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Linking sewage pollution and water quality to spatial patterns of *Porites lobata* growth anomalies in Puako, Hawaii

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ABSTRACT

Sewage pollution threatens the health of coastal populations and ecosystems, including coral reefs. We investigated spatial patterns of sewage pollution in Puako, Hawaii using enterococci concentrations and $\delta^{15}N$ *Ulva fasciata* macroalgal bioassays to assess relationships with the coral disease *Porites lobata* growth anomalies (PGAs). PGA severity and enterococci concentrations were high, spatially variable, and positively related. Bioassay algal $\delta^{15}N$ showed low sewage pollution at the reef edge while high values of resident algae indicated sewage pollution nearshore. Neither $\delta^{15}N$ metric predicted PGA measures, though bioassay $\delta^{15}N$ was negatively related to coral cover. Furthermore, PGA prevalence was much higher than previously recorded in Hawaii and the greater Indo-Pacific, highlighting Puako as an area of concern. Although further work is needed to resolve the relationship between sewage pollution and coral cover and disease, these results implicate sewage pollution as a contributor to diminished reef health.

1. Introduction

Coral disease is a significant factor in declining coral health throughout the world (Ruiz-Moreno et al., 2012), and is often exacerbated by global and local stressors, such as increasing water temperature and sedimentation (rev. in Harvell et al., 2007). One widespread anthropogenic stressor impacting coral reef ecosystems is sewage pollution. Sewage pollution is not a single, simple stressor; rather, it is complex and can introduce diverse pollutants, including nutrients (especially nitrogen and phosphorous), microbial pathogens, and chemical contaminants that themselves can impact coral reefs (Wear and Vega Thurber, 2015).

One component of sewage, nutrient pollution, can be an important facilitator of 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 (Bruno et al., 2003; Voss and Richardson, 2006; Harvell et al., 2007; Vega Thurber et al., 2014). It is important to note that there are several sources of nutrient influx to coral reefs, which include runoff (Fabricius, 2005), fish farm effluents (García-Sanz et al., 2011), and other natural terrestrial and atmospheric contributions (Szmant, 2002). Sewage pollution can also introduce pathogens, as is the case with the human gut microbe, *Serratia marcescens*. This microbe has been considered responsible for white pox disease in the Caribbean and the resulting decimation of the now endangered *Acropora palmata* corals (Sutherland et al., 2010), though this relationship is disputed (Lesser and Jarett, 2014). A third possible contribution of sewage pollution to coral disease is chemical contaminants such as endocrine disruptors, heavy metals, and other toxins (rev. in Wear and Vega Thurber, 2015).

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. The gram-positive *Enterococcus* spp. bacteria are used by the US Environmental Protection Agency (EPA) as a water quality metric and to assess the degree of sewage pollution. Naturally found as facultative anaerobes in human and animal guts, enterococci are relatively persistent bacteria and are able to tolerate both fresh and saline water.

Compared to typical seawater, sewage-polluted water is enriched in ^{15}N relative to ^{14}N, and therefore, has a highly positive $\delta^{15}N$ value (>10 ‰), which distinguishes it from other nitrogen sources such as fertilizers or N_2-fixing plants (~0‰) (Heaton, 1986; Derse et al., 2007; Risk et al., 2009; Dailer et al., 2010). The enrichment of ^{15}N in the environment is reflected in macroalgae and other biological tissues, allowing bioassays to be used to detect enrichment of ^{15}N and thus indicate sewage pollution (Risk et al., 2009). There is a large range of $\delta^{15}N$ values that

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could indicate sewage pollution in macroalgae (3 to >10‰), but levels typically decrease with increasing distance from the pollution source (Heaton, 1986; Costanzo et al., 2001; Risk et al., 2009). In Dailer et al. (2011), the alga *Ulva fasciata* was grown in waters off West Hawaii to produce a sample that integrated levels of nitrogen over a 1-week period. Dailer et al. (2010) also used $\delta^{15}N$ analysis on resident algae to map sewage pollution in Maui, Hawaii. Similar studies have been performed in both temperate and tropical environments; for example, bioassays of the oyster *Crassostrea virginica* and macroalga *Gracilaria* sp. were used to detect sewage and animal waste pollution in coastal Maryland, USA (Fertig et al., 2009) and Moynihan et al. (2012) used a combination of enterococci concentration measurements and $\delta^{15}N$ analysis of seagrass, scleractinian corals, sponges, and macroalgae in Zanzibar, Tanzania.

In Puako, Hawaii, untreated sewage pollution can enter the coral reef ecosystem through the combination of old cesspool systems, highly porous volcanic bedrock, and close proximity to the shore via submarine groundwater discharge (SGD) (Street et al., 2008; Knee et al., 2010). This region of the leeward coast of Hawaii Island (West Hawaii) has particularly well-developed reefs. However, as with many other coastal regions of Hawaii, the Puako region has experienced increased pressures from fishing, land-based pollution, recreational use, development, and likely climate change in recent decades. The consequences of these compounding impacts likely explain the dramatic decline of Puako marine resources over the long term. Data compiled by The Nature Conservancy and the Hawaii State Division of Aquatic Resources show a decrease in fish abundance over a 40-year span (Minton et al., 2012; Walsh, 2013). Coral cover also declined dramatically in Puako, dropping from 80% in the 1970s to 32% in 2010 (Walsh, 2013). Additionally, Couch et al. (2014) identified Puako as one of four regions in West Hawaii 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, it is important to consider the role of coastal pollution in facilitating coral disease and death. Community concern primarily focuses on sewage pollution; however, other pollutants and sources of nutrient inputs may include animal wastes from upland regions, and fertilizers and pesticides used in the area. The nitrogen-fixing tree *Prosopis pallida* is common in the area, but work in anchialine pools elsewhere suggests that its contribution to nitrogen inputs is minimal (Dudley et al., 2014).

Puako reefs are dominated by *Porites lobata* corals, which are also the most vulnerable to disease (Friedlander et al., 2008; Aeby et al., 2011b; Couch et al., 2014). The most prevalent of these diseases is *P. lobata* 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 to grow (Yasuda et al., 2012). As sinks for their colonies' resources and sites of decreased reproductive function, PGAs have the potential to decrease the reproductive ability and immunity of the whole colony (see Burns and Takabayashi, 2011 for *Montipora capitata* growth anomaly impact). Although a viral microbial agent has been investigated (Vega Thurber and Correa, 2011), the causative agent remains unknown and could even include somatic mutation (Irikawa et al., 2011).

Our study examines the relationships between PGAs, enterococci concentrations, and macroalgal $\delta^{15}N$ across 10 sites in Puako to test the hypothesis that coral disease is higher in areas of sewage inputs. Because host properties can also influence disease in addition to environmental pollution, we also investigated the role of colony size in patterns of PGAs.

2. Methods

2.1. The Puako region and study sites

The Puako region is located along the leeward, Kohala Coast of Hawaii Island, Hawaii. The adjacent community is comprised of one hundred sixty-three (163) houses. Cesspools and septic tanks provide the majority of wastewater treatment for the community, except for the condominium which uses an ejection well for primary treated sewage (Minton et al., 2012). The greater census-designated place has 772 residents and 2326 housing units, which includes hotels and condominiums in the resort area nearby to the community of interest (US Census Bureau, 2010).

Ten study sites ranging from Waialea Bay to Paniau Bay were selected to capture variation in coral health and sewage pollution along 3 km of the Puako coast (Fig. 2). Where possible, study sites were selected at shoreline access (SA) locations. SA sites (SA40, SA80, SA88, SA100, SA136, SA152), Condos, and Waialea were all located adjacent to residences. The road through the community hugs the shoreline such that most of the residences are immediately adjacent or across the road (inland) from the shoreline, forming a consistent strip two-houses deep along the SA sites. Paniau and Waialea are both public beaches, PBR is a launching site for small boats, and Condos is near the condominiums complex in the community.

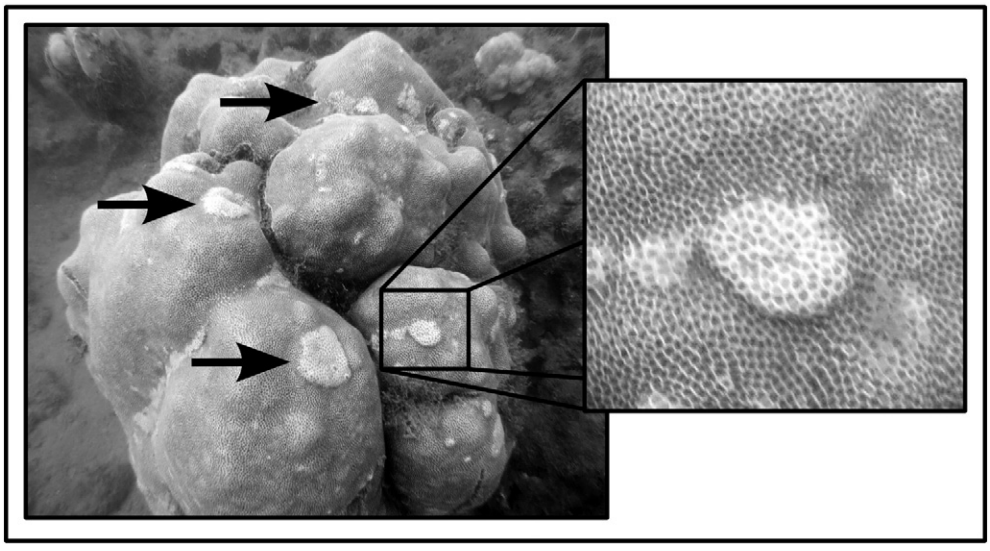

Fig. 1. *Porites* growth anomalies (PGAs) on *Porites lobata*. Arrows indicate several conspicuous PGAs. Note lighter pigmentation, raised surface, and enlarged polyps of PGA as shown in inset.

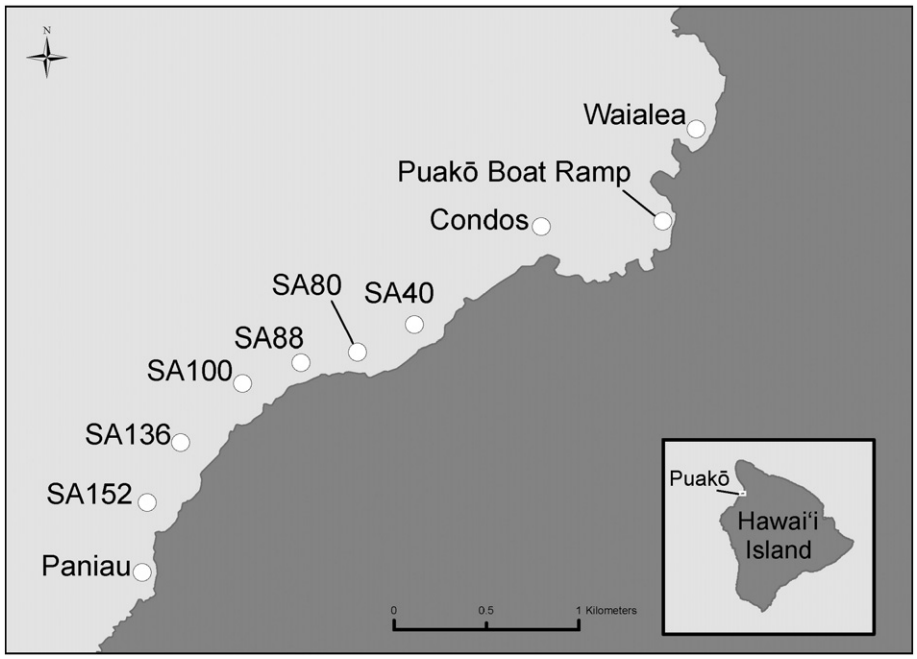

Fig. 2. Puako region and study sites. Ten sites were selected for this study, spanning from Paniau Bay to Waialea Bay. Algal $\delta^{15}N$ bioassays were conducted at Waialea, Condos, SA80, SA136, and Paniau. Inset depicts the entire Hawaii Island with a box indicating the study region.

The region has a dry climate as does most of the leeward coast of the island. Freshwater entering the marine environment is primarily from upland areas and is delivered via underground aquifers through porous volcanic rock. Areas nearshore where the aquifers are exposed can create anchialine pools, which are surficially isolated from the ocean but are connected underground. The pools are mixohaline due to the combined influences of the freshwater aquifers and the ocean, and rise and fall with the tide. Anchialine pools sampled in this study were 102 ± 12 m from the shore and in the same area as the more inland residences.

2.2. Coral cover and disease surveys

Three replicate 15-m transects were laid haphazardly at each of the 10 sites, approximately parallel to the reef edge in 2–3 m deep water to maintain consistency among sites. Ten 0.5 × 0.5-m quadrats were placed along each side of the transect for a total of 20 quadrats per transect. To limit duplicate measures of large colonies spanning the transect line quadrats were placed alternately along 1.5-m intervals on the line. Within each quadrat the number of PGAs on each colony, and the maximum diameter of each colony (here on referred to as "colony size"), and the percent coral cover were recorded. PGAs were identified based on gross appearance of raised skeleton with light pigmentation and large or irregular polyp size. PGA severity was recorded as the number of PGAs per colony and prevalence as the proportion of *P. lobata* colonies afflicted with PGAs. Live coral cover was estimated using 10 × 10-cm grids within the quadrat.

2.3. Enterococci assays

Shortly after low tide, five 100 mL water samples were collected at each site, with three along the open shoreline (25 June 2013) and two in the nearby tide pools, where visible exchange with the ocean was absent or restricted (3 July 2013). Although it would have been ideal to sample the water column directly over the coral survey transects, enterococci concentrations were expected to be too dilute for detection. Five anchialine pools (two samples per pool) were sampled in the Puako area, as these were expected to show a stronger influence of sewage if present, using the same methods as marine samples (28 June 2013). Water samples were kept on ice and processed within 6 h using methods similar to those described in the EPA Method 1600 (US Environmental Protection Agency, 2009) and Baker et al. (2010), except substituting mEI agar with m-*Enterococcus* agar and incubating at 37 °C for 48 h. Additionally, samples were collected in the following winter on 7 January 2014 and 13 January 2014, both after rainfall events (post-rainfall I and post-rainfall II, resp.). Winter samples were collected at five of the 10 original sites (Waialea, Puako Boat Ramp, Condos, SA80, and Paniau, n = 3–6 per site per sampling time). Enterococci colony forming units (CFU) growing on m-*Enterococcus* agar produce red colonies; the number of these colonies divided by the volume of water filtered provided the concentration of enterococci, here on presented as CFU/100 mL.

2.4. $\delta^{15}N$ bioassay with *U. fasciata*

U. fasciata was collected from the shoreline at the Natural Energy Labs of Hawaii Authority due to very low abundance in Puako. To minimize variation in initial algal $\delta^{15}N$ and starve the algae of nitrogen, 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; however, this was not verified. Over the seven-day incubation (based on methods in Dailer et al., 2010, 2011), half of the aquaria water was replaced with fresh seawater every two days.

After incubation, *U. fasciata* was divided into individual fronds, which were placed into cages of plastic fencing and zip ties. Labeled cages were deployed at reef level in five of the sites: Waialea, Condos, SA80, SA136, and Paniau (n = 10 per site). An additional 10 replicate fronds were reserved and processed to serve as initial measurements prior to deployment. Resource limitations did not allow cages to be deployed at all sites; these were selected because they spanned the region and also captured areas of high human use and interest (Waialea, Condos, and Paniau). The *U. fasciata* were left to incubate for eight days, after which cages were collected, the fronds promptly rinsed in commercial bottled distilled freshwater, cleaned of debris, and dried. Dried samples were sent to Ithaca, NY, where they were ground in liquid nitrogen for $\delta^{15}N$ and %N analysis by the Cornell University Stable Isotope Laboratory (COIL), Ithaca, NY.

To determine whether the $\delta^{15}N$ of incubated algae would reflect the $\delta^{15}N$ of resident algae, resident *U. fasciata* were also collected from the shoreline (0-m depth) adjacent to the Condos and SA80 study sites ($n = 5$ per site), which were processed for stable isotope analysis similarly to incubated samples at COIL.

2.5. 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 concentrations among sites. A binomial-family generalized linear mixed model (GLMM, in lme4, Bates et al., 2015) followed with a general linear hypothesis test (glht, in multcomp, Hothorn et al., 2008) was used to examine the site-level differences of PGA prevalence with transect treated as a random effect. PGA severity was investigated similarly using a Poisson-family GLMM and glht. Goodness-of-fit testing against the null model was done with likelihood ratio tests (LRT).

To investigate the impact of sampling time and location on enterococci concentration using the additional winter 2014 samples, a GLM with the negative binomial distribution (Venables and Ripley, 2002) was used as is appropriate for count data with high variance relative to the mean (Crawley, 2007). Sum 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 among sites using glht.

Because macroalgal $\delta^{15}N$ data were non-normal, the Kruskal–Wallis test was used to determine variation in $\delta^{15}N$ among sites. The relationships between PGA measures and colony size, $\delta^{15}N$, and enterococci counts were investigated with linear regressions. In cases where analysis of Cook's distance detected possible outliers or points with high influence, an additional regression excluding those points was performed.

All analyses are performed at significance level $\alpha = 0.05$, except in post hoc tests where Bonferroni-corrected *p*-values are given.

3. Results

3.1. Spatial patterns of P. lobata growth anomalies and coral cover

PGA prevalence and PGA severity varied significantly by site (Fig. 3A, B, prevalence: likelihood ratio test (LRT): $\chi^2(9) = 39.4$, $p < 0.05$, severity: LRT: $\chi^2(9) = 53.0$, $p < 0.05$). Highest values occurred in Waialea, the Puako Boat Ramp, and Paniau (prevalence: 37.5 ± 6.1%, 35.8 ± 3.1%, and 27.6 ± 4.8%, resp.; severity: 1.8 ± 0.2, 2.1 ± 0.4, and 0.9 ± 0.1 PGAs per colony, resp.). The lowest measures of disease were found at SA40, with a prevalence of 13.8 ± 1.1% and a severity of 0.3 ± 0.05 PGAs per colony. Mean PGA severity and prevalence for the entire study region were 20.5 ± 0.7% and 0.63 ± 0.03 PGAs per colony, respectively. Percent coral cover varied significantly by site (Fig. 3C, ANOVA: $F(9,589) = 17.2$, $p < 0.05$), though not in a recognizable geographic pattern. Mean percent coral cover ranged from 13.4 ± 1.6% at the Condos site to 48.3 ± 3.2% at SA88, with an overall mean of 44.6 ± 0.4%.

3.2. Host demographic patterns of P. lobata growth anomalies

PGA prevalence was significantly predicted by colony size (Fig. 4, linear model, $F(1,8) = 9.50$, $p < 0.05$, $R^2 = 0.49$). Notably, omitting an outlier (Waialea) with high influence still yielded a strong, significant relationship (lm, $F(1,7) = 15.88$, $p < 0.05$, $R^2 = 0.65$). Similarly, PGA severity increased with the size of the colony (lm, $F(1,8) = 11.76$, $p < 0.05$, $R^2 = 0.54$). However, this relationship was driven by high disease and large colonies at PBR, suggesting that other, more site-specific factors may play greater roles in determining PGA severity (a linear regression omitting PBR was non-significant: $F(1,7) = 3.41$, $p = 0.11$, $R^2 = 0.23$).

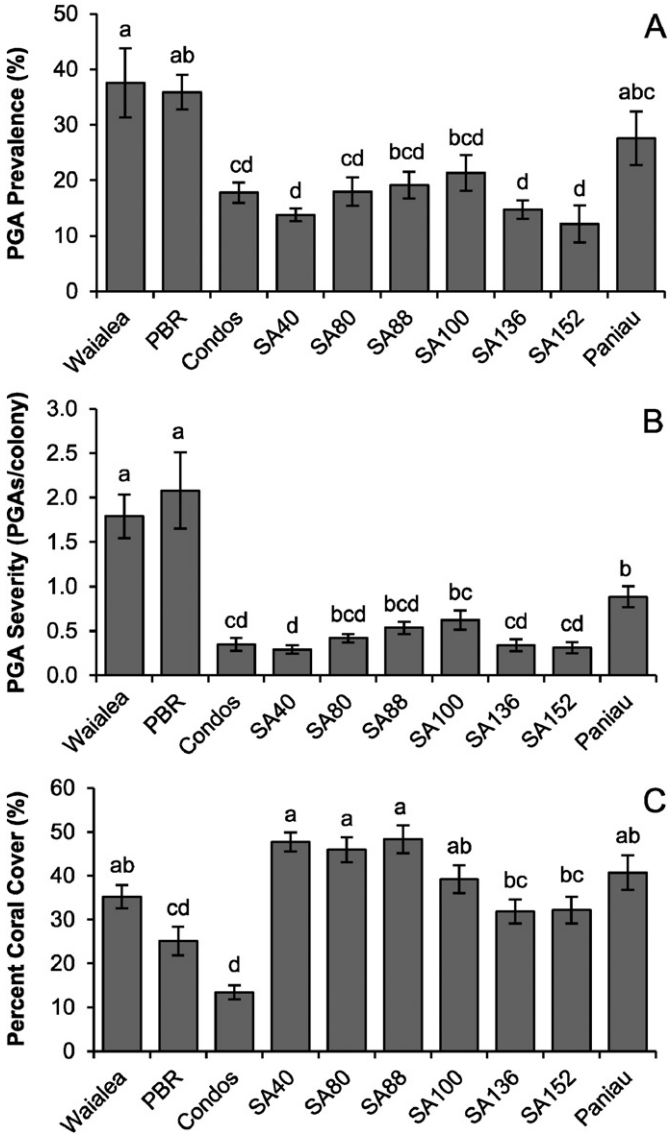

Fig. 3. Mean (±SE) (A) PGA prevalence (binomial-family generalized linear mixed model (GLMM), likelihood ratio test (LRT): $\chi^2(9) = 136.38$, $p < 0.05$), (B) PGA severity (Poisson-family GLMM, LRT: $\chi^2(9) = 1076.1$, $p < 0.05$), and (C) percent coral cover by site (ANOVA: $F(9589) = 17.2$, $p < 0.05$). Shared letters indicate no significant difference. The Puako Boat Ramp is abbreviated as PBR.

3.3. Enterococci concentrations

Enterococci concentration varied greatly within and across study sites (Fig. 5). 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 concentrations among sites. Anchialine pools were also variable, with marginally non-significant differences among sites (Fig. 5, Table 1). The mean enterococci concentrations only exceeded the recommendations of 104 CFU/100 mL for some tide pools. Four of the five anchialine pools had concentrations greatly exceeding the limits of 104 CFU/100 mL.

Incorporating additional data from sampling in January 2014, a model that includes site, sampling time, and the interaction between site and sampling time best predicts enterococci concentration (Fig. 6, LRT: $\chi^2(14) = 85.2$, $p < 0.05$), as the AIC value is much lower than those of the other models (Table 2). The results of the multiple comparisons by sampling time show that enterococci concentration was significantly higher following the first rainfall event (post-rainfall I)

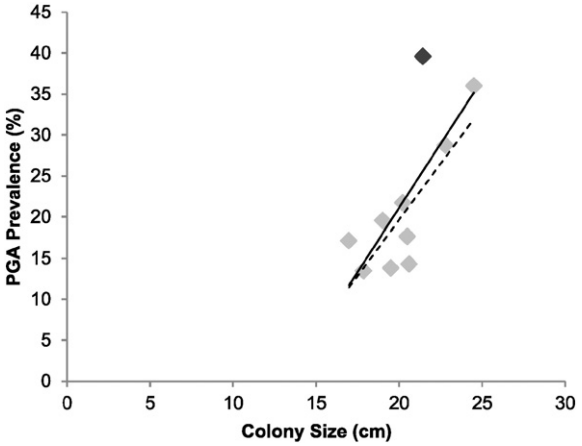

Fig. 4. PGA prevalence versus colony size (linear model, $F(1,8) = 9.50$, $p < 0.05$, $R^2 = 0.49$). Omitting an outlier (dark point, Waialea) with high influence still yielded a significant relationship (lm, $F(1,7) = 15.88$, $p < 0.05$, $R^2 = 0.65$).

compared to both the summer and the second post-rainfall event (post-rainfall II) sampling times ($p < 0.05$ for both). Multiple comparisons by sites indicate overall significant differences among sites (Fig. 6); however, as predicted by the model, these differences are not consistent between sampling times.

3.4. $\delta^{15}N$ analyses

Measures of $\delta^{15}N$ in deployed algae did not vary significantly by site ($\chi^2 = 9.0637$, df = 5, $p = 0.107$), though the initial values (post-starvation, pre-deployment) were on average lower than those of the treatments by 0.4 ± 0.1‰. The Condos site had the greatest $\delta^{15}N$. Percent nitrogen differed significantly by site for deployed algae (ANOVA, $F(5,54) = 14.47$, $p < 0.05$), with Waialea Bay and Condos significantly higher than all sites except Paniau. While these data alone do not point to a source of pollution, 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 SA80 and Condos sites ($t(7.035) = 18.1526$, $p < 0.05$), with the $\delta^{15}N$ of Condos (8.6 ± 0.1‰) being nearly double that of SA80 (4.6 ± 0.2‰) and much higher than the incubated algae.

3.5. Relationship between PGAs and indicators of sewage pollution

$\delta^{15}N$ and enterococci concentration were significantly related as indicated by linear models (Fig. 7, lm, $F(1,3) = 10.66$, $p = 0.047$, $R^2 = 0.71$). However, the slope was small (0.02), two data points were highly influential, and there was a small number of data points ($n = 5$).

Open shoreline enterococci concentration and PGA severity were positively related (Fig. 8, lm, $F(1,8) = 8.632$, $p = 0.019$, $R^2 = 0.46$). This strong relationship should be interpreted cautiously because the relationship is driven largely by the Puako Boat Ramp data point with particularly high enterococci concentrations and PGA severity. While not statistically significant, a similar trend was found in open shoreline enterococci concentration and PGA prevalence (lm, Table 1), and also driven by the same Puako Boat Ramp data point. Omitting the influential Puako Boat Ramp point removed any relationships between enterococci concentration and both PGA severity and prevalence (severity: $F(1,7) = 0.1599$, $p = 0.70$, $R^2 = -0.12$; prevalence: $F(1,7) = 0.3836$,

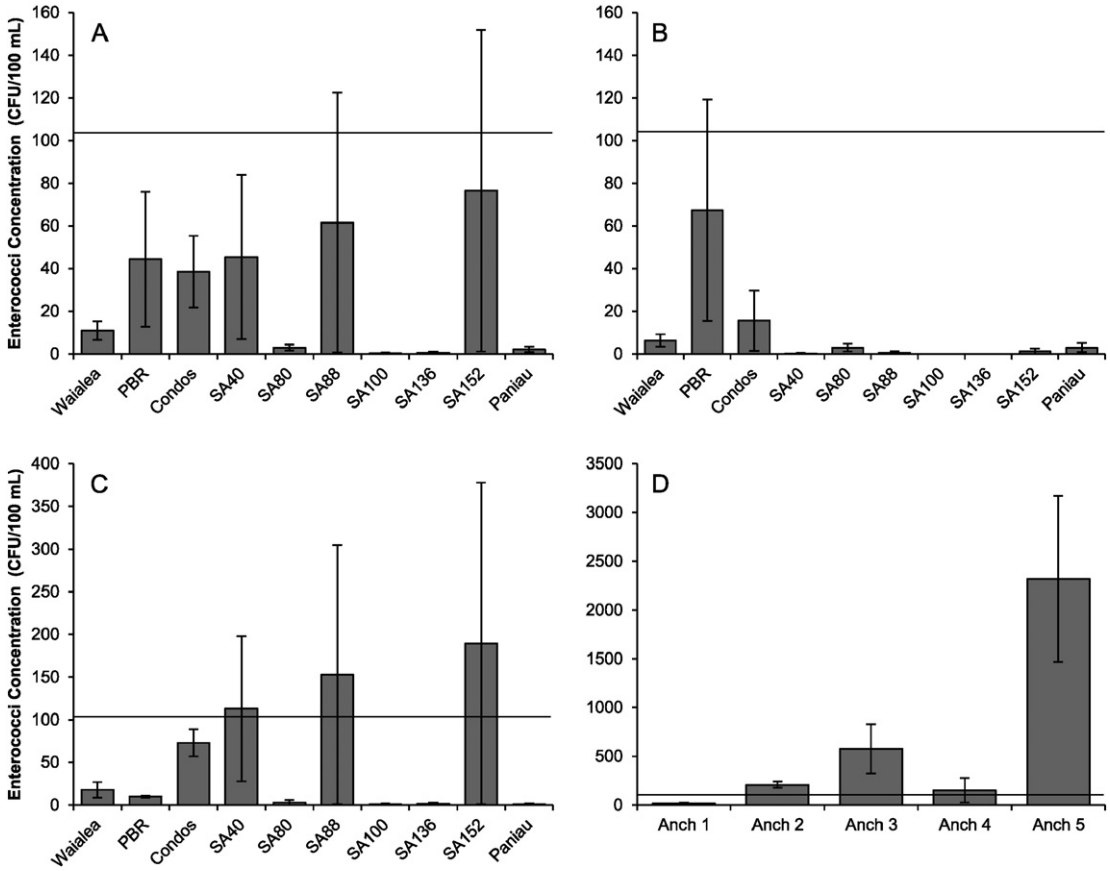

Fig. 5. Enterococci concentration in CFU/100 mL A) overall (combined open shoreline and tide pools), B) open shoreline only, C) tide pools only, and D) anchialine pools. No significant differences were found between sites. Horizontal lines represents Hawaii State Department of Health single sample maximum of 104 CFU/100 mL. The Puako Boat Ramp is abbreviated as PBR.

Table 1
Statistics summary for differences among sites and relationships between predictors of sewage pollution and PGAs. Analysis of variance is abbreviated as ANOVA and linear model/regression as LM.

Relationship	Test	df	F	p	R^2
Overall (combined shoreline and tidepool) enterococci concentration vs site	ANOVA	9,40	0.649	0.749	NA
Anchialine pool enterococci concentration vs site	ANOVA	4,5	5.119	0.051	NA
Severity vs overall enterococci concentration	LM	1,8	0.0796	0.785	−0.1139
PGA prevalence vs overall enterococci concentration	LM	1,8	0.6157	0.455	−0.0446
PGA prevalence vs open shoreline enterococci concentration	LM	1,8	4.063	0.0786	0.2539
% coral cover vs overall enterococci concentration	LM	1,8	0.1393	0.719	−0.1058
% coral cover vs open shoreline enterococci concentration	LM	1,8	2.752	0.136	0.1629
PGA severity vs algal $\delta^{15}N$	LM	1,3	0.254	0.649	−0.2292
PGA prevalence vs algal $\delta^{15}N$	LM	1,3	0.2331	0.662	−0.2372

$p = 0.56$, $R^2 = -0.08$). Additionally, both tide pool and combined (open shoreline + tide pool) enterococci concentrations did not vary significantly with PGA prevalence or severity (lm, Table 1).

No significant relationship was found between $\delta^{15}N$ and PGA prevalence nor severity (lm, Table 1).

3.6. Coral cover and indicators of sewage pollution

Coral cover did not vary significantly with enterococci concentration (lm, Table 1). However, a linear model showed a strong negative relationship between $\delta^{15}N$ and coral cover (Fig. 9, $F(1,3) = 112.4$, $p < 0.05$, $R^2 = 0.97$).

4. Discussion

Sewage pollution is a widespread coastal issue impacting many coral reef environments worldwide. As a multi-faceted stressor, sewage pollution can impact many aspects of coral reef health, including disease (Wear and Vega Thurber, 2015). Despite Puako's reputation as one of Hawaii's healthiest coral reefs, here we provide evidence that sewage pollution is entering the marine environment, is linked to reduced coral cover, and may facilitate the coral disease *Porites* growth anomalies (PGAs).

As snapshot and integrative metrics of sewage pollution, respectively, enterococci concentrations and $\delta^{15}N$ measures are rarely used in conjunction to relate possible sewage impacts on coral reefs. Either one or the other is used, and if both are, they are used to validate the other (Baker et al., 2010; Moynihan et al., 2012). We went further to sample both enterococci concentrations and macroalgal bioassay $\delta^{15}N$ in relation to a highly prevalent coral disease. As our results have shown, enterococci concentrations are intermittent (Fig. 6), changing in spatial pattern following rain events and possibly varying by season, which typically have different levels and patterns of precipitation. It was also only in this temporal–spatial analysis that significant differences between sites in enterococci concentration were significant, not in the summer sampling alone. This highlights the importance of long-term sampling of enterococci to capture the temporal patterns of sewage pollution entering the marine environment, as snapshot sampling is insufficient to elucidate these patterns.

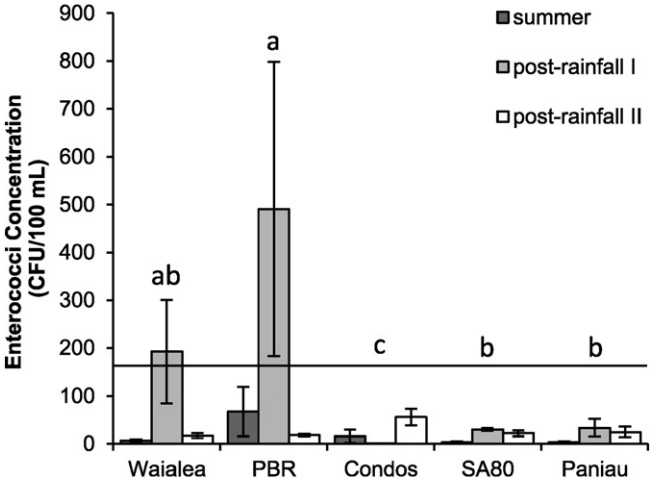

Fig. 6. Mean (±SE) open shoreline enterococci concentrations by site on 25 June 2013 (summer) and on 7 January and 13 January 2014 respectively following two rainfall events (post-rainfall I and II). Shared letters indicate no significant differences among sites overall as determined by multiple comparisons general linear hypothesis testing, but do not indicate any relationships at or between specific sampling times (negative binomial-family general linear model, likelihood ratio test: $\chi^2(14) = 85.2$, $p < 0.05$). Horizontal line indicates single sample maximum of 104 CFU/100 mL as per Hawaii State regulations. The Puako Boat Ramp is abbreviated as PBR.

Although the quantification of enterococci concentrations in seawater is used by US governmental agencies to monitor human health risks and in numerous studies to detect sewage pollution, the method has limitations. Several factors can affect enterococci concentrations in seawater, possibly making them less reliable as indicators of human sewage, including non-human animal wastes (birds, invertebrates, fish, etc.), disruption of environmental reservoirs (sands and other sediments), dilution, and exposure (rev. in Staley et al., 2014; Byappanahalli et al. 2012). One alternative explanation for high enterococci concentrations along the shoreline and tidepools in Puako is visitation by green sea turtles (*Chelonia mydas*), which are common in the area. The high concentrations of enterococci in anchialine pools (which they cannot access) allow us to infer sewage pollution as the source in Puako.

The $\delta^{15}N$ of resident algae collected at the Condos (8.6 ± 0.1‰) and SA40 (4.6 ± 0.2‰) sites were high and indicative of sewage pollution. The $\delta^{15}N$ of the Condos algae were consistent with that found in Dailer et al., 2011. That of SA80 may still reflect sewage pollution (based on minimum values of Costanzo et al., 2001), but falls between values of soil (2.3‰) and surface seawater (7‰) based on values found in Derse et al., 2007. Condos and SA80 resident algae $\delta^{15}N$ were significantly different from each other, which suggests shoreline sampling of resident algae or more nearshore, surface bioassays could be used to detect differences in $\delta^{15}N$ and thus sources of nitrogen along the coast. These finding are consistent with other studies that have shown the efficacy of assessing macroalgal $\delta^{15}N$ to determine sources of nitrogen entering the marine environment (Lapointe et al., 2005;

Table 2
Generalized linear model selection using Akaike information criterion (AIC) values. Lower AIC values are interpreted to represent better models. The difference between the best model AIC and the respective model AIC is shown as dAIC.

Model	AIC	dAIC	df
Enterococci concentration vs site × time	529.7395	0	16
Enterococci concentration vs site + time	563.4427	33.7032	8
Enterococci concentration vs time	569.5475	39.808	4
Enterococci concentration vs site	570.8209	41.0814	6
Enterococci concentration vs 1 (null)	585.9854	56.2459	2

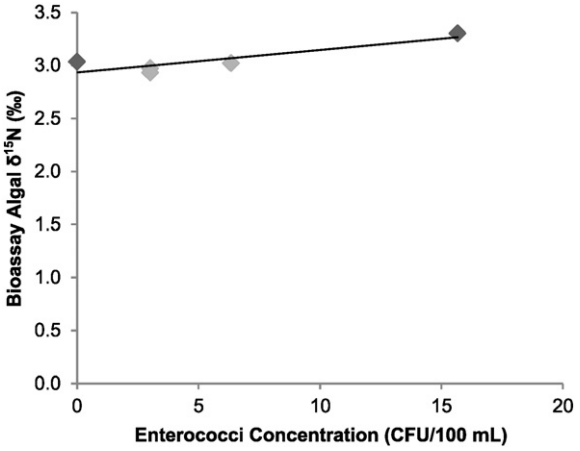

Fig. 7. Bioassay algal $\delta^{15}N$ versus open shoreline enterococci concentration (linear model, $F(1,3) = 10.66$, $p = 0.047$, $R^2 = 0.71$). Darker points are those with high influence (Condos and SA136); a regression omitting those points is not shown as the resulting model would be uninformative.

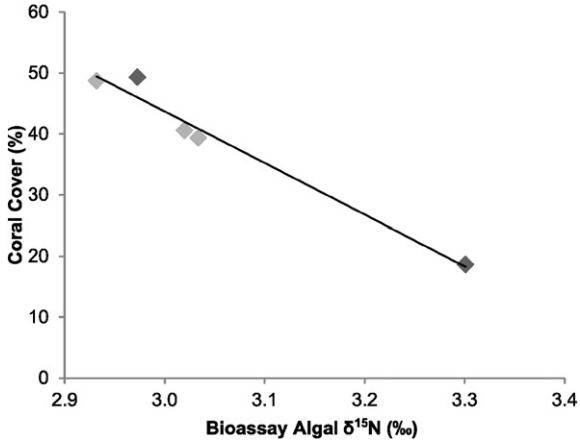

Fig. 9. Percent coral cover versus bioassay algal $\delta^{15}N$ (linear model, $F(1,3) = 112.4$, $p = 0.00179$, $R^2 = 0.9653$). Dark points are those with high influence (Condos and Paniau); a regression omitting these points is not shown as it would be uninformative.

Costanzo et al., 2001, 2005; Baker et al., 2013; Dailer et al., 2010, 2011; Moynihan et al., 2012).

In contrast, $\delta^{15}N$ values found in our macroalgal bioassay were not significantly different among sites and low compared to published values, making them closer to those of fertilizer or biologically-fixed nitrogen (~0‰) than those more indicative of sewage pollution (~10‰ or greater, Heaton, 1986). However, these values are not too low to be influenced by sewage pollution as they could represent 1) lower influence of sewage pollution and greater homogeneity of water more offshore, where our surveys and bioassays were located, 2) limitations to nitrogen uptake at reef-level (consistent with findings of Dailer et al., 2010 that surface-deployed algae have higher %N and $\delta^{15}N$ values than those at depth), and/or 3) a short deployment time. Values of deployed macroalgae in other studies that may indicate sewage pollution vary from 3‰ (Costanzo et al., 2001) to 50.1‰ (Dailer et al., 2010), a large range that reflects variation in the degree of sewage input, location of sampling, and other aspects of the samplings. Dailer et al. (2010) found that surface bioassay algae could reach values comparable to those of collected resident algae in 7 days; however, the location of our assays at depth and the lack of sewage inputs during that time could have required a longer time to do the same.

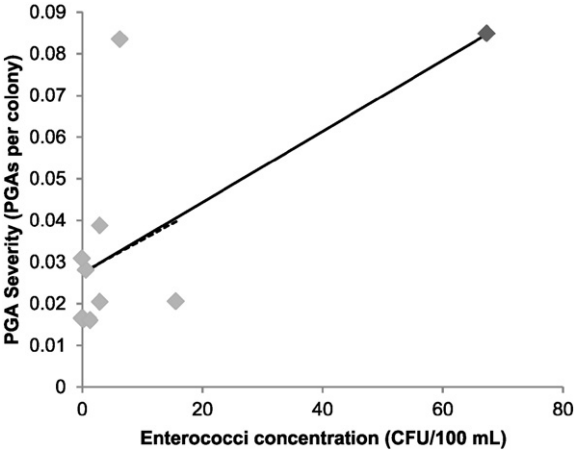

Fig. 8. Mean site PGA severity versus open shoreline enterococci concentration. Darker point is Puako Boat Ramp, which is an outlier with high influence due to its particularly high level of disease and enterococci concentration. The linear model for all sites is shown with the solid regression line ($F(1,8) = 8.632$, $p = 0.019$, $R^2 = 0.46$); a regression omitting the Puako Boat Ramp point is shown as the shorter dashed line ($F(1,7) = 0.1599$, $p = 0.7012$, $R^2 = -0.1173$).

Sewage pollution may affect multiple aspects of coral health, with one main concern often being its nutrient component (rev. in Fabricius, 2005 and Wear and Vega Thurber, 2015). The few studies that have studied the relationship between sewage pollution and coral disease find that sewage pollution exacerbates disease (Kaczmarsky et al., 2005, Sutherland et al., 2010). In our study, PGA severity (number of PGAs per colony) was positively related to enterococci concentration (Fig. 8). However, the relationship was driven by the high-enterococci concentrations and high-disease levels at the Puako Boat ramp. Additionally, distance between the shoreline enterococci samplings and more offshore coral disease surveys complicate the relationship. This is not unexpected, as survey sites were chosen to be further offshore in order to have sufficient coral populations for disease measures. Further inshore, there were fewer colonies and a greater proportion were unhealthy with sedimentation and algal overgrowth (Yoshioka & Kim pers. obs.). This skews our results conservatively, towards less-impacted colonies. $\delta^{15}N$ analyses were less informative, likely due to low sample size and among-site variation. Although no other metrics of sewage pollution were significantly related to coral disease, $\delta^{15}N$ was strongly negatively related with % coral cover, despite low among-site differences (Fig. 9). The small number of points ($n = 5$) requires greater sampling and study to confirm this relationship and its mechanism. Additionally, our bioassays revealed that %N did vary among sites, with highest levels at Waialea Bay and Condos, suggesting that nitrogen inputs into Puako reefs differ along the coastline, regardless of the source.

Variation in host factors can also influence coral disease (Sutherland et al., 2004; Harvell et al., 2007). In this study PGA prevalence and severity were both positively related with colony size, though severity was driven by large colony sizes and high disease at PBR (Fig. 4 for PGA prevalence). Larger colonies are typically older, which theoretically provides a larger spatial and temporal opportunity for disease to occur. Additionally, larger colonies were generally found in more embayed sites such as Waialea, Puako Boat Ramp and to a lesser extent, Paniau. The protection from wave action in embayed sites generally allows for more extensive reefs and larger coral colony sizes (Todd, 2008). 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, 2014; Burns et al., 2011) and a highly significant effect of coral size on PGA (Couch, 2014; Couch et al., 2014). Although it is uncertain whether colony size or water movement is driving high disease measures, the evidence suggests higher risk of disease in embayments and prioritizes the reduction of possible stressors in these areas. Measures of water movement,

such as clod cards, may help to distinguish these environmental and host factors.

PGAs are among the most prevalent coral diseases on Hawaii Island, with Aeby et al. (2011b) finding an average of 0.52 ± 0.2% colonies afflicted for the island and Couch et al. (2014) finding an 26-fold greater (13.7 ± 0.82%) prevalence in West Hawaii. Our site with the lowest disease measures, SA40, had a similar prevalence of 13.8 ± 1.1%. Overall, the prevalence of PGAs in our study was 20.5 ± 0.7%, with a maximum of 37.6 ± 6.2% at Waialea. These measurements dwarf prevalences found by Aeby et al. (2011a) for the entire Indo-Pacific region: an average of 0.21% with a maximum of 16.7% (in the Hawaiian Islands). Even with the possibility of minor differences in sampling and identification of PGAs due to their variable nature, the relatively high prevalence of PGAs in Puako highlights the region as one of concern for Hawaii Island and the Hawaiian Islands in general.

This study adds to the growing body of research implicating sewage pollution as an important factor in declining coral reef health. Sewage pollution, detected through enterococci concentrations and $\delta^{15}N$ macroalgal bioassays, may exacerbate coral diseases and reduce coral cover. Sewage inputs are influenced by rainfall, highly variable over space and time, and challenging to pin down. Furthermore, Puako has been identified as a region of high disease, despite its reputation as a healthy reef. It is important to note that while most samplings fell below the single sample maximum of 104 CFU/100 mL as recommended by the USEPA and Hawaii State Department of Health (Hawaii Administrative Rules §11–54-8, 2013), Waialea and Puako Boat Ramp (post-rainfall I), tidepools at SA40, SA80, and SA152, and four of the five anchialine pools greatly exceeded the limits (Fig. 5), constituting a public health concern. Although the exact relationship between sewage pollution and levels of coral disease remains uncertain in Puako, our work highlights priorities for future studies of sewage pollution and coral reef health there and elsewhere: 1) improve the temporal resolution of sewage pollution metrics to capture variation due to intermittent pulses of sewage pollution, 2) develop and utilize alternative metrics for sewage pollution, such as microbial source tracking, and 3) quantify water movement, especially in embayments where reefs may be particularly affected by decreased water quality. Sewage pollution is a pervasive threat to both human and coral health; improved studies can help us manage health of our coral reefs worldwide.

Author contributions

CDH, CJSK, and RMY designed the study with help from RM. CJSK and RMY conducted most of the fieldwork with assistance from CDH and AMT. RMY and AMT conducted the statistical analyses. RMY and RM produced the figures. RMY, CJSK, and CDH wrote the manuscript with input from AMT and RM.

Conflicts of interest

Authors are not aware of any conflicts of interest. Although support from the Puako Community Association might be considered such, the community was interested in the project's results regardless of outcome, and the support did not influence the direction of results and conclusions.

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