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Isotopic and elemental indicators of nutrient sources and status of coastal habitats in the Caribbean Sea, Yucatan Peninsula, Mexico

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Abstract

Nutrient inputs associated with coastal population growth threaten the integrity of coastal ecosystems around the globe. In order to assess the threat posed by rapid growth in tourism, we analyzed the nutrient concentrations as well as the $\delta^{15}N$ of NO_3^- and macrophytes to detect wastewater nitrogen (N) at 6 locations along a groundwater-dominated coastal seagrass bed on the Caribbean coast of Mexico. We predicted that locations with greater coastal development would have higher concentrations of dissolved inorganic nitrogen (DIN) and phosphorus (P), as well as $\delta^{15}N$ of NO_3^-, reflecting wastewater sources of N. However, concentrations of NO_3^- were not significantly different between developed (3.3 ± 5.3 μM NO_3^-) and undeveloped (1.1 ± 0.7 μM) marine embayments. The most important control on DIN concentration appeared to be mixing of fresh and salt water, with DIN concentrations negatively correlated with salinity. The $\delta^{15}N$ of NO_3^- was elevated at an inland pond (7.0 ± 0.42‰) and a hydrologically-connected tide pool (7.6 ± 0.57‰) approximately 1 km downstream of the pond. The elevated $\delta^{15}N$ of NO_3^- at the pond was paralleled by high $\delta^{15}N$ values of *Cladophora* sp., a ubiquitous green alga (10 ± 1‰). We hypothesize that inputs of nitrogen rich ($NO_3^- > 30$ μM) groundwater, characterized by ^{15}N enriched signatures, flow through localized submarine groundwater discharges (SGD) and contribute to the elevated $\delta^{15}N$ signatures observed in many benthic macrophytes. However, changes in nitrogen concentrations and isotope values over the salinity gradient suggest that other processes (e.g. denitrification) could also be contributing to the ^{15}N enrichments observed in primary producers. More measurements are needed to determine the relative importance of nitrogen transformation processes as a source of ^{15}N to groundwaters; however, it is clear that continued inputs of anthropogenic N via SGD have the potential to severely impact ecologically and economically valuable seagrass meadows and coral reefs along the Caribbean coast of Mexico.

Keywords: $\delta^{15}N$; nutrients; seagrass; coral reefs; eutrophication; wastewater; Mexico; Yucatan

1. Introduction

Estimates indicate that approximately 50% of the world's population currently lives within 200 km of the coast and that percentage is expected to rise (Stegeman and Solow, 2002). As a result, human impacts increasingly threaten productive and diverse coastal ecosystems such as seagrass meadows and coral reefs. One of the greatest threats to these systems is cultural eutrophication. As well, construction of buildings, roads, and aquaculture ponds can affect the biogeochemistry of coastal ecosystems by eliminating an important sink for anthropogenic nutrients as well as eliminating a source of refractory organic matter to nearshore environments (Alongi et al., 1992; Duarte and Cebrian, 1996; Alongi, 2002).

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Anthropogenic nutrients can contribute to declines in seagrass meadows and coral reefs (Short and Wyllie-Echeverria, 1996; Lapointe, 1997) because nutrient enrichment stimulates the growth of algae, which can overgrow coral reefs and limit light availability to seagrasses (Silberstein et al., 1986; Lapointe et al., 2005). Anthropogenic nutrients are the primary factor in the loss of at least 90,000 ha of seagrass meadow in a decade (Short and Wyllie-Echeverria, 1996), and as many as 58% of coral reefs worldwide are at "high" or "medium" risk of degradation due to anthropogenic stress (Bryant et al., 1998). Seagrass meadows and coral reefs are ecologically and economically valuable systems because they enhance biodiversity (Dorenbosch et al., 2005), elevate coastal productivity (Cebrian, 2002), support fisheries (Nagelkerken et al., 2000; Nagelkerken et al., 2002), and generate tourism. Early detection of changes in anthropogenic inputs to tropical coastal ecosystems is imperative to maintain these ecosystem services.

The Caribbean coast of Mexico is experiencing swift population growth and expansion in tourism. In the Riviera Maya alone, tourism and hotel density quadrupled from 1998 to 2004 (595,000 to 2.4 million tourists; Secretaría de Turismo, Quintana Roo, Mexico) (Secretaría de Turismo, 2005), and the trend is likely to continue into the near future. A byproduct of this increase in tourism may be greater wastewater production along the coast. Proper wastewater treatment and disposal in the area are critical because the bedrock of the Yucatan Peninsula consists of karstic limestone. Groundwater easily moves through karst, carrying nutrients from untreated or partially treated wastewater and faulty septic systems (Whitney et al., 2003). These nutrients enter coastal waters by submarine groundwater discharge (SGD) and may contribute to eutrophication of nearby seagrass meadows and coral reefs (Lapointe et al., 1990; Simmons and Lyons, 1994). Discharge of groundwater is often difficult to detect, however, because of its extremely localized distribution (Charette et al., 2001).

The objective of this study was to investigate the presence of anthropogenic N in coastal waters in the vicinity of Akumal, Quintana Roo, Mexico (Fig. 1) by analyzing the $\delta^{15}N$ of NO_3^- and primary producers. Because wastewater N typically has elevated $\delta^{15}N$ values (5–9‰ in untreated wastewater and 10–22‰ in treated wastewater) relative to NO_3^- from natural soils, anthropogenic N inputs to coastal waters and producers can be identified (McClelland et al., 1997; McClelland and Valiela, 1998). Specifically, we predicted that Akumal Bay, which is surrounded by hotels and other tourism-related development, would have greater dissolved inorganic nitrogen (NO_3^- and NH_4^+) and phosphorus (P) levels than at Xaak, an adjacent but comparably much more isolated lagoon. We also predicted that $\delta^{15}N$ of NO_3^- at Akumal Bay would be greater than at Xaak due to the presence of wastewater, and the $\delta^{15}N$ values of primary producers would be elevated, reflecting uptake of anthropogenic (wastewater-derived) N. In addition to samples collected at Akumal Bay and Xaak, we also measured the $\delta^{15}N$ of NO_3^- at several other local water bodies to provide baseline data for future research.

2. Methods

2.1. Study sites

The study was conducted along the Caribbean coast of Quintana Roo, Mexico (Fig. 1). Akumal Bay is a small embayment located adjacent to the resort area of Akumal, Mexico. The Mesoamerican Barrier Reef guards the outer edge of Akumal Bay (up to 4 m deep), protecting seagrass meadows containing *Halodule wrightii* Ascherson, *Syringodium filiforme* Kützing, and *Thalassia testudinum* Banks ex König. The bottom slopes gradually from the shoreline to the barrier reef and there are no surface water inputs into the bay. Numerous resorts, vacation homes, and the village of Akumal surround the bay (Fig. 1). Xaak is a similar marine embayment although it is smaller in size and is surrounded by undeveloped land. Development of the surrounding area is scheduled for the near future. The three seagrass species mentioned above are found within Xaak, and the coral reefs at the site appear relatively healthy with respect to the reefs in Akumal Bay. The water clarity was high in both embayments, and the water column was relatively uniform in salinity and temperature (data not shown).

In addition to samples collected at Akumal Bay and Xaak, water samples were also collected at 4 other local water bodies. Casa Cenote is a sinkhole that formed as the ceiling of an underground cave collapsed and exposed the water under the limestone bedrock. Water enters Casa Cenote via subsurface flow of groundwater underneath mangrove forest and exits through an underground cave that upwells into the Caribbean a few meters offshore. Yal Kú Lagoon is a narrow inlet with a strong marine influence at its seaward end. The southern boundary of the lagoon is lined with vacation homes; however, its northern edge is less developed. Laguna Lagartos is a small, inland body of nearly fresh water in a forested area northeast of Akumal Pueblo and west of the developed coastline. Thick mats of the green alga *Cladophora* sp. cover the surface of Laguna Lagartos. Laguna Lagartos is connected to the inland edge of Yal Kú Lagoon via subsurface flow (K. Riley, Centro Ecológico Akumal, pers. commun.). Along the edge, persistent pools form in deep fissures of the bedrock and are believed to be fed by the subsurface flow between Laguna Lagartos and Yal Kú Lagoon. Yal Kú Chico is a small inlet that receives groundwater discharge from the surrounding undeveloped, forested area. The relatively fresh groundwater forms a distinct layer above the intruding saline waters of the Caribbean Sea in classic salt-wedge stratification. With the exception of the overgrown surface waters of Laguna Lagartos, water clarity at all sites is high. Light easily penetrated to the bottom of all sampling sites, even to depths >7 m in Casa Cenote.

2.2. Sample collection and processing

Water and plant samples were collected in May and June 2005. Four water samples were collected from different locations in Akumal Bay and three were collected from Xaak.

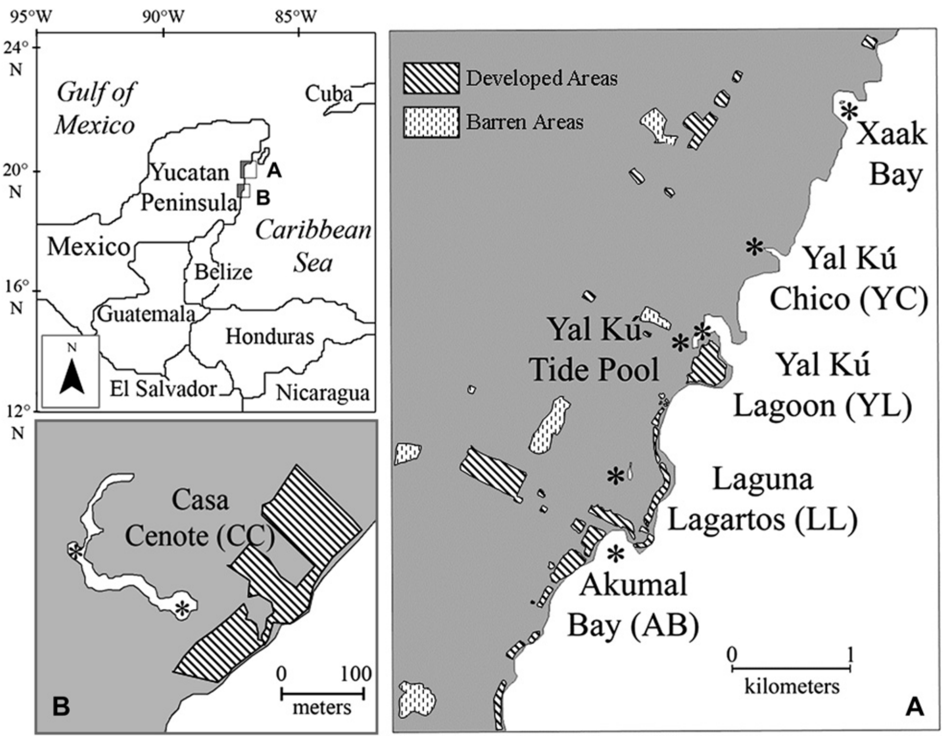

Fig. 1. Location of the study area and sampling sites on the Yucatan Peninsula. Developed areas (hatches) and barren areas (cross-hatches) assessed from aerial imagery (2003–2005) and site visits.

Little spatial variability existed in the water columns of these embayments (Fig. 2 and unpublished data). Additionally, three replicate plant samples of each seagrass species were collected at these two sites. In the remaining sites, sample collection varied depending upon the water column characteristics of the site. At sites with salinity gradients (Table 1), such as Casa Cenote and Yal Kú Chico, individual water samples were collected upstream and downstream, at the surface, and at depth. At Laguna Lagartos, three surface water samples were collected from the same location. Two water samples were collected within Yal Kú Lagoon. One additional sample was taken from a small tidepool at Yal Kú Lagoon located next to the main body of water; however, no water characteristics were measured other than two determinations of $\delta^{15}N$ of NO_3^-.

Salinity, temperature, depth, and pH of the water column were measured *in situ* with a YSI 600 XLM data sonde (YSI Inc., Yellow Springs, OH). Water samples for isotope and nutrient analyses were collected, placed immediately in the dark on ice, and then frozen at −20 °C. Prior to analysis, water samples were thawed and filtered using Whatman GF/F glass fiber filters (0.7 µm pore size). Concentrations of nitrate + nitrite (NO_3^-), ammonium (NH_4^+), and orthophosphate (PO_4^{3-}), were determined on a Lachat QuikChem® 8000 (Loveland, CO).

Nitrogen stable isotopic composition ($\delta^{15}N$) of dissolved NO_3^- was determined according to the method of McIlvin and Altabet (2005), with some modifications to account for low sample volume and NO_3^- concentration. Nitrous oxide produced by this method was analyzed for $\delta^{15}N$ on a Finnegan Trace GC with a preconcentration unit coupled to a Finnegan MAT Delta+ isotope ratio mass spectrometer at the University of Texas Marine Science Institute. The precision of this method for N isotopes is 0.2‰ or better.

Plant tissues were collected and placed on ice in the field. In the lab, seagrass leaves were rinsed with deionized H_2O and scraped to remove epiphytes. Algal tissues were also rinsed, and calcareous species were acidified with 10% HCl to remove carbonates. All tissues were then dried in an oven at 60 °C and pulverized with a Wig-L-Bug (Rinn Corp., Elgin, IL). Tissue $\delta^{15}N$ and %C and %N were determined with a Carlo Erba 2500 elemental analyzer coupled to a Finnegan MAT Delta+ isotope ratio mass spectrometer at the University of Texas Marine Science Institute. Stable isotope ratios are presented as $\delta^{15}N$ relative to atmospheric N_2. Elemental content of seagrass tissues was calculated using the dry weight, and molar C:N ratios were determined.

Phosphorus (P) content of seagrass tissue was determined following the method outlined by Fourqurean et al. (1992). Known amounts of ground tissues were dry oxidized in a muffle furnace, and P was extracted by acid hydrolysis. Phosphate concentration was determined by colorimetric analysis.

2.3. Statistical analysis

Statistical analyses were performed using Statistical Analysis System version 9.1 (SAS, Cary, NC). One-way and two-way analyses of variance (ANOVA) were performed with location and species as factors when appropriate. Residuals of the fitted models were tested for homogeneity of

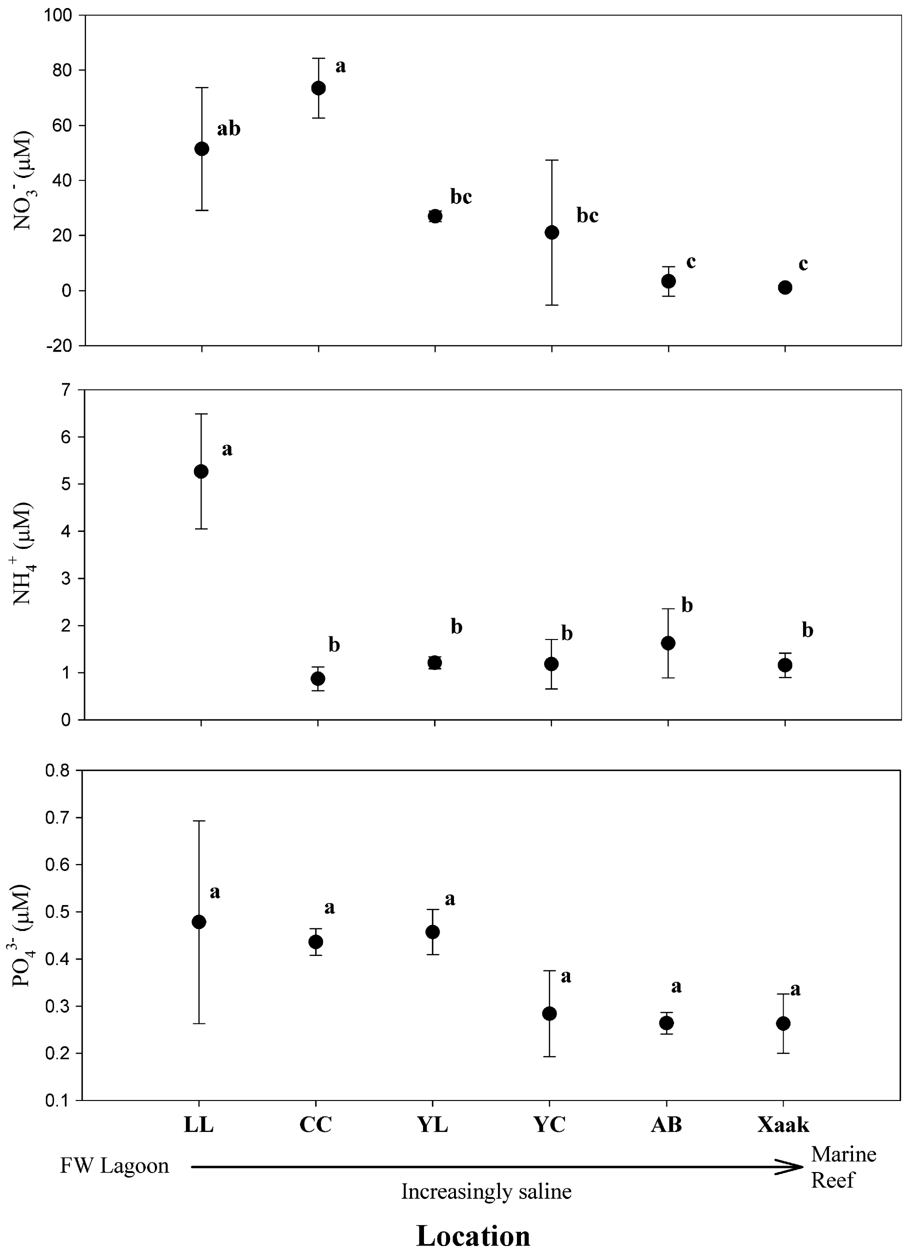

Fig. 2. Water column nutrients at each of the six study sites (means ± SD). Values with the same letter are not statistically different ($p > 0.05$). Top panel, NO_3^-; middle panel, NH_4^+; bottom panel, PO_4^{3-}. For site abbreviations, see Fig. 1.

variance and departures from normality. Kolmogorov–Smirnov tests indicated no departures from normality ($p < 0.05$); therefore, no data transformations were necessary. *Post hoc* Tukey Honestly Significantly Different tests were performed to identify differences among treatment combinations. For all analyses, alpha was set at 0.05.

3. Results

3.1. Water chemistry

Water column NO_3^- and NH_4^+ concentrations differed significantly among locations ($p = 0.0001$ and $p < 0.0001$, respectively; Fig. 2). The general trend was a decrease in N concentration with increasing salinity. Nitrate concentrations were significantly higher in Casa Cenote (73.5 ± 10.9 µM; mean ± standard deviation; $n = 4$; $p < 0.05$ in all significant comparisons) than all sites except Laguna Lagartos (51.4 ± 22.4 µM; $n = 3$). Nitrate concentrations at Laguna Lagartos were significantly higher than Akumal Bay (3.3 ± 5.3 µM; $n = 4$; $p = 0.014$) and Xaak (1.1 ± 0.7 µM; $n = 3$; $p = 0.017$) but not Yal Kú Chico (21.0 ± 26.3 µM; $n = 4$; $p = 0.19$) or Yal Kú Lagoon (26.9 ± 2.0 µM; $n = 2$; $p = 0.56$). Nitrate concentrations at Yal Kú Chico were extremely variable due to stratification of the water column. Ammonium concentrations were significantly higher at Laguna Lagartos (5.3 ± 1.2 µM; $n = 3$; $p < 0.0001$ in all comparisons) than at all other sites. Orthophosphate

Table 1
Water column characteristics at sampling sites based on instantaneous *in situ* measurements with a YSI 600 XLM data sonde. n.d., not determined

Location	Depth (m)	Salinity	Temperature (°C)	pH
Akumal Bay	1	36.5	29.9	7.9
Casa Cenote				
upstream	0	11.5	26	6.6
	7	12.4	26.1	6.6
downstream	0	11.6	26	6.6
	6	23	26.7	6.6
Laguna Lagartos	0	6	n.d.	n.d.
Xaak	0	36.6	30	7.9
Yal Kú Chico				
upstream	0	11.4	26.2	7
downstream	0	32.1	26.7	7.4
	2.6	37	20.1	7.9
Yal Kú Lagoon	0	17	n.d.	n.d.

concentrations were not significantly different among locations ($p > 0.05$ in all comparisons; Fig. 2).

3.2. $\delta^{15}N$ of NO_3^-

The trend of $\delta^{15}N$ of NO_3^- was similar to that of NO_3^- concentration (Fig. 3). Sites with greater freshwater influence had higher $\delta^{15}N$ of NO_3^- than marine sites. In addition, $\delta^{15}N$ differed significantly among locations ($p < 0.0016$; Fig. 3). Mean $\delta^{15}N$ values were significantly higher at Laguna Lagartos (7.0 ± 0.42‰; $n = 2$) and the tide pool at Yal Kú Lagoon (7.6 ± 0.57‰; $n = 2$) than at both marine reef end members, Akumal Bay (4.1 ± 1.26‰; $n = 3$) and Xaak (3.9 ± 1.18‰; $n = 2$; $p < 0.03$ in each case). Isotope values for NO_3^- at the tide pool at Yal Kú Lagoon (YLTP; 7.6 ± 0.6‰; $n = 2$) were also significantly higher than those at Yal Kú Chico (4.8 ± 0.6‰; $n = 4$; $p < 0.007$).

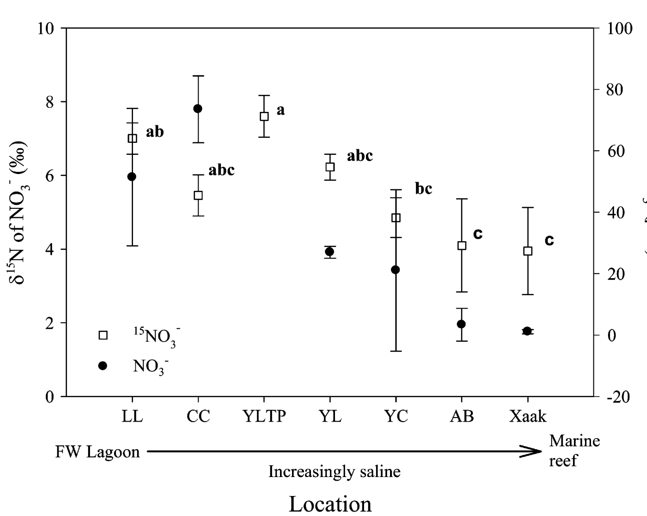

Fig. 3. Concentration and $\delta^{15}N$ of dissolved nitrate at the various sampling sites (means ± SD). Values with the same letter (for $\delta^{15}N$) are not statistically different ($p > 0.05$). For site abbreviations, see Fig. 1. Position of Yal Kú Lagoon tide pool (YLTP) on *x*-axis estimated based on its location between Laguna Lagartos and Yal Kú Lagoon.

3.3. Plant tissue nutrient content and isotope values

Isotope values for plants collected in coastal systems are summarized in Table 2. The mean $\delta^{15}N$ value of *Cladophora* sp. collected from Laguna Lagartos was 10 ± 1‰. This value reflects the relatively high $\delta^{15}N$ of NO_3^- at the site (Fig. 4). $\delta^{15}N$ values of algae from other locations are also similar to $\delta^{15}N$ of NO_3^- at the respective site.

Analysis of seagrass $\delta^{15}N$ values at Akumal Bay and Xaak indicated significant differences among species ($p < 0.0001$ and $p = 0.0007$, respectively) but not between sites.

Analysis of the N, P, and C content of seagrass at Akumal Bay and Xaak indicated significant interactions between location and species ($p = 0.011$, $p = 0.015$, and $p = 0.012$, respectively). *Post hoc* Tukey tests indicate that the only differences in N and P content were higher values for *Halodule wrightii* than *Thalassia testudinum* at Akumal Bay ($p = 0.022$ and $p = 0.018$, respectively; Table 2). There were no significant differences between the two sampling locations. The C content of *Syringodium filiforme* was significantly lower than the other seagrass species within each site ($p < 0.02$ in all cases). Additionally, the C content of *T. testudinum* at Akumal Bay was significantly lower than at Xaak ($p = 0.02$), and it was also lower than the C content of *H. wrightii* at Akumal Bay ($p = 0.013$).

4. Discussion

Contrary to our expectation, nutrient concentrations were not different between the more-developed Akumal Bay and less-developed Xaak. Nutrient levels and $\delta^{15}N$ of NO_3^- tended to be higher in systems with greater freshwater influence, regardless of development. This relationship was particularly evident at Yal Kú Chico, which exhibited highly variable NO_3^- concentrations. During sampling, the water column at Yal Kú Chico was stratified, and lower salinity surface waters had higher NO_3^- concentrations than corresponding samples taken at depth. The $\delta^{15}N$ of NO_3^- at Yal Kú Chico (4.8 ± 0.6‰), however, was not as high as the Yal Kú Lagoon tide pool (7.6 ± 0.6‰) which may suggest different nitrogen sources between the two systems.

Both the extensive growth of *Cladophora* sp. at Laguna Lagartos and the elevated $\delta^{15}N$ of NO_3^- and producers at Laguna Lagartos and the tide pool at Yal Kú Lagoon suggest the presence of terrestrial N inputs into these systems. The $\delta^{15}N$ of untreated wastewater ranges from 5‰ to 9‰ (Aravena et al., 1993; Waldron et al., 2001), but denitrification during treatment increases $\delta^{15}N$ values to between 10‰ and 22‰ (Kreitler and Browning, 1983; Aravena et al., 1993). Laguna Lagartos and Yal Kú Lagoon, which are hydrologically connected, are located near the village of Akumal Pueblo, numerous vacation homes, and resorts. High $\delta^{15}N$ values at these two sites may result from anthropogenic N inputs into the groundwater from septic systems, untreated wastewater, and injection wells associated with these residential areas. The groundwater then discharges into Yal Kú Lagoon, and the $\delta^{15}N$ of NO_3^- declines as N inputs mix with marine waters

Table 2
Elemental composition and $\delta^{15}N$ values (means ± SD) of algal and seagrass tissues collected at four study sites. Values with the same superscript letter are not significantly different ($p > 0.05$)

Location	Species (*n*)	$\delta^{15}N$ (‰)	%C	%N	%P	C:N	N:P	C:N:P
Akumal Bay	*Halodule wrightii* (3)	5 ± 1^a	36 ± 0.6^a	2 ± 0.2^a	0.16 ± 0.06^a	20 ± 2^a	32 ± 14^a	626:32:1
	Syringodium filiforme (3)	3 ± 1^b	29 ± 0.6^b	2 ± 0.2^{ab}	0.08 ± 0.02^{ab}	21 ± 3^{ab}	46 ± 15^a	955:46:1
	Thalassia testudinum (3)	7 ± 1^a	32 ± 2^c	1 ± 0.3^b	0.06 ± 0.01^b	29 ± 5^b	47 ± 6^a	1336:47:1
	Halimeda sp. (2)	3 ± 0.1	17 ± 2	1 ± 0.04		26 ± 10		
	Penicillus sp. (2)	4 ± 0.2	31 ± 6	2 ± 1		15 ± 3		
	Rhipocephalus sp. (2)	5 ± 0.1	31 ± 3	2 ± 0.3		18 ± 1		
Xaak	*H. wrightii* (3)	4 ± 0.2^a	36 ± 2^a	2 ± 0.2^{ab}	0.1 ± 0.01^{ab}	25 ± 2^{ab}	36 ± 2^a	910:36:1
	S. filiforme (3)	1 ± 3^b	28 ± 0.5^b	2 ± 0.2^{ab}	0.13 ± 0.02^{ab}	20 ± 3^a	29 ± 2^a	582:29:1
	T. testudinum (3)	6 ± 1^a	36 ± 0.2^a	2 ± 0.2^{ab}	0.11 ± 0.03^{ab}	22 ± 2^{ab}	40 ± 10^a	867:40:1
Laguna Lagartos	*Cladophora* sp.(2)	10 ± 1	36 ± 3	4 ± 1		12 ± 2		
Yal Kú Lagoon	*Bryopsis* sp. (2)	6 ± 0.3	46 ± 0.01	4 ± 0.3		13 ± 1		

from the Caribbean. Previous research has shown that submarine groundwater discharge (SGD) can be a significant source of nutrients to the coastal zone and coral reefs. Paytan et al. (2006) used radium isotopes to document significant N inputs from SGD to coral reefs around the world. In the Florida Keys, they estimated that SGD supplies about 0.46 g N m^{-2} d^{-1} to the water along Key Largo (Paytan et al., 2006).

$\delta^{15}N$ and nutrient concentrations decreased along the fresh to salt water continuum (Fig. 3). Laguna Lagartos and the tide pool at Yal Kú Lagoon had elevated $\delta^{15}N$ of NO_3^- (7–7.6‰) compared to the most marine coastal sites (3.9–4.1‰). Intermediate between these two extremes were the isotopic signatures for Yal Kú Lagoon, Casa Cenote, and Yal Kú Chico (6.2–4.8‰), which reflected the dilution of nutrient enriched brackish waters with the oligotrophic waters of the Caribbean Sea. Laguna Lagartos is hydrologically connected via subsurface groundwater to Yal Kú Lagoon and ultimately the marine endmember represented by Akumal Bay. Thus, one possible explanation for the patterns in NO_3^- and $\delta^{15}N$ of NO_3^- is that water with a high $\delta^{15}N$ of NO_3^- observed in Laguna Lagartos and the tide pool at Yal Kú Lagoon enters Yal Kú Lagoon and is diluted by lower $\delta^{15}N$ NO_3^- from marine sources. Alternatively, elevated $\delta^{15}N$ of NO_3^- in brackish sites could arise from progressive enrichment of $\delta^{15}N$ of NO_3^- due to denitrification in sediments and during groundwater transport. Gradients in $\delta^{15}N$ values must be interpreted with caution as patterns that are consistent with conservative mixing may result from spatial differences in heterotrophic nitrogen processing (Fourqurean et al., 1997).

Because there were no surface water inputs into any of the study areas, the dominant N and P sources into marine systems are likely to be terrigenous dissolved inorganic nutrients in subsurface groundwater. This does not necessarily mean, however, that N is transported conservatively in groundwater from terrestrial to marine ecosystems in this system. While NO_3^- and $\delta^{15}N$ are both higher in freshwater sources (Fig. 5), plots of $\delta^{15}N$ vs. the reciprocal of NO_3^- and the natural log of NO_3^- yield ambiguous results regarding conservative mixing from Laguna Lagartos to the marine endmember (Fig. 6). The linear relationship ($r^2 = 0.85$) between $\delta^{15}N$ and the reciprocal of

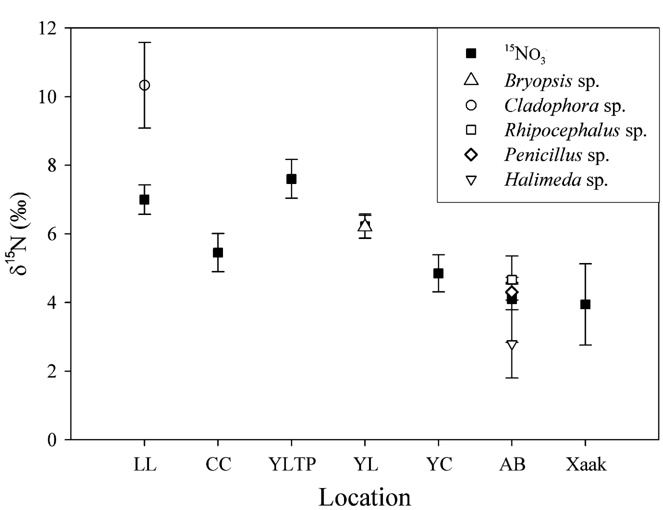

Fig. 4. Nitrate concentration and $\delta^{15}N$ values of algal tissues at the various sampling sites (means ± SD). For site abbreviations, see Fig. 1. Position of Yal Kú Lagoon tide pool (YLTP) on *x*-axis estimated based on its location between Laguna Lagartos and Yal Kú Lagoon.

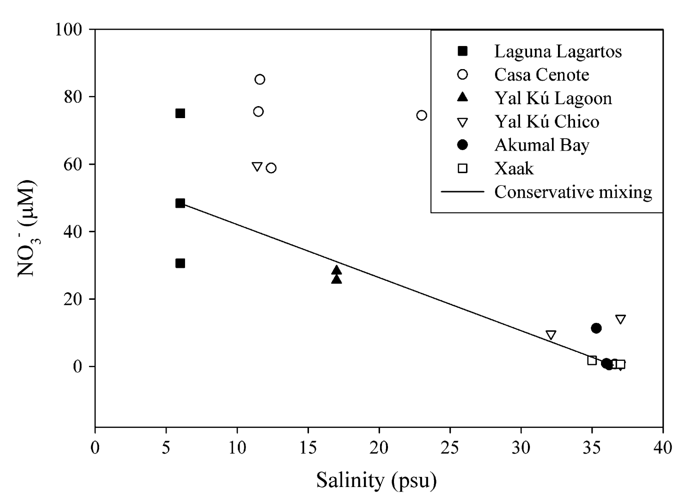

Fig. 5. NO_3^- concentration as a function of salinity at six sampling sites (raw data). Line shows conservative mixing between hydrologically connected lagoons from the freshwater Laguna Lagartos, to brackish Yal Kú Lagoon, and the marine reef end member (Akumal Bay).

NO_3^- concentration suggests that conservative mixing may indeed occur; however, when the natural log of NO_3^- concentration is plotted against $\delta^{15}N$ (NO_3^-), the resulting relationship ($r^2 = 0.9$), suggests that other transformation processes (e.g. denitrification) are contributing to the enrichment of $\delta^{15}N$ (Kendall, 1998; Fig. 6). Non-conservative mixing of N in the Laguna Lagartos/Yal Kú Lagoon system is consistent with results from studies in the geologically similar Florida Keys, where NO_3^- may be removed by denitrification or dissimilatory NO_3^- reduction to NH_4^+ during groundwater transport (Corbett et al., 2000; Griggs et al., 2003). A more intense sampling effort is needed to determine whether N is transported conservatively in groundwater to marine systems in this area. Such an analysis is necessary to reliably predict the effects of increased anthropogenic N loading to these systems.

$\delta^{15}N$ values of plants exhibit a similar pattern to the NO_3^- concentration and ^{15}N values of NO_3^-. High values for *Cladophora* sp. in Laguna Lagartos mirror the high $\delta^{15}N$ values of

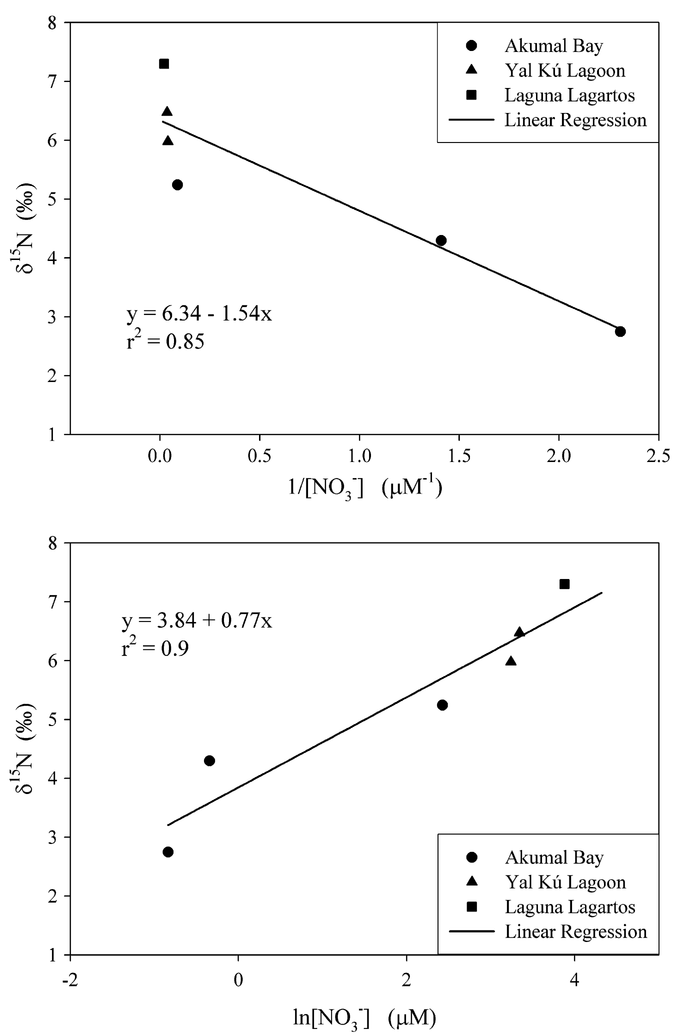

Fig. 6. $\delta^{15}N$ of NO_3^- plotted versus the reciprocal of NO_3^- concentration (top panel) and natural log of NO_3^- concentration (bottom panel) for identical water samples. Points represent raw data from hydrologically connected Laguna Lagartos, Yal Kú Lagoon, and Akumal Bay. For explanation, see text.

NO_3^- at this site, whereas, the algae and two species of seagrasses (*Halodule wrightii* and *Syringodium filiforme*) collected from Akumal Bay and Xaak (range 1–5‰) reflect the relatively low $\delta^{15}N$ values of NO_3^- (4‰) at these sites. Although the $\delta^{15}N$ values for *Thalassia testudinum* at these bay sites are somewhat elevated (6–7‰), they fall within the range reported elsewhere for the species (Carruthers et al., 2005; Fourqurean et al., 2005). In fact, $\delta^{15}N$ values of seagrasses at Akumal Bay and Xaak are similar to values at Nichupte Lagoon (5.49–9.06‰) in the northern Yucatan Peninsula which is believed to be impacted by wastewater inputs (Carruthers et al., 2005). As mentioned above, however, it is unclear whether these values result from elevated $\delta^{15}N$ of NO_3^- associated with patchy SGD (Fourqurean et al., 2005) or ^{15}N enrichment due to denitrification within the carbonate bedrock or marine sediments. Corbett et al. (1999) documented heavier $\delta^{15}N$ values of macrophytes (6–13‰) along the bay side of the Florida Keys. Enrichment in ^{15}N in other locations is believed to result from SGD that is ^{15}N-enriched due to both denitrification in groundwater and from inputs of human wastewater (McClelland and Valiela, 1998; Corbett et al., 1999).

Tissue nutrient contents for seagrasses in this study are similar to those found at other locations in the Yucatan Peninsula and Florida Bay. Carruthers et al. (2005) reported that %P for *Thalassia testudinum* ranged from 0.12–0.14% for Nichupte Lagoon and Puerto Morelos Lagoon; however, values were higher near springs which release freshwater into the Puerto Morelos lagoon (0.18–0.38%). The authors interpreted these high %P values as products of increased P availability due to SGD inputs. In this study, average P content of *T. testudinum* was relatively low (Table 2) compared to values reported by van Tussenbroek et al. (1996) and Carruthers et al. (2005) and was similar to values for plants that were distant from P sources on Porjoe Key in Florida (0.078 ± 0.001 to 0.120 ± 0.015%; Fourqurean et al., 1992). In general, seagrass tissue N and P content for the three seagrass species are similar to values reported in the literature for non-eutrophic sites (Fourqurean et al., 1992; van Tussenbroek et al., 1996; Carruthers et al., 2005), and suggest that P is more likely to be limiting in Xaak and Akumal Bay (Duarte, 1990). These data do not indicate that seagrasses are experiencing excessive nutrient availability, but incipient increases in nutrient loading due to anthropogenic activity may result in changes in biogeochemistry of these systems as observed elsewhere.

Although we did not detect isotopic enrichment in Akumal Bay and Xaak, other studies have successfully documented nutrient increases to coral reef ecosystems using isotopes (Heikoop et al., 2000; Yamamuro et al., 2003; Paytan et al., 2006). Our inability to detect anthropogenic inputs at the bay sites, despite their presence in local brackish waters, may result from: (1) rapid dilution of freshwater inputs by marine N sources due to low residence times in these systems (Herrera-Silveira et al., 2002); (2) small-scale point sources of SGD in the coastal zone that were undetected in our sampling; (3) short-term pulses of nutrient inputs; or (4) N removal during groundwater transport. A more intensive sampling effort is required to characterize the temporal and spatial nature of potential inputs directly into marine systems.

Identification of anthropogenic nutrient inputs in the watershed surrounding Yal Kú Lagoon underscores the need for effective wastewater management and ecosystem monitoring in the Yucatan Peninsula. The seagrass meadows and coral reefs of this region are adapted to low nutrient conditions, and even small changes to the nutrient regime may have a large impact on these systems (Lapointe et al., 1992; van Tussenbroek et al., 1996), including a reduction in seagrass diversity in the area (Kamermans et al., 2002). High nutrient concentrations, elevated $\delta^{15}N$ of NO_3^-, and extensive growth of *Cladophora* sp. in Laguna Lagartos provide evidence that changes may already be occurring. Isotope and seagrass nutrient content analyses may be particularly useful in monitoring the nutrient inputs and status of tropical systems, especially when coupled with estimates of total system productivity and trophic indices (Herrera-Silveira et al., 2002).

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