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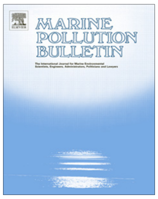

Link between sewage-derived nitrogen pollution and coral disease severity in Guam

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ABSTRACT

The goals of this study were to evaluate the contribution of sewage-derived N to reef flat communities in Guam and to assess the impact of N inputs on coral disease. We used stable isotope analysis of macroalgae and a soft coral, sampled bimonthly, as a proxy for N dynamics, and surveyed *Porites* spp., a dominant coral taxon on Guam's reefs, for white syndrome disease severity. Results showed a strong influence of sewage-derived N in nearshore waters, with $\delta^{15}N$ values varying as a function of species sampled, site, and sampling date. Increases in sewage-derived N correlated significantly with increases in the severity of disease among *Porites* spp., with $\delta^{15}N$ values accounting for more than 48% of the variation in changes in disease severity. The anticipated military realignment and related population increase in Guam are expected to lead to increased white syndrome infections and other coral diseases.

1. Introduction

In nearshore systems, sources of nitrogen (N) include land-based run-off, offshore upwelling, and atmospheric deposition. As in many places around the world, the last century has seen an unprecedented increase in N inputs from land-based sources to the nearshore waters around Guam, first from synthetic fertilizer use in agriculture beginning in the 1950s and more recently from land-use shifts resulting in huge increases in sewage inputs from expanding coastal populations (Baker et al., 2010). N pollution in nearshore systems leads to eutrophication, which can alter ecosystem structure and function (Howarth et al., 2000), including phase shifts in which reefs once dominated by corals become dominated by algae (Hughes et al., 2007). In addition, there is evidence to suggest that corals are directly affected by elevated N in the environment. In a review of the literature, Fabricius (2005) noted that nutrients (both N and P) negatively affect coral physiology by reducing calcification rates, fecundity, fertilization success, and larval development.

N has also been suggested as an important influence on coral diseases. For instance, proximity to sources of sewage-derived material is thought to be responsible for increases in the severity of black-band and white plague diseases of scleractinian corals (Kaczmarsky et al., 2005; Walker and Ormond, 1982). Similarly, Kim and Harvell (2002) suggested that aspergillosis of sea fans was correlated with dissolved inorganic nitrogen (DIN), and Kuta and 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. Similarly, Baker et al. (2007) found a positive relationship between disease severity and the ratio between dissolved inorganic nitrogen and total phosphate (DIN:TP).

Determining the provenance of N from various point and non-point sources is necessary for managing and controlling the impacts of N pollution on health of coastal environments. Quantifying the ratio of the isotopes of N ($^{15}N/^{14}N$) relative to a standard (i.e., $\delta^{15}N$) has become a particularly useful tool in this regard because trophic enrichment and microbial processing result in high $\delta^{15}N$ in human sewage effluents (Savage, 2005), whereas nitrogenous fertilizers and atmospheric deposition yield ^{15}N-depleted

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compounds that result in lower δ^{15}N values (Barile, 2004; Marion et al., 2005). For example, in a retrospective analysis of corals from the ENCORE experiments conducted on One Tree Island, Australia, Hoegh-Guldberg et al. (2004) documented a significant drop in δ^{15}N, from 3.5‰ to 1.0‰ in both corals and their symbionts, from exposure to synthetic nitrogenous fertilizers. Nitrate in raw or partially treated sewage-contaminated groundwater can have δ^{15}N values much greater than 10‰ (Katz et al., 2004). In contrast, N from upwelling has lower δ^{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, δ^{15}N can be used to track N pollution on coral reefs (Risk, 2009; Risk et al., 2009), and sewage-derived N can be easily distinguished from natural marine sources and fertilizer, especially when sewage N comprises a major proportion of the total N pool. It should be noted that interpreting δ^{15}N values requires some understanding of N sources contributing to the N pool in a given area. In particular, Baker et al. (2010) note that in order to use δ^{15}N as a correlate for N source, one must assume that the local pool of N is derived from a single dominant source; otherwise, mixing among sewage, agricultural effluents and other N sources can make interpreting δ^{15}N problematic.

The main objectives of the current study were to evaluate the contribution of sewage-derived N to coastal dynamics of Guam's reef flat communities using stable isotope analysis and to assess the impact of elevated N on white syndrome, a coral disease. Guam is the southernmost island of the Mariana Islands chain, and is the largest (541 km^2) and most populated of this island chain, with more than 180,000 residents (Fig. 1). Guam supports more than 100 km^2 of fringing, patch, and barrier reefs that encircle the island, as well as over 100 km^2 of coral on offshore banks (Kirkendale, 2003). These reefs are home to more than 400 species of corals and 1000 species of fish (Myers and Donaldson, 2003). In total the coral reefs of Guam comprise an economic value of US$127 million a year in tourism and fishing revenue (van Beukering et al., 2007). Despite the economic importance of Guam's reefs, the past 40 years have witnessed a steady decline in their health and vitality (Bruno et al., 2007; Colgan, 1987). The most urgent threats are low water quality, predator outbreaks (e.g., Crown-of-Thorns), overfishing, and, more recently, disease (Burdick et al., 2008).

The primary reef builder and most abundant coral in Guam's reefs is the genus *Porites*; it is also the genus most affected by

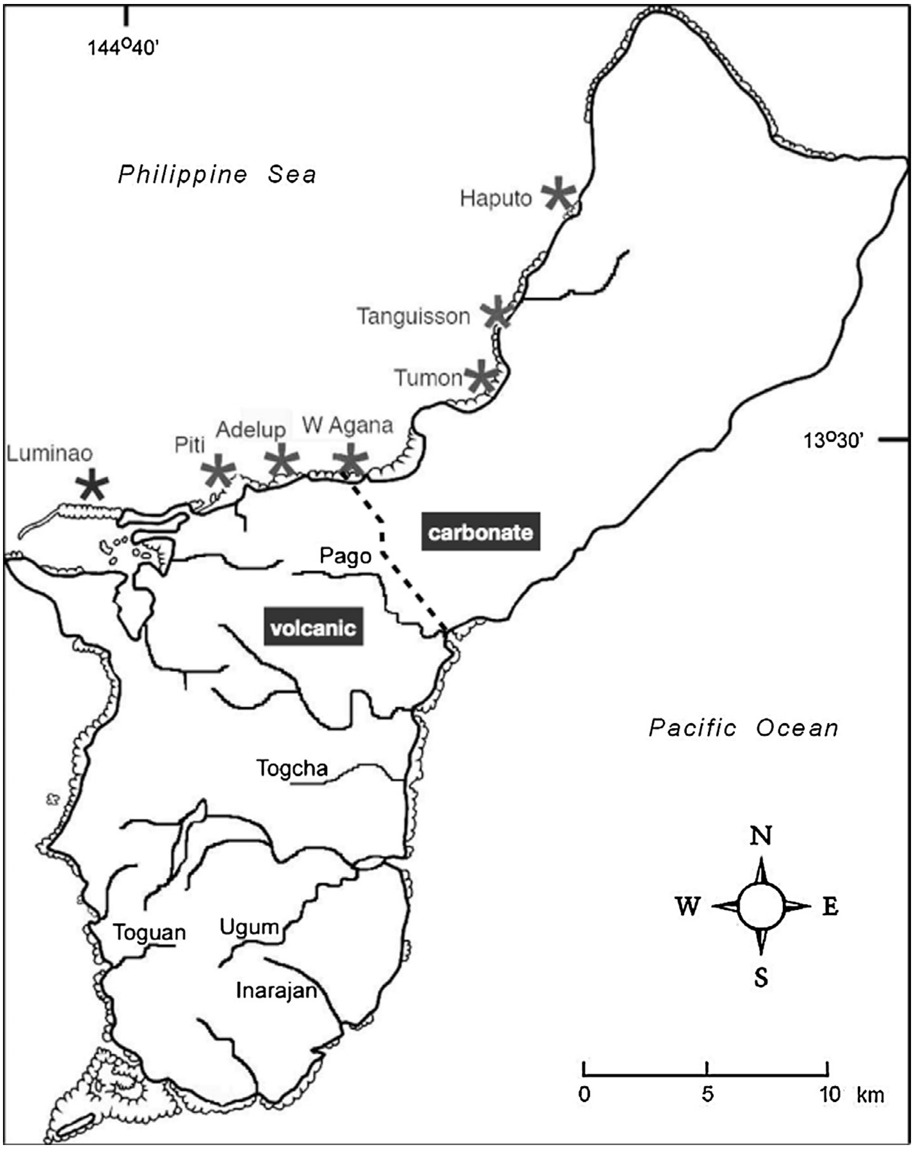

Fig. 1. Map of Guam showing location of monitoring sites. Site abbreviations: Luminao (LUM), Piti (LIT), Adelupe (ADE), West Agana (WAG), Tumon (TUM), Tanguisson (TAN), Haputo (HAP). Dashed line notes the approximate boundary between carbonate and volcanic terrain. (see Taborosi et al., in press).

disease (Burdick et al., 2008; Myers and Raymundo, 2009). Average coral cover on Guam in the 1960s was roughly 50% (Randall, 1971), but it had declined to 26.1% ± 3.6% (mean ± SE) by 2005 (Burdick et al., 2008). Because of repeated Crown-of-Thorns outbreaks on fore reefs, most of Guam's remaining coral communities are limited to reef flat zones, where they are most vulnerable to land-based influences. Approximately 3.7% of the landmass of Guam is used as harvested cropland, thus there should not be a large influx of fertilizer to its coastal waters. However, Guam's wastewater is subject only to primary- or secondary-treatment (i.e., no nutrient removal) and thus, the N pool in coastal waters is likely to be dominated by sewage-derived N.

2. Materials and methods

2.1. Study sites

Geologically, Guam consists of exposed volcanic rock in the southern part of the island and exposed limestone plateau in the north (Gingerich, 2003). Because of the higher permeability of the limestone plateau, there are no permanent rivers or other surface drainage sites in the northern half of the island. This area is comprised of large federal land holdings and is home to approximately 43% of the island's residents (Crossett et al., 2008). Central Guam is the most developed and urbanized, and includes the core tourist area at Tumon Bay. The Tamuning district, which is adjacent to Tumon, is home to 11% of Guam's population (Crossett et al., 2008). In contrast, the southern portion of Guam has major rivers and embayments. Poor land use practices in the headwaters of these watersheds are the primary source of silt and sediment that have impacted southern reefs.

To avoid the confounding effect of siltation from rivers, we focused this study on reefs along the north-western coast of Guam (Fig. 1). Luminao is the most "off-shore site," located on the seaward margin of Apra Harbor, the main port in Guam. Piti, Adelup, West Agana, and Tumon are near highly populated areas. For instance, Tumon Bay, known as "hotel row," is the center of tourism; it is also one of Guam's five marine preserve sites. The northernmost site, Haputo, is situated in an Ecological Reserve Area adjacent to land that is largely uninhabited and difficult to access. Thus, we predicted that Luminao and Haputo would be the least affected by sewage-derived N. West Agana and Tanguisson Reefs are each close to the ocean outfall of waste water treatment plants. Both plants provide primary treatment of sewage (thus no treatment for nutrients), discharging effluent through newly constructed outfall pipes into the Philippine Sea approximately 640 m offshore at a depths of 45 m (Navy, 2010).

2.2. Study species, sample preparation and analysis

Macroalgae have been used in nutrient pollution studies employing stable isotope analysis because their relatively fast growth rates and high tissue turnover rates mean that they provide a snapshot of water quality at the time of sampling and can reveal short-term changes in nitrogen associated with episodic rainfall or peak tourist visits (Gartner et al., 2002; Lapointe et al., 2004). Soft corals, on the other hand, have relatively slower growth and turnover rates, and thus provide a longer-term picture of N dynamics. Sampling a combination of the two groups provides information on both short- and long-term incorporation of nutrients into tissues and, therefore, both short- and long-term assessments of predominant N sources (Risk et al., 2009).

Preliminary surveys of the seven study sites showed that the macroalgae *Caulerpa serrulata* and *Halimeda micronesica* were found in all locations. The soft coral *Sinularia polydactyla* was found in five out of the seven sites; no other soft coral was present consistently. Starting in February 2009, and continuing approximately every other month for one year, *C. serrulata*, *H. micronesica*, and *S. polydactyla* were sampled ($n = 3$ per species) at each of the reef study sites (all less than 2 m depth). The samples were oven-dried for 48 h at 50 °C, vacuum-packed, and stored at −20 °C until all samples were collected. This allowed the samples to be analyzed at the same time, thus reducing random sources of variation during analysis.

Each sample was individually ground with a mortar and pestle until homogenized into powder. Then the fine powder was weighed into a 4×6 mm silver capsule to contain approximately 1.0 mg (±0.15) of coral tissue or 2.0 (±0.15) mg of macroalgal tissue. The unpackaged silver capsules were fumigated in a glass desiccator containing 12 N hydrochloric acid for 48 h to remove the carbonates from the samples (Hedges and Stern, 1984). The efficacy of the treatment was tested by looking for bubbling when HCl was added drop-wise directly to the samples. The samples were dried at 80 °C for 48 h prior to combustion in a Carlo-Erba NC2500 elemental analyzer coupled, through a Conflo III open-split interface, to a ThermoDelta V Isotope Ratio Mass Spectrometer. This portion of the work was carried out at the Carnegie Institution of Washington in Washington D.C., USA. Results are reported as δ^{15}N values relative to atmospheric N_2; precision was determined by analysis of an in-house acetanilide standard (ACET-5) which was <0.2‰.

2.3. Coral disease surveys

The coral disease severity data were derived from an on-going effort in Guam (Myers and Raymundo, 2009). Three belt transects (20 m × 2 m) were permanently established within the reef flat zone at each site. The transects were laid parallel to shore in areas of highest coral cover at 1–4 m depth and were spaced approximately 10–20 m apart. We focused on disease severity because the effects of N pollution appear to manifest in increased disease severity (i.e., area of tissues affected by disease) but not prevalence (i.e., proportion of corals with disease signs) (Baker et al., 2007; Bruno et al., 2003). Disease signs include multifocal, irregular lesions characterized by rapid tissue loss that exposes bare skeleton which is rapidly colonized by turfing algae.

Disease severity was assessed at each site by identifying and tagging diseased *Porites* spp. colonies and determining the percent of each tagged colony affected by disease (total number of colonies monitored: $n = 128$). Colonies were then monitored every other month in order to determine the change in disease severity from the previous census. Species observed within our sites were *Porites cylindrica*, *P. rus*, *P. annae*, and massive *Porites*, most likely *P. lutea* (Randall and Myers, 1983). We did not attempt to identify the massive colonies to species for this study. Disease severity data were used to calculate change in severity from each monitoring census to the next (averaged by site). The change in disease severity for each sequential set of censuses was analyzed as a function of δ^{15}N values of algae and soft coral samples collected at the same site during first monitoring census of that set.

2.4. Rain data

Monthly precipitation data were provided by the National Climatic Data Center and collected at Guam International Airport (centrally located relative to our reef sites).

2.5. Statistical analysis

Replicate δ^{15}N values from algae and soft coral samples were averaged as a function of species ($n = 3$), reef site ($n = 7$ for macroalgae *C. serrulata* and *H. micronesica*; $n = 5$ for soft coral *S. polydactyla*), and sampling period ($n = 7$), resulting in a sample size of 121. These data were tested for homogeneity of variance (Levene Test) and normality (Kolmogorov) and log-transformed prior to statistical analyses, including Pearson's *r*, ANOVA and regression analyses. For ANOVA, we first used a 3-way (species × site × date) model with full interactions. However, none of the interaction terms were significant. Therefore, based on the recommendation of Underwood (1997), we removed terms with *p*-values greater than 0.25 (i.e., species × date and species × date × site, but not species × site) and repeated the ANOVA.

3. Results

3.1. Isotope analysis and nitrogen pollution

Stable isotope analyses of soft coral and macroalgae samples suggested varying levels of N enrichment in the nearshore waters of Guam and a strong influence of sewage-derived N. ANOVA indicated δ^{15}N values varied as a function of species ($F = 25.0$, $p < 0.001$), site ($F = 50.1$, $p < 0.001$), and date ($F = 3.42$, $p = 0.004$). δ^{15}N values ranged from a low of 0.9‰ (for *Caulerpa serrulata*, Piti, October-2009) to a high of 8.3‰ (for *Sinularia polydactyla*, Tumon, October-2009). On average, the soft coral *S. polydactyla* was the most enriched (mean ± SEM; 5.2‰ ± 0.20‰, $n = 89$) followed by *C. serrulata* (4.0‰ ± 0.12‰) and *H. micronesica* (3.4‰ ± 0.13‰) (Fig. 2).

Samples from Tumon (near the center of tourism on the island) were consistently the most enriched, across all species; δ^{15}N values declined both northward and southward along the west coast of Guam (Fig. 2). There was a greater than two to threefold difference, depending on the species examined, in δ^{15}N values between the most (Tumon) and least (Luminao) enriched sites. δ^{15}N values also varied temporally. The three species showed some correspondence in δ^{15}N values over the course of the sampling period, with peaks occurring in the months of February and July–August of 2009 (Fig. 3).

Isotopically, *H. micronesica* and *S. polydactyla* were quite similar ($r = 0.879$, $n = 31$, $p < 0.001$), albeit marked by an isotopic shift of +1.7‰ for the soft coral. The relationships for other combinations of species are as follows: *H. micronesica* and *C. serrulata* ($r = 0.673$, $n = 43$, $p < 0.001$), *S. polydactyla* and *C. cerrulata* ($r = 0.608$, $n = 27$, $p = 0.001$). All pair-wise comparisons, therefore, showed some level of consistency in δ^{15}N values.

Finally, we found that rainfall was positively correlated with δ^{15}N values (Fig. 4). However, this effect was only significant for *S. polydactyla* samples.

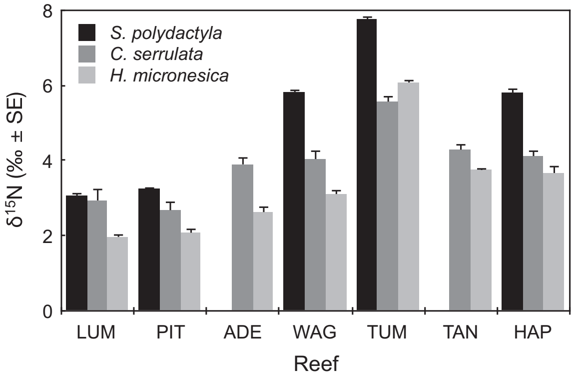

Fig. 2. δ^{15}N values by species and across sites. The sites are arranged from south to north (refer to Fig. 1).

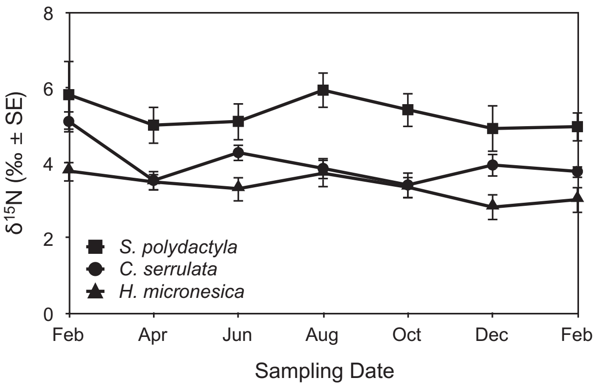

Fig. 3. δ^{15}N values by species and across time, for seven bimonthly sampling periods.

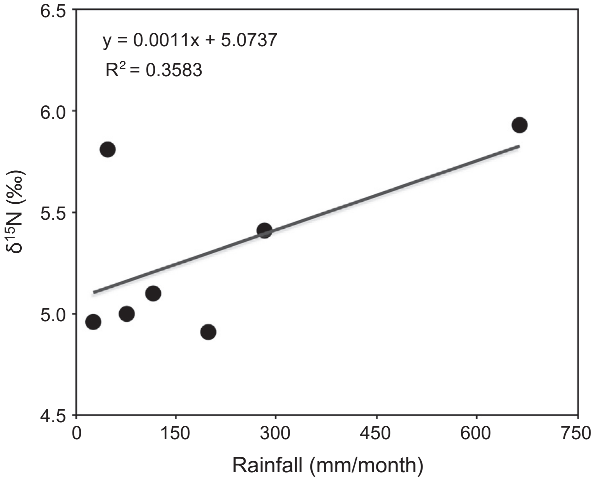

Fig. 4. Relationship between rainfall and stable isotope values for *Sinularia*. Note the high value off the regression line is for October 2009 when typhoon Melor passed in close proximity to Guam. Although this storm did not result in greatly increased rainfall, it did cause greater than normal wave action and turbulence along the coast.

3.2. Disease severity and δ^{15}N

For all three species sampled for isotope analyses, significant positive relationships were detected between δ^{15}N values and disease severity in *Porites* species (Fig. 5). In particular, δ^{15}N values for *Sinularia polydactyla* explained more than 48% of the variation in disease severity over time.

4. Discussion

The goals of this study were to investigate the extent to which sewage-derived nitrogen (N) was entering and impacting the coral reefs in Guam. The results point to a strong influence of sewage-derived N that is worsening the impact of disease on the corals. Land-based nutrients have been influencing coastal fringing reefs in Guam for decades. One of the earliest studies examined the terrestrial inputs of nitrogen and phosphorus to reefs and determined that groundwater seepage could be an important source of terrestrial nutrients, particularly from the northern karst topography (Marsh, 1977). Indeed, Matson (1991) concluded that nutrient dynamics in the northwest region of Guam were dominated by discharge from aquifers. At present, the inadequate and poorly maintained wastewater infrastructure, coupled with increasing

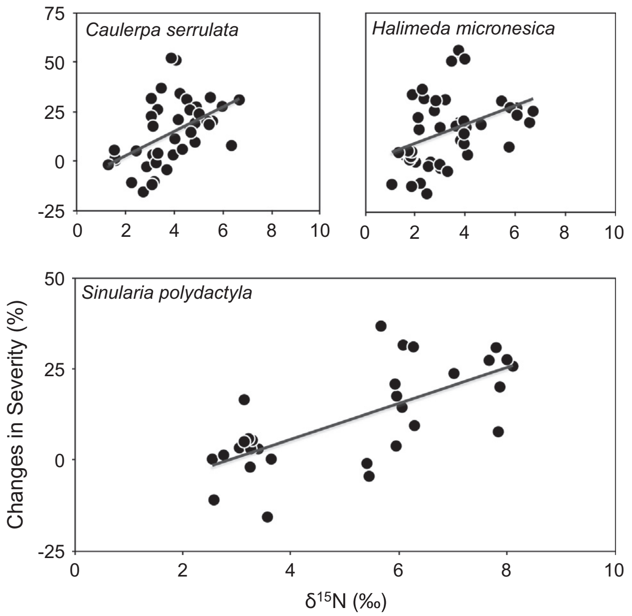

Fig. 5. Changes in disease severity in *Porites* spp. (%) as a function of isotopic value. Positive values for changes in disease severity reflect increase in tissue affected by disease from one survey to the next and negative values indicate a decrease in affected tissue (i.e., recovery). Regression statistics are as follows: *C. serrulata* ($y = 6.16x + 9.67$, $R^2 = 0.244$, $p < 0.001$); *H. micronesica* ($y = 4.74x + 0.014$, $R^2 = 0.151$, $p = 0.002$); *S. polydactyla* ($y = 4.94x + 14.1$, $R^2 = 0.484$, $p < 0.001$).

population, has resulted in accelerated stress to the near-shore environment. In spite of recent infrastructure upgrades, including replacing leaky sewage pipes with new pipes extending farther offshore, and the proximity of Guam's reef flats to open ocean waters and flushing from daily tidal activity, δ^{15}N values documented in this study point to significant inputs of sewage-derived N from terrestrial sources.

4.1. Species variation in δ^{15}N

We sampled several species common to the reefs of Guam to evaluate N sources in Guam's coastal water. For algae, values >3.0‰ have been suggested as a threshold for sewage-derived N (Lapointe et al., 2004). The threshold for the soft coral is higher (>4.0‰) because of the mixotrophic nature of this soft coral species (Fabricius and Klumpp, 1995). If these thresholds are applied, there is strong evidence of dominance by sewage-derived N on the coral reefs we studied, especially in Tumon (Figs. 2 and 3). From Tumon, δ^{15}N values declined both north- and southward along the coastline, with the least enriched samples from two southernmost sites, Luminao and Piti. The strongest signal of sewage-derived N did not occur nearest the two sewage outfalls—at West Agana and Tanguisson reefs—as might be expected (Risk et al., 2009). Current patterns along the coast may explain this. According to Wolanski et al. (2003), this coast of Guam is dominated by longshore currents that drive coastal surface waters south of Tumon Bay in a southwestern direction and coastal surface waters north of Tumon Bay in a northeastern direction. Thus, sewage from the Hagatna wastewater treatment plant, which is discharged via an offshore outfall near our West Agana site, is diverted southward along the coast, away from our site, while water from the Tanguisson outfall, which is discharged via an offshore outfall near our Tanguisson site, would be driven north, toward Haputo. These longshore currents would serve to dilute the sewage-derived N discharged from the wastewater treatment outfalls.

High δ^{15}N values at the Tumon site most likely result from additional and highly localized inputs of sewage-derived N, including a combination of terrestrial run-off, documented overflows from the inadequate sewage delivery system from this highly populated and developed tourism district (J. Shane, Guam Waterworks Authority, pers. comm., 2009), and groundwater seeps and springs, which manifest visibly throughout this large, shallow embayment (Taborosi et al., 2004). It is well documented that Tumon Bay is subject to periodic blooms of the filamentous green alga, *Enteromorpha* (now *Ulva*) *intestinalis*, with N concentrations reaching as high as 8.4 mg L^{-1} (Denton et al., 2005). Tumon is situated on a permeable karst limestone through which groundwater crosses numerous septic tanks, injection wells, and leaky sewer lines before entering the ocean (Denton et al., 2005; Marsh, 1977; Taborosi et al., 2004). The discharges are maximal during the wet season (Matson, 1993) and can contribute more than 150 million L of groundwater into the bay each day (Jocson, 1998). In comparison, the two primary wastewater treatment plants were designed to treat 15 million L d^{-1} (GWA, 2007). The significant role of groundwater in Tumon as well as other northern coastal areas is supported by our finding that δ^{15}N values were still relatively high in Haputo, a site with little or no human influence or river inputs, but with groundwater seeps and springs. Further, the significant correlation with rainfall suggests other paths of entry for sewage into coastal systems, such as septic tank leakage and overflow from inadequate and poorly maintained sewage pipes, both of which are transported to coastal areas by runoff, and groundwater seeps or river discharge during heavy rains. In contrast, Luminao which had the lowest δ^{15}N values is adjacent to a causeway and thus, least affected by surface or ground water discharges.

In addition to providing information about N sources on the reefs, we wanted to assess the utility of the three species as integrators of N fluxes on times scales relevant to ecological processes such as coral disease dynamics. Although we found correspondence between disease severity of *Porites* and δ^{15}N values for all three species (*r* values ranging from 0.608 to 0.879; Fig. 5), only δ^{15}N values from the soft coral *Sinularia polydactyla* were significant predictors of disease severity. This finding likely reflects the mismatch between the relatively short δ^{15}N turnover rates in the algae, which can probably grow several millimeters per day (Li et al., 1998; Drew, 1983), and the bimonthly frequency of our disease monitoring efforts. In contrast, members of the soft coral genus *Sinularia* probably grow only a few centimeters per year (Bastidas et al., 2004; Fabricius, 1995). Given that white syndrome can take up to two weeks to develop in laboratory transmission experiments and progresses at an average rate of 0.7 cm^2 d^{-1} (Lozada, 2011), a longer-term integrator of N dynamics, like *Sinularia*, is better suited to the bimonthly monitoring and sampling regime used in this study.

4.2. δ^{15}N and coral disease

There is growing evidence that N pollution has a direct effect on the health of coral reefs. Here, we showed δ^{15}N values are a strong predictor of white syndrome disease severity in *Porites* (Fig. 5). This finding is consistent with previous studies linking nutrient pollution and disease progression and severity (Baker et al., 2007; Bruno et al., 2003).

The mechanism by which elevated nitrogen directly affects coral health remains poorly understood. An emerging hypothesis, proposed by Wooldridge (2010), is based on the premise that the mutualism between the coral host and zooxanthellae is a "controlled parasitism" in which zooxanthellae are farmed for their photosynthates and anything that undermines host control can lead to the breakdown in the relationship. Wooldridge (2010) contends that factors such as increased light, water temperature, inorganic nitrogen, or pCO$_2$ can directly enhance zooxanthellae growth and reproduction, leading to loss of host control of the symbionts.

The prediction that elevated N can directly undermine the symbiotic relationship was borne out in a long-term dataset from the Great Barrier Reef which revealed that reefs with chronically elevated levels of DIN had bleaching thresholds that were 2.5 °C lower than those less affected by nutrients (Wooldridge and Done, 2009).

Although the reefs in Guam are clearly in decline (Burdick et al., 2008), the role of diseases in this decline is difficult to quantify. For instance, the prevalence of white syndrome seems relatively low at approximately 9% (Myers and Raymundo, 2009) compared to approximately 31% for aspergillosis, a fungal disease affecting Caribbean sea fan corals (Kim and Harvell, 2004). However, within reef prevalence can be very high. Monitoring has revealed white syndrome prevalence in one site (Piti) was over 30% for three out of the seven census periods (Raymundo et al., 2011). Thus, while nominal on average over the course of a year, the overall impact of chronic white syndrome infections and other coral diseases may result in significant coral loss over time. Such impacts are expected to increase over the longer term because of the anticipated population increase with the realignment of military personnel currently stationed in Japan. At the height of the build-up, Guam's population may rise by nearly 80,000, an increase of 45%, with the influx of military personnel, dependents, contractors, etc. (Navy, 2010). It is unclear whether Guam's wastewater infrastructure will be able to adequately handle this population increase. Even an anticipated upgrade to secondary treatment for Guam's two wastewater treatment plants will do little to reduce N inputs, via outfall or groundwater discharge, into the coastal ecosystem.

Guam currently has designated five marine parks to help protect its reef resources, especially from overfishing. Although overfishing is a major concern, Guam's reefs face many additional stressors, both global and local. While N pollution is a local stressor and, therefore, potentially locally managed, we have focused this study on the role of N because the this local problem affects reefs around the globe (NRC, 2000). Climate change, sea level rise, and ocean acidification also affect reefs on a global scale, but they are difficult or impossible to protect from and manage locally. Furthermore, as noted by Knowlton and Jackson (2008), the existence of luxuriant and healthy reefs in some parts of the world suggests that the impact of rapid global change may be alleviated by reducing local stressors. The decline of coral reefs in Guam, as in many other places, is not driven by a single factor but by many factors acting synergistically or additively. Given the substantial ecosystem services and food security afforded by healthy and vibrant coral reefs, efforts to reduce local stressors and improve water quality must be a top priority.

5. Uncited references

Taborosi et al. (in press); Walker and Ormond (1982).

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