

# Reconstruction of nitrogen sources on coral reefs: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in gorgonians from Florida Reef Tract

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**ABSTRACT:** The gorgonians *Plexaura* spp. occur throughout the Florida Reef Tract, and lay down annual bands of a tough protein, gorgonin, in their skeletons. We analyzed stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) from individual annual bands in skeletons of *Plexaura homomalla* and *P. flexuosa* from 10 locations on the Florida Reef Tract, producing a proxy record of nutrient inputs dating from the mid-1970s to 2002. Isotope data were compared with tissue from offshore Belize reefs, and inshore reefs from Xel-Ha, Mexico (Yucatan Peninsula), collected in 2003. Based on previous nutrient work, Florida sites were classified as 'clean' (relatively low nutrient levels) and 'dirty' (elevated nutrient levels). Samples from clean and dirty sites had significantly different nitrogen isotope values, which were correlated with the average total nitrogen of the seawater. Dirty sites ( $3.9 \pm 0.2\text{‰}$   $\delta^{15}\text{N}$ ) were isotopically enriched over clean sites ( $2.9 \pm 0.1\text{‰}$ ) throughout the entire duration of the study. Dirty sites had an enrichment of  $0.8\text{‰}$   $\delta^{15}\text{N}$  throughout the study period, with the greatest increase in the 10 yr from 1993 to 2002 inclusive. Clean sites showed no significant change since 1974. Levels and trends in  $\delta^{13}\text{C}$  showed a similar picture of high and increasing eutrophication stress. Clean sites had lower  $\delta^{13}\text{C}$  values, consistent with predominantly autotrophic nutrition; dirty sites had higher values, suggesting a shift to a more heterotrophic mode of feeding. The methods outlined herein suggest that long-term records of organic pollution on coral reefs may be retrieved from gorgonian skeletons.

**KEY WORDS:** Florida Keys · Belize · *Plexaura* spp. · Gorgonian ·  $\delta^{15}\text{N}$  ·  $\delta^{13}\text{C}$  · Sewage · Water quality

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## INTRODUCTION

Worldwide, coral reefs provide food, income and a barrier against coastal erosion. As the human population increases, anthropogenic impacts increase and compound by impacts of global climate change. Increased development of coastal zones surrounding coral reefs decreases mangrove and seagrass habitats, which provide natural cleansing for the reef. Thus, coral reefs become even more susceptible to localized land-based sources of pollution; namely sedimentation (Cortes & Risk 1985) and nutrient-loading (Smith et al. 1981, Dustan & Halas 1987, Porter et al. 1999, Lapointe et al. 2004).

Serious damage to reefs from excess nutrient-loading has been documented in a variety of studies (Maragos et al. 1985, Rose & Risk 1985, Tomascik & Sander

1985, Lapointe & Matzie 1996). Nutrients and bacteria from sewage result in increased coral disease (white pox disease, Patterson et al. 2002, and black-band disease, Fouke et al. 2001), increased bioerosion by boring sponges (Rose & Risk 1985, Ward-Paige 2003, Ward-Paige & Risk in press), altered coral reproductive behaviour (Tomascik & Sander 1987) and an overall decline in coral health (Tomascik & Sander 1985, Lapointe et al. 1993). These effects contribute to coral death, especially in times of bleaching and other stresses (Lapointe et al. 1993, Bell & Tomascik 1993, Fouke et al. 2001).

The Florida Keys consist of an archipelago of tropical islands extending from the southern tip of the Florida Mainland to the Dry Tortugas. More than 100 000 permanent and/or winter residents and 3 million visitors to the Florida Keys each year impinge on the reef ecosys-

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tem via their sewage. Lapointe et al. (1990, 1993) found elevated nutrient (total nitrogen, chlorophyll *a*, and total phosphorus) levels as a result of discharge from septic and cesspit effluent in the nearshore waters of the Florida Keys. Sewage enters marine waters via 25 000 septic systems, 4000 'unknown systems' (probably cesspits), and 350 shallow injection wells (Kruczynski 1999 and 2002 pers. comm.). Szmant & Forrester (1996) have suggested that the levels of sewage-loading are constrained to within 0.5 km of shore and pose no risk to reefs at greater distance. These authors claim that concern over sewage-loading overlooks 'natural nutrient enrichment of the Florida Keys' (e.g. inputs from Florida Bay, upwelling, gyres, phytoplankton blooms and seagrass decay; Szmant & Forrester 1996). Low levels of sewage may not be detectable in the water column in water-quality tests; however, organisms that use ambient water to build tissue retain the isotopic signature of ambient seawater over long-term periods. Contrary to the assertions of Szmant & Forrester (1996), using  $\delta^{15}\text{N}$  as a tracer, several previous studies have shown that the signal of polluted coastal waters can be traced offshore more than 10 km (Risk et al. 1994, 1989, Mendes et al. 1997, Lapointe et al. 2004).

In a previous study,  $\delta^{15}\text{N}$  in boring sponge tissue was found to increase with total nitrogen (TN; Ward-Paige 2003). Nitrogen isotope values of sponge tissue provide insight into regional inputs of sewage-loading because of a ~3‰ stepwise increase in  $\delta^{15}\text{N}$  values up the food chain (DeNiro & Epstein 1981). Transformations occurring during discharge of human wastes, including denitrification of nitrate, nitrification of ammonia and ammonia volatilization, leave residual dissolved inorganic nitrogen with elevated  $\delta^{15}\text{N}$  (typically 10 to 20‰; Heaton 1986, Jordan et al. 1997, Heikoop et al. 2000). Geochemical analyses of coral tissue have also been used to track sewage stress on reefs (Burriss 1983, Dunn 1993, Yamamuro & Minagawa 1995, Heikoop 1997). In a recent study, Lapointe et al. (2004) used  $\delta^{15}\text{N}$  techniques to document the effects of both sewage and agricultural sources of land-based runoff on blooms of macroalgae in the Lower Florida Keys.

Gorgonian corals (sea fans) are common in the Florida Keys National Marine Sanctuary. These corals form concentric layers in their skeletons (much like tree rings), composed of a tough protein, gorgonin, which is preserved throughout the lifespan of the colony (Goldberg & Hamilton 1974, Goldberg 1976). The concentric layers in many gorgonian species have been shown to be annual (Grigg 1974, Opresko 1974, Goldberg 1976, Mitchell et al. 1993, Andrews et al. 2002, Risk 2002), and we assume that this is the case for *Plexaura* spp. as well. The annual bands can be isolated for isotopic analysis, thus providing a time-

history of environmental conditions (Heikoop et al. 1998, 2002, Sherwood & Ward-Paige 2003).

Here we focus on the gorgonians *Plexaura homomalla* and *P. flexuosa*. Isotope analyses of the gorgonin layers are used to reconstruct the history of nutrient-loading in the Florida Keys from 1976 to 2002 inclusive. Regional analysis was made by comparing  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of Florida gorgonians with isotope values of waters at 2 comparison sites, Mexico and Belize. These organisms may live for more than 30 yr (Opresko 1974), and have the potential to provide retrospective data on oceanographic nutrient conditions over the length of the gorgonian's life.

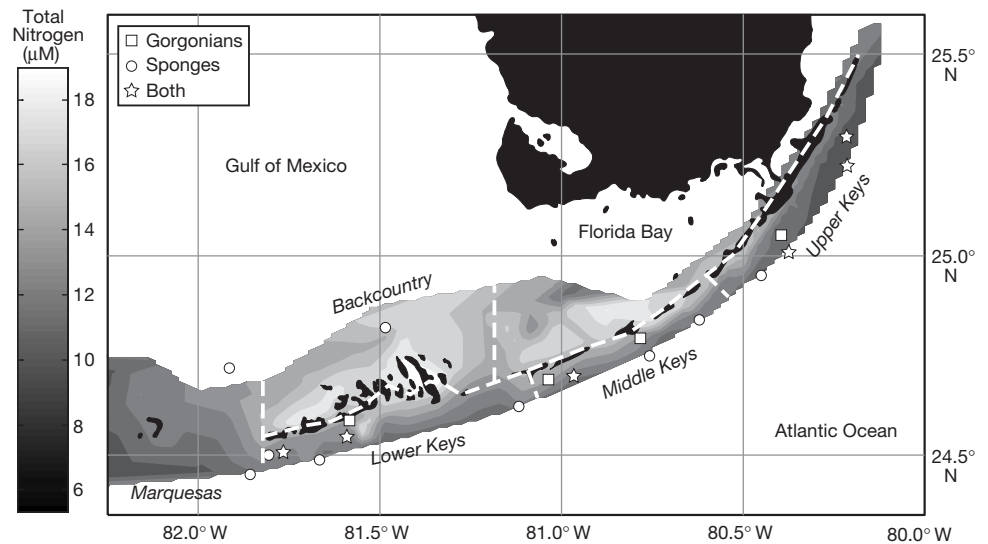
## MATERIALS AND METHODS

**Site selection and characterization.** *Plexaura homomalla* and *P. flexuosa* were collected on the Atlantic Ocean side of the Lower, Middle, and Upper Keys of the Florida Keys National Marine Sanctuary (FKNMS), USA, in the summer months of 2001 and 2002 (Fig. 1). We chose 10 sites at random from the 43 sites examined by the Coral Reef Monitoring Project (CRMP)/Florida Marine Research Institute (FMRI; see Porter et al. 2002). The Dry Tortugas, Marquesas, and Backcountry were excluded because of logistical difficulties. The comparison sites comprised (1) a site <0.2 km off the eastern shore of the Yucatan Peninsula, by Xel-Ha, Mexico, and (2) a site ~75 km off the coast of Belize, near Lighthouse Reef. The Xel-Ha site was near a large tourist region <200 m from shore, within the barrier reef. Lighthouse Reef is inhabited by few people; a number of boats visit the reef daily.

Boyer (2003) examined water quality at 154 sites in the FKNMS between 1995 and 2001, and statistically delineated these sites into 8 clusters. Each site in this study was matched to the corresponding Boyer (2003) site and cluster number. The sites with the highest nutrient concentrations were generally in the Lower Keys and Backcountry, while those with the lowest nutrients were in the Upper Keys. For further description of FKNMS sites see Porter et al. (2002). Belize water quality is discussed in Lapointe et al. (2004).

Specimens were collected from depths ranging from 3 to 10 m. We obtained 3 colonies of *Plexaura* spp. at each site by cutting the colony at the base, where living tissue meets the holdfast. Colony height was measured from the cut base to the tallest tips. A 5 cm long section was removed from the base, along with three 5 cm long tips (tissue and axis). The remainder of the colony was reattached to the seafloor by drilling a hole into the non-living substrate, filling the hole with epoxy and attaching the colony. Samples were kept on ice, in the dark, for transport to the laboratory.

Fig. 1. Florida Keys region, showing stations sampled for gorgonians *Plexaura* spp., sponges *Cliona* spp. and both. Contour shading, encompassing approximate extent of Florida Keys National Marine Sanctuary, shows average total nitrogen concentrations in surface water for period 1995 to 2001 (data from Boyer 2003). Dashed white lines delineate the different water-quality segments



**Sample preparation.** Samples were dried at 60°C and transported to McMaster University (Hamilton, Ontario). Thick sections (~1 to 2 cm) were taken from the bases of colonies using a low-speed ISOMET™ saw. In preliminary trials, the water lubricant used in sawing caused the dried gorgonin to swell, causing difficulties with sectioning. Ethanol (95%) replaced water as the lubricant in the saw in all subsequent operations to prevent this problem. Thick-sections were polished and photographed using a digital camera attached to a binocular microscope. Thick sections and tips were decalcified in 5% HCl to remove calcium carbonate sediment inclusions and calcite spicules. Concentric annual layers were separated under a dissecting microscope with scalpel and tweezers, starting at the outermost layer and moving in toward the center of the section. Photos of the adjacent gorgonian section were used as a 'map' to guide sampling. The chronological error associated with this procedure is estimated at ±2 yr. Individual layers were placed in vials with 5% HCl for an additional 24 h to ensure complete decalcification. The samples were then rinsed in deionized water and centrifuged for 3 min in triplicate. Cleaned samples were then dried in a vacuum. Tissue and axes of colony tips were prepared in the same way. Samples were sent to GEOTOP, Université du Québec,

Montréal, for nitrogen and carbon isotope analysis. Reproducibility, determined by repeat-analysis of the same material was ±0.2‰ for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ .

## RESULTS

### Geographic distribution of dissolved nitrogen

Of the 10 Florida study sites (Fig. 1), 5 fell within Water-Quality Cluster 3, and 5 within Clusters 5 and 6 (Table 1; see also Boyer 2003). Cluster 3 had the lowest

Table 1. Site locations, water-quality clusters, total nitrogen (TN) and  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotope values for gorgonians *Plexaura* spp. and sponges *Cliona* spp. Cluster/Site and TN data from Boyer (2003); TN are average benthic values for 1995 to 2002; gorgonian isotope data is average for last 5 yr. -: not sampled

Site	Latitude, longitude	Cluster no./ site no.	TN ( $\mu\text{M}$ )	Gorgonian $\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Sponge $\delta^{15}\text{N}$ (‰)
Turtle Patch	25° 17', 80° 13'	3/212	8.9	2.7 (0.5)	-16.8 (0.3)	5.2
Carysfort Reef	25° 13', 80° 12'	3/216	9.2	3.2 (0.2)	-15.9 (0.5)	4.7
Admiral Patch	25° 02', 80° 23'	3/224	10.0	3.0 (0.1)	-15.2 (0.3)	-
Molasses	25° 00', 80° 22'	3/225	9.0	2.9 (0.3)	-15.8 (0.3)	5.0
Conch Key Deep	24° 57', 80° 27'	3/228	9.6	-	-	5.4
Long Key	24° 47', 80° 47'	5/241	14.8	4.6 (0.5)	-15.0 (0.3)	-
Tennessee Deep	24° 45', 80° 45'	3/243	9.9	-	-	5.1
Dustan Rocks	24° 41', 81° 01'	5/247	14.4	3.8 (0.6)	-17.7 (0.4)	-
West Turtle Shoal	24° 41', 80° 58'	3/248	10.0	3.4 (0.2)	-15.8 (0.2)	4.4
Sombrero Deep	24° 37', 81° 06'	6/402	11.9	-	-	4.9
Alligator Deep	24° 50', 80° 37'	3/237	10.6	-	-	5.1
Jaap Reef	24° 35', 81° 34'	5/268	13.2	4.7 (0.3)	-15.5 (0.4)	-
West Washerwoman	24° 32', 81° 35'	6/269	11.4	3.9 (0.4)	-17.9 (0.5)	4.8
Eastern Sambo	24° 29', 81° 39'	3/273	9.8	-	-	5.3
Cliff Green	24° 30', 81° 46'	6/275	12.3	3.8 (0.3)	-17.3 (0.8)	5.5
Western Head	24° 29', 81° 48'	6/278	12.7	-	-	5.1
Rock Key Deep	24° 27', 81° 51'	6/280	13.5	-	-	5.6
Content Key	24° 49', 81° 29'	8/302	14.5	-	-	7.0



Fig. 2. *Plexaura homomalla*. Cross-section showing annual growth bands. Based on growth-band counts, this specimen is estimated to have lived for 26 yr

nutrient values of all 8 clusters, and 4 of these 5 sites were located in the Upper Keys region. The other clusters, 5 and 6, had relatively high levels of nutrients and were in the Lower Keys. Herein we refer to sites in

Cluster 3 as 'clean' sites, and those in Clusters 5 and 6 as 'dirty' sites. These terms are chosen for convenience and refer only to differences in nutrient levels. Values of surface water total nitrogen (TN), averaged over 1995 to 2002, ranged from 8.9 to 10.6  $\mu\text{M}$  at the clean sites and from 11.4 to 14.8  $\mu\text{M}$  at the dirty sites (Boyer 2003).

### Gorgonian growth rates

Colony heights ranged from 14 to 54 cm. The number of growth bands, which appeared as light and dark brown couplets in sections, ranged from 5 to 24 (Fig. 2). Comparison of the colony height and number of growth bands measured by Opresko (1974) showed a strong positive relationship; measurements in this study showed the same trend (Fig. 3). Assuming that each band represents 1 yr, the average yearly bandwidth increase of *Plexaura* spp. measured in this study was 0.6 mm,  $\text{SD} = 0.3 \text{ yr}^{-1}$ , and the average increase in height was about 2 cm  $\text{yr}^{-1}$ .

### Relationship of $\delta^{15}\text{N}$ with dissolved nitrogen

Average gorgonian  $\delta^{15}\text{N}$  isotope data were calculated for the 5 yr from 1998 to 2002 inclusive and compared with average total nitrogen (TN) in surface water averaged over 1995 to 2002 (Boyer 2003); it showed a significant positive relationship (Fig. 4). Data from Clionid sponges (*Cliona deletrix* and *C. lampa*) presented by Ward-Paige (2003) showed the same trend; however, relative to gorgonian tissue, sponge tissue was enriched in  $\delta^{15}\text{N}$  by 1.7‰.

### Trends in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

The  $\delta^{15}\text{N}$  values of gorgonian tissue samples from the dirty sites were significantly more enriched than the clean sites (Student's *t*-test:  $p \ll 0.0001$ ) throughout the sampling period (Fig. 5). Clean sites showed no significant change in  $\delta^{15}\text{N}$  since 1978, while the dirty sites had a significant trend of increasing  $\delta^{15}\text{N}$  (average increase of 0.8‰;  $p < 0.05$ ) between 1974 and 2001, with most of the increase occurring since 1993. Data for Jaap Reef did not fit the trends for either clean or dirty sites. These values were significantly higher (about 5‰) than at all the other sites and there was an overall decrease in  $\delta^{15}\text{N}$  over the study period.

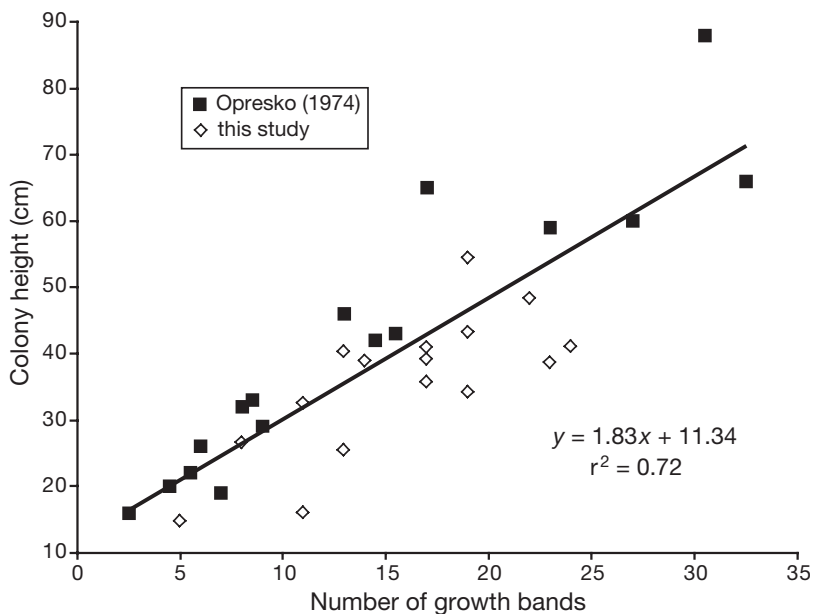


Fig. 3. *Plexaura* spp. Colony height versus number of growth bands. Agreement between data of Opresko (1974) and present study supports hypothesis that concentric growth bands are annual

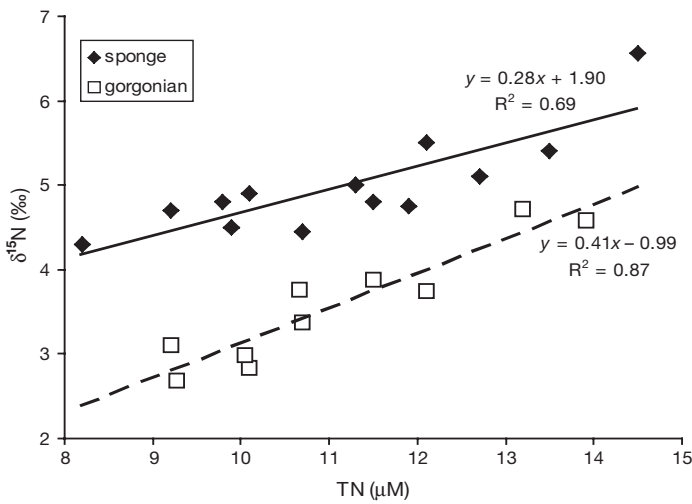


Fig. 4. *Plexaura* spp. and *Cliona* spp. Average  $\delta^{15}\text{N}$  of gorgonian and sponge tissue for the 5 yr 1998 to 2002 vs. average total nitrogen (TN) at each site from 1995 to 2001 (data from Boyer 2003)

$\delta^{13}\text{C}$  values of gorgonian tissue decreased between 1974 and 2001 at both the clean and dirty sites (Fig. 6). Clean sites were isotopically enriched relative to dirty sites throughout the sampling period.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were positively correlated (Fig. 7). Compared with the Florida results,  $\delta^{15}\text{N}$  was higher at Xel-Ha, Mexico, and lower at Belize. In addition,  $\delta^{13}\text{C}$  was lowest at Xel-Ha and highest at Belize. The  $\delta^{13}\text{C}$  data for Florida spanned the range between these Xel-Ha and Belize end-members.

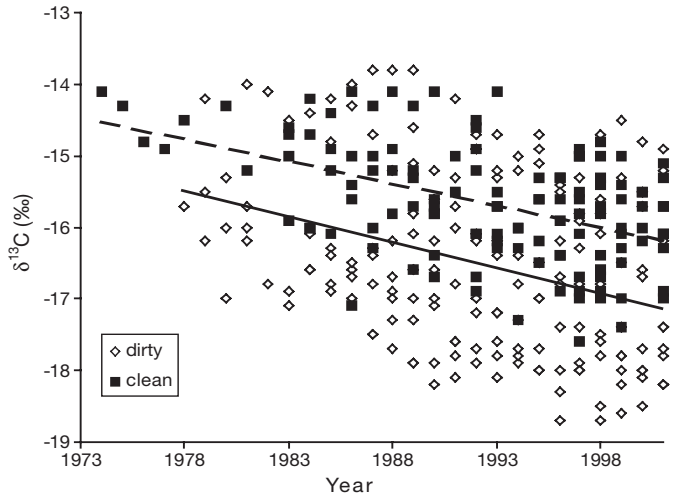


Fig. 6. *Plexaura* spp. Trends in  $\delta^{13}\text{C}$  of gorgonian tissue layers over time at clean and dirty sites. Both trends are significant ( $p < 0.001$ ). Estimated chronological error  $\pm 2$  yr

## DISCUSSION

### Spatial variation in land-based pollution

Division of sites into clusters showed a trend of clean sites in the Upper Keys and dirty sites in the Lower Keys, with overlap in the Middle Keys (Fig. 1). This is consistent with other nutrient and water-quality studies, indicating that anthropogenic nutrient-loading is relatively low in the Upper Keys (Ogden et al. 1994,

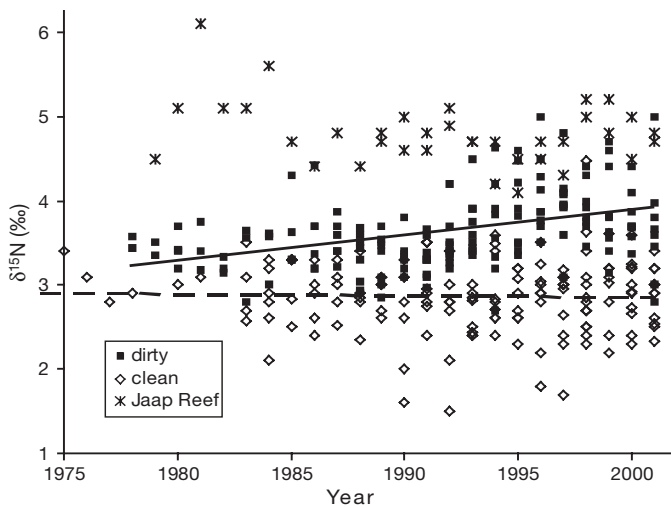


Fig. 5. *Plexaura* spp. Trends in  $\delta^{15}\text{N}$  of gorgonian tissue layers from Florida over time, showing significant ( $p < 0.05$ ) increase in  $\delta^{15}\text{N}$  at dirty sites, but not at clean sites. Data for Jaap Reef are shown separately because they do not fit the trends for either clean or dirty sites. Estimated chronological error  $\pm 2$  yr

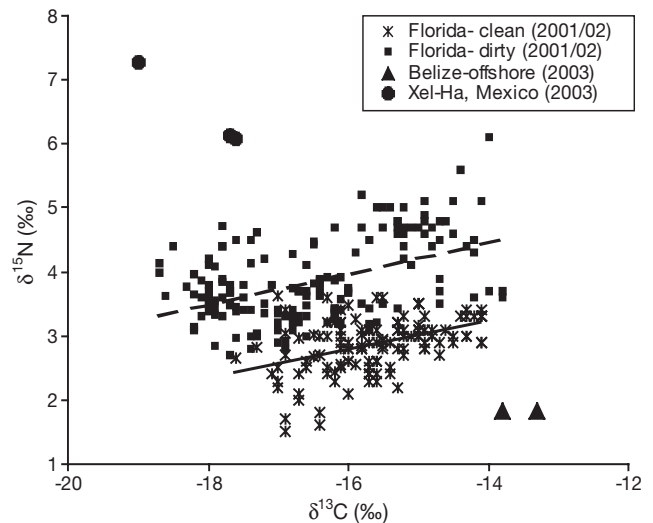


Fig. 7. *Plexaura* spp.  $\delta^{15}\text{N}$  versus  $\delta^{13}\text{C}$  of gorgonian tissue at clean and dirty Florida sites, and at offshore Belize sites and inshore Mexico sites



Szmant & Forrester 1996), and is greater in the Middle and Lower Keys (Lapointe & Matzie 1996, Szmant & Forrester 1996). Everglades runoff via the Shark River Slough (primarily from oxidation of peat from sugar cane farms) accelerates eutrophication of the Lower Keys compared to the Upper Keys (Lapointe et al. 2002, 2004). The trend in the Middle Keys (dirty sites inshore and clean sites offshore) is comparable to results presented by Szmant & Forrester (1996), Boyer & Jones (2002) and Lapointe et al. (2002), whereby a decrease in inshore–offshore nutrient levels was found, probably as a result of dilution with Atlantic Ocean waters (Boyer & Jones 2002, Lapointe et al. 2002). Regional differences in water quality may occur as a result of a combination of differences in Florida Current influences and natural and anthropogenic nutrient inputs.

Waters in the Upper Keys are mixed with Atlantic Ocean waters via the Florida Current (Smith & Pitts 2002). There are only limited passages that allow exchange of Florida Bay water with the Atlantic Ocean in the Upper Keys, which isolates the Atlantic Ocean side of the Upper Keys from nitrogen inputs from Florida Bay, Gulf of Mexico, and their watersheds (Smith & Pitts 2002). The Lower Keys and Middle Keys are not greatly impacted by the Florida Current; instead, they are influenced by the Tortugas Gyre, an eddy of the Loop Current from the Gulf of Mexico (Lee et al. 1992, 1994, 2002), and water from the SW Florida Shelf (Lapointe et al. 2002). There are 2 main passages in the Lower and Middle Keys that allow unrestricted exchange of the Gulf of Mexico and west Florida Shelf water to the reefs.

Natural sources of nutrients in the Florida Keys include (1) resuspension of nutrient-rich Florida Bay sediments (Richardson & Zimba 2002), (2) upwelling of deep Atlantic Ocean waters (Szmant & Forrester 1996, Richardson & Zimba 2002), (3) seagrass decay, (4) phytoplankton blooms, and (5) nitrogen fixation/nitrification within mangroves. Lapointe & Smith (1987), Szmant & Forrester (1996), and Leichter et al. (2003) described inshore transport of upwelled, nutrient-rich waters over the Florida shelf. This brings with it waters enriched in  $\delta^{15}\text{N}$ , as described by Sammarco et al. (1999) for the Great Barrier Reef. It is possible that much of the 'noise' in our long-term data set is generated by input of  $\delta^{15}\text{N}$ -enriched waters from short-lived upwelling events; however, upwelling primarily affects reefs >30 m depth (Lapointe et al. 2004). Upwelling is also more frequent and stronger in the Upper Keys than in the Lower Keys (Lapointe et al. 2004), so this does not support the spatial pattern of our  $\delta^{15}\text{N}$  data.

In addition to natural nutrient inputs, anthropogenic sources have resulted in nutrient enrichment in near-shore waters throughout the Florida Keys (Lapointe et al. 1990, Lapointe & Clark 1992, Lapointe & Matzie

1996). Lapointe et al. (2004) showed that anthropogenic pollution extends to the offshore reefs as well. Human activities doubled in the early 1990s compared to the early 1970s (Lapointe & Clark 1992), and 65% of human wastewater is disposed in onsite sewage disposal systems (OSDS; Lapointe et al. 1990). Sediment-core studies have documented the increase in terrestrial runoff (Nelsen et al. 2002). Approximately 80% of OSDS are in high-density zoned subdivisions that have man-made finger-canal systems, resulting in a short distance (25 m) separating OSDS from naturally oligotrophic marine waters (Lapointe et al. 1993).

Change in water management practices in the Everglades, where significant amounts of nitrogen derive from agricultural runoff, has led to increased nitrogen inputs into eastern Florida Bay. The major N discharges from the Everglades are from Shark River Slough (up to 3 million acre feet  $\text{yr}^{-1}$  and about 4500 tons TN in 1995), and these impact the western regions of Florida Bay as well as the Middle and Lower Florida Keys (Lapointe et al. 2002). Lower flows and TN loads come from Taylor Slough into central Florida Bay, and also the C-111 canal discharges bring freshwater and TN into eastern Florida Bay (Lapointe et al. 2002).

Lapointe & Matzie (1996) tracked nutrient-enriched water from neighbouring septic-tank drainage fields to adjacent waters. A 2- to 3-order-of-magnitude increase in nutrient concentrations has been found in waters adjacent to OSDS compared to comparison sites (Lapointe et al. 1990). Lapointe & Matzie (1996) found a 5000-fold nutrient enrichment in groundwaters contiguous to OSDS on Big Pine Key, and the zone of eutrophication could be traced up to 9 km offshore. Lipp et al. (2002) found evidence of human fecal contamination on the surface of coral heads in the Florida Keys.

### Gorgonian biology and growth

*Plexaura* spp. in the Florida Keys are exposed to seasonal environmental changes including air and water temperature, precipitation, storm activity, salinity, sedimentation and terrestrial nutrient-loading. *Plexaura* spp. colonies undergo changes in sexual orientation throughout the year, and only reproduce in late summer (Kinzie 1974). Each of these factors contributes to different feeding and energy budgets in *Plexaura* spp. throughout the year, resulting in differential growth rates and producing annual growth bands.

Opresko (1974) determined that *Plexaura homomalla* colonies had an average increase of 5 to 12  $\text{g yr}^{-1}$ , and concluded that age of a colony could be predicted by its weight. Data from Opresko (1974) and the present

study show a significant positive relationship between colony height and number of growth bands ( $r^2 = 0.72$ ) and indicate that colony height and/or number of growth bands can be used to predict age. The combined data show that *Plexaura* spp. grows in height by  $1.8 \text{ cm band}^{-1} \text{ yr}^{-1}$ . The irregular shape and number of branches of gorgonians make it difficult to determine vertical growth rates; however, Kinzie (1974) estimated the average annual vertical growth rate of *Plexaura* spp. to be  $2 \text{ cm yr}^{-1}$  (range 0.13 to 4.2). Therefore, our assumption of annual growth-band formation seems reasonable.

### Isotope content as a function of TN

$\delta^{15}\text{N}$  of both boring gorgonian and sponge tissue is significantly correlated with TN throughout the FKNMS.  $\delta^{15}\text{N}$  values in sponges were  $1.7\text{‰}$  higher than in gorgonians, but the slopes were very similar (Fig. 4). The clean sites had the lowest TN and  $\delta^{15}\text{N}$  values, while the dirty sites had the highest. These results show that geographic variation in  $\delta^{15}\text{N}$  parallels water quality, even among different organisms. Higher  $\delta^{15}\text{N}$  values in sponges relative to gorgonians can be explained by differences in feeding habits. Lacking photosymbionts, *Cliona deletrix* and *C. lampa* feed at a higher trophic level, and therefore have higher  $\delta^{15}\text{N}$  signatures (DeNiro & Epstein 1981).

Lapointe & Clark (1992) found that the least-developed portion of the Upper Keys (clean sites) exhibited the lowest concentrations of nitrate and ammonium, which could be evidence of a local anthropogenic source for both variables along the ocean side of the Florida Keys. Although dissolved inorganic nitrogen ( $\text{DIN} = \text{NO}_3 + \text{NO}_2 + \text{NH}_4$ ) accounts for the greatest contribution of TN in canals, dissolved organic nitrogen (DON) dominates TN at offshore bank reefs (Lapointe & Clark 1992). It is highly unlikely that DIN would result from a natural source such as N-fixation or the decomposition of leguminous matter (Lapointe et al. 1990), because predominance of ammonium results from suboxic and anoxic conditions that limit nitrification (Lapointe et al. 1990). Ammonium is the major form of N in sewage, which is introduced by submarine groundwater discharge (Lapointe & Clark 1992).  $\delta^{15}\text{N}$  values indicate that clean and dirty sites received at least part of their nitrogen from different sources. Since  $\delta^{15}\text{N}$  is enriched up the food chain by  $\sim 3\text{‰}$  per step, trends in  $\delta^{15}\text{N}$  are of great utility in tracing sources of organic matter. Enrichment in the heavy isotope,  $^{15}\text{N}$ , at dirty sites indicates that dirty sites receive more nutrients from human sewage than clean sites. Lapointe et al. (2004) suggested that  $\delta^{15}\text{N}$  values higher than  $+3\text{‰}$  could indicate the influence of sewage-derived nitrogen.

### Temporal trends in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

Both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  showed significant changes within the period covered by our sampling.  $\delta^{15}\text{N}$  of clean sites (around 2 to 3‰) showed a weak, non-significant decrease between 1974 and 2001 (Fig. 5). At the dirty sites, the  $\delta^{15}\text{N}$  was elevated compared to the clean sites ( $>3\text{‰}$ ) and there was a  $0.8\text{‰}$  increase over the sampling period. Most of this increase occurred after 1993. The increase in  $\delta^{15}\text{N}$  at dirty sites around 1993 coincided approximately with major seagrass die-offs (Robblee 1991) and phytoplankton blooms in Florida Bay (Richardson & Zimba 2002). At this point, however, our isotopic time-series were too imprecise to link them with specific events; perhaps future work will improve on this. Jaap Reef appears to be under the greatest sewage-stress, as indicated by  $\delta^{15}\text{N}$  data around 5 to 6‰; this is consistent with its close proximity to the shore and its high level of TN ( $13.2 \mu\text{M}$ ). The reason for the decrease in  $\delta^{15}\text{N}$  values from Jaap Reef around 1985 is unclear. Overall, these data suggest that sewage-derived nitrogen inputs have persisted at the dirty sites since the beginning of our sampling period and have increased in recent years. Risk (2002) has suggested that an elevation in  $\delta^{15}\text{N}$  of  $1\text{‰}$  is sufficient evidence of eutrophication to warrant policy intervention.

In addition to  $\delta^{15}\text{N}$ , the trends in  $\delta^{13}\text{C}$  indicate a steady decline over the 30 yr from 1993 to 2002 inclusive (Fig. 6). Trends for both clean and dirty sites are parallel, with the clean sites consistently elevated by about  $1\text{‰}$ . Risk et al. (1994) showed that the  $\delta^{13}\text{C}$  of corals on the Great Barrier Reef decreases inshore due to increased utilization of terrestrial carbon (heterotrophic feeding). Our data suggest that gorgonians at both clean and dirty sites are utilizing increasing amounts of terrestrial dissolved organic matter, more so at the dirty sites.

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  data combined present a picture of an ecosystem that has been under stress from terrestrial sources for at least 30 yr, with the degree of stress increasing markedly in the last decade (from 1993 to 2002). The generally lower values in the Keys reflect massive inputs of agricultural TN derived from Everglades peat, which has a  $\delta^{15}\text{N}$  signature of 1 to 3‰ and results in generally lower  $\delta^{15}\text{N}$  values, but is still anthropogenic in origin (mobilized by drainage and farming practices; Fig. 7). In comparison, the Xel-Ha reefs are not impacted by organic TN from such agricultural practices and reflect primarily sewage N from a burgeoning tourism industry, and the sewage-source signature is elevated well above that of peat and in the range expected for this sewage source (Fig. 7). The Belize reefs, relatively remote from either agriculture or sewage inputs, have the lowest values of all and are close to that of natural N fixation (Fig. 7).

## CONCLUSIONS

$\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  measured in the annual bands of gorgonian corals have provided a 30 yr retrospective analysis of water quality in the Florida Keys. Sewage-derived nitrogen sources have persisted at the Backcountry, Lower Keys and Middle Keys reef sites since the 1970s and there has been a marked increase in  $\delta^{15}\text{N}$  since ca. 1993, suggesting even greater insult to the reef ecosystem from land-based sources of pollution. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  data from other reefs in the Caribbean suggest that land-based pollution in the Florida Keys is intermediate between conditions at a pristine reef (Belize) and those at a heavily impacted reef (Xel-Ha, Mexico).

**Acknowledgements.** We thank the Coral Reef Monitoring project (M. Callahan, W. Jaap, V. Kosmynin, M. Lybolt, J. Porter, J. Wheaton) for their support, and ideas in the field. Special thanks to W. Jaap for collections, support, and advice, and C. Torres for additional collection of specimens in our absence. We also thank J. Wade, B. Williams, D. Wilson, E. Webb, M. Knyf, and J. McKay for helping with laboratory analyses, and M. McField and A. Salazar for their assistance in Belize and Mexico. We thank 3 anonymous reviewers whose comments have strengthened the manuscript. This work was supported by FMRI and NSERC grants to M.J.R. Collections and research was conducted under National Marine Sanctuary Permits FKNMS-2001-016, FKNMS-2001-016, and FKNMS(LR)-04-95-02.

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