FINAL REPORT for Puakō Community Association

Linking sewage pollution and water quality to spatial patterns of *Porites* growth anomalies in Puakō, Hawai‘i

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Catherine J.S. Kim, Reyn M. Yoshioka, Allison M. Tracy, and C. Drew Harvell
Cornell University
Contact: cdh5@cornell.edu

Abstract

The Puakō region of West Hawai‘i is known for its healthy coral reef resources. However, studies have revealed a recent decline in coral cover and reef health (Minton et al 2012). While there are a number of possible contributors to this trend, coral disease exacerbated by land-based pollution is a potentially important factor. Recent work has shown the most abundant corals in West Hawai‘i, *Porites lobata* and *P. compressa*, are also the most susceptible to disease (Couch PhD 2014). The most common disease of these corals are *Porites* growth anomalies, or PGAs, which are misshapen, tumorous lesions that harm afflicted corals. In the Puakō region, sewage pollution entering coral reefs through submarine groundwater discharge could alter disease risk by increasing nutrients or other pollutants that could compromise coral immunity or introduce pathogens that could cause disease. In this study, we measured coral health and inferred the spatial patterns of sewage pollution through enterococci fecal bacteria counts and $\delta^{15}N$ bioassays using the alga *Ulva fasciata.* PGA disease varied strongly among the 10 sites we studied, with highest severity of disease at Waialea Bay and Puakō Boat Ramp. Counts of enterococci were highly variable within and between sites, but were unusually high and indicated significant sewage pollution at some times and places. PGA severity and enterococci abundance in open shore water were positively associated, suggesting that higher levels of coral disease co-occur at sites with higher sewage pollution, for example at embayed sites like Puakō Boat Ramp or Waialea Bay. Additionally, percent coral cover and %N were strongly negatively correlated, although $\delta^{15}N$ did not predict PGA measures. However, great spatial and temporal variation, such as in timing of rainfall events and submarine ground water discharge, present large challenges that require further reef and pollution monitoring to improve confidence in pinpointing causes of poor coral health. Future work will focus on increasing our understanding of the variability in spatial and temporal abundances of enterococci and sampling $\delta^{15}N$ closer to shore, where inputs are thought to be highest.

Introduction

The Puakō region of the leeward coast of Hawai‘i Island (also known as West Hawai‘i or WHI) is known for having particularly well-developed reefs. However, as with many other coastal regions of Hawai‘i, the Puakō region has experienced increased pressures from fishing, land-based pollution, recreational use, development, and likely climate change in the recent decades. The consequences of these synergistic impacts likely explain the dramatic decline of Puakō marine resources over the long term. Data compiled by The Nature Conservancy and the Hawai‘i State Division of Aquatic Resources show a decrease in fish abundance over a 40-year

span (Walsh 2013; Minton et al 2012). Coral cover also declined dramatically in Puakō, dropping from 80% in the 1970s to 32% in 2010 (Walsh 2013). Additionally, Couch et al (2014) identified Puakō as one of four regions in West Hawai‘i warranting special concern based on a 12% reduction in coral cover between 2003 and 2011. While there are likely many contributing factors in this decline, one that should not be ignored is coastal pollution facilitating coral disease and death.

Coral disease is a significant driver in coral decline throughout the world (Burge et al 2014, Aeby et al 2011, Harvell et al 2007). Along West Hawai‘i, most coral reefs are dominated by *Porites* corals. While being the most abundant corals in the region, *Porites* corals are also the most vulnerable to disease (Couch et al 2014, Aeby et al 2011, Friedlander et al 2008). The most prevalent of these diseases is *Porites* Growth Anomalies (PGAs) which are identified as gross lesions of tumor-like tissue with lighter pigmentation, raised tissue, and enlarged or variable polyp (calyx) size (fig. 1). The lighter pigmentation of PGAs is the result of lower densities of symbiotic dinoflagellates in PGA tissue. As a result, PGAs are likely unable to produce enough energy to sustain themselves and must rely on resources of healthy portions of the host in order to grow (Yasuda et al 2012). As sinks for their colonies' resources as well as having decreased reproductive function, PGAs likely decrease the reproductive ability of the whole colonies and likely their immunity (see Burns and Takabayashi 2011 for *Montipora* growth anomaly impact). Lastly, PGAs have less dense skeletons making them likely more vulnerable to burrowing organism such as shrimp and gastropods. Although potentially viral (Vega Thurber and Correa 2011), the causative agent remains unknown and could even include somatic mutation (Irikawa et al 2011).

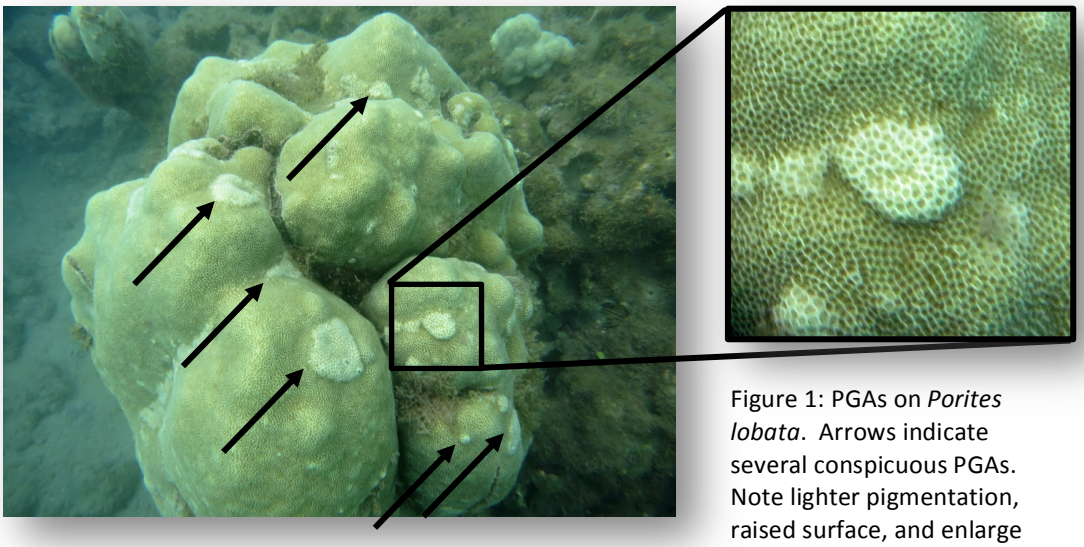


Figure 1: PGAs on *Porites lobata*. Arrows indicate several conspicuous PGAs. Note lighter pigmentation, raised surface, and enlarge corallites.

Disruptions in the interactions of coral host, pathogen, and the environment, shift the balance between the host and the pathogen, potential causing or exacerbating disease (Harvell et al 2007). Nutrient pollution can be an important factor in coral disease. Couch et al (2008) suggest that nutrient enrichment may favor growth of coral microbial associates, which indirectly modifies

host coral immunity by requiring an elevated immune response. Increases in nutrient loading can increase the severity and prevalence of coral disease, possibly by favoring pathogen growth or inhibiting resistance (Vega Thurber et al 2014, Harvell et al 2007, Bruno et al 2003).

In Puakō, untreated sewage pollution can enter the coral reef ecosystem through the combination of old cesspool systems, highly porous volcanic bedrock, and community's close proximity to the shore. Sewage may introduce a number of pollutants: nutrients (especially nitrogen and phosphorous), chemical contaminants, and microbes. In the Caribbean, sewage pollution introduced the human gut microbe, *Serratia marcescens*, into coral reefs, where it is responsible for the decimating white pox disease of Elkhorn corals (*Acropora palmata*) (Sutherland et al., 2010). Other human gut bacteria can also enter the marine environment through sewage pollution. While not necessarily pathogenic, these bacteria are of interest as indicators of untreated sewage entering coral reef ecosystems. Namely, the gram-positive *Enterococcus* spp. bacteria can be used to assess the degree of sewage pollution and is used by the USEPA as a water quality metric. Naturally found as facultative anaerobes in human and animal gut, enterococci are relatively persistent bacteria and are able to tolerate both fresh and saline water. The recommendation for enterococci in coastal waters established by the Hawai‘i State law and the EPA is a geometric mean of 35 colony-forming units (CFU) per 100 mL of water for five or more samples per 25-30 days with a single sample maximum (ssm) of 104 CFUs per 100 mL. For inland recreational waters there is an ssm of 89 CFUs per 100 mL of water (Hawai‘i Administrative Rules §11-54-8, 2013). However, preliminary study indicated levels higher than the EPA standards along Puakō's shoreline and anchialine ponds.

To further clarify nitrogen-based inputs to the nearshore coastal waters in Puakō, we also tested levels of $\delta^{15}N$. The most common isotope of nitrogen is ^{14}N; however, ^{15}N exists in trace quantities throughout the environment. Biological processes, such as nutrient uptake in tissues, nitrification and denitrification, and those in the animal gut tend to enrich ^{15}N, the heavier isotope. The enrichment of ^{15}N relative to ^{14}N compared to atmospheric nitrogen is measured as $\delta^{15}N$; higher $\delta^{15}N$ values indicate greater relative quantities of ^{15}N and suggest greater biological processing of nitrogen. Sewage pollution can be inferred through high $\delta^{15}N$ values; as relatively high trophic level organisms, humans enrich ^{15}N in tissues and feces. Furthermore, microbial and physical processing of sewage also leaves it enriched through the preferential removal of the lighter (^{14}N) isotopes of nitrogen. In contrast, $\delta^{15}N$ of other sources such as synthetic fertilizers and biological fixation tend to be low (Dailer et al 2010, Derse et al 2007). Bioassays can be used to detect enrichment of ^{15}N (Risk et al., 2009). In Dailer et al (2011) the alga *Ulva fasciata* was grown in waters off West Hawai‘i to produce a sample that integrated levels of nitrogen over a 2 week period. Baker et al (2010) performed ^{15}N isotope analyses on sea fans (*Gorgonia ventalina*) to detect human sewage impacts in the Mexican Caribbean. Dailer et al (2010) also used $\delta^{15}N$ analysis on resident algae to map sewage pollution in Maui, Hawai‘i.

This study aimed to 1) investigate the spatial patterns of the coral disease *Porites* growth anomalies in the Puakō region of West Hawai‘i and 2) evaluate the potential for relationships between sewage pollution, nutrient pollution and occurrence of coral disease.

Methods

Coral cover and disease surveys
Ten sites ranging from Waialea Bay to Paniau were selected to capture variation of coral cover and health along the Puakō coast (fig. 2). Where possible, sites were selected at mile-marker shore access (SA) locations. At each site, three replicate 50 ft (~15 m) transects were established. Transect locations were haphazardly laid parallel to the reef edge in depths around 5-10 ft. in order to maintain consistency and sampling between sites. In each transect, 20 0.5x0.5m quadrats were laid,with 10 quadrats alternating on each side of the transect tape. Within each quadrat, coral cover (measured as number of the 25 10x10cm grids containing live coral), the number of *P. lobata* colonies, and the number of PGAs on each colony were recorded.

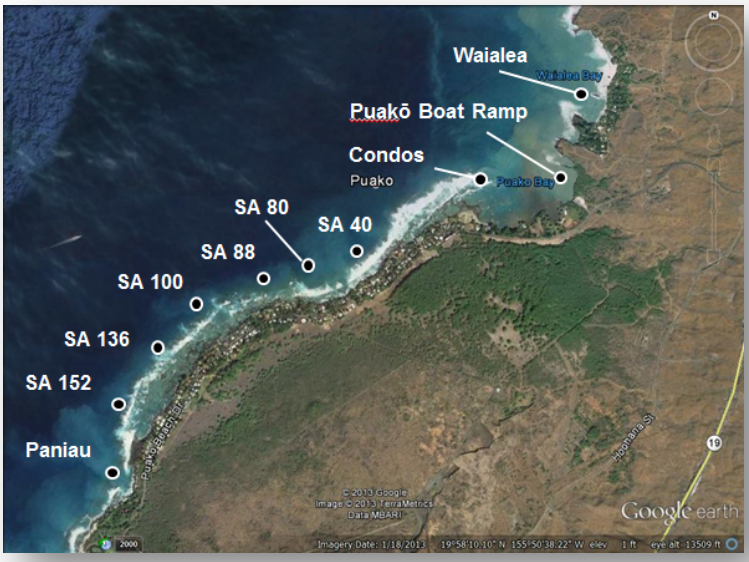


Figure 2: Puakō region and study sites. Ten sites were selected for this study, spanning from Paniau bay to Waialea bay. $\delta^{15}N$ bioassays were conducted at Waialea, Condos, SA80, SA136, and Paniau.

Enterococci assays
Shortly after low tide, five 100 ml water samples were collected at each site, with three along the open shoreline (on 25 June 2013) and two in the nearby tide pools (on 3 July 2013, some entirely enclosed with rock while others not). Enterococci water samples were processed within 6 hours of collection using methods described in the EPA Method 1600 and Baker *et al*. 2010 using m-Enterococcus agar. Using the same methods, additional samplings were performed in the following winter on 13 January 2014 and 16 January 2014, during and following a rainfall event at five of the 10 original sites (Waialea, PBR, Condos, SA80, and Paniau, n=3-6 per site per sampling time).

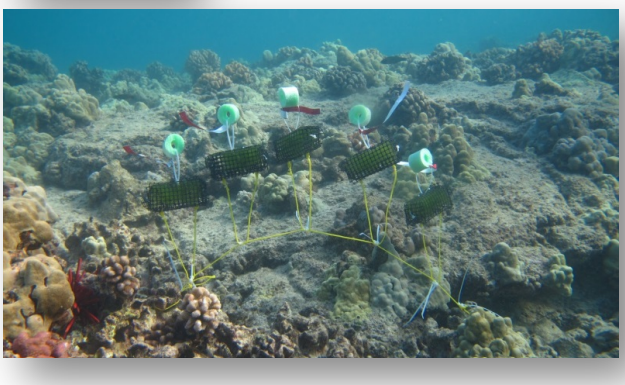
Two samples were also taken each from five anchialine pools in the Puakō area, using the same methods as marine samples (on 28 June 2013).

Positive controls were performed by swabbing skin of one of the researchers with a deionized water-dampened filter and plating it onto m-Enterococcus agar plates. Negative controls were performed as described for the samples but using deionized water in place of collected water.

$\delta^{15}N$ Bioassay with Ulva fasciata
Due to the very low abundance in Puakō, *Ulva fasciata* was collected from shoreline at the Natural Energy Labs of Hawai‘i Authority (NELHA). To minimize variation and to starve the *Ulva* of nitrogen, the samples were incubated onshore in naturally-lit aquaria in seawater collected ~1 km offshore. It was assumed that the open ocean water would have sufficiently low

nutrient concentrations to achieve nitrogen starvation. During the seven day incubation, half of the water was changed out with fresh seawater every two days.

Figure 3: *Ulva fasciata* (photo: Bishop Museum), and deployed cages for $\delta^{15}N$ bioassay.



After incubation, the *Ulva* were divided into individual fronds and placed into cages (see fig. 3) composed of plastic fencing and zip ties. Each cage was labeled and deployed at reef level in five of the aforementioned sites: Waialea, Condos, SA80, SA136, and Paniau. The *Ulva* were left to incubate for eight days, after which the cages were promptly collected and the *Ulva* cleaned of debris and dried. The dried samples were sent to Ithaca, NY, prepared (ground in liquid nitrogen) for $\delta^{15}N$ analysis at the Cornell University Stable Isotope Laboratory (COIL). To determine whether the $\delta^{15}N$ incubated *Ulva* would reflect those of resident algae, resident *Ulva* was collected from the Condos and SA80 sites and processed for stable isotope analysis in the same way as the incubated samples.

Data analysis

All data were analyzed in R version 3.0.1. Analysis of variance (ANOVA) with Tukey's post hoc HSD was used to determine differences in enterococci CFU between sites. A binary-family generalized linear model (glm) followed with a general linear hypothesis test (glht) in multcomp (Torsten et al. 2008) was used to study the site-level differences of PGA prevalence. PGA severity was investigated similarly using a poisson-family glm and glht. To investigate the impact of sampling time and location on enterococci abundance using the additional winter 2014 samples, we used a glm with the negative binomial distribution (glm.nb, Venables et al. 2002) as is appropriate for count data with high variance relative to the mean (Crawley et al. 2007). Treatment contrasts were used for both site and sampling time. The Akaike information criterion (AIC) determined the selection of the best-fit model. Tukey's all-way, pairwise comparisons were conducted between sampling times and sites using glht in multcomp. The multiple comparisons were run both with covariate_average=TRUE and covariate_average=FALSE to ensure that the presence of any significant interactions between predictors did not skew the results.

Because $\delta^{15}N$ data were non-normal, we used the non-parametric Kruskal-Wallis test to determine variation in $\delta^{15}N$ between sites. Linear regressions were used to investigate the relationship between PGA prevalence, severity, $\delta^{15}N$, and enterococci counts.
All analyses are performed at significance level $\alpha=0.05$, except in post hoc tests where adjusted p-values are given.

Results

Spatial patterns of Porites growth anomalies and coral cover

Spatial patterns of PGA prevalence (proportion of colonies afflicted with PGAs per site) and severity (mean number of PGAs per colony per site) are shown in figure 4. PGA prevalence varied significantly by site (likelihood ratio test: $\chi^2(9)=136.38$, $p<0.001$). Also, PGA severity varied significantly by site in a similar pattern (LRT: $\chi^2(9)=1076.1$, $p<0.001$). Highest values occurred in Waialea, the Puakō Boat Ramp, and Paniau. Percent coral cover varied significantly by site (ANOVA: $F(9,589)=17.2$, $p<0.001$) though not in a recognizable pattern.

Host demographic patterns of Porites growth anomalies

The number of PGAs per colony increased with the size of the colony (linear model, $F(1,3781)=424.5$, $p<0.001$). However, the strength of this relationship was weak ($r^2=0.1007$), suggesting that other factors play much greater roles in PGA severity. Similarly, PGA prevalence was significantly predicted by colony size (lm, $F(1,28)=17.24$, $p<0.001$, $r^2=0.36$, and omitting point with high leverage still revealed significant relationship: $F(1,27)=13.15$, $p=0.001$, $r^2=0.30$).

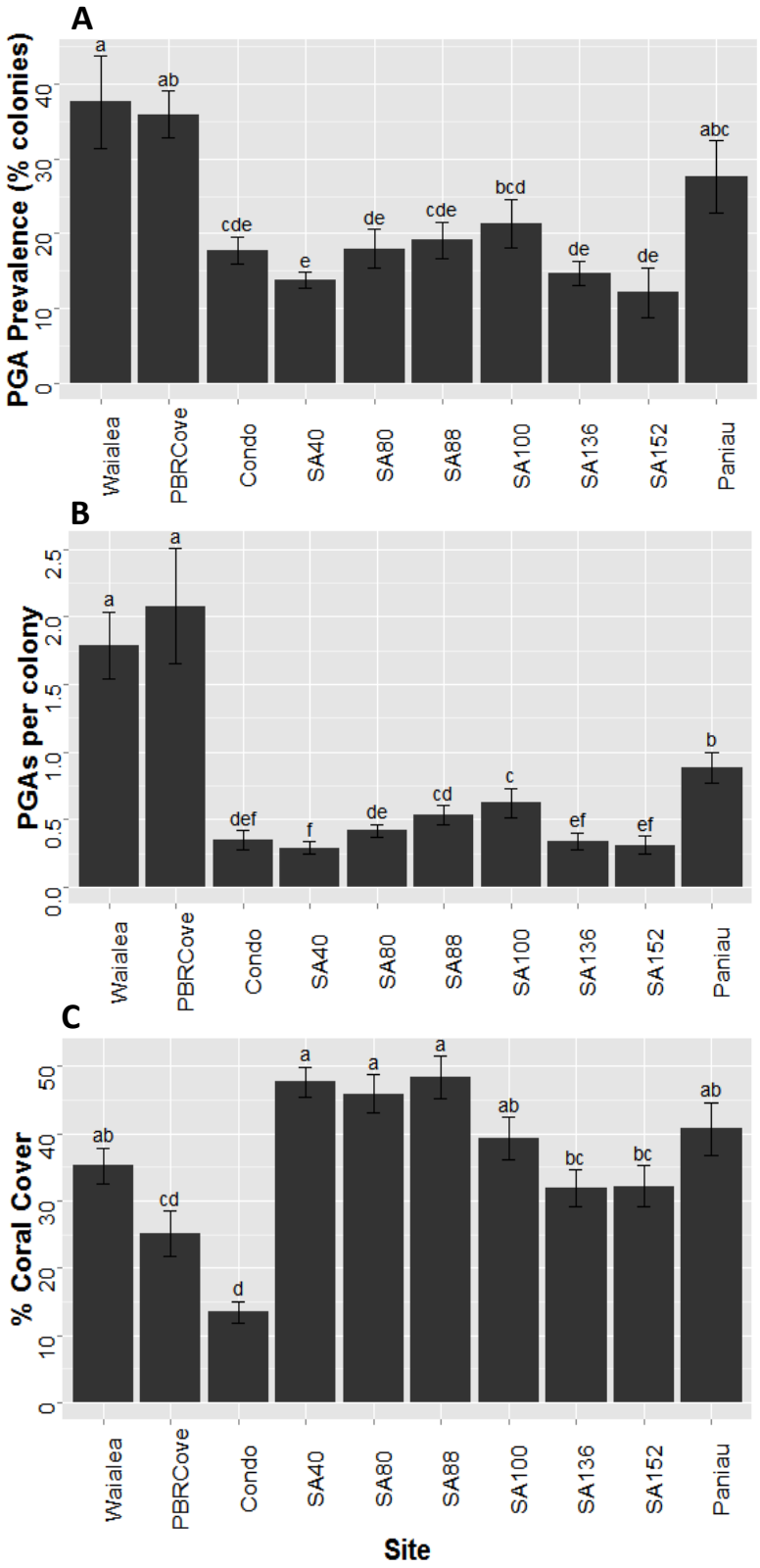


Figure 4: (A) PGA prevalence, (B) severity, and (C) % coral cover by site. Shared letters indicate no significant difference.

Enterococci abundance in Puakō

Enterococci abundance varied greatly within study sites and across study sites. However, due to the high variability within sites (such as differences in local SGD input, tide, circulation, sediments, and wave protection) there were no significant differences in enterococci CFUs found between sites. Mean enterococci CFUs between sites are shown in Figure 5. Anchialine pools were also variable, with close to significant differences between sites (fig. 5, table 2). Summary statistics are presented in table 2. Only mean CFUs for some tide pools exceeded the recommendations of 104 CFU per100 mL. All anchialine pools had CFU nearing or greatly exceeding the limits of 89 CFU per 100 mL.

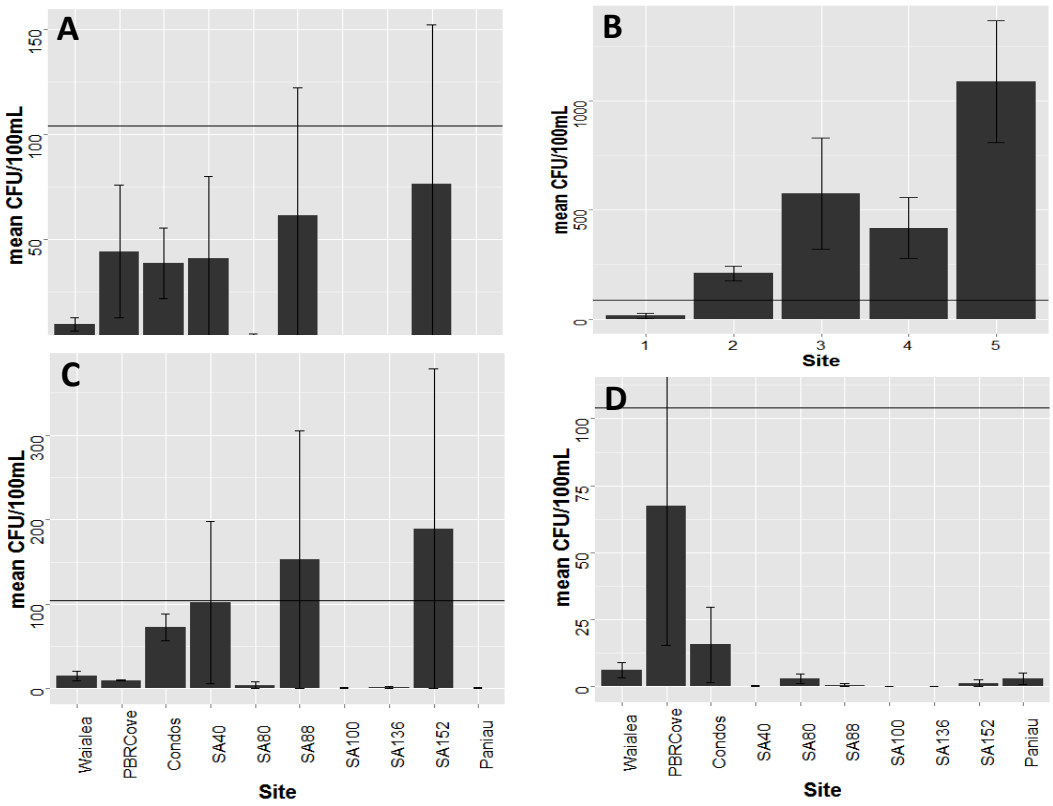


Figure 5: enterococci abundance in CFU/100mL a) overall (open shore and tide pools), b) anchialine pools, c) tide pools, and d) open shore only. Horizontal lines represents Hawai'i State single sample maxima 104 CFU/100mL (shore samplings, a, c, d) or 89 CFU/100mL (anchialine pools, b).

Using the additional data from sampling in January 2014, the model that includes site, sampling time, and the interaction between site and sampling time best predicts the CFU counts (see fig. 6, LRT: $\chi^2(14)=85.2$, $p<0.001$). It is unambiguously the best-fit model as the AIC value is much lower than those of the other models (Table 1). The results of the multiple comparisons (glht) by sampling time show that CFU counts are significantly higher during rainfall compared to both the summer and post-rainfall sampling times ($p<0.001$ for both). Multiple comparisons of site indicate overall significant differences between sites (fig.6); however, as predicted by the model, these differences are not consistent between sampling times.

Model	AIC	df
CFU~site x time	529.7395	16
CFU~site + time	563.4427	8
CFU~time	569.5475	4
CFU~site	570.8209	6
CFU~1 (null)	585.9854	2

Table 1: Model selection using Akaike information criterion (AIC) values. Lower AIC values indicate better models.

All positive controls were successful with limited contamination (1-2 colonies) of some negative controls on the edges of filters. This was likely due to inadequate sterilization of equipment between samples, but likely does not discount results, as CFUs counted from the samples were typically more abundant and more centrally located.

$\delta^{15}N$ analyses

Measures of $\delta^{15}N$ in incubated algae did not vary significantly by site (χ^2=9.0637, df = 5, p=0.1066, fig. 7), though it appears that the initial were lower than the treatments, and that the condos had the greatest $\delta^{15}N$, which could be caused by sewage pollution. Percent nitrogen did differ significantly by site (ANOVA, $F(5,54)=14.47$, $p=5.49\times10^{-9}$, Fig.7), with Waialea Bay and Condos significantly higher than all sites except Paniau. While these data alone do not indicate the source, the differences in %N indicate that there is variation in the nitrogen load at the different sites. Additionally, $\delta^{15}N$ analysis on resident *Ulva* found significant differences between the SA 80 and Condos sites ($t(7.035)=18.1526$, $p<0.0001$), with the $\delta^{15}N$ of Condos (8.60±0.12‰) being nearly double that of SA 80 (4.64±0.18‰).

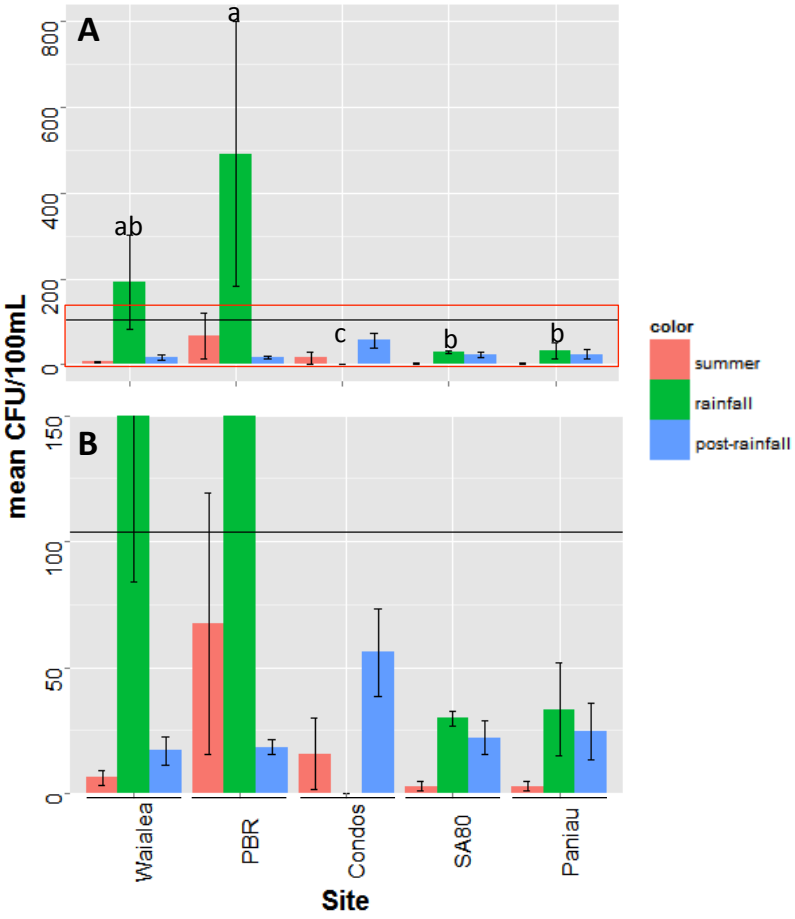


Figure 6: Mean CFU/100 mL by site in the summer, immediately following rainfall event, and post-rainfall (A). Fig. 6B shows enlarged area of A boxed in red to highlight finer scale differences between sites at different times (note vertical scale differences). Shared letters indicate no significant differences between sites overall as determined by multiple comparisons glht, but **do not** indicate any relationships at or between different sampling times. Horizontal line indicates single sample maximum of 104 CFU/100mL as per Hawai'i State regulations. Error bars indicate ±1 standard error.

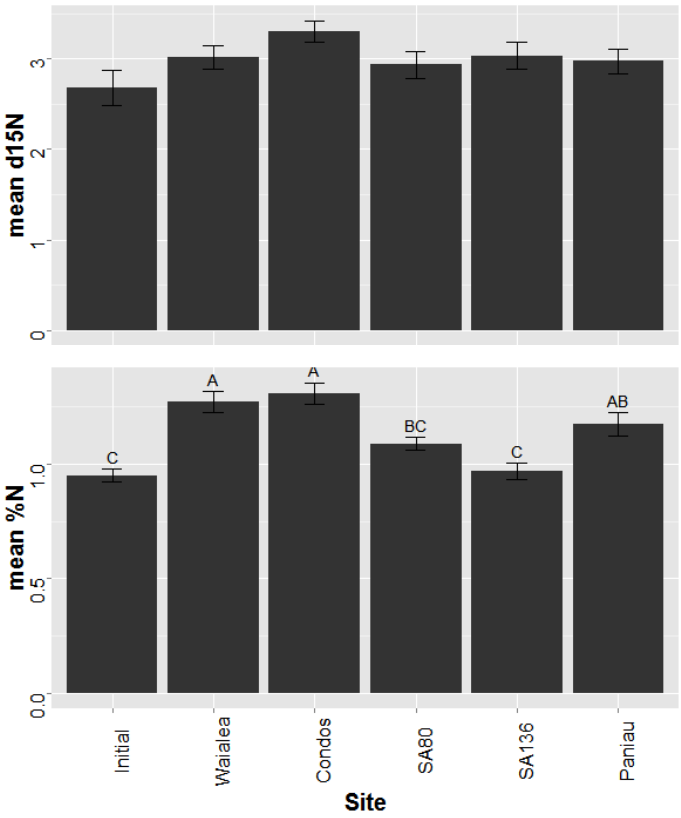


Figure 7: Results of nitrogen analyses. Mean $\delta^{15}N$ (‰) and mean %N for five sites and initial Ulva samples. Error bars indicate ± 1 SE. Statistically significant differences were not found for $\delta^{15}N$, but were found for %N, where shared letters indicate no significant difference.

Interaction of PGAs and indicators of sewage pollution

Linear models indicated a significant relationship between $\delta^{15}N$ and enterococci abundance (lm, $F(1,3)=10.66$, $p=0.047$, $r^2=0.71$) . However, high leverage of two data points and small number of points (n=5) renders interpretations preliminary.

There was a significant positive relationship between open shore CFU and PGA severity (lm, $F(1,8)=8.632$, $p=0.019$, $r^2=0.46$). This is a strong relationship, but should be interpreted cautiously because the relationship is driven largely by the Puakō Boat Ramp data point with particularly high CFUs and PGA severity. While not statistically significant, a similar trend was found in open shore CFU and PGA prevalence (lm, table 2, asterisk*), and also driven by the same PBR data point. Omitting the influential PBR point removed any relationships between enterococci and both PGA severity and prevalence (severity: $F(1,7)=0.1599$, $p=0.7012$, $r^2=-0.1173$; prevalence: $F(1,7)=0.3836$, $p=0.5553$, $r^2=-0.08348$). Additionally, linear models indicate no relationship between tide pool enterococci abundance and PGA measures, as both tide pool and combined CFUs did not vary significantly with PGA prevalence or severity (linear model, table 2).

No significant relationship was found between $\delta^{15}N$ and PGA prevalence or severity (lm, table 2).

Coral cover and indicators of sewage pollution

Enterococci abundance did not vary significantly with coral cover (lm, table 2). However, a linear model showed a strong association between $\delta^{15}N$ and coral cover ($F(1,3)=112.4$, $p=0.00179$, $r^2=0.9653$), but the high leverage of few points, and the small number of data points, make additional data useful.

Relationship	Test	df=	F=	p=	R^2=
overall CFU/100mL ~ Site	ANOVA	9,40	0.649	0.749	NA
anchialine pool CFU/100mL ~ Site	ANOVA	4,5	5.119	0.0513	NA
severity ~ overall CFU/100mL	LM	1,8	0.0796	0.785	-0.1139
PGA prevalence ~ overall CFU/100mL	LM	1,8	0.6157	0.4553	-0.04461
PGA prevalence ~ open shore CFU/100mL	LM	1,8	4.063	0.07859	0.2539
% coral cover ~ overall CFU/100mL	LM	1,8	0.1393	0.7187	-0.1058
% coral cover ~ open shore CFU/100mL	LM	1,8	2.752	0.1357	0.1629
PGA severity ~ δ^{15}N	LM	1,3	0.254	0.649	-0.2292
PGA prevalence ~ δ^{15}N	LM	1,3	0.2331	0.6622	-0.2372

Table 2: Statistics summary for differences across sites (ANOVA) and linear models. Asterisk (*) indicates near significant or significant relationship**.**

Discussion

Our study showed strong site-level variation in *Porites* growth anomaly prevalence and severity in the Puakō region. The highest levels were found in Waialea and the Puakō Boat Ramp area. While there were some statistical differences between the other sites measured, their general overlap shows they are all similarly lower in PGA prevalence and severity than the top two sites.

Enterococci abundance appeared to vary across sites; however, these differences were not statistically significant during summer 2013. Even within sites, enterococci abundance was highly variable and is likely dependent on a number of factors that affect the release and persistence of the bacteria in the water. In spite of within-site variation, our analysis of summer 2013 data with additional samples taken in winter 2014 showed that both site and time of sampling were important factors in enterococci abundance. These results, consistent with Cornell intern data from spring 2013, show that enterococci abundance is affected by rain events, which likely flush more enterococci into the ocean via groundwater and runoff. This data encourages future, seasonal monitoring of enterococci abundance in water along the Puakō coast to capture the spatial and temporal variation in sewage pollution, especially following rain events. Despite taking five replicate samples per site, the variability is high enough that an even larger sample size is needed to rank sites in levels of bacterial pollution. However, it is worth noting that very high levels were detected at SA 40, 88 and 152 and SA 80 and PBR showed extremely high levels immediately following rainfall.

The high levels of enterococci in anchialine pools (fig. 5D) suggest high levels of sewage pollution in the Puakō community. The pools are likely closer to the pollution sources than the shoreline, and molecular typing of enterococci found in the pools and along the shore could provide a connection between them. While it can be suggested that turtle feces could contribute to high levels of Enterococcus in tidepools, this does not explain the extremely high levels found in anchialine pools, which turtles cannot access. However, the long residency time of water and possible visitation by birds or terrestrial mammals may partially account for the high enterococci abundance in anchialine pools. Nonetheless, the exceptionally high levels of enterococci are indicative of human sewage pollution.

The highest values of PGA severity and prevalence occurred in Waialea and the Puakō Boat Ramp area. These areas, while having differing enterococci abundances, are similar in being embayments, which suggests limited water movement. In other studies, higher levels of coral disease also occurred in sites with limited water circulation (Couch PhD 2014). Incorporating a water movement measure, such as dissolving clod cards, into future studies would provide valuable information on the degree of water movement and verification that these are more sensitive areas with higher risk for coral disease.

We are only beginning to understand the role that sewage pollution plays in coral health in the Puakō region. There is an indication that PGA severity is correlated with open shore enterococci counts, but the high degree of variability in enterococci make it challenging to detect consistently and the relatively low r^2 value (0.46) indicate that other factors likely play into spatial patterns of PGAs. Furthermore, the relationships we found were driven largely by the highly degraded Puakō Boat Ramp site, and the removal of this data point dissolved the relationships. Although enterococci levels were very high in tidepools at some sites, perhaps indicating high inputs, the measured corals were over 50 meters away. Because enterococci was not sampled in the exact locations that the corals were surveyed, the observed strength of the relationship between sewage pollution and PGAs may underestimate the true relationship, as sewage pollution entering the marine environment would be subject to the hydrology between the SGD input and the corals. We suggest that the next step would be direct sampling from coral mucus to investigate a connection between sewage pollution and coral disease in Puakō.

There were no significant differences in our measured $\delta^{15}N$ between sites, which likely reflect the short incubation and outplanting times for our bioassays. Our samples were only incubated for 8 days and at sites directly on the reef, not nearshore where sewage pollution would be expected to be highest. Furthermore, the $\delta^{15}N$ levels we measured were much lower than those detected in an earlier study by Dailer et al (2010), which incubated algae in surface waters. In contrast, our algae were incubated at reef level, which could have limited nitrogen uptake due to limited light availability. Analysis of near shore samples of *Ulva* collected from Condos and SA80 showed significant differences between sites; levels of 8.6‰ at Condos are consistent with $\delta^{15}N$ enrichment associated with sewage pollution, while levels at SA80 at that time fall within a more normal range. Earlier analyses on resident algae from two sites revealed levels that were much higher than the bioassay algae and were consistent with sewage pollution in SA88 and possibly the Condos (based on results from Derse et al 2007). The significant difference in $\delta^{15}N$ in resident algae between the two sites suggests that site-level differences of $\delta^{15}N$ along the Puakō coast could be detected; however, the inconsistent distribution of *Ulva* along the region limits such sampling. Nonetheless, despite relatively low levels in $\delta^{15}N$ of incubated algae, our bioassays of incubated algae revealed that %N did vary among sites, with highest levels at Waialea Bay and Condos. This variation was also predictive of % coral cover in our study: coral cover was negatively correlated with %N. Our data indicates that nitrogen isotope analyses of algae can be a powerful tool in assessing water quality in Puakō, with the addition of longer incubation times, resident algae, and the analysis of sources (soil, seawater, cesspool sludge, etc.) for comparison.

Coral colony size was the most significant predictor of disease measures in our study, though the relationship was somewhat weak (severity: r^2=0.1007, prevalence: r^2=0.36). This relationship

can interact with the effects of water movement on coral growth. While low water movement can be detrimental to coral health, the protection from wave action that is provided by embayed sites, such as Waialea, Puakō Boat Ramp and to a lesser extent, Paniau, allow for extensive reefs and large coral colony sizes. However, if embayments limit water movement and the flushing of contaminants and pathogens, then they may also exacerbate disease (Couch et al 2014). Other studies have found negative relationships between water movement and coral disease (Couch PhD 2014, Burns et al 2011) Thus, the evidence suggests to us that risk of impacts to coral health is higher in the embayments along Puakō Reef.

This study provides evidence of a strong, but intermittent sewage input into the Puakō coastal waters, as evidenced by spikes of high abundances of fecal coliform bacteria. The extremely high levels of enterococci in anchialine ponds, often exceeding public health limits, supports the hypothesis that terrestrial sources are contributing to shoreline enterococci levels, not sea turtles. It is likely that these are human sources, but we cannot be certain if other animals (birds or mammals) may be contributing to the counts. In near-shore waters, the dilution effect makes it difficult to pick up a strong signal at the coral reef at all times and places, but there are intermittent pulses of high levels and data are consistent with sewage pollution affecting levels of coral disease in some locations. For example, Waialea Bay is more enclosed with less flushing and the high levels of coral disease there might be reflective of a sewage pollution impact. The caveats associated with enterococci count methods, including within-site variability and potential environmental reservoirs (sediments, soils, non-human biota, reviewed in Staley et al 2014), may warrant the development of microbial source tracking techniques (MST) in the future. MST are involved molecular methods that use DNA markers to trace sewage pollution to specific sources (Harwood et al 2013).

These results provide a strong case encouraging further study to successfully detect a relationship between coral health and sewage pollution, despite the complexity of the dynamics of the submarine groundwater discharge. Increased samplings per site and over time will provide better understanding of the differences between different sites along Puakō and may allow greater statistical power. Also, it may be of interest to add a study site on the south edge of Puakō Point, where there is a particularly large groundwater plume. Ultimately, this study provides a strong first step in pointing towards an impact of sewage pollution on Puakō's reef health.

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