

Relationship between water quality, $\delta^{15}\text{N}$, and aspergillosis of Caribbean sea fan corals

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ABSTRACT: It has been hypothesized that excess nitrogen (N) contributes to coral reef decline by exacerbating the impact of disease. We tested the relationship between N concentration and the prevalence and severity of aspergillosis of the sea fan coral *Gorgonia ventalina* at 9 reefs along the Florida Keys Reef Tract. Quarterly water quality data, averaged over 1 and 4 yr prior to a disease survey, were used to examine whether aspergillosis dynamics reflected short- or long-term N concentrations. A positive relationship was detected between prevalence of aspergillosis and long-term total nitrogen (TN) concentration; in contrast, disease severity was positively related to the ratio between dissolved inorganic nitrogen and total phosphate (DIN:TP) over both short- and long-terms. These results may reflect the differential influence of N on the host and pathogen. We also tested whether N isotope analysis ($\delta^{15}\text{N}$) of sea fan coral tissue and skeleton could be used to assess the relative exposure to anthropogenic N inputs and its impact on disease. There was no relationship between $\delta^{15}\text{N}$ and aspergillosis (either prevalence or severity). Furthermore, there was no relationship between $\delta^{15}\text{N}$ and environmental concentrations of N. It is possible that the source of N (e.g. anthropogenic) does not affect the dynamics of sea fan aspergillosis, or that the $\delta^{15}\text{N}$ signatures were suppressed by agricultural effluents and other N sources, thus confounding our analyses with disease.

KEY WORDS: Sea fan coral · *Gorgonia ventalina* · Water quality · Aspergillosis · Disease · Nitrogen · $\delta^{15}\text{N}$

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INTRODUCTION

According to the National Research Council (2000), elevated levels of nutrients, particularly nitrogen (N), are responsible for eutrophication of coastal marine ecosystems. In these environments, sources of N include land-based run-off and offshore upwelling in addition to atmospheric deposition. Coastal development and concomitant population growth have increased the amounts of wastewater and agricultural effluents entering the environment (Nixon 1995). Laden with organic and inorganic N, these effluents have contributed to hypoxia, harmful algal blooms and, in some instances, shifts in the structure and composition of benthic communities (Howarth et al. 2000). In Chesapeake Bay, nutrient loading has been attributed to blooms of the dinoflagellate *Pfiesteria* spp. that

have been linked to fish-kills and human intoxication (Glibert et al. 2005). In the Gulf of Mexico, the nutrient-rich waters of the Mississippi River have promoted the formation of hypoxic zones, leading to habitat degradation and loss of biodiversity (Scavia & Bricker 2006). In Kaneohe Bay, Hawai'i, USA, untreated sewage discharged onto the reef released algae and members of fouling communities from nutrient limitation, leading to overgrowth and smothering of the hard corals (Pastorok & Bilyard 1985). Subsequent diversion of 2 of the 3 outfalls away from the shallow reefs resulted in the return of corals.

Recently, N has been suggested as an important factor in diseases of corals. For instance, the occurrence of sewage-derived material was thought to be responsible for increases in the severity of black-band and white plague disease of scleractinian corals

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(Walker & Ormand 1982, Kaczmarek et al. 2005). Similarly, Kim & Harvell (2002) suggested that aspergillosis of sea fans was correlated with dissolved inorganic nitrogen (DIN), and Kuta & Richardson (2002) noted higher levels of nitrite associated with prevalence of black-band disease. Experimental evidence linking nutrients and coral disease was provided by Bruno et al. (2003), who showed that the severity (% tissue affected) of aspergillosis of sea fans and yellow-band disease of a scleractinian coral increased in the presence of elevated DIN and phosphate (P) concentrations.

However, testing the role of nutrients, in particular N, in the dynamics (spatial and temporal patterns) of coral diseases has been problematic. This has been due in large part to the fact that water quality data are often limited in spatial and temporal resolution (Kim & Harvell 2002, Szmant 2002). Even relatively comprehensive monitoring, such as the Southeast Environmental Research Center's (SERC) Water Quality Monitoring Network in the Florida Keys, USA, sample only 4 times per year. Such datasets are useful indicators of seasonal and longer-term variation in nutrient regimes that may be important in disease dynamics, but they may miss short-term changes and spikes in nutrient loadings (Leichter et al. 2003).

In coral reef ecosystems, stable nitrogen isotope analysis ($\delta^{15}\text{N}$, a ratio of ^{15}N to ^{14}N relative to a standard) has been used to identify sewage-impacted sites (Heikoop et al. 2000). Sewage becomes enriched with ^{15}N via ammonium volatilization followed by nitrification to nitrate (NO_3^-), with $\delta^{15}\text{N}$ often exceeding 20‰ (Heaton 1986). In comparison, ^{15}N from agricultural fertilizers or from fixation is generally 'depleted', having a $\delta^{15}\text{N}$ similar to atmospheric N_2 (~0‰). Because corals are long-lived and accumulate N as they grow, coral tissue $\delta^{15}\text{N}$ should provide a temporally integrated view of environmental nutrient conditions. Furthermore, while $\delta^{15}\text{N}$ is typically used as a tracer for sources of N, Ward-Paige et al. (2005) noted a correlation between 5 yr mean $\delta^{15}\text{N}$ of the gorgonian *Plexaura* spp. and both total N and ammonium concentrations for the Florida Keys Reef Tract. Thus, $\delta^{15}\text{N}$ may also be used as a proxy of source and concentration of N.

Aspergillosis of gorgonian corals is one of the best-characterized coral diseases: long-term datasets document the disease impact (Kim & Harvell 2004), the pathogen—the common soil fungus *Apergillus sydowii* (Geiser et al. 1998)—has been identified and is cultivatable, and a number of studies document various aspects of host–pathogen interaction (see Kim et al. 2006). In this study, we investigated the relationship between aspergillosis of sea fan corals *Gorgonia ventalina* L. and N. Specifically, we used 2 approaches—water column nutrient concentrations and stable iso-

tope signatures—to assess the role of nutrient concentrations and sources as potential drivers of disease prevalence and severity.

MATERIALS AND METHODS

Study species and sites. Sea fans *Gorgonia* spp. and other gorgonians are common in many shallow water reefs throughout the Caribbean. In the Florida Keys, sea fans alone can occur at densities of 1 m^{-2} (Kim & Harvell 2004). Because they are long-lived, gorgonian corals can serve as temporally integrated proxies of environmental conditions (Ward-Paige et al. 2005). In addition, gorgonians, unlike scleractinians, possess a protein-rich axial skeleton that provides substantial material for ^{15}N analysis from a small ($<2 \times 2\text{ cm}$) sample.

The study was carried out on shallow reefs ($<7\text{ m}$) in the Florida Keys, USA (Fig. 1). The Florida Keys are highly developed, with a population exceeding 65 000 and large seasonal fluctuations of tourists (Turgeon et al. 2002). Population densities in Key West and Key Largo are among the highest, with 600 to 800 km^{-2} . In 2002, the Florida Department of Health reported over 26 000 on-site septic systems in Monroe County, of which approximately 7900 had no sewage disposal method. Additionally, approximately 4000 lots in the county used illegal cesspits, exposing ground and surface waters to untreated sewage (Lapointe 1990). Owing to the porous limestone substrate in the Florida Keys, very little nitrogen is removed by denitrification (~4%), and the resulting N-rich groundwater eventually makes its way into canals, sea grass beds, and coral reefs (Corbett et al. 2000).

Disease survey and sampling. Nine reefs (all ~5 m depth) along the Florida Keys were sampled in May 1999: Alligator (24.853° N, 80.622° W), Carysfort (25.205° N, 80.244° W), Conch (24.977° N, 80.450° W), Little Grecian (24.107° N, 80.306° W), Looe (24.548° N, 81.397° W), Marquesas (24.454° N, 82.224° W), Molasses (25.007° N, 80.370° W), Sombrero (24.630° N, 81.112° W), and Western Dry Rocks (25.510° N, 80.165° W) (Fig. 1). These reefs are found within ~10 km of shore, with the exception of Marquesas, which is ~45 km from Key West. At each reef, all sea fans within 3 haphazardly located $25 \times 2\text{ m}$ transects were visually examined (see Kim & Harvell 2004). For each site, mean disease prevalence (% of individuals infected) and severity (% of colony area affected by disease, actual error based on image analysis = $\pm 2.5\%$, $N = 18$ sea fans) were determined. In addition, sea fan samples ($N = 3$ from a single transect per reef) were taken haphazardly from each reef for stable isotope analysis (see below).

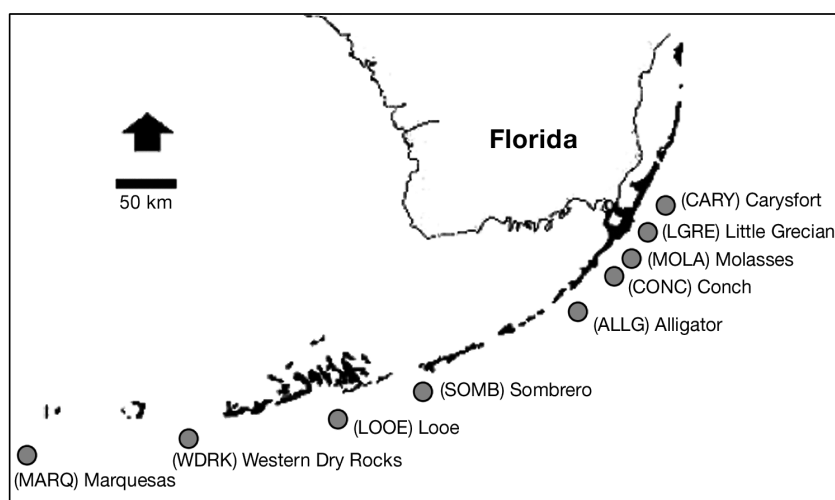


Fig.1. Sample sites in the Florida Keys Reef Tract. (see 'Materials and methods; Disease survey and sampling' for coordinates)

Nutrient data. The SERC provided water quality data (<http://serc.fiu.edu/wqmnetwork>). Nutrient parameters used for disease correlations were total nitrogen (TN); DIN; the DIN:TP (total phosphate) ratio; and total organic carbon (TOC). TN and DIN were selected based on their use in other studies that examined the role of nutrients in disease (Bruno et al. 2003) and gorgonian coral isotopic analysis (Ward-Paige et al. 2005). The DIN:TP ratio was selected because it is a biologically relevant measure of the relative availability of N to primary productivity (R. Howarth pers. comm.). TOC was selected as a proxy for dissolved organic carbon (DOC), which has been linked to coral mortality (Kline et al. 2006). DOC is the dominant form of TOC in the oceans (Hansell 2002), and in the Florida Keys there is no significant difference between TOC and DOC (J. Boyer pers. comm.). Surface data were used for all parameters. In 2 cases (Sombrero and Molasses Reef), the SERC stations corresponding to the survey sites were only recently installed, resulting in an incomplete dataset (Jones & Boyer 2000). Therefore, the nearest station with a complete water quality dataset was used.

Statistical analysis. Each of the nutrient parameters was summarized as follows: 1 yr average (i.e. average of the 4 sampling events in the year immediately prior to May 1999), and 4 yr average (i.e. average of the 15 sampling events in the 4 yr prior to May 1999) (5 yr averages, such as those used by Ward-Paige et al. [2005], could not be determined owing to the lack of available data). The 1 and 4 yr averages were used to assess whether short- or longer-term nutrient conditions were related to disease dynamics. The averaged nutrient parameters were used in re-

gression analyses (JMP 5.0) with the following disease parameters: prevalence (% of individuals infected), and severity (% of colony area affected by disease) (see below). Therefore, each reef was associated with a combination of nutrients (short- and long-term averages) \times disease (prevalence and severity). Sequential Bonferroni corrections were used to control for multiple tests (Rice 1989). For each set of nutrient and disease combination ($k = 4$), a 'table-wide' α level of 0.0125 was used (i.e. $\alpha = 0.05/4$).

Stable isotope analysis. Sea fan samples were separated into tissue and axial components by grinding with a mortar and pestle, and then using a dental tool to scrape excess

dried tissue from the axis. Residual tissue was completely removed from the axis with a 30 min treatment of 50% commercial bleach (diluted in de-ionized water). The bleach treatment did not have an effect on isotope values (Wilcoxon's signed-rank test, $N = 6$, $p = 0.844$). The axis was washed with de-ionized water, air-dried overnight, and pulverized to a powder form. The dried tissue, comprising coenenchyme, polyp tissue, sclerites, and zooxanthellae, was also pulverized to a powder. Axis and tissue samples were examined separately. Approximately 1.0 mg (± 0.15) of the sample was placed into 5×9 mm tin capsules for isotope analysis at the Davis Stable Isotope Facility, University of California. The facility uses a Europa Hydra 20/20 (PDZ Europa) with a continuous-flow isotope ratio mass spectrometer with a precision of $\pm 0.1\%$. $\delta^{15}\text{N}$ values are relative to an air standard ($\delta^{15}\text{N}_{\text{AIR}}$).

Data were checked for normality and homogeneity of variance prior to statistical analyses (Kolmogorov-Smirnov and Levene's tests, respectively). Reef-to-reef variation and variation in $\delta^{15}\text{N}$ values between tissue and axis were tested using a nested ANOVA (source of material nested within colony). Regression analyses were used to test for relationships between nutrient parameters and $\delta^{15}\text{N}$ values. Given that the 2×2 cm colony edge samples represent approximately 1 yr of growth (Cary 1914), $\delta^{15}\text{N}$ values were compared to 1 yr averages of nutrient parameters. We also examined the relationship between $\delta^{15}\text{N}$ values and disease in order to assess the applicability of using biological proxies of nutrient conditions (see Ward-Paige et al. 2005). Sequential Bonferroni tests were applied as described above.

RESULTS

Nutrients and disease

Significant relationships between nutrient levels and disease were detected; however, disease prevalence and severity were related to different nutrient parameters over different timeframes (Fig. 2). Disease prevalence increased with TN, but only when the 4 yr average was used. There was no relationship between the 1 yr average of TN and disease prevalence. In contrast, disease severity (a measure of how much of the sea fan is affected by disease) increased with the DIN:TP ratio when both 1 and 4 yr averages were used. Neither of the disease parameters was related to DIN or TOC.

 $\delta^{15}\text{N}$, nutrient levels and disease

$\delta^{15}\text{N}$ of sea fans ranged from +3.2 to 4.3‰ for the axis and from +3.2 to 4.2‰ for tissue (Fig. 3). A nested ANOVA revealed significant reef-to-reef variation in $\delta^{15}\text{N}$ values ($F = 8.032$, $df = 8$, $p = 0.0001$) but not between tissue and axis ($F = 0.471$, $df = 27$, $p = 0.963$). Therefore, for subsequent analyses, comparisons are based on $\delta^{15}\text{N}$ values of the axis. In general, sea fans from the lower Keys (e.g. Looe Key) and Marquesas were among the most enriched with ^{15}N , while sea fans from the upper Keys (e.g. Carysfort and Conch) were among the most depleted. However, the isotopic values were not related to either 1 or 4 yr averages of nutrient levels or disease (regression analyses, $p > 0.05$).

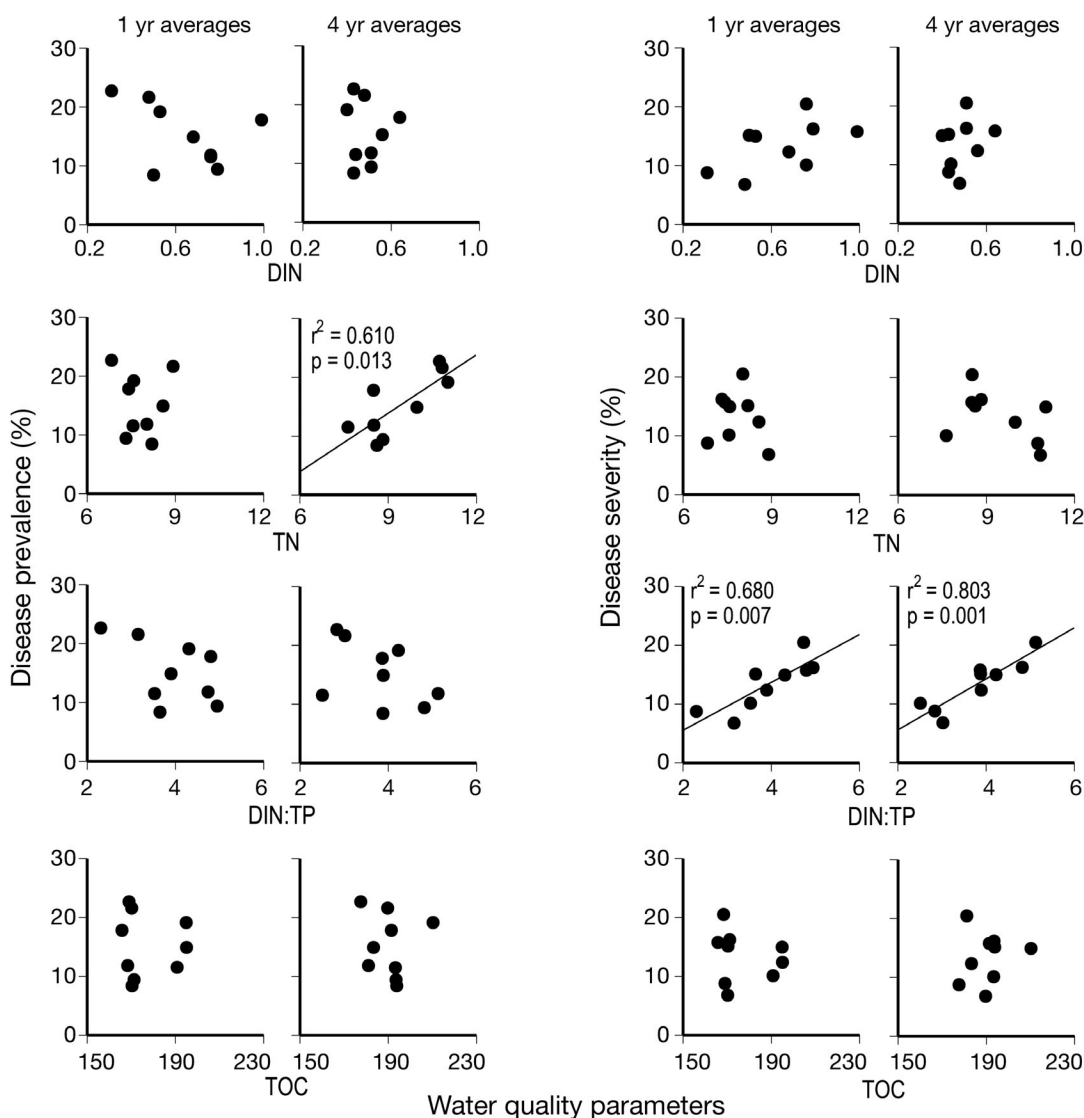


Fig. 2. Aspergilliosis prevalence and severity as a function of 1 and 4 yr mean water quality variables: DIN, TN, TOC (all in μM) and the DIN:TP ratio

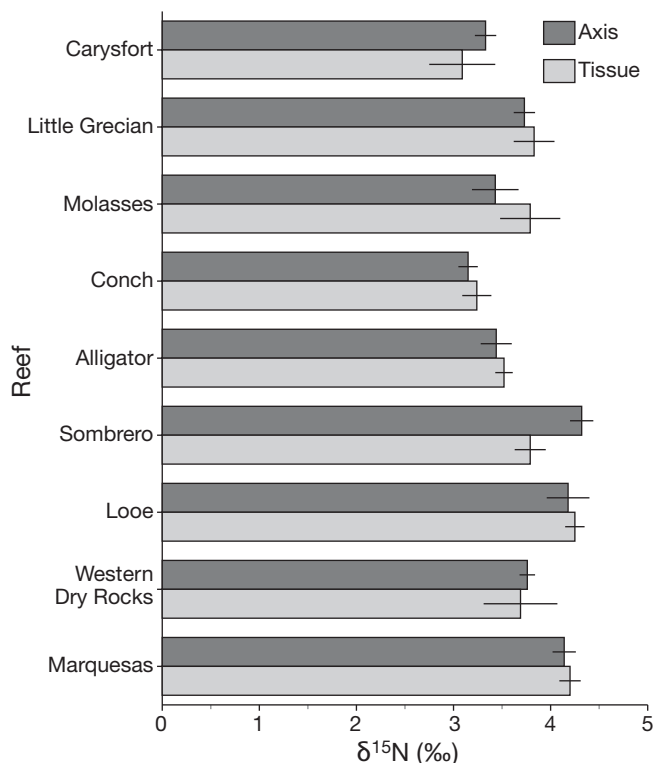


Fig. 3. Mean \pm SE tissue and axis $\delta^{15}\text{N}$ for 9 sites in the Florida Keys reef tract, sorted from upper to lower Keys

DISCUSSION

Environmental drivers of aspergillosis

For terrestrial systems (plants and animals), the direct impacts of N availability on the virulence of fungal and bacterial pathogens, and the molecular genetic mechanisms involved, are well documented (reviewed by Snoeijs et al. 2000). These studies show that nitrogen limitation, which can occur as a result of a lack of N availability in the environment, can decrease disease development in plant–pathogen interactions. Recently, Bruno et al. (2003) showed that disease progress in corals *Gorgonia ventalina* and *Montastrea* spp. increased with elevated nutrient levels. Although this study provides support for the hypothesis linking N and diseases in corals, the unusually high concentrations (as much as 40 \times for nitrate and ammonium, and >40 \times for P over 3 mo) are problematic for the extrapolation of these results in a non-experimental (i.e. ‘natural’) environment.

Our study shows that, in the Florida Keys, sea fan aspergillosis is related to N concentration and relative availability: disease prevalence increased with increasing TN, while disease severity increased with an increasing DIN:TP ratio (Fig. 2). We suggest that

this complex interaction reflects the differential utilization of N and the variety of forms available in the environment to the pathogen and host. For instance, a positive relationship between TN concentration and disease prevalence is consistent with what is known about the role of N on host–pathogen interactions (see above). Given the metabolic diversity of fungi with respect to N (Snoeijs et al. 2000), the high levels of N in the water column likely promote the growth of *Aspergillus sydowii*, and thus increase the likelihood of colonization of susceptible hosts (i.e. disease prevalence).

Once established in the coral host, *Aspergillus sydowii* migrates into the axial skeleton (Petes et al. 2003) where it derives N and other nutrients from the degradation of host tissue/skeleton. Thus, water column TN levels are unlikely available to the fungus, and are therefore not important for disease progress (i.e. disease severity) (Fig. 2). Instead, we hypothesize that elevated nutrient levels directly affect the coral host. The positive relationship between the DIN:TP ratio and disease severity likely reflects increasing stress on the coral–zooxanthellae mutualism as forms of N available to the zooxanthellae—nitrate and ammonium—become more abundant. The generally nutrient-limited zooxanthellae appear to favor growth under N (but not P) enrichment, utilizing the fixed carbon rather than translocating it to the host (Muscatine et al. 1989). This shift is thought to result in nutritional stress in the host, and is consistent with the finding of increased mortality under nutrient enrichment in the Caribbean scleractinian *Acropora cervicornis* (Rene-gar & Riegl 2005).

Alternatively, the relationship between disease and N may not be causative but correlative. For instance, if *Aspergillus sydowii* enters via land-based sources of pollution, then coastal waters with high terrestrial inputs would contain a greater abundance of the pathogen in addition to higher concentrations of N. For instance, wastewater was found to carry *Serratia marscecens*, a human enteric bacterium, which was discovered to be the causative agent of white pox disease of Caribbean acroporid corals (Patterson et al. 2002). In addition, terrestrial inputs may contain toxins, pharmaceuticals, and other microbes that may increase disease prevalence and severity (Pastorok & Bilyard 1985, Costanzo et al. 2005).

Recent evidence suggests that organic carbon may compromise coral health. Kline et al. (2006) suggested that simple sugars and starch increase coral mortality by disrupting the coral surface microbial community. However, we did not detect a significant relationship between TOC and either prevalence or severity. One possible explanation for our finding is that we used TOC rather than DOC levels; however, given that

there is no significant difference between TOC and DOC in the Florida Keys (J. Boyer pers. comm.), we believe this issue to be of little relevance. Perhaps more important to this discussion is the difference in organic carbon concentrations between experimental and natural conditions. In their work, Kline et al. (2006) used DOC concentrations that are ~2 to 10× (415 to 2083 μM) higher than TOC concentrations recorded at our sites in the Florida Keys (Table 1). Although there may be pulses of organic carbon that are substantially higher than the means reported in this study, such pulses are rare in the Florida Keys. For instance, TOC measurements of >400 and >2000 μM represent 2.7 and 0%, respectively, of the 6698 measurements taken in the Florida Keys National Marine Sanctuary between 1995 and 2006 (see <http://serc.fiu.edu/wqmnetwork>). While simple sugars and starch may disrupt coral microbiota and cause mortality in a laboratory setting, it is unlikely that similar conditions (i.e. 30 d at 2000 μM DOC) would occur in the natural environment and be detrimental to corals.

$\delta^{15}\text{N}$, nutrient levels and disease

In the Florida Keys, N contributions to shallow water habitats (<30 m in depth) along the reef tract are dominated by sewage-derived N from poor on-site sewage disposal coupled with highly seasonal populations (Lapointe 2004). According to Lapointe (2004), $\delta^{15}\text{N} > 3.0\text{‰}$ in macroalgae is indicative of wastewater N. The $\delta^{15}\text{N}$ of sea fan tissue and axis recorded in this study (all >3.0‰) supports the hypothesis that Florida sea fans are exposed to sewage-derived N. This is further supported by comparison of these results with the $\delta^{15}\text{N}$ of *Gorgonia ventalina* from a more pristine location, San Salvador, Bahamas, which were relatively depleted by 1.1‰ on average (D. M. Baker unpubl. data).

Several studies have shown that severity of coral disease can be exacerbated by sewage-derived material (Walker & Ormond 1982, Kaczmarek et al. 2005). However, when $\delta^{15}\text{N}$ of *Gorgonia ventalina* was used as an indicator of sewage inputs (e.g. Heikoop et al. 2000), a relationship between sewage and either disease prevalence or severity was not detected. This lack of fit suggests that perhaps the sewage inputs in the Florida Keys do not play a role in the dynamics of aspergillosis. If this is correct, given the link between disease and N (Fig. 2), it would mean that the sewage inputs are not significant sources of N on these reefs. However, it is possible that $\delta^{15}\text{N}$ levels in sea fans do not accurately reflect levels of sewage N inputs in the environment. In the Florida Keys, sea fans can assimilate N from a variety of sources including upwellings (enriched in ^{15}N), agricultural run-off (depleted), and wastewater (highly enriched). Although Lapointe (2004) concluded that sewage-derived N is dominant in all shallow water areas (<30 m), increased agricultural effluents that enter the Florida Bay during the rainy season can suppress $\delta^{15}\text{N}$ values, particularly among sea fans along the western portions of the reef tract (i.e. middle to lower Keys) (Lee et al. 2002). Our data also suggest that local N limitation may affect isotopic discrimination during uptake by *G. ventalina*. Sea fans from the Marquesas were among the most enriched while also being the farthest offshore (~45 km west of Key West) and exposed to lowest levels of N (Table 1). This pattern suggests N limitation that reduces discrimination against ^{15}N during uptake and assimilation and results in higher $\delta^{15}\text{N}$ values.

The possibility of using $\delta^{15}\text{N}$ values as proxies for environmental concentrations of TN was suggested by Ward-Paige et al. (2005), who found that $\delta^{15}\text{N}$ of the gorgonian coral *Plexaura* spp. was strongly related to 5 yr averages of TN in the Florida Keys. We did not find this to be the case for *Gorgonia ventalina*.

Table 1. Site-specific water quality, aspergillosis, and sea fan $\delta^{15}\text{N}$ data for 9 reefs along the Florida Keys reef tract. Water quality data are 15 survey (4 yr) averages from Southeast Environmental Research Center (see <http://serc.fiu.edu/wqmnetwork>). Prev: prevalence; Sev: severity. Errors are \pm SE of mean

Reef	TN (μM)	DIN (μM)	TOC (μM)	DIN:TP ratio	Prev (%)	Sev (%)	$\delta^{15}\text{N}$ axis (‰)	$\delta^{15}\text{N}$ tissue (‰)
Carysfort	8.83 \pm 0.93	0.51 \pm 0.09	194 \pm 17	4.8 \pm 1.8	9.4 \pm 0.6	16.2 \pm 6.2	3.3 \pm 0.11	3.4 \pm 0.04
Little Grecian	8.62 \pm 0.62	0.43 \pm 0.04	194 \pm 14	3.9 \pm 0.9	8.4 \pm 1.0	15.1 \pm 5.9	3.7 \pm 0.11	3.8 \pm 0.21
Molasses	8.52 \pm 0.59	0.51 \pm 0.07	181 \pm 12	5.1 \pm 2.0	11.8 \pm 4.1	20.5 \pm 2.7	3.7 \pm 0.04	3.8 \pm 0.31
Conch	8.51 \pm 0.74	0.64 \pm 0.14	191 \pm 20	3.9 \pm 0.8	17.8 \pm 1.9	15.7 \pm 3.8	3.2 \pm 0.14	3.2 \pm 0.15
Alligator	10.84 \pm 0.84	0.48 \pm 0.08	190 \pm 15	3.0 \pm 0.5	21.6 \pm 5.1	6.8 \pm 1.0	3.4 \pm 0.16	3.5 \pm 0.09
Sombrero	10.75 \pm 0.82	0.43 \pm 0.06	178 \pm 12	2.8 \pm 0.4	22.7 \pm 3.9	8.8 \pm 3.5	4.3 \pm 0.12	3.8 \pm 0.16
Looe	9.98 \pm 0.77	0.56 \pm 0.13	183 \pm 12	3.9 \pm 1.0	14.9 \pm 8.6	12.4 \pm 4.5	4.2 \pm 0.22	4.2 \pm 0.16
Western Dry Rocks	11.03 \pm 0.78	0.40 \pm 0.05	210 \pm 12	4.2 \pm 1.4	19.1 \pm 2.6	14.9 \pm 1.6	3.8 \pm 0.03	4.1 \pm 0.16
Marquesas	7.64 \pm 0.81	0.44 \pm 0.07	193 \pm 27	2.5 \pm 0.4	11.5 \pm 0.6	10.1 \pm 0.1	4.1 \pm 0.12	4.2 \pm 0.11

and 4 yr averages of TN. It is unclear what is responsible for the lack of congruence between the 2 studies, but it may owe to the interactions between N sources and concentrations, and species-specific differences in ^{15}N discrimination. Thus, the use of $\delta^{15}\text{N}$ values as proxies for environmental concentrations of N should await additional support. Nonetheless, the ability to reconstruct historical trends using growth rings (Ward-Paige et al. 2005) or museum samples makes isotopic analysis of gorgonian corals a potentially valuable tool with which to improve our understanding of changes in anthropogenic inputs of N in coastal marine ecosystems.

Acknowledgements. This study was supported by NSF-OCE-9818830, NURC-UNCW 9821 (from NOAA's Undersea Research Center at the University of North Carolina at Wilmington pursuant to NOAA award number NA03OAR4300088), an American University Senate Grant to K.K., and an American University Department of Biology Helmlinge Student Research Award to D.M.B. Collections were carried out under US National Marine Sanctuary Permit FKNMS-286-97 and Florida Department of Environmental Protection Permit 98S-334. Water quality data were provided by the SERC-FIU Water Quality Monitoring Network, supported by SFWMD/SERC Cooperative Agreements No. C-10244 and C-13178 and EPA Agreement No. X994621-94-0. Comments from and discussions with C. Harvell, R. Howarth, E. Kim, S. Macko, J. Sparks, J. Andras, K. Rypien, and J. Ward are greatly appreciated.

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Editorial responsibility: Charles Birkeland (Contributing Editor), Honolulu, Hawaii, USA

*Submitted: September 5, 2006; Accepted: February 15, 2007
Proofs received from author(s): July 24, 2007*