994), whereas nitrate associated with sewage input is typically more enriched (Kendall et al. 2001).

The relatively shallow depths of the offshore thermocline (typically varying from ~50 to 80 m) create a hydrographic structure conducive to interactions of this nutrient pool with the seaward margin of the Florida Keys reef tract. Indeed, the water masses and inorganic nutrients associated with the offshore thermocline frequently come into contact with the Florida Keys reef tract (Leichter et al. 2003), and the  $\delta^{15}\text{N}$  values of 3.5–5.5‰ measured for  $\text{NO}_3^-$  within and below the offshore thermocline are consistent with values observed in benthic algal tissue on the offshore reef slope. For the green alga *Codium isthmocladum*, Leichter et al. (2003) found tissue  $\delta^{15}\text{N}$  values of 2.5–5.0‰ across depths of 9–35 m and a strong relationship between tissue  $\delta^{15}\text{N}$  values and depth-specific time of exposure to water <25°C.  $\delta^{15}\text{N}$  values for a suite of additional algal taxa found seaward of the reef slope at 35–60 m depth are also in the same range (J. Leichter unpubl. data). The present data support the interpretation that benthic macroalgae use the offshore nutrient pool available during high-frequency upwelling events and that gradients with depth in the isotopic composition of benthic algal tissue N reflect variation in exposure to this subsurface nutrient pool. The present data also point to the underlying variability that can exist in isotopic composition within and among water masses, likely reflecting small-scale patchiness of N cycling within the water column. These data also suggest

that, in some cases, isotopic signatures of terrestrial and anthropogenic nitrogen sources might be indistinguishable from natural sources. However, direct measurement of the isotopic composition of nitrogen sources in groundwater and runoff specific to each site of interest are needed to differentiate potential sources reaching the offshore environment.

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