

# Links Between Deteriorating Coral Health and Sewage Pollution of Guam Reef Flats

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## INTRODUCTION

Coral reefs, which generally flourish in shallow oligotrophic tropical seas, are in global decline (Hughes et al. 2003) and nutrient loading is often mooted as a main cause (Szmant 2002), with other stressors such as coral diseases involved as secondary causes. Consequently, understanding the effects of increased nutrient loads on coral reef ecosystems has become a global research focus, and interest in coral diseases is growing (Ward and Lafferty 2004). Nutrients of particular interest are dissolved inorganic nitrogen (N) and phosphorus (P), which have a demonstrated effect on fertilization rates, embryonic development, and survival in several coral species (Koop et al. 2001). Elevated nutrients can also affect the rate of disease progression in diseased corals (Bruno et al. 2003). Both frequency and magnitude of coral disease have increased over the past 30 years (Hughes et al. 2003, Ward and Lafferty 2004; Reshef et al. 2006). These trends, coupled with positive associations between land-based effluents and coral disease prevalence (Kaczmarsky et al. 2005), have generated a significant research focus: understanding the relationship between coral disease and nutrient load. Because poor water quality is generally the result of local-scale processes (e.g., lack of adequate sewage treatment) a better understanding of its impacts could directly inform management policy and be put to immediate good use to improve the health of nearshore reef communities, with benefits for tourism, human health and fisheries (Coles and Ruddy 1995; Arkoosh et al. 1998; Denton et al. 2005; Sutherland et al. 2010).

In Guam, land-based influences on coastal fringing reefs have been studied for decades, and one of the earliest studies examined the terrestrial inputs of nitrogen and phosphorus on reefs (Marsh, 1977) and determined that underground seepage could be an important source of terrestrial nutrients. The effects of thermal effluent were studied first by Neudecker (1976), followed by Birkeland et al. (1979) who studied the effects of thermal effluent on coral transplants. A primary objective of that project was to reintroduce coral into an area from which it had died off. The first comprehensive description of nutrient concentrations in Guam's coastal waters was conducted by Matson (1991). Since then, much progress has been made by the Water and Environmental Research Institute (WERI) to establish assessment and monitoring of both nutrient loads and toxin levels (Denton et al. 1999; Denton et al. 2005) in Guam's coastal and ground water. Guam EPA regularly monitors various parameters for water quality in Guam's aquatic and nearshore coastal ecosystems (A. Leon Guerrero, pers comm.; GEPA 2010). The 2010 assessment listed 11 out of 24 assessed bays as "impaired", citing various fish advisories, though most of Guam's marine waters are classified as being of "good" quality (sufficient for recreational activities and harvesting). In spite of this overall classification, 752 swimming advisories were issued for 2009, based on bacterial counts in nearshore waters (GEPA 2010). Guam Waterworks Authority, the agency with responsibility over wastewater treatment, reported that the Agaña treatment plant is non-compliant with National Pollution Discharge Elimination System (NPDES) regulations for certain parameters 58% of the time. The 12 mil gal/day maximum flow rate is exceeded 25% of the time. The Tanguisson (Northern District) treatment plant exceeds all NPDES standards for all parameters from 8% to 100% of the time (GWA 2006). Furthermore, 41% of Guam homes are not on a sewage collection system and rely on septic tanks. Information about their quality, maintenance and

proper construction is unknown. It is clear that, in spite of the importance of Guam's coral reefs to its economy, links between land-based effluents and coral disease have not been assessed until recently and the limited data that we do have suggests that water quality continues to decline and is having an undocumented impact on nearshore ecosystems.

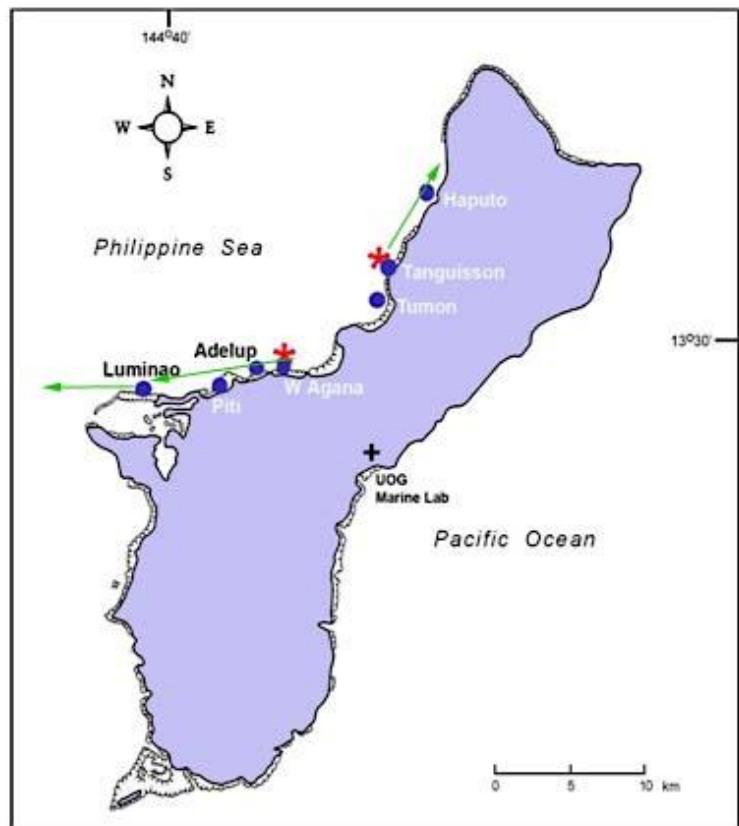
Guam's reefs are currently affected by six coral diseases, with some reefs showing over 10% of assessed corals affected by at least one disease (Myers & Raymundo 2009). Given the potential for anthropogenic nutrients to exacerbate disease severity and increase the rate of tissue loss, and in light of future increases in Guam's population, management concerns about reef health and longevity have been expressed. With the advent of a large-scale military build-up, estimated to increase Guam's population by 40,000, anthropogenic stress to Guam's reefs will increase over the next decade. In 2009, Guam Waterworks Authority renovated two primary sewage treatment plants that previously leaked untreated sewage into Guam's coastal waters. These renovations, however, only involved repair of leaks and extension of outfall pipes to deeper offshore water. Plans for updates to secondary treatment in the future are uncertain. Local managers expressed an interest in examining the impacts of sewage nutrients on nearshore coral reefs, which this study has attempted to address.

The main objectives of this project were: 1) to identify and quantify associations between nutrient load and coral health and disease on Guam reef flats, and 2) to monitor for improvements in coral health over time, as a result of the extension of the offshore outfalls. This project serves as a baseline assessment of land-based sewage impacts to coral health and disease and will hopefully provide information on the impacts of nutrient-loading on reef communities that will help guide future management practices on Guam.

## METHODS

### *Site selection and benthic composition assessment*

Seven sites, with predicted variable nutrient inputs, were selected along the western coast of Guam and monitored for 14 months (Figure 1,



**Figure 1.** Map of Guam showing seven reef flat monitoring sites (blue dots), two sewage outfall sites (red stars) and net coastal current patterns (green arrows; modified from Wolanski et al.

Table 1). Sites were originally selected to represent a potential gradient in either direction inshore from the two outfall pipes. Sites from Tumon to Luminao are all heavily influenced by anthropogenic inputs as this stretch of coastline is highly populated, and lies on predominantly volcanic bedrock, containing several small rivers that empty directly into coastal waters. Additional non-point sources of pollution along this coastline may include road run-off and septic tank and sewage line overflows into rivers. Tanguisson and Haputo, in contrast, are along a stretch of coastline that is largely uninhabited, relatively inaccessible, and without river influences, though the characteristic karst topography dominating in the northern half of the island is known to feature multiple seeps and springs (GEPA 2010). Sewage outfalls extend offshore of Tanguisson and West Agaña.

Wolanski et al. (2003) characterized the general net near-surface current pattern along this coast as a northward flowing current from Tanguisson to Haputo, and a southwestern flowing current from West Agaña Bay past Luminao. Tumon Bay represents a separation point between these two currents, where water may be deflected offshore during certain times of the year. Localized eddies created in the many shallow bays along this coast may entrain dissolved and suspended material and larvae and provide a mechanism for self-seeding, but also for pollutants to be retained locally.

**Table 1.** Site descriptions of reef flats monitored for nutrient load and coral health impacts.

| Site                           | Location                     | Distance to nearest inputs  |
|--------------------------------|------------------------------|---|
| Haputo (HAP)                   | N13° 34.655;<br>E144° 49.831 | 4.25 km from Tanguisson outfall, groundwater seeps on reef flat   |
| Tanguisson (TAN)               | N13° 32.953;<br>E144° 48.615 | 0.13 km inshore from outfall, groundwater seeps on reef flat  |
| Tumon Bay Marine Reserve (TUM) | N13° 31.009;<br>E144° 48.139 | 0.27 km from nonpoint runoff from hotels, seasonal stormwater channels from road runoff, groundwater seeps on reef flat |
| West Agaña (WAG)               | N13° 28.936;<br>E144° 44.796 | 0.51 km inshore from outfall, road runoff   |
| Adelup (ADE)                   | N13° 28.818;<br>E144° 43.690 | 2.2 km from outfall, 1.46 km from Asan River, road runoff   |
| Piti Marine Reserve (PIT)      | N13° 28.385;<br>E144° 42.261 | 1.13 km from Masso, Tatgua Rivers, 1.51 km from Asan River, road runoff   |
| Luminao (LUM)                  | N13° 27.912;<br>E144° 38.839 | 11.3 km from WAG outfall, 4.83 km from Masso, Tatgua Rivers   |

Three belt transects, each 20 m long and 2 m, wide were permanently established within the reef flat zone at each site. The transects were generally laid parallel to shore among areas of highest coral cover at 1-4 m depth and were spaced approximately 10 to 20 m apart. Coral community composition, coral disease prevalence, other coral health impacts, and disease severity were assessed along each transect bimonthly, from February 2009 to February 2010. Water temperature was monitored at three sites



(Luminao, Piti and Haputo) using submersible Hobo Tidbit data loggers<sup>®</sup>. Selected benthic organisms were sampled bimonthly for N isotope analysis (described below).

Benthic composition was assessed initially and at the end of one year using the line intercept transect (LIT) method (English et al. 1997). The following categories were quantified in terms of percent cover: live hard coral (identified to species), dead standing coral, coral rubble, pavement/rock with turf algae, soft coral, fleshy macroalgae, sand, and other benthos (sponges, ascidians).

### ***Coral health impacts assessment***

Coral disease prevalence was assessed within the 2 m belt along each transect. During bimonthly censusing, all coral colonies within the belt were counted and identified to species; colonies on the belt margin were only included if >50% of the colony lay within the belt. All diseases observed within the

belt were noted, and their hosts recorded (see Figure 2, for diseases recorded from Guam reefs). In addition to described diseases, bleaching, predation, and competitive overgrowth interactions were also assessed, following Raymundo et al. (2008). Prevalence of health impacts was quantified along belt transects using the following formula: [# colonies showing a particular impact/total # of colonies counted] x 100.

Disease severity was assessed by monitoring selected diseased *Porites* spp. colonies within each transect over the 14 month period. Colonies were tagged during the second month of monitoring and the following characteristics were noted: coral species, colony size, disease state, number of lesions, and percent of colony affected. Colonies were then monitored every month for disease, number of lesions, and percent of colony affected, to determine patterns of colony recovery, disease progression to mortality, or infection stasis over time. The genus *Porites* was selected as it is a dominant component of the community at each site, and because it has been demonstrated to be susceptible to five out of the six diseases described in Guam (Myers and Raymundo 2009). *Porites* species observed within our sites were *Porites cylindrica*, *P. rus*, *P. annae*, and massive *Porites*. Massives cannot be identified *in situ*, though previous work by R. Randall established that *P. lutea* was then the most common massive growth form on Guam reef flats (Randall and Myers 1983; Randall, pers. comm.), a situation that qualitative observations suggest has persisted until the present.

### ***Nutrient isotope sampling and analysis***

Stable isotope analysis has become a widely used and valuable tool for examining questions of nutrient enrichment, because it represents materials incorporated into the tissues of living organisms that have been directly exposed to elevated nutrient levels. Careful use of the method, along with knowledge of the nature of terrestrial impacts, can provide quantifiable information regarding the extent of nutrient enrichment from different sources. There is little agricultural activity on Guam, and most is limited to small plots in the central portions of the island. Therefore, it can be presumed that most anthropogenic N entering nearshore communities is from sewage inputs. Many Guam homes utilize septic tank systems that may be poorly maintained, and sewage pipes of Guam Waterworks Authority may also be

inadequate to accommodate sudden increases in volume, resulting in overflows or leaks into waterways or coastal areas (J. Shane, GWA 2009, pers. comm.; GWA 2011).

Preliminary surveys of the seven sites showed that the macroalgae *Caulerpa serrulata* and *Halimeda micronesica* were found in all sites, while the soft coral *Sinularia polydactyla* was found in five out of the seven, and no other soft coral was present consistently. As algae generally have faster growth and turnover rates than soft corals, 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 levels of eutrophication (Risk et al. 2009). Samples were collected simultaneously with bimonthly monitoring, placed in separate whirlpaks with fresh seawater, and transported immediately on ice in a cooler to the UOG Marine Lab. They were then cleaned of epiphytes, oven-dried at 40°C for two days, and frozen in a -80°C freezer, where they were stored until shipment to Carnegie Institute for analysis. Samples were then homogenized by grinding using a mortar and pestle. To remove carbonates, the resulting powder was weighed into 4 x 6 mm silver capsules, and treated with 2 x 20 uL of HCl. Samples were then oven-dried at 60°C for 24 hr. Samples were combusted in a Carlo-Erba NC2500 elemental analyzer and analyzed by a Thermo Delta V isotope ratio mass spectrometer. Reported  $\delta^{15}\text{N}$  values are relative to atmospheric  $\text{N}_2$ . Precision was quantified by an in-house acetanilide standard (STDEV  $\pm$  0.02 ‰), calibrated against IAEA international standards.

### ***Nutrient dosing experiment***

To further examine a link between nutrient enrichment and coral disease impacts, a controlled experiment was set up in July 2009 at the UOGML (Figure 3). Two experiments were run simultaneously: one examined the impact of elevated nutrients on disease progression in previously-infected corals and the other examined the rate of transmission of disease to healthy corals in elevated nutrient conditions. *Porites cylindrica* was used as the test coral, and white syndrome, the most common disease affecting corals on Guam, was used as the test disease. Eight replicate aquaria were set up per treatment, with treatments as follows:

#### *Progression study:*

Diseased coral fragments in ambient nutrient concentrations

Diseased coral fragments in elevated nutrient concentrations

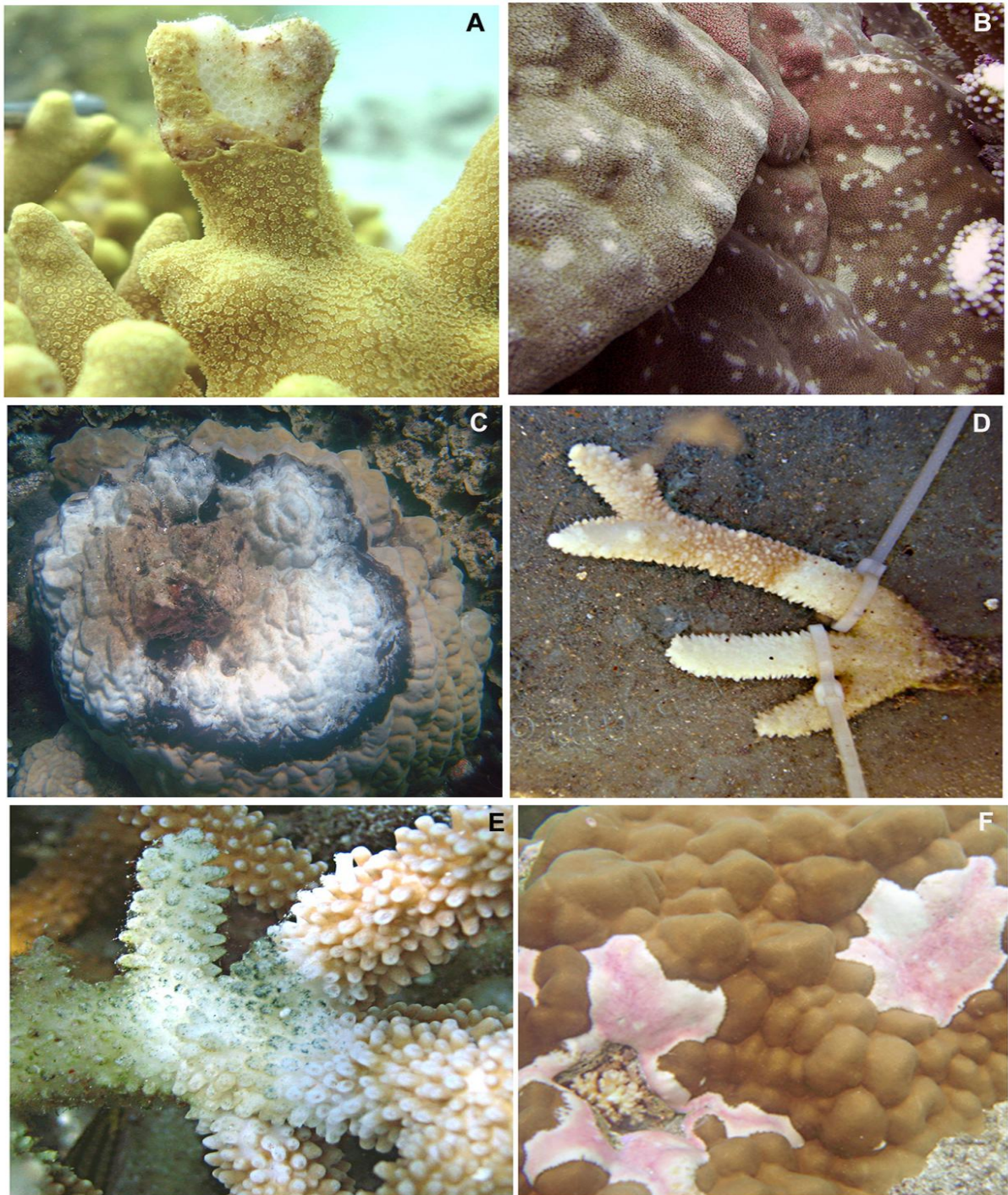
#### *Transmission study:*

Diseased-healthy paired fragments (direct contact) in ambient nutrient concentration

Diseased-healthy paired fragments (direct contact) in elevated nutrient concentration



**Figure 3.** Experimental set up for nutrient dosing experiment.



**Figure 2.** Photographs of six diseases recorded from Guam reefs: A) White syndrome; B) Ulcerative white spots; C) Black band disease; D) Brown band disease; E) Skeletal eroding band; F) Growth anomalies. After Myers and Raymundo 2009. Photos by D. Burdick and L. Raymundo.

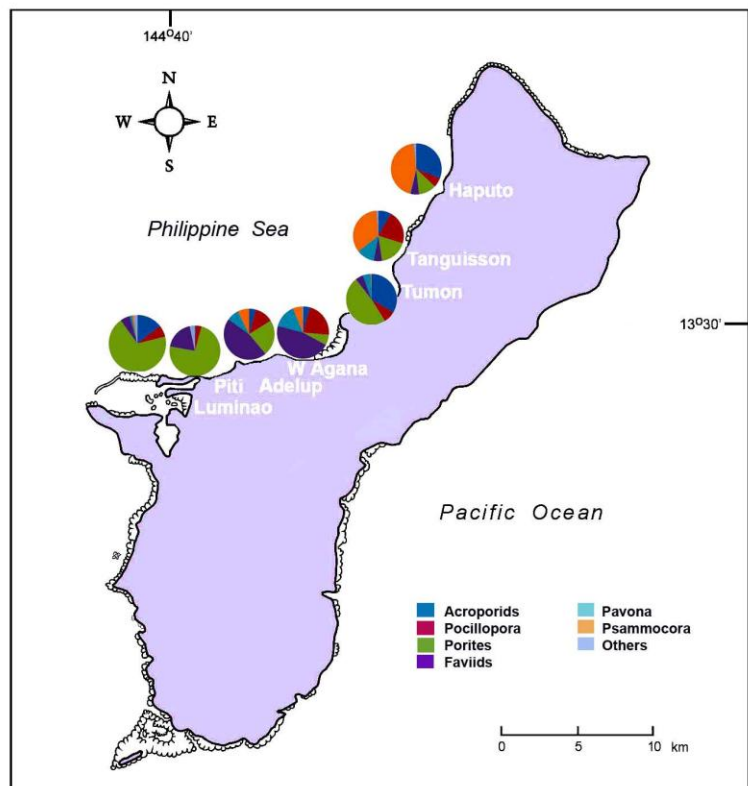
Ambient concentrations (0.1-0.9 mgNO<sub>2</sub>-NO<sub>3</sub>/L) were established by maintaining fragments in 2 L aquaria with natural seawater from the UOG ML seawater system, which receives coastal water from an intake pipe on the northern margin of Pago Bay. Enriched concentrations were achieved by adding 20 g of Osmocote fertilizer® (19:6:12, N:P:K) to this water source. Aquaria were maintained with aeration at a temperature of 28°C. Water was changed and new fertilizer added every 3 days, and water samples were taken before and after each water change to be analyzed for NO<sub>2</sub>-NO<sub>3</sub> concentration by the Water and Environmental Research Institute. Corals were censused daily for changes in lesion appearance and signs of transmission of the disease from diseased to healthy fragments.

## RESULTS AND DISCUSSION

### Benthic Composition

Reef flat communities in the seven sites were generally of three types: a) dominated by *Porites* (*P. cylindrica*, massive *Porites*, *P. rus*); b) mixed communities dominated by faviids (*Leptastrea*, *Goniastrea*), *Pocillopora damicornis* and *Acropora* spp. (*A. muricata*, *A. azurea*); and c) mixed communities dominated by *Psammocora* and *Pavona* (Figure 4). Generally, most of these species were present at all sites, which facilitated inter-site comparisons and allowed examination of the effect of coral host abundance on disease prevalence.

There were significant differences in live coral cover between sites, as was expected (GLM  $F=10.56$ ;  $p<0.001$ ); interestingly, West Agaña and Tanguisson, the two outfall sites, had the lowest hard coral cover (Figure 5). This may reflect the effect of chronic exposure to elevated nutrients, as the outfalls have been operating since 1979. Over this length of time, coral community composition would be expected to shift toward species that can tolerate high nutrient load, and more sensitive species would die out.



**Figure 4.** Coral community composition per site in each of the seven monitored sites along Guam’s western coast.

However, as no previous information exists for these communities, these changes can only be speculated on.

Hard coral cover declined significantly from 2009 to 2010 (GLM  $F=7.57$ ;  $p=0.0123$ ), and is summarized in Figure 5. Only two sites, Luminao and Adelup, showed slight and insignificant increases in live coral cover. Five out of seven sites showed a decrease in live hard coral, with the most profound difference occurring in Tanguisson, with a 10% drop in cover, followed by Tumon with an 8.4% drop and Haputo with a 7.2% drop. We observed seasonal bleaching in August 2009, which resulted in widespread mortality of *Pavona decussata* (dominant in Tanguisson), *Acropora muricata* (dominant in Tumon) and *Acropora azurea* (dominant in Haputo). This event accounts for much of the decrease in coral cover over this year, and was corroborated by simultaneous increases in recently-killed coral and rubble at each site. The dominant species in each of the sites showing measurable loss appeared highly susceptible to bleaching (discussed below). Other sites, such as Luminao and Piti, are dominated by *Porites*, which is known to be less susceptible to bleaching (Hueerkamp et al. 2001) and consequently showed less bleaching-related mortality. However, the overall significant trend in live coral cover decrease over a

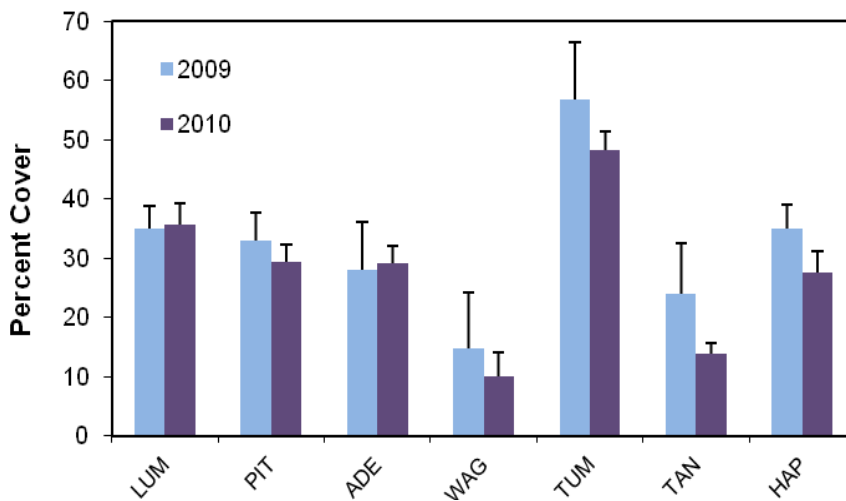
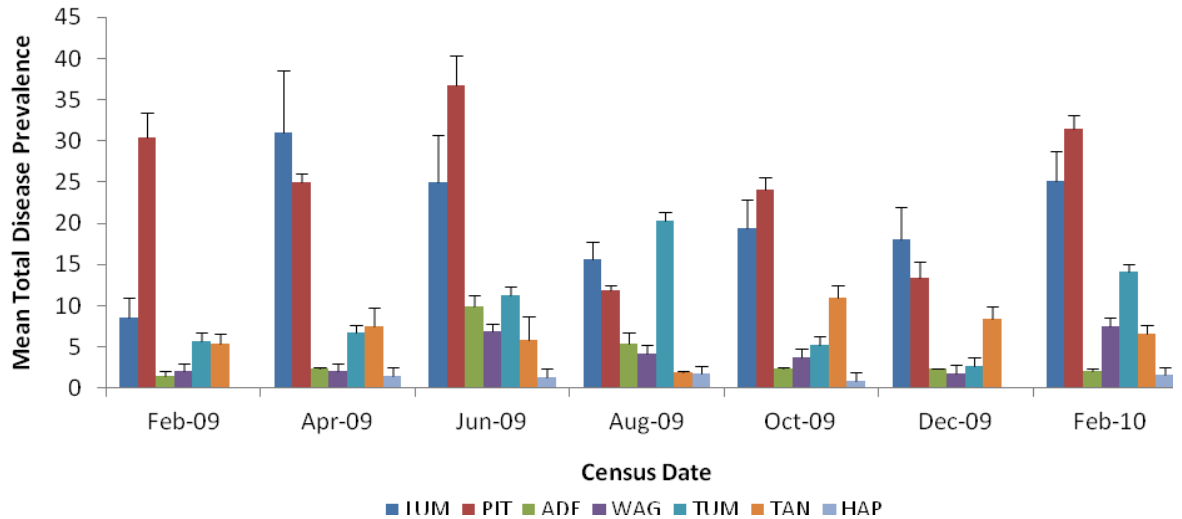


Figure 5. Changes in live hard coral cover from 2009 to 2010 in seven monitored sites. X $\pm$  SD; n=3 transects per site.

single year clearly identifies a management issue which would benefit from monitoring. Even minor decreases in coral cover may accumulate over time to result in major changes in reef flat community structure if recovery cannot compensate for tissue loss or whole colony mortality, regardless of the cause of mortality.

### Coral health impacts

Five of the six diseases recorded from Guam (Myers and Raymundo 2009) were observed within the monitored sites over the study period: white syndrome, black band disease, growth anomalies, brown band disease and skeletal eroding band (refer to Fig. 2). Disease prevalence patterns showed high variability between sites and survey dates, though within-site variability (i.e., between-transects, within census periods) was generally low. The Piti Marine Reserve showed the highest disease prevalence throughout the monitoring period, followed by Luminao (Figure 6). The least disturbed site, Haputo, consistently showed the lowest disease prevalence throughout the monitoring period.



**Figure 6.** Mean total disease prevalence per bimonthly census period for seven monitored sites.  $X \pm SE$ ;  $n=3$  transects per site.

Variation in total disease prevalence between census periods was high, and there was no evidence of decreasing prevalence over time to suggest recovery correlated with improved water quality. In addition, there was no spatial pattern to suggest that sites in closest proximity to the outfalls showed the largest densities of diseased corals. If anything, the spatial patterns of disease incidence suggested seasonal influences, although temperature is likely to have differential effects on different diseases. It was therefore necessary to examine possible correlations with temperature per individual disease. Surface water temperature peaked during the summer months ( $>30^{\circ}\text{C}$ ), from June through August, with lowest temperatures in January through March ( $27^{\circ}\text{C}$ ). Figures 7A-C summarize the prevalence of three health impacts, two with documented seasonal patterns (bleaching and black band disease; Brown 1997; Boyette et al. 2007) and one (white syndrome) for which seasonal patterns have not yet been clearly established.

White syndrome (WS; Figs. 4A and 8) was, by far, the most common health impact noted, followed by bleaching. WS is an infectious disease (Lozada, unpubl. data) and affected 14 species along our transects, including all of the dominant species present at all sites. It was observed at the majority of the sites at all census periods. It is characterized by rapid and progressive tissue loss, leaving bare skeleton which becomes colonized by algae (Willis et al. 2004). Disease borders may be irregular and are often accompanied by a zone of bleached tissue and mucous secretion (Figure 8). The disease is currently thought to be caused by several pathogens within the genus *Vibrio* (Sussman et al. 2008; Lozada unpubl. data) and is found on a wide range of host species. Consistent with findings from Hawaii (G. Aeby, pers. comm.) our results do not suggest either a clear link with seasonal temperature changes, or broad patterns of WS outbreaks such as have been observed on the Great Barrier Reef (Bruno et al. 2007).

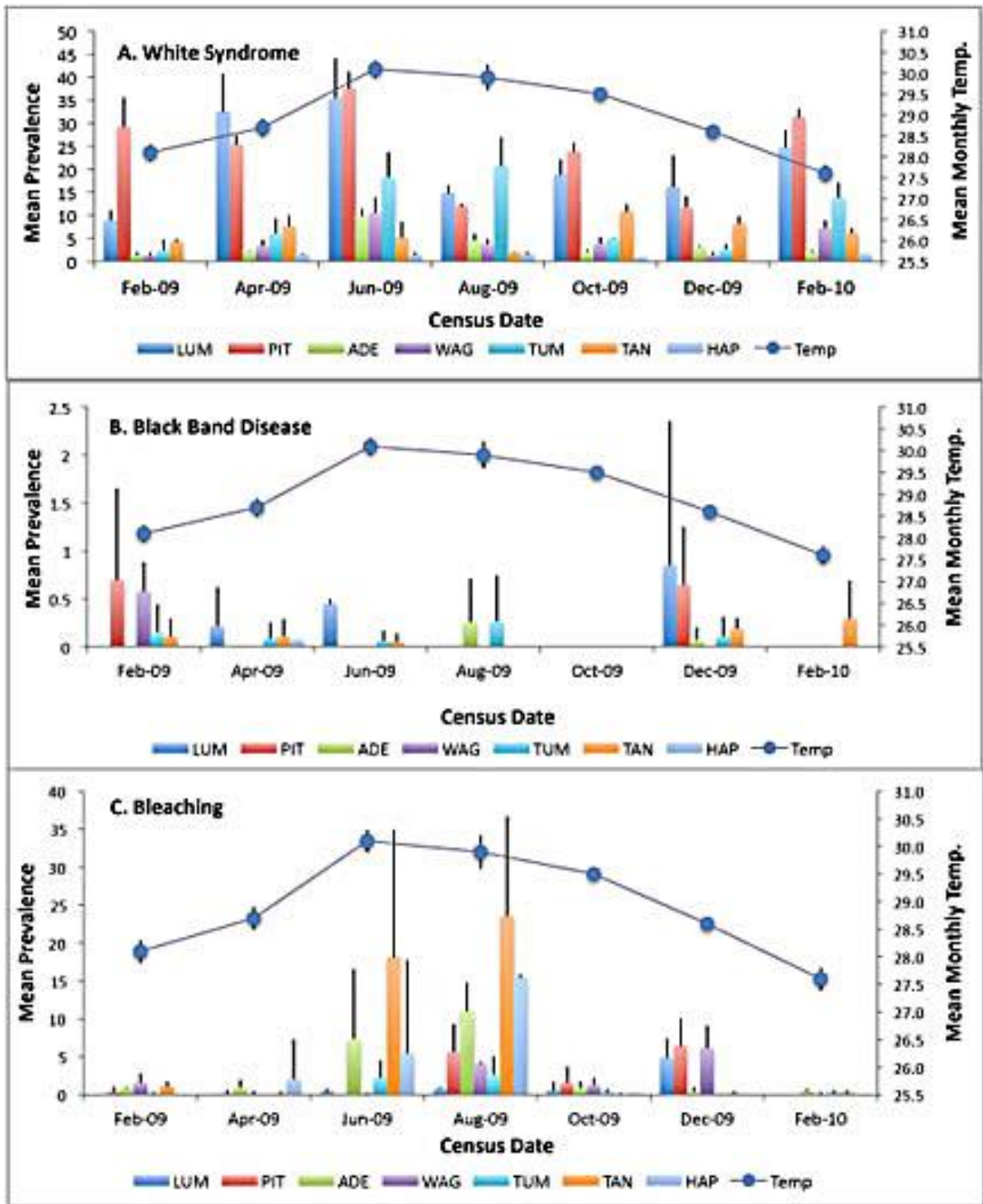
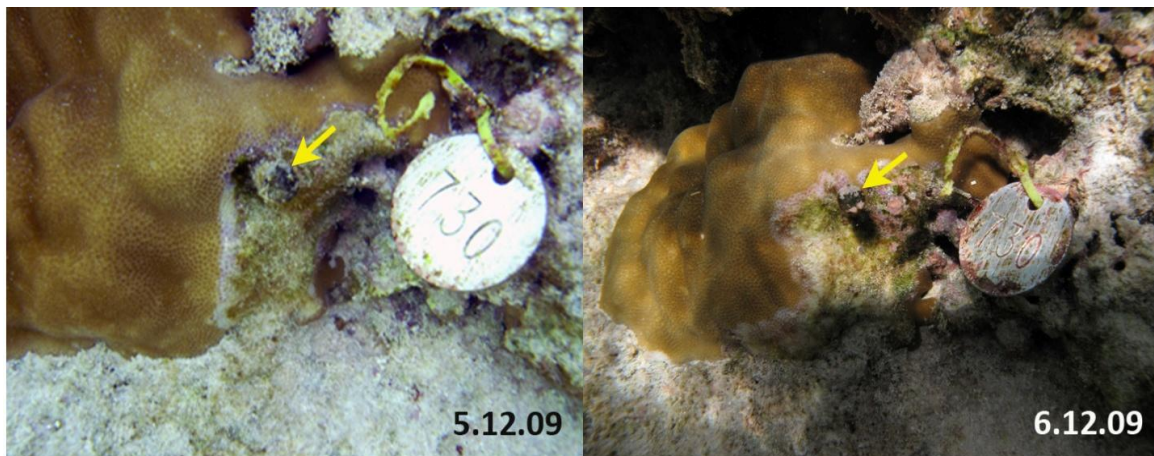


Figure 7. Relationships between the three most common health impacts to corals and mean surface seawater temperature, in °C.

Black band disease was the second most common infectious disease noted, though prevalence was much lower (Fig. 7B) than observed for white syndrome. Although BBD prevalence has been linked with seasonally high temperatures (Boyette et al. 2007; Sato et al. 2009), we found no clear evidence that this was the case at our sites. However, given the observed low prevalence of the disease, our method of evaluating seasonality may not have provided sufficient resolution to make this determination. Bleaching, however, was clearly associated with seasonally warm temperatures (Fig. 7C), and strongly suggests that most of the bleaching observed on Guam is temperature stress-related, though bacterial involvement cannot be ruled out (Kushmaro et al. 1996).

Bleaching is an annual event observed to affect several common species on Guam reef flats, notably, staghorn *Acropora*, *Porites rus*, and *Millepora* spp. (L. Raymundo and D. Burdick, pers. obs.). *P. rus* shows widespread recovery within 3 months of bleaching (L. Raymundo, unpubl. data), while partially killed *Acropora* spp. resheet over dead skeleton as water temperatures cool. However, given that sublethal bleaching events are stressors which may cause patchy tissue loss and impact fecundity (Cox 2007), changes in community structure and recruitment patterns may occur slowly. Therefore, monitoring over longer time scales is essential to determine rates of tissue loss vs. recovery and whether these incremental losses of coral cover result in the die-out of more susceptible species and their replacement with scleractinian corals or other organisms such as soft corals or algae.



**Figure 8.** Time series of Colony 730, a massive *Porites*, with white syndrome. Photos show disease front (yellow arrows) with progressing tissue loss over one month.

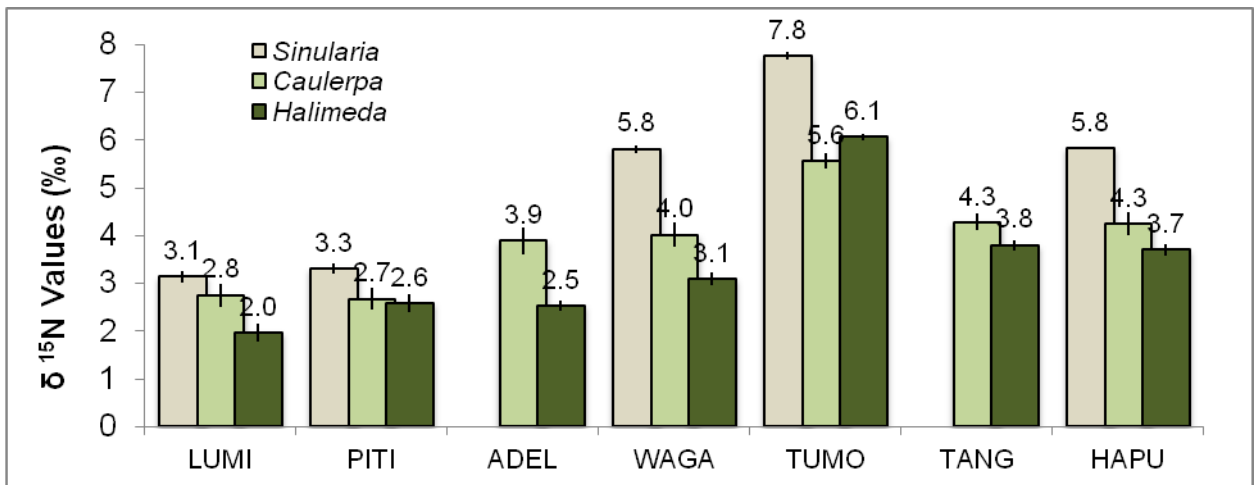
### ***Sewage nutrient analyses***

Stable isotope analyses of the soft coral and algae samples suggested varying levels of N enrichment of nearshore waters of Guam. There was reasonably good congruence between the three species we tested. As indicated in Figure 9, waters of Tumon were the most enriched, with  $\delta^{15}\text{N}$  values declining both northward (Tanguisson and Haputo) and southward along the west coast of Guam. Given the proximity of these reefs to oceanic waters, these relatively high values suggest a strong terrestrial influence. Given the geology of the island (i.e., carbonate platform in the north and volcanic in the



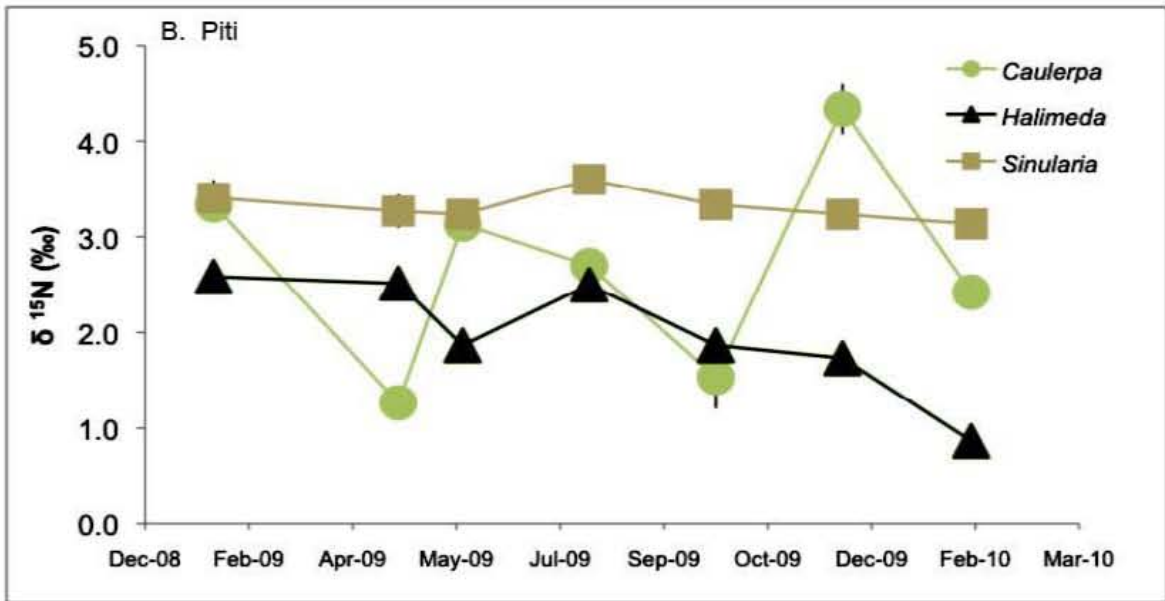
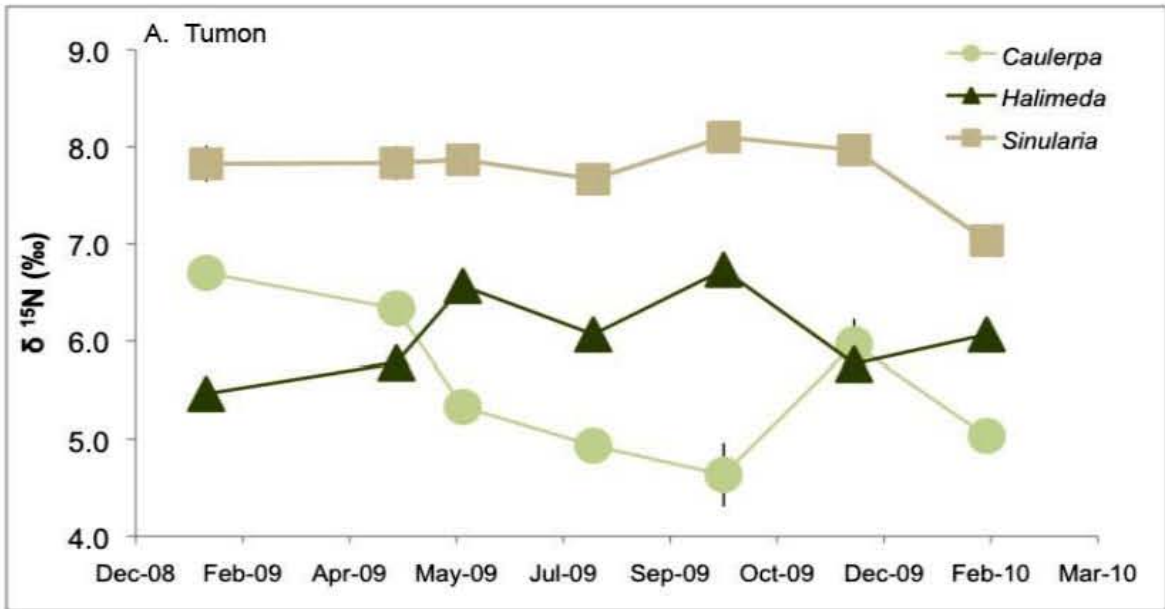
south), it is likely that Tumon and sites northward are influenced by a combination of groundwater discharge (Denton et al. 2005) and direct sewage inputs via wastewater treatment plants and surface run-off, whereas the southern sites are likely affected by surface inputs only. The isotope values also suggest some seasonality in the N inputs, especially among the two algal species (Fig 10A & B).

For soft corals, values >4.0 ‰ are likely due to sewage N sources (Baker *et al.* 2010). This threshold identifies Tumon as the most polluted site, followed by West Agaña and Haputo (Figure 9). Luminao had the lowest  $\delta^{15}\text{N}$ , which may indicate a reduced or absent contribution of sewage derived N. For algae, values >3.0 ‰ have been suggested as a threshold for sewage-derived N (Lapointe *et al.* 2004). Again sites above this value include Tumon, West Agaña, Haputo, in addition to Adelup. Luminao had the lowest  $\delta^{15}\text{N}$  for both species sampled.



**Figure 9.** Mean isotope values from three sampled benthic organisms at seven sites along the west coast of Guam. Data are time-averaged (7 samples per sites between February 2009 to February 2010; Mean +/- SD).

Annual variation of  $\delta^{15}\text{N}$  in soft corals was low in Tumon (Figure 10A) but nonetheless might have been correlated with peak tourism and rainfall in the area. Algal  $\delta^{15}\text{N}$  levels were slightly more variable, ranging about 2.0 ‰ over the year. Hints of seasonality appear in the algae dataset, but  $\delta^{15}\text{N}$  levels in each species behaved differently: *Halimeda* showed enrichment and *Caulerpa*, a depletion in the summer. Enrichment would be expected during peak productivity (high light and water temperatures). *Caulerpa* is a rapidly growing fleshy algae, whereas *Halimeda* is a slower growing calcareous species with a larger standing stock of biomass which is more resistant to isotopic change. Fleshy macroalgae have been shown to be more rapid integrators of N sources on the order of several days, while calcareous species integrate over larger time frames (Gardner *et al.* 2004). Therefore, the *Caulerpa* data may represent a series of snapshots that are easily influenced by ephemeral inputs from various N sources (e.g., the data are “noisy”). This point is applicable to the Piti Marine Reserve dataset as well (Figure 10B). *Caulerpa* values varied widely through the year, whereas *Halimeda* values were more stable. The >1.0‰ depletion in *Halimeda* over the year may be significant if fewer tourists visited the area, or increased proportions of isotopically depleted sources were introduced.

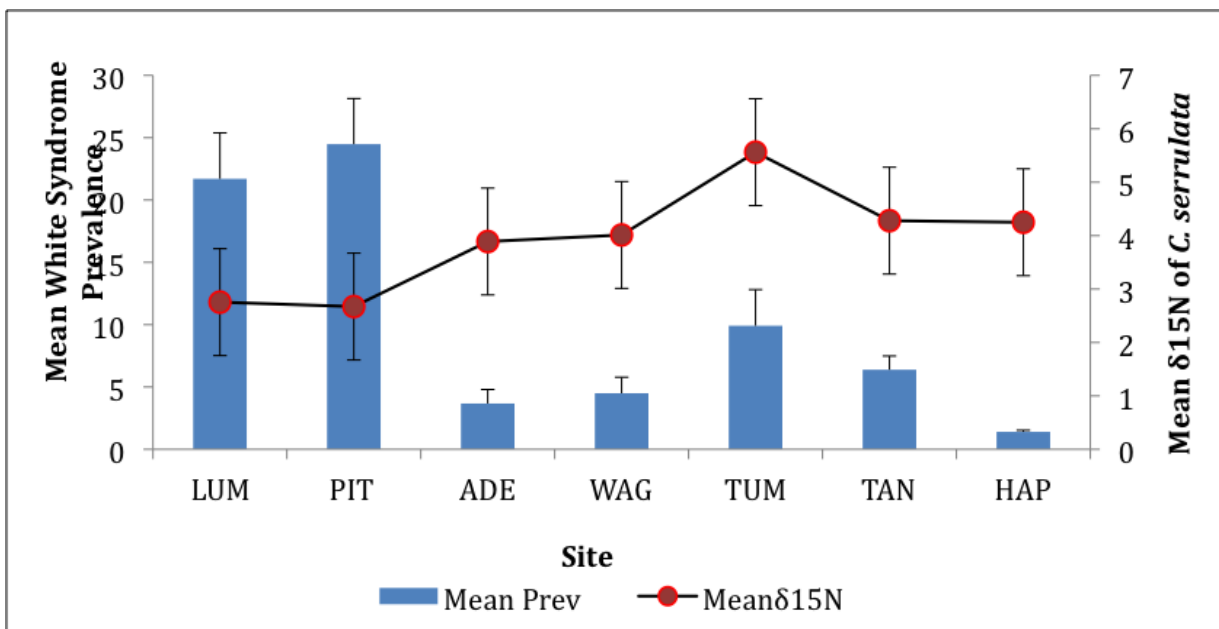


**Figure 10.** Temporal variation in  $\delta^{15}\text{N}$  for (A) Tumon and (B) Piti Marine Reserves for a soft coral (*Sinularia*) and two species of macroalgae (*Caulerpa* and *Sinularia*). Note the differences in the vertical scale between A & B.

## Associations between disease and nutrient load

### In situ monitoring

There was no evidence of a gradient in disease impacts initiated at the two outfall sites (WAG and TAN) and lessening with distance from these sites, though our results did suggest a gradient initiated at Tumon and lessening to the north and south. In five of the seven monitored sites, there was reasonable congruence between the prevalence of white syndrome in *Porites* and sewage nutrient levels (Figure 11). However, in the two most southern sites, Piti and Luminao,  $\delta^{15}\text{N}$  levels were the lowest, yet disease prevalence was 2X-3X higher than in all other sites. In contrast, the northernmost and least populated site, Haputo, consistently displayed the lowest disease prevalence as we predicted, although  $\delta^{15}\text{N}$ , surprisingly, was not. Given the geology of this area, it is reasonable to speculate that groundwater seepage may be a source of sewage inputs to the reef flat at this site.



**Figure 11.** Association between white syndrome prevalence within the *Porites* population and sewage-derived nitrogen within *Caulerpa serrulata*, at each of seven sites. Mean values obtained from bimonthly samplings of both disease and algal tissue over a 12-month period.

These results suggest additional factors influencing disease prevalence patterns, such as high host density (*Porites* dominates at both the southernmost sites, but is rare in Haputo; Fig. 4) and current patterns flowing northward and southward from Tumon Bay, as discussed previously (Wolanski et al. 2003). Other short-term, localized events may play a role as well. Guam EPA reported a wastewater spill whereby raw sewage was flowing into one of the two rivers which empty into Piti and had been for an unknown period of time. The spill was due to a malfunctioning manhole and delivered raw sewage at a rate of 20 gals/min (Guam Waterworks Authority 2011; river in question not identified in the report).

Other spills, leaks and breaks in sewer lines may go undocumented for extended periods of time, resulting in unknown quantities of raw sewage deposited from point and non-point sources into coastal waters.

The dosing we used averaged two orders of magnitude higher than known enriched concentrations reported for nearshore Guam waters (1-3.4 mgNO<sub>2</sub>-NO<sub>3</sub>/L; Denton et al. 2005). Therefore, future dosing experiments will refine this concentration to represent more realistic levels, to determine if the effects of nutrients on disease dynamics are consistent with our current findings. In addition, a temperature factor will be incorporated into these experiments, to examine synergisms between enrichment and temperature.

**Nutrient dosing experiment**

White syndrome-diseased *Porites cylindrica* fragments maintained in water enriched with Osmocote showed higher mortality and less healing during the experiment than those maintained in seawater with ambient nutrient concentrations (Table 2). However, the number of successful transmissions of disease to healthy fragments did not differ significantly between the two treatments. This is consistent with the findings of Bruno et al. (2003), who found faster progression of Yellow Band Disease in *Montastraea* in enriched conditions, but no effect on transmission.

**Table 2.** Summary of performance of *Porites cylindrica* fragments with white syndrome, maintained in ambient and elevated nutrient conditions (28°C)

| Performance Parameter<br>(n=8 fragments/treatment; 1 mo census) | Ambient nutrients | Enriched nutrients |
|---|-------------------|--------------------|
| Percent fragment mortality                                      | 6.25              | 50                 |
| Percent transmission to healthy fragment                        | 37.5              | 25                 |
| Percent of fragments with healed lesions                        | 31.25             | 6.25               |

**CONCLUSIONS AND RECOMMENDATIONS**

- There is clear evidence of sewage-related enrichment of Guam’s coastal waters.
- There is congruence between the amount of sewage enrichment and coral health, though other factors such as the density of susceptible species, nearshore surface current patterns, localized events involving sewage spills or leaks, and seasonal temperature variations may also be influencing the disease prevalence patterns observed.
- There is no evidence of a gradient in coral health impacts with distance from the two point sources of sewage input, but some evidence of gradients to the north and south of Tumon Bay. However,

given the numerous non-point sources (road run-off, springs, leaky septic tanks) and point (small rivers, streams, canals) sources, it is likely that these other sources are influencing nearshore communities at least as much as the outfalls may be.

- There was no improvement observed in coral health during the census period. Bleaching-related mortality was high in certain sites.
- Continuation of monitoring on these reef flats is recommended and has, in fact, been undertaken with the support of the local managing agencies. Of particular importance is monitoring bleaching-related coral loss and recovery and the identification of vulnerable species.
- Substantial efforts to decrease the amount of untreated sewage delivered to nearshore coral communities should be undertaken. To this end, secondary sewage treatment and improvements in septic tank construction and sewage transport pipes are highly recommended.

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