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Sea fan corals provide a stable isotope baseline for assessing sewage pollution in the Mexican Caribbean

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Abstract

We compared stable nitrogen isotope ($\delta^{15}N$) values from the common Caribbean sea fan *Gorgonia ventalina*, collected from a developed and undeveloped coastline, to test the hypothesis that sewage-derived nitrogen (N) inputs are detectable and more severe in developed areas along the Mesoamerican barrier reef of Mexico. The Akumal coast was selected as the developed site since this area is inhabited by thousands of local residents and has a significant flux of tourists; it was compared to a relatively undeveloped shoreline south of Mahahual, a small town with a few hundred residents and sewage treatment infrastructure. Gorgonians sampled from Akumal were relatively enriched in $\delta^{15}N$ (as high as 7.7‰ nearshore) and were $\sim$ 3.5‰ greater than sea fans from Mahahual collected at similar depths. While previous work has shown that water column N concentrations are uniform around Akumal, $\delta^{15}N$ values of sea fans sampled parallel to shore were variable, indicating that sewage-derived N inputs are spotty along the coast. $\delta^{15}N$ values were positively correlated with fecal *Enterococcus* counts from seawater, confirming that these enrichments are associated with sewage and not denitrification. We suggest that the data from Mahahual can be used as an isotopic baseline for monitoring the Mesoamerican barrier reef at sites where increased development is planned or underway.

With burgeoning coastal populations, sewage pollution is a serious threat to coastal marine systems because it disrupts ecosystem function through the input of nutrients, pathogens, and other contaminants (Camargo and Alonso 2006). Sewage-derived nutrients, particularly nitrogen (N) and phosphate (P), alter the fundamental structure and function of marine systems by increasing primary productivity, exacerbating disease, and altering food web structure (Howarth et al. 2000). Generally oligotrophic, tropical marine systems are particularly disturbed by increased nutrient inputs from land. Indirectly, nutrients increase the proliferation of algae, epiphytes, suspension feeders, and bioeroders, which collectively smother, foul, and prevent recruitment of critical species such as seagrasses and reef-building corals (Ward-Paige et al. 2005; Waycott et al. 2009). Directly, elevated nutrient concentrations disrupt the symbiotic association between corals and their zooxanthellae and reduce fecundity (Fabricius 2005). Taken together, the direct and indirect effects of nutrient pollution can induce a shift toward an algal-dominated benthos, which is characterized by a severe loss of biodiversity and ecosystem services (Knowlton 2004; Littler et al. 2006). In addition to N and P, sewage effluents contain pathogens and contaminants that have been linked to coral disease and mortality (Patterson et al. 2002). These effects are particularly damaging to developing regions, where public well-being and economic growth are dependant upon ecosystem health (Fabricius 2005; Kaczmarsky et al. 2005; Murray 2007).

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Stable isotope analysis is a promising tool for monitoring changes in the contribution of human N sources to nearby ecosystems, especially where comprehensive water quality monitoring programs are not established. Specifically, the isotopic ratio of $^{15}N:^{14}N$ is regarded as an effective and direct indicator of human N pollution (Risk 2009; Risk et al. 2009*b*). Generally, enriched isotope values (having relatively more ^{15}N) arise from the accumulation and degradation of human and animal wastes. For example, nitrate in raw or partially treated sewage-contaminated groundwater can have $\delta^{15}N$ values that are much higher than 10‰ (Katz 2004), although fractionations are dependent on the method of sewage treatment. In contrast, N from upwelling has lower $\delta^{15}N$, averaging 4–7‰ (Leichter et al. 2007), while N fixed by diazotrophs is relatively depleted, averaging -1‰ to 0‰ (Karl et al. 2002). Thus, sewage-derived N can be easily distinguished from natural marine sources, especially when sewage N comprises a major proportion of the total N pool.

Simultaneous determination of %N, % carbon (%C) (and thus C : N), and $\delta^{13}C$ by elemental analyzer–isotope ratio mass spectrometry provides further information for the identification of sewage pollution by revealing the potential for N limitation to confound interpretation of $\delta^{15}N$ values and by offering a secondary isotopic indicator of sewage pollution, respectively. As a measure of a coral's nutritional status, C : N can identify physiological differences between individuals that may account for observed variations in isotope ratios (Alamaru et al. 2009). Furthermore, if N were limiting within the environment, we would expect a negative relationship between C : N and $\delta^{15}N$, since an overall scarcity of N reduces isotopic discrimination against ^{15}N, thus increasing $\delta^{15}N$ of the organism toward isotopic equilibrium with its N source.

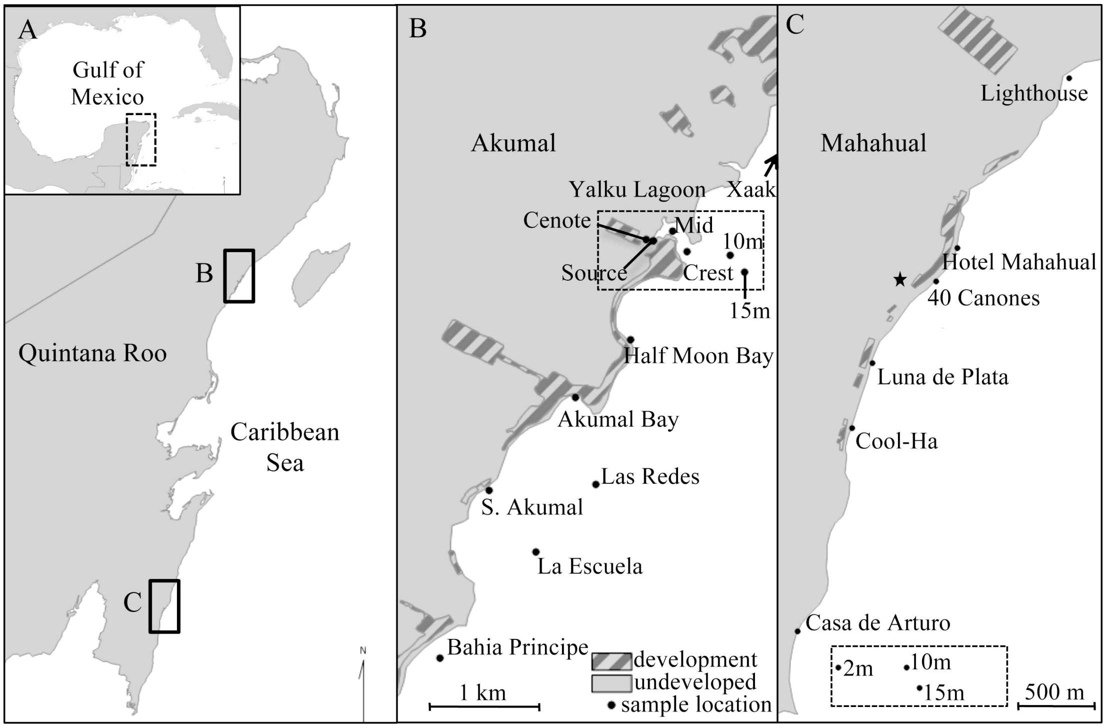

Fig. 1. (A) Map of the study area, Yucatan Peninsula and the state of Quintana Roo, Mexico, (B) highlighting the developed coastline of Akumal and (C) the relatively undeveloped coastline of Mahahual. Points represent sampling sites. Dashed boxes illustrate the location of the nearshore to offshore transects (perpendicular sampling). The star represents the location of the sewage treatment facility in Mahahual.

Lastly, $\delta^{13}C$ can corroborate inputs of particulate matter from sewage ($\delta^{13}C = -19$ to -27; Wayland and Hobson 2001; Rogers 2003; Ramirez-Alvarez et al. 2007). Rogers (2003) showed that mussels consuming sewage particulates were up to 3.4‰ depleted relative to control sites.

While isotope ratios provide information as to the source of N within an ecosystem, local factors can complicate the interpretation and the general applicability of conclusions. Several studies tracing N sources in highly polluted locations have illustrated significant relationships between $\delta^{15}N$ and proximity to those sources (Risk and Erdmann 2000; Marion et al. 2005). However, these examples may be exceptional, as most developed coastlines are likely to have multiple sources of both natural and anthropogenic N (Lapointe et al. 2004). Moreover, it has been shown that light is an important factor in influencing isotope values through metabolic fractionations sensitive to photosynthetic rate (Heikoop et al. 1998) and should be considered in isotope studies of obligate and facultative autotrophs. While it has been stressed that attention should be given to characterizing local sources (upwelling, deposition, fixation, etc.) and identifying environmental transformations of N (denitrification, nitrification, etc.), there is also a need to corroborate isotopic signals of anthropogenic N sources with other environmental data.

The Yucatan Peninsula is among the most rapidly developing regions in the world, situated along the coast of the State of Quintana Roo (Fig. 1A). This region attracts more than 5 million tourists per year and provides a unique opportunity to examine the effects of coastal development and intensified sewage inputs to the coastal marine environment (Murray 2007). Quintana Roo is characterized by a karst geology, which is so permeated with subterranean channels that there are few instances of surface waters in the region. Typically, groundwater is conducted from inland and discharges through benthic sinkholes into the coastal marine environment. En route to the sea, groundwater is easily contaminated by surface pollutants, particularly from agriculture and wastewater effluents from homes and resorts. Many local residences lack the means to properly dispose of sewage, while resorts are responsible for on-site wastewater treatment (Pacheco et al. 2001). Much of the groundwater research in this region has been focused on the study of wells and sinkholes ('cenotes'), with concern for microbial and chemical threats to human health. Comparatively little attention has been given to the marine environment, where polluted groundwater encounters sensitive seagrass and coral reef ecosystems. This is a critical area of study given the concentration of development adjacent to the sea, poor wastewater treatment practices, lack of temporally resolved marine water quality monitoring, and the potential consequences for the benthic marine environment, upon which the tourism industry relies heavily.

Here we examine stable isotope ratios of gorgonian corals and the abundance of enteric bacteria in the water column to test the hypothesis that reefs adjacent to developed coastlines are polluted by sewage-contaminated groundwater. We ask the following: (1) Do developed coastlines have higher $\delta^{15}N$ nearshore and offshore as a result of greater sewage N inputs? (2) Can we identify sites < 1 km apart that have greater sewage inputs alongshore? (3) Are high gorgonian $\delta^{15}N$ values a result N assimilation from sewage, or do they indicate natural microbial denitrification? The answers to these questions are critical to developing cost-effective monitoring in rapidly developing coastal areas worldwide.

Methods

Regional comparisons—Perpendicular sampling—To test the hypothesis that development generates sewage pollution adjacent to developed coastlines, we sampled sea fan corals (*Gorgonia ventalina* L.) perpendicular to the shoreline along a nearshore–offshore gradient from 2 m to 15 m in depth in two separate regions of the Mesoamerican Barrier Reef (Fig. 1A). The Akumal coast (n = 90) was selected as the developed site. This area is located $\sim$ 100 km to the south of Cancun and has a local population $>$ 3000, with a significant flux of tourists (Fig. 1B). The undeveloped coastline was adjacent to Mahahual (n = 30), a historically small fishing village located $\sim$ 200 km to the south of Akumal, now under new development pressures. In addition to the large population differences, Mahahual maintains a sewer and wastewater treatment system and has a much smaller inland community (Fig. 1C). Both regions have the same karst geology, few instances of surface waters, and an abundance of benthic sinkholes and sandy depressions, through which groundwater percolates into the marine environment. Furthermore, the reef and coral community structure are similar between these regions (E. Jordán-Dahlgren unpubl.). In both Akumal and Mahahual, the 15-m reefs sampled are $\sim$ 1 km from shore; however, corals are more abundant, and cover is slightly higher in Mahahual (15%) vs. Akumal (13.8%; E. Jordán-Dahlgren unpubl.).

In Akumal there is a coastal lagoon formed by a geological fracture named 'Yalku,' from which a substantial groundwater source has eroded the surface limestone and terminates at a well-formed reef crest, where it meets the sea. Beginning in the middle of the lagoon, where sea fans are present (Mid), and ending offshore at 15 m, five sea fans were sampled at approximately every 1.5-m depth interval (Fig. 1B). In Mahahual, three sites were sampled from nearshore to offshore: backreef (2 m), 10 m, and 15 m (Fig. 1C). Sampling began with the first occurrence of sea fans in shallow waters and ended approximately 1 km from the shoreline at the 15-m sites. All samples were collected in August of 2008.

In 2008, we sampled sea fans from the north-facing wall of Yalku Lagoon, approximately every 20 m from the first specimen to the reef crest, to determine fine-scale variation in isotope values. All samples were collected from $<$ 2 m in depth.

Identifying point sources of N pollution—Parallel sampling—In the summers of 2004 through 2008, we sampled sea fans from the shallow forereef parallel to shore to identify areas of sewage pollution, as evidenced by enriched $\delta^{15}N$ and depleted $\delta^{13}C$. In Akumal, eight sites were sampled at various depths parallel to shore: Yalku Lagoon, Xa'ak, La Escuela, Las Redes, Half Moon Bay, Akumal Bay, Bahia Principe, and South Akumal (Fig. 1B). We did not collect samples parallel to shore in Mahahual, as most gorgonians had been uprooted and killed by Hurricane Dean (category 5) prior to our sampling in 2008.

At each site approximately five 2-cm^2 fragments of sea fan were sampled from the colony edge with scissors using a self-contained underwater breathing apparatus or snorkel. This area likely represents the previous year of growth (Cary 1914). All samples were immediately taken to shore, where they were air-dried and stored prior to shipping to Cornell University.

Stable isotope analyses—Each sample was homogenized by grinding in a SPEX Certiprep cryogrinder using liquid N. The resulting powder, consisting of the skeletal axis, coenenchyme, polyps, and zooxanthellae, was weighed into 4 $\times$ 6-mm tin capsules and combusted in a Carlo–Erba elemental analyzer and analyzed by a Finnegan-MAT Delta Plus isotope ratio mass spectrometer. Reported $\delta^{15}N$ and $\delta^{13}C$ values are relative to atmospheric N_2 and Vienna Pee Dee Belemnite, respectively. Precision for $\delta^{15}N$ and $\delta^{13}C$ was quantified by two in-house standards (cabbage [0.08‰ and 0.06‰, respectively] and methionine [0.09‰ and 0.1‰, respectively]) calibrated against international standards. Additionally, we used an in-house homogenized sea fan standard, which had a precision for $\delta^{15}N$ and $\delta^{13}C$ of 0.05‰ and 0.25‰, respectively.

Enterococcus *assays*—To determine if N isotope values are correlated with sewage pollution along the coast, we quantified the prevalence of fecal *Enterococcus* bacteria in surface waters adjacent to sea fans sampled at 10 sites, seven of which were nearshore in the protected backreef (Yalku Mid, Half Moon Bay, South Akumal, and four sites from the lagoon in Puerto Morelos) and three sites that were just behind the reef crest (Akumal Bay, Bahia Principe, and Yalku Crest). Three 100-mL seawater samples were collected from each site in sterile Whirl-paks and transported to the lab on ice. The samples were allowed to settle, and an 80-mL aliquot was vacuum filtered through a 47-mm, sterile, grid-marked membrane filter (0.45-μm pore size). The filter was removed with sterile forceps and placed into a 9 $\times$ 50-mm petri dish containing $\sim$ 5 mL of m-Enterococcus agar (Difco; Environmental Protection Agency Method 1600). This media selectively cultures bacteria from the fecal *Streptococci* subgroup of *Enterococci*, including *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, and *Enterococcus avium*, which appear red on the membrane surface. The plates were incubated at 37°C for at least 48 h, or until colonies were observed and countable. Individual colonies were counted using a magnifying glass under fluorescent light. Each set of incubations was conducted with replicate negative controls (sterile deionized water) and positive controls using a pure culture of *E. faecalis*. Data reported are measured in colony-forming units (CFU) per 100 mL.

Statistical analyses—Prior to analysis, all data were screened for normality and homoscedasticity using Shapiro–Wilk and Levene tests, respectively. We used analysis of covariance (ANCOVA) on log-transformed data to test the null hypotheses that sea fan δ^{15}N, δ^{13}C, and %N, %C, and C : N; (1) are not different between regions, (2) do not vary with depth, and (3) change over depth at the same rate between regions (depth × region).

Within each region we tested the null hypothesis that the δ^{15}N and δ^{13}C values of sea fans sampled at individual sites (reefs or hard-bottom communities) are similar. ANOVA was used to test for an effect of site on isotope ratios. Where the outcome of the ANOVA was significant, we determined which sites were statistically different using pairwise Student's *t*-tests post hoc.

Within each region bacteria counts from each site were compared after a Box–Cox transformation to meet the assumptions of normality and homoscedasticity for parametric analyses. Differences among sites within a region were determined using ANOVA. Specific differences between sites were determined using post hoc pairwise Student's *t*-tests. The relationship between δ^{15}N and *Enterococcus* counts was determined using linear regression. All statistical analyses were conducted in JMP 7.0 (SAS Institute).

Results

Isotopes and elemental ratios along perpendicular transects—Overall, the δ^{15}N and δ^{13}C values of *G. ventalina* ranged from 0.9‰ to 7.7‰ and from −17.6‰ to −6.6‰, respectively, across the depth gradients from nearshore to offshore in both regions. Sea fans from shallow sites nearshore were consistently enriched in both isotopes, relative to deeper, offshore sites. In Akumal, sea fan δ^{15}N ranged from 7.7‰ closest to the groundwater source in Yalku Lagoon (1-m depth; 'Source' in Fig. 1C) to 2.1‰ at '15 m,' ~ 1 km offshore (Fig. 2A). Samples from Mahahual showed a similar trend, although a smaller range, from 3.6‰ at 2 m in depth to 1.5‰ at 15 m in depth (Fig. 2A). C : N trends were similar from nearshore to offshore at both sites, increasing with depth (Fig. 2B).

To test for the effect of depth, region, and depth × region on %C, %N, C : N, δ^{15}N, and δ^{13}C, we used an ANCOVA on log-transformed factors (Table 1). There was a significant effect of depth on all factors. δ^{15}N, %C, and %N all decreased with increasing depth, while δ^{13}C and C : N increased over the depth gradient. %C was highest nearshore and decreased with depth, ranging from 22.4% to 26.4% in Akumal and from 21.1% to 25.6% in Mahahual. Similar declines with depth were seen in %N, which ranged from 2.5% to 3.9% in Akumal and from 2.9% to 4.0% in Mahahual. There was no difference between Akumal and Mahahual, nor was there a difference in the rate of change over depth between regions for either % element (Table 1). There were significant differences between regions, with Akumal having higher C : N and δ^{15}N and lower δ^{13}C than Mahahual. However, there were no differences detected in either %N or %C between the regions. Only δ^{13}C produced a significant depth × region interaction, which was driven by very low values in samples from the shallow waters of Yalku Lagoon, which were relatively depleted by 2.2‰ compared to samples from the Mahahual backreef, thus increasing the slope of the regression.

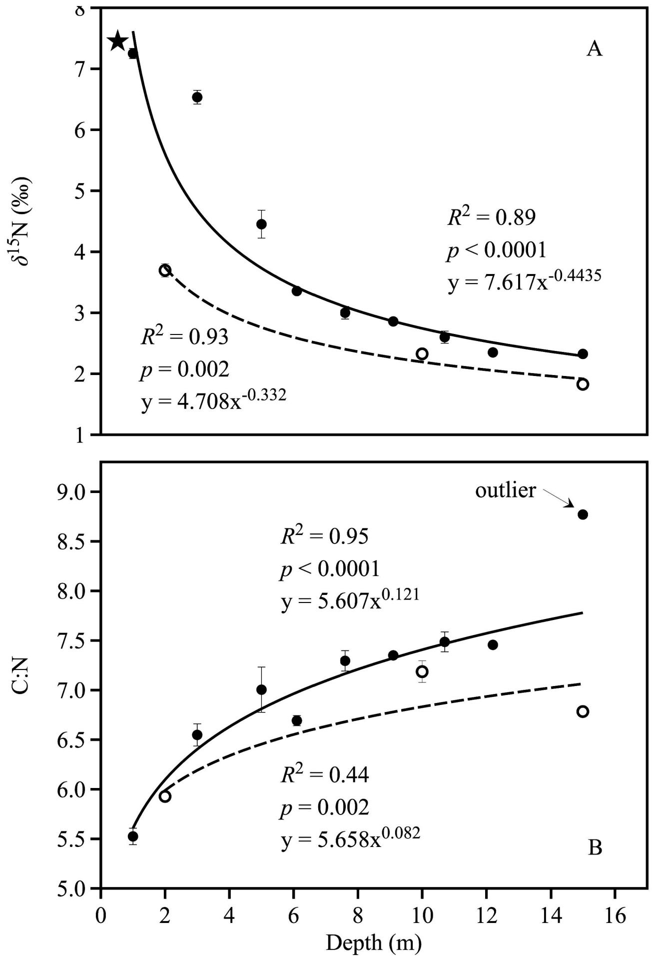

Fig. 2. Mean (A) δ^{15}N and (B) C : N as a function of depth in Akumal (closed circles) and Mahahual (open circles) of *G. ventalina* collected in 2008. Bars represent standard error. Lines represent significant power (log–log) regressions. The star represents the mean δ^{15}N of nitrate in Yalku Lagoon, as reported by Mutchler et al. (2007). Note that the 15-m locations are ~ 1 km from shore in both transects.

Fine-scale isotope and elemental trends in Akumal—δ^{15}N and δ^{13}C varied among sea fans sampled along a 160-m transect in Yalku Lagoon. δ^{15}N was highest in sea fans located closest to the groundwater source (7.7‰), although there was another spike in δ^{15}N at 140 m, followed by a gradual decline toward the reef crest (6.9‰). However, the slope of this decline was not different from zero (R^2 = 0.33, p = 0.10; Fig. 3A). There was a significant increase in δ^{13}C along the same transect, from −17.6‰ to −13.8‰ (R^2 = 0.67, p = 0.006; Fig. 3A). C : N ratios increased along the transect, although this trend was not statistically significant (R^2 = 0.38, p = 0.07; Fig. 3B). Variation in C : N was driven by changes in %N (R^2 = 0.67, p = 0.007), not %C (R^2 = 0.00, p = 0.99).

Table 1. Results of ANCOVA with log-transformed factors. Bold values indicate statistical significance at α=0.05. The last column (Akumal [AK] vs. Mahahual [MH]) represents the relative differences between factor means between regions using post hoc Student's *t*-test. nd, no statistical difference.

Factor	Source	df	Sum of squares	*F*-ratio	Probability$>F$	AK vs. MH
%C	Region	1	0.005	1.368	0.246	nd
	Depth	1	0.092	22.59	**<0.0001**	—
	Region×depth	1	0.002	0.396	0.531	—
%N	Region	1	0.039	2.891	0.094	nd
	Depth	1	1.029	76.83	**<0.0001**	—
	Region×depth	1	0.002	0.119	0.731	—
C : N	Region	1	0.087	13.94	**0.0001**	$>$
	Depth	1	0.522	83.84	**0.012**	—
	Region×depth	1	0.008	1.299	0.447	—
δ^{15}N	Region	1	0.908	46.19	**<0.0001**	$>$
	Depth	1	6.703	380.8	**<0.0001**	—
	Region×depth	1	0.071	3.621	0.061	—
δ^{13}C	Region	1	0.006	10.54	**0.0019**	$<$
	Depth	1	0.068	11.35	**0.0013**	—
	Region×depth	1	0.039	6.569	**0.0127**	—

Stable isotope variation along the Akumal coast—Although we sampled some sites in different years, there were few instances of significant year-to-year variation in isotope ratios. For example, δ^{15}N values from sea fans collected from the 10-m site outside of Yalku Lagoon varied by only 0.7‰ among the 3 yr sampled. Thus, all data for samples collected from sites sampled in multiple years were pooled. Among sites in Akumal sampled parallel to shore, there was a significant effect of site on δ^{13}C (ANOVA; n = 77, F_7 = 17.04, $p < 0.0001$; Fig. 4A). However, post hoc tests illustrate that this difference was driven by relatively low δ^{13}C values in Yalku Lagoon, while other sites were statistically indistinguishable. There was a similar effect of site on δ^{15}N (ANOVA; n = 77, F_7 = 50.33, $p < 0.0001$; Fig. 4B) and greater variation among sites. Yalku Lagoon was significantly enriched in ^{15}N relative to other sites, followed by Akumal Bay and Bahia Principe. Las Redes and Xaak had the lowest δ^{15}N values.

Enterococcus—Groundwater originating in Yalku Lagoon was a source of *Enterococci* (mean ± SE = 59 ± 3.5 CFU 100 mL^{-1}), although the presence of culturable bacteria declined with distance from the source, with less than 1 CFU 100 mL^{-1} at the reef crest (Fig. 5). Throughout Akumal, there were differences among sites with respect to the prevalence of *Enterococci* in surface waters (ANOVA; n = 15, F_4 = 10.7, p = 0.0012; Fig. 6). Water samples from Yalku Lagoon and Half Moon Bay yielded significantly more bacterial colonies than did samples from Akumal Bay, South Akumal, or Bahia Principe (Fig. 6).

In Mahahual, there were differences between sites in terms of *Enterococcus* abundance (ANOVA; n = 18, F_5 = 25.5, $p < 0.0001$; Fig. 7). This was driven by high CFU in samples adjacent to the Luna de Plata site (28 ± 5.3 CFU 100 mL^{-1}), which is just beyond the end of the town's sewer line. This site was unique among the other sites sampled from the sewered areas in town and from more remote sites (Fig. 7). Bacterial counts were low in the town center, which typically has the highest density of swimmers and bathers (D. Baker pers. obs.).

There was a significant positive correlation between δ^{15}N and *Enterococcus* counts from sites sampled from low-energy lagoon areas (n = 7, R^2 = 0.66, p = 0.025; Fig. 8). There was no correlation among high-energy reef crest sites, although these sites were still relatively enriched in ^{15}N.

Discussion

We detected clear differences in the predominant N sources assimilated by sea fan corals along the coast of Quintana Roo, consistent with the known isotopic influence of sewage-derived N. Elevated isotope ratios were observed along the developed coastline and were correlated with the abundance of enteric bacteria, which supports our hypothesis that these inputs are from sewage and not the result of microbial denitrification of natural groundwater N sources. In support of our hypothesis, the δ^{15}N of sea fans from Akumal revealed significant enrichment, relative to Mahahual (Fig. 2A). The observed difference between nearshore sea fans was ~ 3.5‰, which is equivalent to the average difference observed between trophic levels within a food web (Deniro and Epstein 1981; Minagawa and Wada 1984).

Based on previous work on the diet of gorgonians, this enrichment is not likely to be due to feeding upon a naturally enriched N source. Gorgonians primarily consume dissolved and particulate organic matter (Lasker 1981) in such low proportion that heterotrophy alone is not sufficient to meet N demands for growth and reproduction (Ribes et al. 1998). Instead, these corals likely assimilate inorganic N via direct uptake by their symbiotic zooxanthellae; thus, the differences in δ^{15}N can be attributed to an inorganic N source. Mahahual's low isotope ratios nearshore ($<$ 4‰) are similar to values reported for peat N (Hoering 1955; Heaton 1986; Lapointe et al. 2004), which originates from mangrove swamps and leaches into

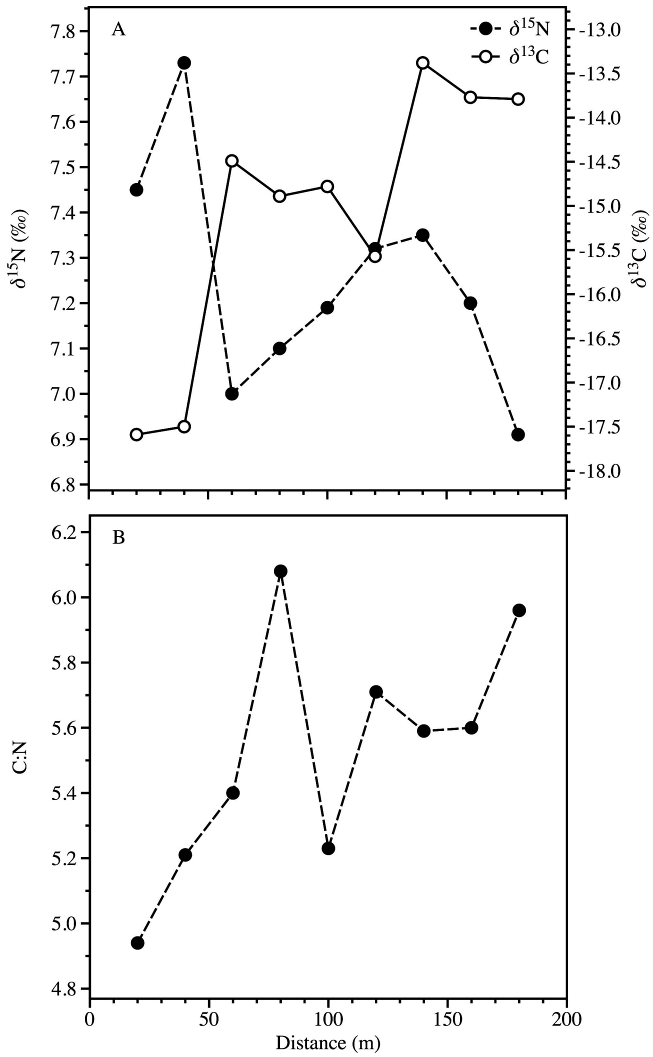

Fig. 3. Trends in (A) $\delta^{15}N$ and $\delta^{13}C$ values and (B) C : N from *G. ventalina* sampled over a 160-m transect in Yalku Lagoon, away from a groundwater source, in 2008. Points represent individual samples. There was a significant correlation between $\delta^{13}C$ and distance, but not among the other parameters.

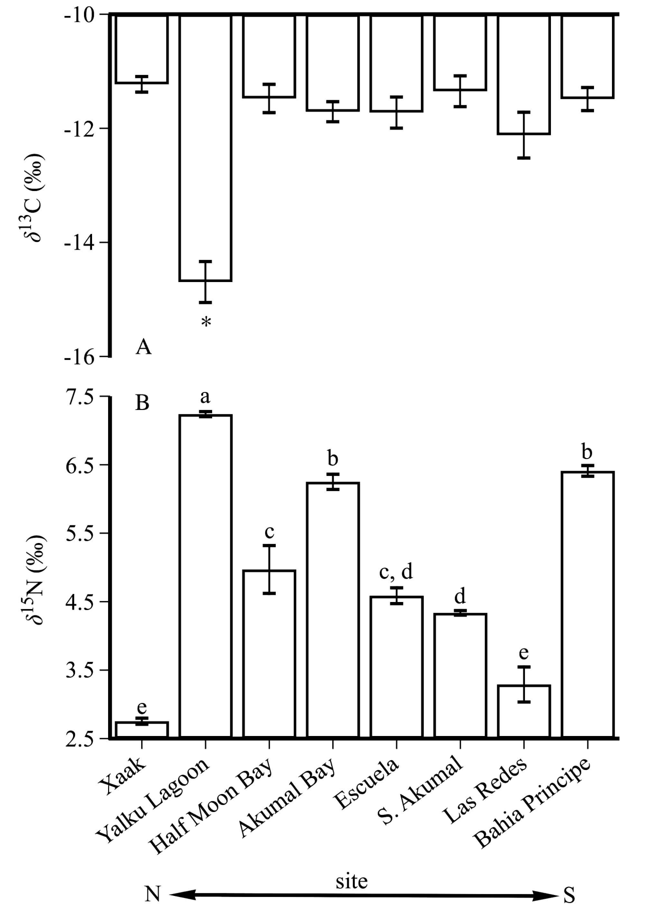

Fig. 4. Mean (A) $\delta^{13}C$ and (B) $\delta^{15}N$ among *G. ventalina* sampled at sites parallel to shore in Akumal averaged for all years sampled between 2004 and 2008. Bars represent standard error. Groups not sharing letters (or asterisk) were significantly different.

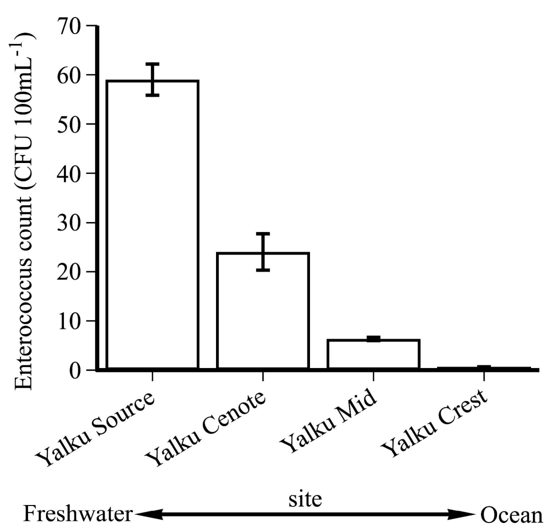

Fig. 5. Mean *Enterococci* colonies cultured from triplicate 100-mL water samples along a freshwater to saltwater gradient within Yalku Lagoon. Note that the mean CFU from the lagoon source are nearly two times higher than the USEPA limit for recreational waters. Bars represent standard error.

the marine environment, becoming an important source of N. Within Akumal, three nearshore sites had $\delta^{15}N$ values greater than 6‰. We hypothesized that sewage pollution in Akumal was the driver of this isotopic enrichment, given the relatively low $\delta^{15}N$ values of nearshore corals in Mahahual, adjacent to nearly pristine areas. Thus, $\delta^{15}N$ values of sea fans along the depth gradient in Mahahual are a useful baseline from a relatively pristine environment, which we can use to compare affected areas along the coast.

Within Akumal, we chose Yalku Lagoon as a target for increased spatial sampling to characterize the relationship between $\delta^{15}N$, distance, and depth as a result of the enrichments seen there (up to 7.7‰). Yalku Lagoon is a known source of N to the coastal marine environment, with NO_3^- concentrations of > 25 μmol L^{-1} (Mutchler et al. 2007). Mutchler et al. (2007) conducted isotope analyses of NO_3^- in Yalku Lagoon, reporting $\delta^{15}N$ values of 7.6‰.

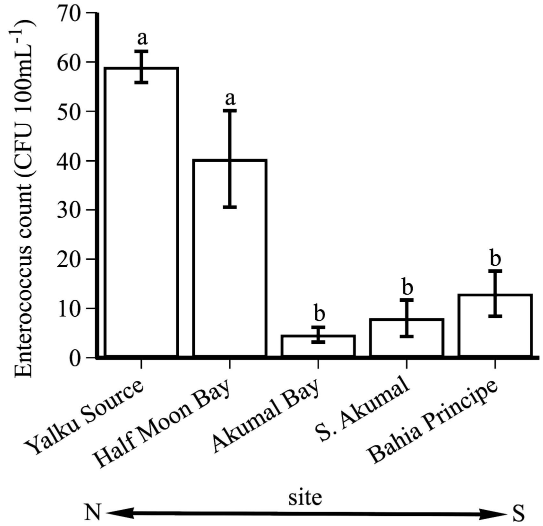

Fig. 6. Mean *Enterococci* colonies cultured from triplicate 100-mL water samples parallel to shore, from north to south, in Akumal. Columns with different letters signify significant differences between means. Bars represent standard error.

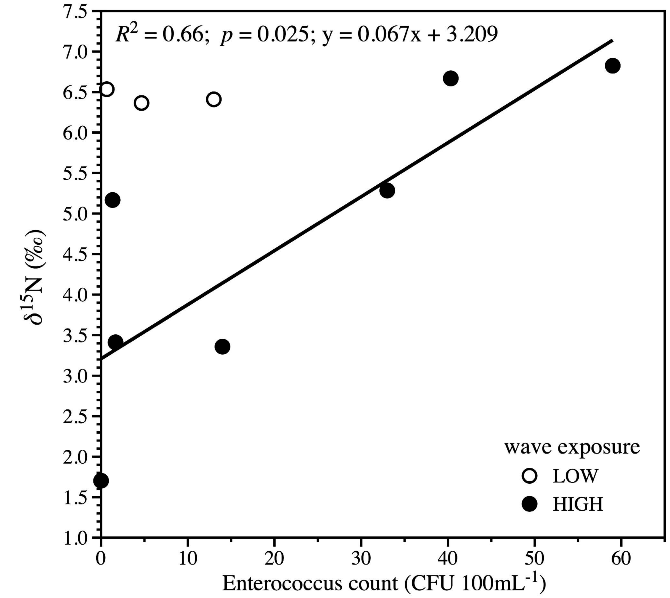

Fig. 8. Mean δ^{15}N of *G. ventalina* as a function of *Enterococci* cultured from an adjacent water sample from reef crest (open circles) and lagoon (closed circles) sites. Black line represents a significant linear relationship between the two values from relatively calm waters.

That the δ^{15}N values of *G. ventalina* nearest to the groundwater source were as high as 7.7‰ indicates that sea fans are assimilating NO_3^- as an N source, with no fractionation. In comparison to Mahahual, the relative ^{15}N enrichment among sea fans from Akumal persisted along the depth gradient and was still higher, by 0.5‰, at 15-m depth, illustrating that sources of N from land are affecting reefs nearly 1 km from the shoreline (Fig. 2B) and that simple dilution by seawater is not effective at mitigating the affect of N pollution. Reports of enriched δ^{15}N along this developed coastline indicate that sewage contamination is widespread. Ward-Paige et al. (2005) recorded δ^{15}N values of > 7‰ off of Xel-Ha, a major tourist attraction just 7 km to the south of our southernmost site in Akumal. Using Mahahual as a pristine baseline, isotope ratios of > 4‰ may be an indication of sewage pollution in similar karst-based tropical marine environments, although this threshold should not be extended to other areas of the Caribbean that present different geology, hydrology, or land-use characteristics.

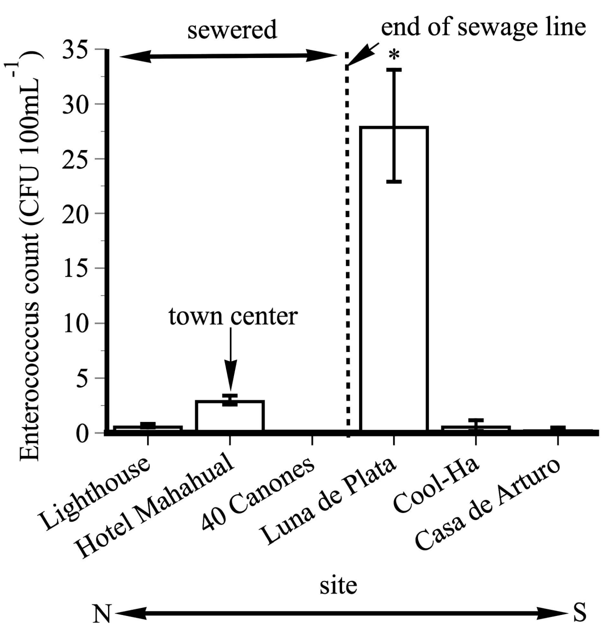

Fig. 7. Mean *Enterococci* colonies cultured per 100-mL water sampled parallel to shore, from north to south, in Mahahual. The column with an asterisk signifies a significant difference in average colony counts. Bars represent standard error. The dashed line represents the separation of sites sampled inside and outside the municipal septic line.

The bulk C isotope ratios of gorgonians were not as sensitive to sewage pollution as was δ^{15}N. δ^{13}C values were depleted in Yalku Lagoon relative to other sampling locations. This indicates that corals close to the groundwater source in Yalku Lagoon are consuming dissolved and particulate organic matter from sewage, which can carry values of −19‰ to −26‰ (Wayland and Hobson 2001; Rogers 2003; Ramirez-Alvarez et al. 2007). δ^{13}C also changed rapidly along a 160-m transect in Yalku Lagoon, becoming progressively enriched, by ~ 4.2‰ (Fig. 3A), relative to δ^{15}N, which decreased by 0.8‰ over the same distance. That the range in δ^{13}C was much greater than that of δ^{15}N indicates that marine C sources quickly dilute any C isotopic signal from sewage, which is not the case for N. This may be why δ^{13}C was not significantly different among other sites sampled parallel to shore (Fig. 4A) that were significantly different with respect to δ^{15}N (Fig. 4B). We did not remove lipid or carbonates from these samples, which makes interpretation of the dietary source of C difficult.

Fine-scale patterns in C and N isotope and elemental ratios of sea fans within Yalku Lagoon are clearly driven by a sewage source (Fig. 4), not by light-mediated isotope effects (Heikoop et al. 1998) or denitrification, as proposed by Mutchler et al. (2007). Light-dependent isotope fractionation would manifest as progressively declining isotope ratios with depth, as we have reported. However, sea fan $\delta^{15}N$ from within Yalku Lagoon declined with distance from the source, even though samples were collected from the same depth (Fig. 4A). Furthermore, denitrification within the sediments would enrich $\delta^{15}N$ with distance, which we did not observe. Perhaps the spike in $\delta^{15}N$ mid-transect is a result of denitrification in the sandy area in the center of the lagoon, or it could be evidence of a point-source wastewater input from residences built upon the lagoon wall. Although C : N did not vary significantly along the wall, there was a general trend of increasing C : N away from the groundwater source. This variability was driven entirely by the variation in %N, which was highest near the source, and not %C. A recent study by Alamaru et al. (2009) demonstrated that C : N, as an indicator of stored lipids, declined over a depth gradient in the hexacoral *Stylophora pistillata*. However, a similar trend was not seen in *Favia favus*, which the authors ascribed to consistent heterotrophy at all depths. Their study concluded that high C : N in shallow waters is a reflection of autotrophy, and low C : N at depth an indication of heterotrophy (Alamaru et al. 2009). The data we present here are completely contrary to this finding, as the lowest C : N was consistently found closest to shore and increased with depth. Given the results of Alamaru et al. (2009), the fact that sea fan C : N varied with depth confirms that *G. ventalina*, like *S. pistillata*, is relatively autotrophic. However, we did not set out to specifically quantify the C : N of lipids, nor did we remove lipids prior to isotope analyses; thus, we do not claim that the observed trends in C : N over the nearshore–offshore gradients in this study are due to lipid content. Since variation in C : N was correlated to %N, not %C, we suggest that N does not limit nearshore corals as they are growing in a high-N environment. Furthermore, these data indicate that N is not limiting to corals in either Akumal or Mahahual; thus, the differences we see in $\delta^{15}N$ between these regions are not due to the fractionating effects of N limitation but rather are due to different N sources.

Gorgonian corals are important targets for isotope analyses, as they sequester N within their tissues and skeletal elements, thus preserving a weighted average of the predominant N sources over time, and therefore they may be better suited for assessing long-term sewage stress than measuring water column N concentrations or isotope ratios of ephemeral benthic organisms like macroalgae (Risk 2009; Risk et al. 2009*b*). For example, our data show that $\delta^{15}N$ from corals in Akumal Bay are 3.5‰ enriched relative to Xaak. This indicates that there is relatively less sewage N present in Xaak, despite the similar NO_3^- concentrations reported by Mutchler et al. (2007), who, using N to P ratios and NO_3^- concentrations in seawater, concluded that Akumal's nearshore marine habitats are not N limited, which is typical for tropical lagoon systems (Corredor et al. 1999). Despite differences in local development (i.e., between Akumal Bay and Xaak), they found no significant differences in NO_3^- concentrations, and they concluded that there was no difference in the N sources between Akumal Bay and Xaak. Our sea fan $\delta^{15}N$ data clearly show distinct differences between sources of N reaching bays and reefs throughout Akumal (Fig. 4B). Sites sampled in Akumal Bay and Bahia Principe, both of which are heavily developed with hotels and resorts, were the most enriched sites, next to Yalku Lagoon. Mutchler et al. (2007) claimed an inability to detect anthropogenic inputs at bay sites as a result of mixing, the small scale of sources, ephemeral inputs, and N removal during transport. One possible reason for the lack of congruence between our study and that of Mutchler et al. (2007) is that macroalgae, having higher rates of N turnover, are better suited to serve as a short-term integrator (days to weeks) of ^{15}N than are sea fans, which integrate N over seasonal to annual time-scales (Gartner et al. 2002; Risk 2009; Risk et al. 2009*a*). Thus, macroalgae may capture short-term spikes in sewage-derived N subsequent to episodic rainfall or peak tourist visitation and, conversely, show rapid returns to marine ^{15}N signals when sewage inputs wane (Lapointe et al. 2004).

With N isotope studies such as ours, it is critical to recognize that natural processes can produce enriched isotope ratios within the expected range for sewage-derived N. Denitrification is a logical alternative explanation for high $\delta^{15}N$ values in groundwater. Therefore, it was important to corroborate the stable isotope data with another metric to support our hypothesis that observed ^{15}N enrichments were due to sewage-derived N, as opposed to a residual N pool that has reduced ^{14}N as a result of denitrifying microbes. The results of the fecal enterococcus assay confirmed that the groundwater source flowing into Yalku Lagoon is significantly contaminated by sewage. Surface waters collected from the origin of the lagoon had the highest *Enterococcus* counts in this study ($\sim$ 59 CFU 100 mL^{-1}). The presence of elevated fecal *Enterococci* well above the U.S. Environmental Protection Agency (USEPA) standard for recreational waters (35 CFU 100 mL^{-1}) is disconcerting, as this lagoon is a popular destination for tourists. It is possible that enteric bacteria are originating from bathers, yet high colony counts were recorded from samples from the nearby cenote, where there are no swimmers, confirming that the bacteria are present in groundwater. Furthermore, Akumal Bay has the highest concentrations of bathers and swimmers, yet it had comparatively low culturable *Enterococci*, indicating that contamination from swimmers is minimal. The inland colonia and residences are probable sources for this contamination, as nearly 20% of habitations in the region are not connected to any sewage system.

In contrast to Akumal, Mahahual maintains a sewage treatment center that connects the first kilometer of residences and businesses to a sewage treatment facility (C. Lucio pers. comm.; Fig. 1C). *Enterococci* were low in Mahahual's town center, despite the high abundance of swimmers in the water, and all sewered sites sampled were indistinguishable from remote areas (Lighthouse and Casa de Arturo). Interestingly, there were significantly more bacteria cultured from the Luna de Plata site, which is situated just beyond the end of the septic line. Because the Luna de Plata is a hotel and restaurant, elevated *Enterococci* here could be coming directly from its wastewater, or there could be leakage from the sewage pipe. Still, Mahahual had significantly lower bacterial counts than did Akumal, and in no instance was the count above the USEPA standard. Continued monitoring, especially around the Luna de Plata site, is crucial for detecting major increases in sewage contamination. We were unable to determine exactly how the sewage was being treated in Mahahual, but our bacterial evidence indicates that the septic line is effective at maintaining low levels of bacterial contamination in the nearshore waters adjacent to the most densely populated areas, which we assume is achieved simply by preventing leaching into the ground. Given the interest in developing Mahahual, these data could be used in support of continued investment in sewage treatment infrastructure.

The correlation between $\delta^{15}N$ and *Enterococcus* shows that in calm waters, high sea fan $\delta^{15}N$ was associated with high concentrations of bacteria in surface waters. The low abundance of bacteria cultured from reef crest waters could be due to progressive dilution of contaminated groundwater with seawater, bacterial mortality, mixing, or settlement to sediments. Future work should focus on quantifying the presence of enteric bacteria from sediments as a more integrated measure of contamination (Fries et al. 2006). In high-energy sites, we see that enteric bacteria are not a reliable indicator alone, as they attenuate rapidly over space, while sewage-derived N travels further and is detectable offshore, perhaps as a result of saturation of N in this environment. Previous studies have shown that sheltered lagoons along the coast retain water for several days (Coronado et al. 2007). This may allow enteric bacteria to accumulate, increasing the probability of sampling culturable bacteria (relative to findings in well-mixed areas).

Coastal populations in the Caribbean are expected to grow 40% by 2050 (http://www.un.org/esa/population/publications/sixbillion/sixbillion.htm). As a result, sewage pollution will worsen and will accelerate the degradation of sensitive marine habitats. Thus, the challenge of cost-effective environmental monitoring will be met by studies measuring the isotopic ratios of benthic marine organisms as recorders of increasing pollution. This study contributes further evidence that isotope ratios from gorgonian corals are a particularly valuable tool for detecting sewage pollution in the absence of water quality monitoring; they are also more useful than algae for detecting pollution among sites with similar N concentrations. Through time-series sampling and relative comparisons between pristine and affected environments, gorgonian corals can effectively capture perturbations in the ambient N pool due to human development. We have shown that coral reefs along the developed Mexican Caribbean are receiving an enriched N source, up to 1 km from shore, and that these sources correspond with elevated levels of enteric bacteria. Furthermore, elevated $\delta^{15}N$ was correlated with high bacteria counts, but only in nearshore lagoon sites, where bacterial dilution and mortality are likely lowest. Therefore, $\delta^{15}N$ is sensitive to sewage pollution, even in areas where fecal bacteria cannot be detected by conventional means.

The $\delta^{15}N$ data we present here are key to understanding coral decline in the Mesoamerican barrier reef. Since 2006, comprehensive assessments of coral diseases among high- and low-development areas within the Mexican Yucatan peninsula have shown significant differences in the prevalence of Yellow Band Syndrome (YBS) among the *Montastrea annularis* species complex, now the dominant reef-building corals in the Caribbean. YBS is nearly twice as prevalent in Akumal than in Mahahual, and these stable isotope data indicate that there are greater sewage inputs to reefs along developed coastlines (E. Jordán-Dahlgren unpubl.). Since in situ experimentation has shown the direct association between nutrients and YBS severity (Bruno et al. 2003), and because YBS is more prevalent in Akumal, the hypothesis that the high disease-related mortality of these corals is facilitated by sewage pollution is indicated. Moreover, remediation of sewage pollution should ameliorate the decline of these reefs from disease and increase their resilience to global climate change. Mahahual offers a unique setting for documenting the effects of development on nearshore marine habitats, as the government has targeted this town for accelerated tourism development. Long-term monitoring of both the coral community and the isotope biogeochemistry of gorgonians in Mahahual will allow us to document the effect of development as it occurs, as we now have a baseline with which we can compare future data. Stable isotope analysis of gorgonian corals is simple and cost effective. They can be collected from shallow waters by free diving with scissors, air-dried, homogenized by hand, and analyzed for $<$ US\$10 per sample. Given the power to detect relative differences among sites with approximately five samples, and given the informative power to monitor sewage pollution, we support the call for these types of analyses to be included in environmental monitoring efforts in both developed and developing nations (Risk 2009; Risk et al. 2009*b*).

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