Understanding the consequences of land-based pollutants on coral health in South Kohala

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Photo by: Rebecca Most

Executive Summary

Located in Hawai'i Island's South Kohala district, the Puakō-Mauna Lani reef system is one of the most well-developed fringing reefs on the island of Hawai'i, providing countless ecological, economic and cultural resources to Hawai'i. The South Kohala Conservation Action Plan (SKCAP) was developed in 2012 to identify important coral reef resources to protect, threats to those resources, and management strategies to abate threats.

During the last 50 years, Puakō has experienced substantial changes in overall reef health, with a 50% decline in coral cover since the 1970's. While the causes of this decline are poorly understood, land-based pollution (LBP) is thought to be a major contributor to the degradation of coral health. LBP is of particular concern along the Puakō-Mauna Lani coastline of South Kohala due to extensive land use change, high recreational use, ineffective sewage treatment and vulnerability to pollution due to its highly porous rock and submarine groundwater discharge (SGD).

To better understand the contribution of LBP to overall coral health and address a priority management issue outlined in the SKCAP we: (1) identified 12 sites across regions of "low" to "high" SGD/terrestrial input and characterized spatial patterns in water quality and coral health between November 2013 to September 2014; (2) tested the correlation between coral health and water quality; and (3) prioritized regions for corrective action. Water quality data included monthly temperature, salinity and chlorophyll-*a* measurements, and quarterly sampling with additional dissolved inorganic nutrient and fecal indicator bacteria (*Enterococcus)* monitoring. Coral health surveys included prevalence and severity assessments of all coral diseases and compromised health states. To determine whether patterns in overall reef health were consistent across the study region, we surveyed benthic and fish assemblages across 37 randomly selected sites in March and April 2014, and conducted coral disease rapid assessments at a subset of 24 of the sites.

SGD was pronounced across the study region and may serve as a delivery mechanism of LBP. Water quality was highly variable across the year, emphasizing the importance of incorporating temporal variability when assessing vulnerability to LBP. Dissolved inorganic nutrient concentrations were generally low compared to other West Hawai'i studies, but highly variable between sites. Silica and nitrite + nitrate concentrations were elevated in surface waters at sites 1 (near Ohai Point), 5 (east of Waima Point), and most notably at site 13 (Pau'oa Bay) compared to the other study sites. *Enterococcus* CFUs/100ml were 7 times higher in knee-deep shoreline waters compared to reef waters. Levels exceeded the US Environmental Protection Agency's standard for recreational marine waters (35 CFU/100mL) for 9 of the 12 shoreline sites and were particularly high at sites 2 (Condos), 6 (east of Waima Point) and 10 & 11 (near Paniau).

Twenty-four percent of corals were diseased, and 27% showed signs of compromised health aside from disease, with coral health primarily driven by growth anomalies and algal overgrowth, each affecting 20% of colonies. We also recorded signs of trematodiasis and tissue loss syndrome, discoloration, bleaching, physical damage, gastropod predation and crown-of-thorns predation. Across the 37 randomly selected

sites, coral cover was 34%, with the coral genus *Porites* dominating coral communities and also the most affected by disease. We identified four areas of concern for coral health including: the reef between sites 4 and 6, as well as Pau'oa Bay/Mauna Lani (near 13 and 14), and, to a lesser degree, the reef between sites 2 and 3 as well as Paniau.

Overall, environment played a stronger role in coral health patterns than coral demography (% cover, colony density and colony size). Nutrient concentration was the strongest environmental diver of disease, namely growth anomalies, with higher growth anomaly pressure (a measure of both prevalence and severity) on reefs with elevated silica and nitrate + nitrate concentration. This suggests that nutrients or another component of the groundwater uncounted for in this study (e.g. chemical pollutants) are increasing disease levels. While *Enterococcus* was not a strong predictor of coral health, additional indicators of human sewage pollution are needed to more accurately detect sewage contamination and assess its affects on coral health. These results also indicate that other watershed nutrient inputs in addition to sewage may play an equally if not more important role in reef health.

Introduction

South Kohala's coral reef ecosystems provide tremendous cultural, economic, fisheries, shoreline protection, and recreational benefit to Hawai'i. In light of the importance of these resources and health of its coral reef ecosystems, South Kohala was selected as a high priority for management by the Hawai'i Coral Program (Hawai'i Coral Reef Working Group 2010) and was recently designated a Habitat Blueprint site under the National Oceanographic and Atmospheric Administration. In 2011, a multi-stakeholder group developed the South Kohala Conservation Action Plan (SKCAP) to identify important coral reef resources to protect, threats to those resources, and management strategies to abate those threats (The Nature Conservancy 2012). Similar to other regions in Hawai'i, South Kohala's terrestrial and marine ecosystems are experiencing a variety of threats associated with increased human use (The Nature Conservancy 2012). In the marine environment, overfishing, invasive species, and land-based pollution (LBP) pose a significant threat to overall reef health from the ecosystem to the organismal scale.

The Puakō-Mauna Lani reef system is one of the most well-developed fringing reefs on the island of Hawai'i, yet has experienced substantial changes in overall reef health during the last 50 years (Minton et al. 2012). These reefs have seen complex changes in reef fish communities likely due to changes in fishing pressure and marine protection status. With the implementation of the Puakō Reef Fisheries Management Area in 1985 (HDAR 2011), many of Puakō's fish species targeted for the aquarium trade have increased (Tissot et al. 2004; Williams et al. 2009). However, the maximum size of five fish species targeted by fishermen decreased by 15% since 1982, whereas the maximum size of non-target species increased (Hayes et al. 1982; Giddens 2010). As of 2010, "prime spawners" (resource fish larger than 70% of the maximum size reported for the species) were rare at Puakō compared to other Kona coast reefs (Minton et al. 2012). Benthic communities have also been affected, with a recent analysis conducted by The Nature Conservancy (TNC) of over 40 years of data from the Puakō reef tract shows an

approximately 50% decline in coral cover over that time (Fig. 1; Minton et al. 2012). These results are consistent with coral cover declines elsewhere in South Kohala (Walsh et al. 2013).
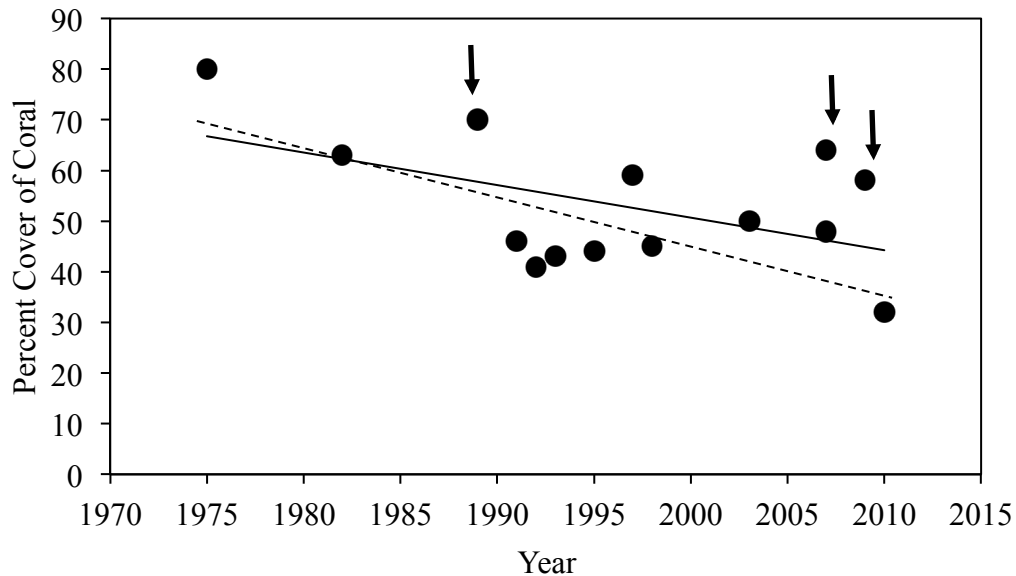

Figure 1. Percent cover of coral at Puakō shows a downward trend (solid line) between 1975 and 2010. Coral cover estimates for 1989, 2007 and 2009 (shown by arrows) were collected as part of fish behavioral studies and were likely conducted in areas of naturally high coral cover. Removing the 1989, 2007 and 2009 data results in a significant (p=0.007) downward trend (dotted line) in percent cover of coral. (From Minton et al. 2012)

The causes of these declines in coral cover are poorly understood, but land-based pollutants and nutrients (LBP) are thought to be a major contributor to the degradation of coral health and the loss of coral cover on the reefs of South Kohala (The Nature Conservancy 2012, Walsh 2013). Research in other locales strongly suggests that degraded water quality can impair coral health by sedimentation events directly smothering and killing corals (Fabricius 2005) and nutrient inputs promoting macroalgal overgrowth of corals when associated with reduced herbivory (Hughes 1994; Rogers and Miller 2006). Altered water quality can also affect disease susceptibility by altering host-pathogen interactions. Increased prevalence and severity of coral disease has been linked to eutrophication (Bruno et al. 2003; Kaczmarsky and Richardson 2010; Vega Thurber et al. 2014) or heavy sedimentation (Haapkylä et al. 2011; Pollock et al. 2014). Sewage pollution has also been implicated in the introduction of coral pathogens and increased disease levels in the Caribbean (Sutherland et al. 2010). While previous studies suggest that sections of the Puakō-Mauna Lani reef system have been affected by sedimentation, high algal overgrowth and coral disease (Couch et al. 2014; Smith and Lillycrop 2014), the extent of these conditions and their drivers remains unknown, which is a critical step necessary for informing management actions.

Land-based pollution is of particular concern along the Puakō-Mauna Lani coastline as South Kohala is a fast-growing region of Hawai'i Island, with substantial changes in land-use (The Nature Conservancy 2012). Following the installation of public access roads in 1957, Puakō experienced substantial residential growth and now contains 163 individual houses, a boat launch facility, a small residential hotel, condos, two churches, and a general store, with 4,000 new homes slated to be built above Puakō's this existing residential community (Minton et al. 2012). With easy shoreline access, this region has also become a popular recreational use area (Lamson 2010). Just south of and adjacent to Puakō, the Mauna Lani coastline has become one of South Kohala's major resort communities with two resorts, condos, golf courses, and high tourism activity.

Compounding the potential impact of these considerable land use changes, Hawai'i Island's highly porous basaltic rock also renders this region particularly susceptible to LBP (Oki 1999). With few perennial streams, freshwater quickly percolates through this porous basalt into underground aquifers, mixes with seawater, and is released into the coastal regions as submarine groundwater discharge (SGD) (Street et al. 2008). Although nutrient levels are naturally elevated in SGD (Umezawa et al. 2002; Johnson et al. 2008; Street et al. 2008), the island's highly porous rock also makes SGD especially susceptible to anthropogenic-related contamination (Knee et al. 2010).

While Puakō residents and marine managers raised concerns about coastal development and overfishing as early as the 1970's (Hayes et al. 1982), more recently sewage pollution into coastal regions has become a major concern. Due to distance from municipal sewage treatment, Puakō relies on a combination of septic systems, cesspools and one injection well (Schott 2010). While all three methods are potentially problematic for nearshore water quality, cesspools have become a major concern in coastal regions across the state. With approximately a third of the Puakō houses using cesspools (Schott et al. 2010, Puakō Community Association unpubl.), the Puakō Community Association (PCA) has raised concerns and become actively involved in assessing the effects of sewage pollution on coastal ecosystems and developing remediation plans. Unlike Puakō, the Mauna Lani resorts use an aerated lagoon wastewater treatment system to reduce microbial and nutrient pollution (Schott 2010). In Puakō, elevated $\delta^{15}N$ (Dailer et al. 2011) and enterococci levels (a widely used fecal indicator bacteria) (Kim et al. 2014) along the shoreline suggests that sewage contamination is leaching into the marine environment. Due to both human and coral health concerns from sewage inputs, the Puakō community has begun to actively seek solutions to improve nearshore water quality, including potentially upgrading existing cesspool systems to reduce nutrient and LBP sources into the coastal ecosystems. Considering the documented precipitous declines in coral cover and high disease prevalence at Puakō and the ongoing land use changes in South Kohala, it is essential to expand spatial assessments of coral health and more directly examine the links between LBP and coral health. To achieve this, we addressed the following objectives:

1. Identify regions with "low" and "high" SGD/terrestrial input and characterize water quality (temperature, salinity, turbidity, nutrients, chlorophyll-a, and enterococci levels) across these regions.

2. Characterize spatial patterns in coral disease and compromised coral health and determine whether these patterns are correlated with water quality parameters.
3. Establish patterns of coral reef condition among the reefs of Puakō and Mauna Lani.
4. Identify and prioritize regions for corrective action.

Methods

Fine-scale Coral Health and Water Quality Surveys

Detailed coral health and water quality surveys were performed at 12 sites selected to represent a spectrum in water quality parameters to both identify regions for corrective action across the Puakō-Mauna Lani reef system and determine whether spatial patterns in coral health are correlated with various water quality and coral demography parameters.

Study Sites

Given that SGD is both widespread across this reef system and one of the primary mechanisms by which land-based inputs are delivered onto West Hawai'i's reefs, sites were established in relation to exposure to SGD. Due to West Hawai'i's unique geology and hydrology, it is difficult to establish gradients parallel to shore away from one point source of LBP without encountering a SGD plume nearby. Alternatively, we identified sites with "low" and "high" SGD/terrestrial input (Fig. 2). Using a combination of initial temperature and salinity surveys in November 2013 (see Water Quality section below), high-resolution salinity mapping (see Water Quality section below), available aerial infrared imagery of SGD plumes (Johnson 2008), feedback from the Puakō Community Association, and preliminary δ15N data (PCA and Harvell preliminary data), we established 6 sites with "low" and "high" terrestrial input (total of 12 sites). To minimize the potential confounding role of coral demography and community composition in coral health patterns, we surveyed sections of the reef crest (2-3m in depth) at each site that had comparable coral community structure across all sites.
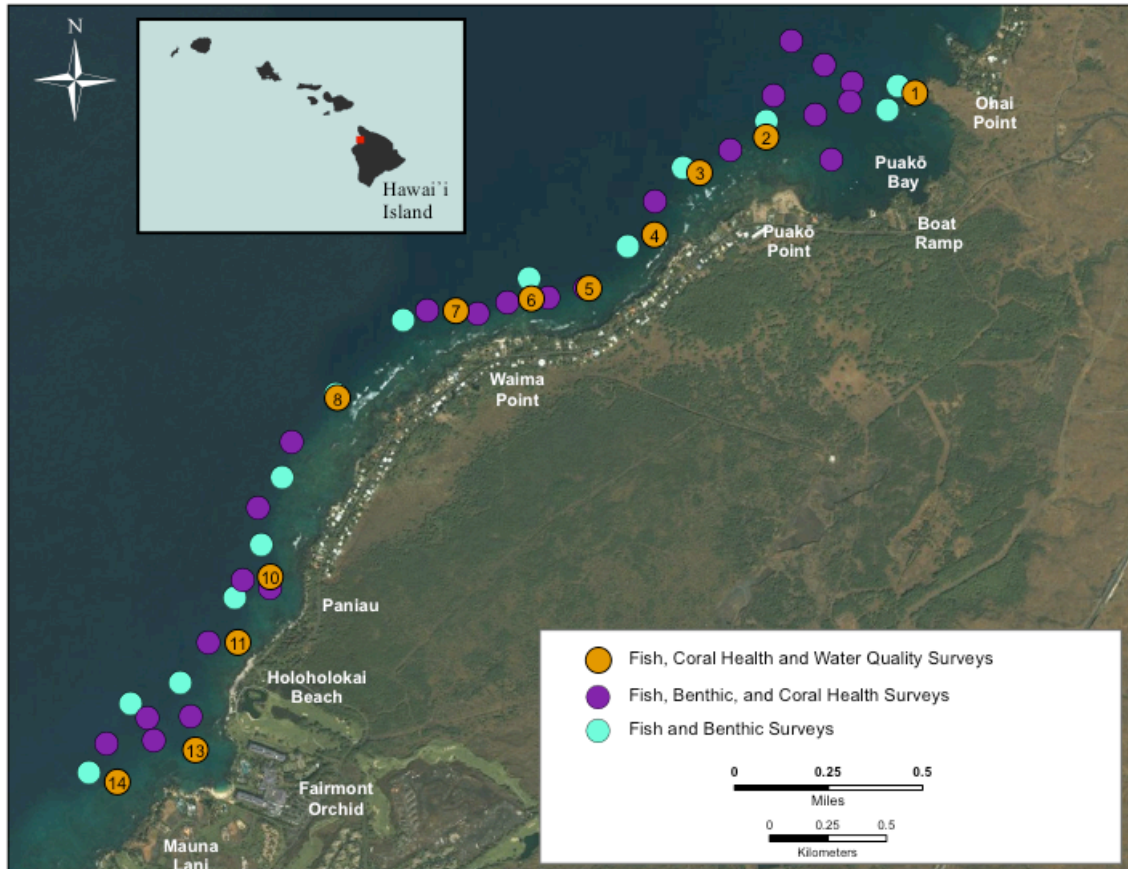
Figure 2. Survey stations between Puakō and Mauna Lani, Hawaiʻi. Coral reef benthic and fish communities were surveyed at all 37 sites between March and April 2014. Coral health surveys were conducted at 24 of the 37 sites and 12 shallow stations (see below). *Note:* sites 9 and 12 were dropped after December sampling due to time constraints and are not included on the map.

Water Quality

Salinity Mapping

In December 2013, we generated a high-resolution map of surface salinity to provide additional data on the spatial variability of SGD plumes and improve site selection (Fig. 3). The study region was divided into two sections and surveyed at low tide on December 16th (north of the Puakō boat ramp to Paniau) and December 17th (Paniau to Mauna Lani). A Yellow Springs Incorporated sonde (YSI 6920 V2) was attached horizontally to the bottom of a kayak and towed across the study region in a grid pattern from shore to reef. When the shoreline could not be accessed by kayak, the YSI was towed by hand after the kayak surveys. Continuous salinity data were collected at a 5 second interval while simultaneously recording the location with a handheld GPS. The composite map was created by interpolating 5753 survey points using the Kriging method in ArcMap.

Monthly Sampling

On a monthly basis at low tide, temperature, salinity and chlorophyll-*a* concentration were surveyed at each site. Turbidity was also measured, but not included in this report due to data inconsistencies attributed to wave action. A YSI sonde 6920 V2 was used to measure temperature and salinity for 2 minutes (5 sec interval) in the surface and benthic water each month. Duplicate or triplicate 500 ml water samples were collected at the surface and benthos at each site, filtered through GF/F Whatman filters, and stored in aluminum foil at - 20° C. Samples were shipped to the Hawai'i Institute of Marine Biology and processed for chlorophyll-a concentration (pheophytin-corrected) using the EPA Method 445.0 with 90% acetone and a Turner® Type 10AU fluorometer.

Quarterly Sampling

To characterize dissolved inorganic nutrient concentration and abundance of fecal indicator bacteria at each site, more extensive surveys were conducted on a quarterly basis at low tide (12/18-19/2013, 3/1/2014, 6/26-27/2014, and 9/9-10/2014). During these sampling periods, temperature and salinity were measured as described above. In addition, duplicate 500 ml water samples were collected in acid washed Nalgene bottles at the surface and benthos for nutrient analyses, and triplicate 500 ml water samples were collected from the surface and benthos at each site to quantify enterococci abundance. All samples were transported to the Natural Energy Laboratory of Hawai'i and nutrient samples were filtered through GF/F Whatman filters. The filtrate was collected in triple-rinsed acid washed bottles, refrigerated and processed within 24-48 hours for dissolved inorganic nutrients (nitrate + nitrite, ammonium, orthophosphate, silica). The filter was frozen and processed for chlorophyll-*a* concentration (see above). Dissolved inorganic nutrients were analyzed using flow-injection analysis on an Astoria Pacific Instruments autoanalyzer. *Enterococcus* samples were filtered through membrane filters and incubated for 24 hours on Difco m-*enterococcus* selective media following EPA Method 1600. One hundred (shoreline) to 200 (reef) ml of each sample was vacuum filtered through a 47mm, sterile, grid-marked membrane filter (0.45mm pore size). The filter was removed with sterile forceps and placed into a 9 x 50mm petri dish containing 5 mL of m-Enterococcus selective agar (Difco; Environmental Protection Agency Method 1600). Negative controls with sterile deionized water were conducted during each sampling. Plates were incubated at 42°C for 24 hours and data were calculated as number of colony forming units/100ml.

To better capture human sewage input associated with shoreline SGD seeps, water samples were also collected on March 4th (following a 2-day massive rain event) and June 29th (dry-season) in knee-deep water along the shoreline adjacent to each site and processed for enterococci abundance. Samples were processed as described above.

Coral Health and Disease:

To capture variability in coral health across seasons, coral health and disease surveys were conducted in winter (February-March 2014) and summer (July 2014). During the winter surveys, 3 haphazard points were chosen at each site at 2-3m depth. At each point,

a 10x1m belt transect was established and marked with temporary cable ties (on dead substrate), photographs and GPS points to locate in future surveys. During winter and summer surveys, all coral colonies within the 10x1m belt were identified to species, sized (maximum colony diameter) and counted. Each colony was inspected for signs of disease (growth anomalies, trematodiasis, and tissue loss syndrome), compromised health (algal overgrowth, discoloration, bleaching and physical damage), gastropod predation and crown-of-thorns predation (see Fig 3 for condition descriptions). Overall disease and compromised health prevalence was calculated as follows: # of colonies with at least 1 type of disease or compromised health lesion/# of total colonies. Overall disease prevalence without trematodiasis was also calculated because trematodiasis is more strongly driven by the abundance and distribution of coral and butterflyfish (known host and disease vector) rather than environmental conditions (Aeby 2007; Williams et al. 2010). The prevalence of each condition was also calculated (# of colonies condition/total # of colonies; growth anomalies: # of colonies growth anomalies/total # of *Porites, Montipora* and *Pocillopora* colonies; trematodiasis: # of colonies trematodiasis/total # of *Porites* colonies). In summer 2014, condition severity was also recorded by estimating the % of each colony affected by a certain condition.
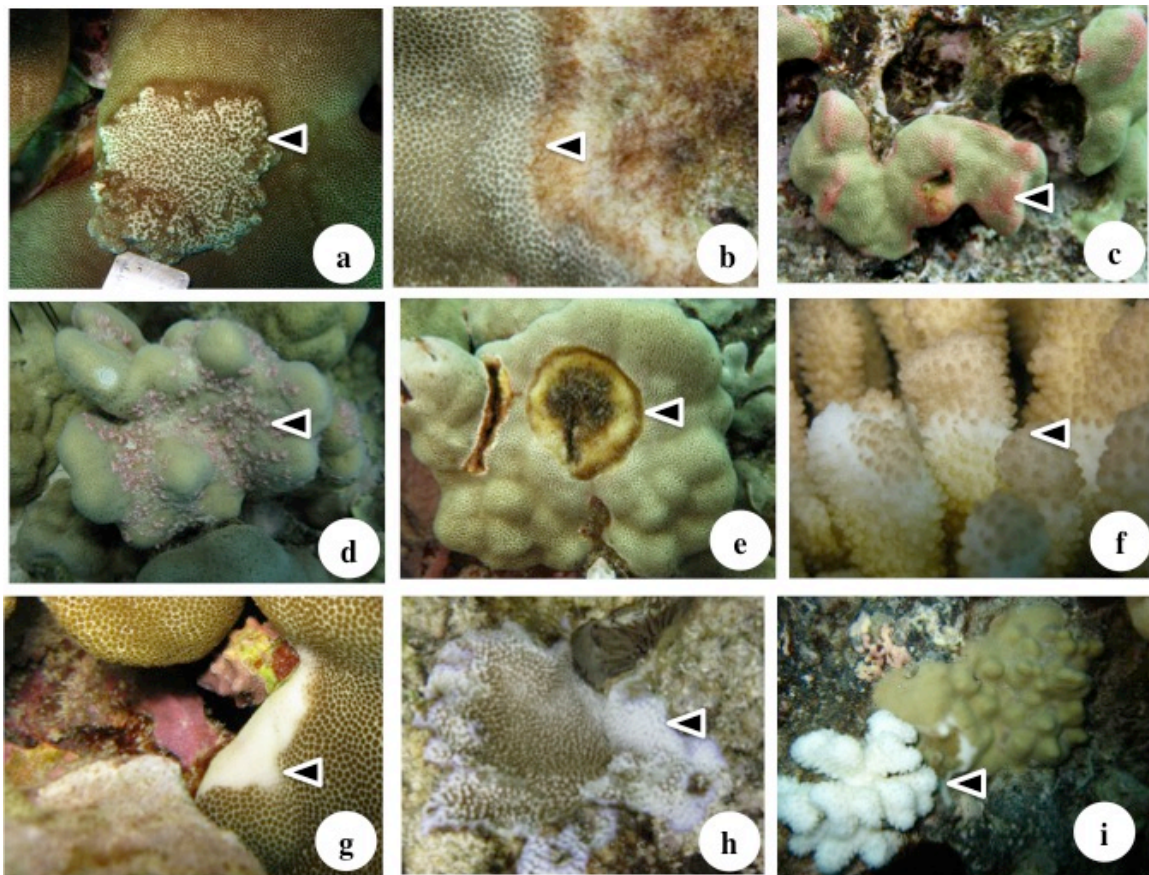

Figure 3. Coral health conditions observed at 12 study sites between February to July 2014. Disease categories include growth anomalies, trematodiasis, and tissue loss syndrome. Compromised health states include algal overgrowth, discoloration, bleaching, and physical damage. Conditions were identified as follows: (a) Growth anomalies: protuberant growths of skeleton accompanied by aberrant calyx formation; (b) Algal overgrowth: areas where macroalgae, turf algae or cyanobacteria actively overgrows, abrades

and/or kills underlying coral tissue; (c) Discoloration: areas of discolored and/or swollen tissue not associated with other lesion categories; (d) Trematodiasis: multiple small (~5 mm) swollen pink to white nodules on *Porites*; (e) Tissue loss disease on *Porites*: distinct areas of tissue loss revealing intact white skeleton progressing basally to a algal patina or multiple variably-sized areas of tissue loss; (f) Tissue loss disease on *Pocillopora*: distinct areas of tissue loss revealing intact white skeleton progressing basally to a algal patina or multiple variably-sized areas of tissue loss; (g) gastropod predation: recent predation scars from *Drupella cornus* or *Coralliphilia violacea*; Physical damage: broken branches, abrasion or fishing line damage; (h) Bleaching: partial or complete loss of tissue pigmentation; (i) Crown-of-thorns: predation recently denuded skeleton caused by *Acanthaster planci*.

Data analysis

All data were analyzed in R version 3.0.2, checked for normality and equal variances and log or square root transformed if needed. Spatial patterns in water quality parameters were characterized by averaging across monthly and quarterly samplings. Linear regressions were used to test the relationship between certain water quality parameters, such as *Enterococcus* and salinity. Overall and individual condition prevalence were averaged across both seasons for each site. Condition pressure (average prevalence x severity per transect and site) was also calculated to obtain an integrated measure of the prevalence and severity.

Linear mixed effect models were used to determine whether coral health was correlated with a combination of demographic (colony density and size) and environmental (temperature, salinity, chlorophyll-*a,* dissolved inorganic nutrient concentration, and enterococci abundance) variables. All predictor variables were checked for multicollinearity (correlation coefficient > 0.8). Due to the high correlation between the nutrient parameters, a Principle Components Analysis (PCA) was used to condense nutrient concentration onto one principle component, which accounted for 80% of the variance in nutrients (data not shown). Treating site as a random effect and all the predictor variables as fixed effects, 23 hierarchical or single-factor models were built for overall prevalence (without trematodiasis), compromised health, growth anomalies and algal overgrowth pressure. Growth anomalies and algal overgrowth targeted because they drove most of the patterns in coral health (see Fig. 14) Model selection comparing Akaike's information criterion (AICc, ΔAICc and AIC weight) were used to determine which factor or combination of factors best fit each condition (Burnham and Anderson 2002). ΔAIC > suggests substantial evidence for the model and Akaike weights (w_i) provide another measure of the strength of evidence for each model (Burnham and Anderson 2002).

Broad-scale coral reef health surveys

<u>Survey Sites</u>

The survey area at Puakō was delineated in ArcGIS (Fig. 2), covering approximately 4.5 km of coastline and including coral reef habitat between 3 and 15 m deep. Within this area, 37 sites were randomly selected via ArcGIS for SCUBA surveys. The survey team navigated to each predetermined site using a Garmin GPS unit. Once on-site, the survey team descended directly to the bottom, where divers established two transect start points approximately 10 m apart. From each start-point, divers deployed a 25-m transect line

along a predetermined compass heading, parallel to each other. Surveys were conducted between March 24 and April 3, 2014.

Benthic Community Surveys

To better target regions of concern and determine how well the 12 shallow sites represented coral disease dynamics along this reef system, coral disease surveys were conducted at 24 of the 37 sites. Due to the time required to complete a thorough assessment of all condition, we focused these surveys on growth anomalies, the most prevalent disease in this region. All coral colonies within a 8 x 1m belt were identified to species and counted. Each colony was inspected and the presence of growth anomalies. The data collected were used to calculate prevalence as follows: # of colonies growth anomalies/total # of *Porites, Montipora* and *Pocillopora* colonies.

Benthic photoquad surveys were designed to collect quantitative data on specific taxa, primarily individual coral species, algae at higher taxonomic resolution (*e.g*., red, green, brown, turf, crustose coralline, etc.), and abiotic substratum type when the bottom was something other than hard substratum. At each survey site, benthic photographs were collected at 1-m intervals along one of the two 25-m transect lines. Photographs were taken with a Canon G11 camera mounted on a 0.8-m long monopod, resulting in images that covered approximately 0.8 x 0.6 m of the bottom. Prior to photographing each transect, the camera was white balanced to improve photograph quality. A 5-cm scale bar marked in 1-cm increments was included in all photographs.

Each photograph was imported into Adobe Photoshop CS5 where its color, contrast, and tone were autobalanced to improve photo quality prior to analysis using the Coral Point Count program with Excel extension (CPCe) developed by the National Coral Reef Institute (Kohler and Gill 2006). Using CPCe, 30 random points were overlaid on each digital photograph, and the benthic component under each point was identified to the lowest possible taxonomic level. To reduce observer variability, all photographs were processed by a single individual. The raw point data from all photographs on a transect line were combined to calculate the percent cover of each benthic component for the entire belt transect.

Fish Community Surveys

All fish within or passing through a 5 m wide belt along each of the two 25 m transects deployed at each survey site were identified to species and sized into 5 cm bins (*i.e*., 0-5 cm, >5-10 cm, >10-15 cm, etc.). Divers moved slowly along the transects, taking between 10 and 15 minutes to complete each belt survey. This method closely corresponds with that used by Dr. Alan Friedlander and colleagues for the "Fish Habitat Utilization Study" (FHUS), and provides comparable data (Friedlander et al. 2007a; Friedlander et al. 2007b).

Data Analysis

Individual fish biomass (wet weight of fish per m^2 of reef area) was calculated from estimated lengths using size to weight conversion parameters from FishBase (Froese and Pauly, 2011) or the Hawai'i Cooperative Fisheries Research Unit (HCFRU) at the University of Hawai'i (UH). For analyses among survey sites, fish survey data were pooled into several broad categories, including: (1) all fishes, excluding manta rays; (2) target fishes[1], which are reef species targeted or regularly harvested by fishers; (3) prime spawners[2], which are target fishes larger than 70% of the maximum size reported for the species; and (4) non-target fishes, which are species not targeted by fishers to any significant degree (see Minton et al. 2012 for list of resource fish). In addition, data were pooled by family for parrotfish and target surgeonfish. Those abundant and conspicuous fishes provide important ecosystem services (*i.e.*, herbivory).

To determine whether fish commonly associated with injury of coral tissue (Jayewardene et al. 2009) may indirectly increase disease levels, we conducted a linear regression between growth anomaly prevalence and barred filefish *Cantherhines dumerilii*, spotted puffer *Arothron meleagris* and parrotfish.

Results

<u>Water Quality</u>

Salinity, Temperature and Chlorophyll-a
> At low tide under calm trade wind conditions, a variety of pronounced SGD plumes were identified across the study region as evidenced by the steep salinity gradients in surface waters from shore to reef (Fig. 4).

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[1] Nearly all fish species are taken by some fishers at some time in Hawai'i, therefore designating a fish species as either 'targeted' or 'non-targeted' is oftentimes difficult. These two groupings are intended to represent the high and low ends of the fishing pressure continuum. The majority of fish biomass at most sites is comprised of species that fall somewhere in the middle of this continuum, and these species were not included in either group for this analysis.

[2] Large target fishes are generally heavily targeted by fishers. In addition, fishes at the high end of their size range tend to be a disproportionately important component of total stock breeding potential due to greater fecundity of large individuals, and higher survivorship of larvae produced by large fishes (Williams *et al*. 2008). Therefore 'prime spawner' biomass is likely to be a good indicator of fishing impacts, and represents an important component of ecological function (*i.e.*, population breeding potential).
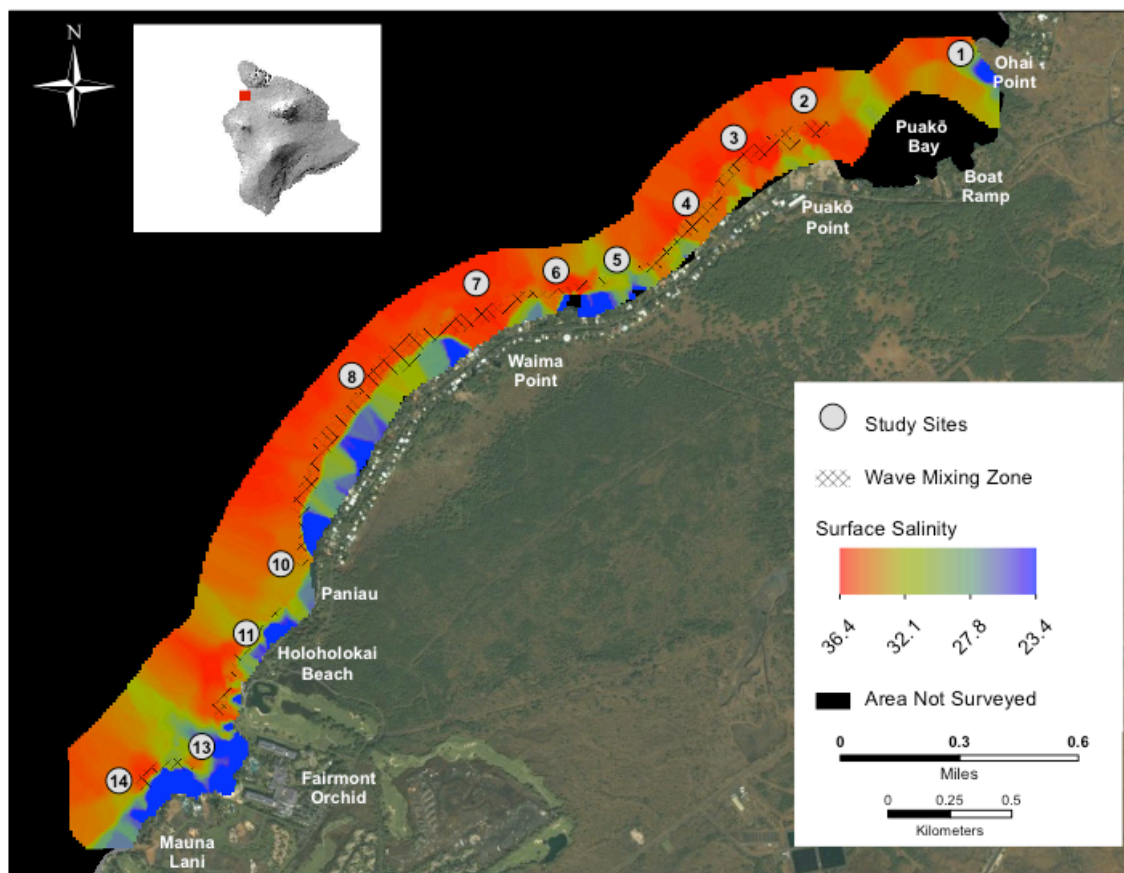
Figure 4. Surface salinity map created from YSI sonde tows in December 2013. Map also includes 12 coral health and water quality study sites. *Note:* sites 9 and 12 were dropped after December sampling due to time constraints and are not included on the map.

These SGD plumes were most pronounced near Ohai Point, just east of Waima Point, and between Waima Point and Mauna Lani. Qualitatively, wave action played an important role in SGD mixing with sheltered embayments near sites 1, 5 and 13 experiencing gradual reefward dilution of low salinity water and high energy sites near sites 6, 7, and 8 well-mixed and experiencing oceanic salinity levels. Strong spatial patterns in surface salinity across the study sites were present and generally consistent between November 2013 and September 2014 with sites 5 and 13 experiencing chronic SGD input, but more uniform at the benthos (Fig. 5 & 11). Puakō Point (site 2) did experience low salinity in surface water during several months not detected in the December sondes tows. Salinity was slightly lower in surface waters (34.47 ± 0.29) compared to benthic (35.00 ± 0.06) water, due to fresh water's lower density. In contrast, surface and benthic temperatures averaged across the year were relatively uniform across the sites (Fig. 5 & 11). Chlorophyll-*a* concentration (surface: 26.19°C ± 0.05, benthic: 26.15°C ± 0.03) varied moderately across the study region, with sites 4, 5 and 6 having slightly elevated concentration in surface and/or benthic waters compared to the other sites (Fig. 5 & 11).
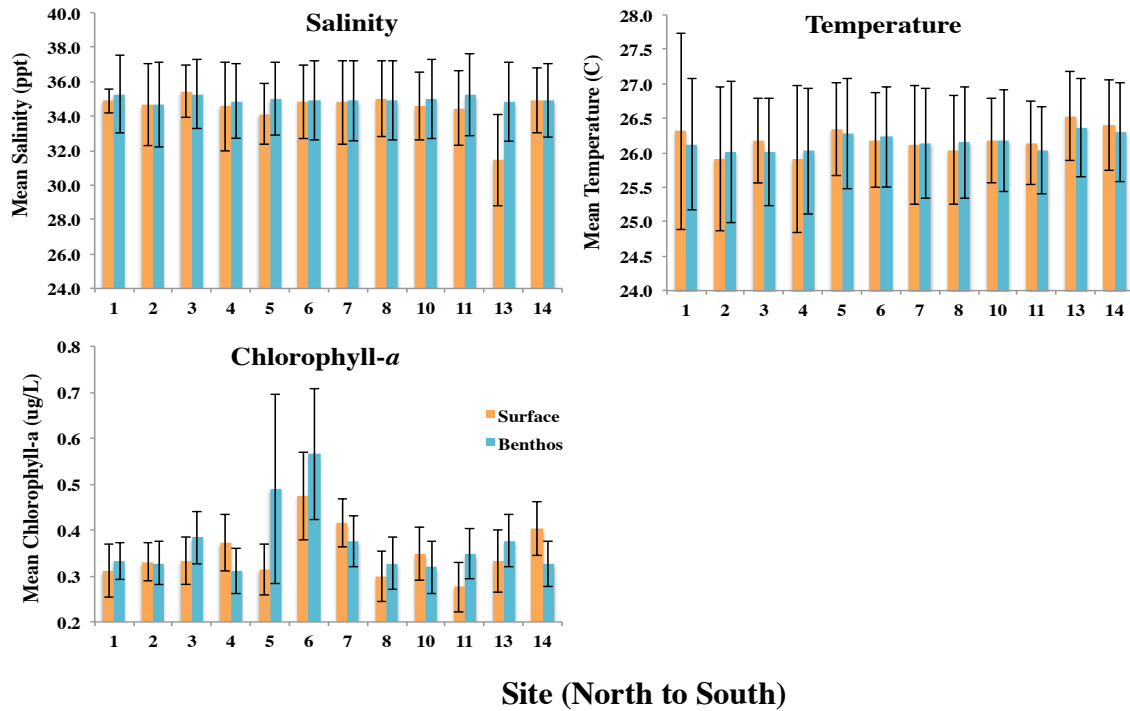
Figure 5. Mean values (± standard deviation) in surface and benthic temperature, salinity and chlorophyll-*a* across 12 shallow study sites. Temperature and salinity represent 2-minute average during each monthly sampling (n= 9 months). Chlorophyll-*a* represents 2-3 replicate water samples/depth/month (n=10 months).

Salinity, temperature and chlorophyll-*a* concentration all varied considerably across the year with a large drop in salinity and an increase in chlorophyll-*a* in April, which is consistent with the heavy rainfall and visible SGD during these sampling periods (Fig. 6). The seasonal changes in temperature are consistent with significant warming and reduced trade winds during early and late summer. Chlorophyll-*a* concentration was not significantly correlated with salinity ($R^2 = 0.0212$, p= 0.4981) suggesting that sources other than SGD are contributing more to primary productivity.

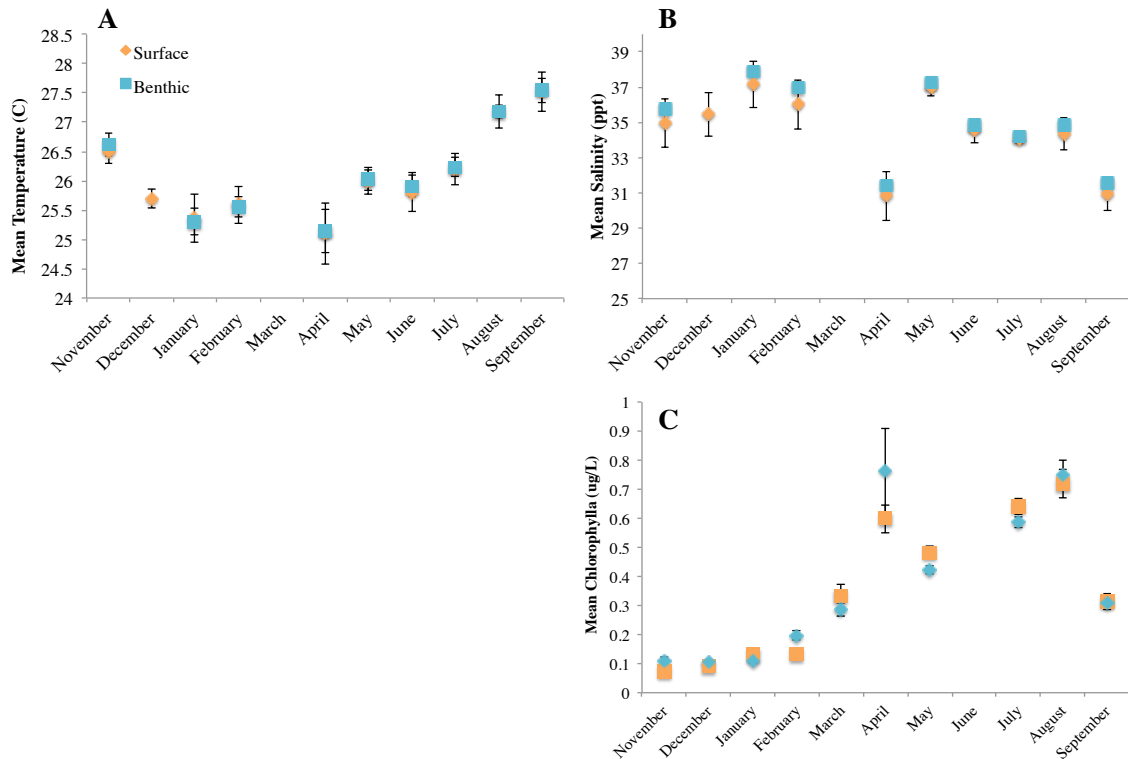**Figure 6.** A) Mean (± SD) temperature in surface and benthic water by month (n=2 minute/depth/month), (B) Mean (± SD) salinity in surface and benthic water by month (n=2 minute/depth/month), (C) Mean (± SE) chlorophyll-*a* concentration in surface and benthic water by month (n=2-3 replicates/depth/month). *Note:* Temperature and salinity data are not included for December and March during to equipment malfunction. December temperature and salinity represent 1 snapshot reading at the surface.

Nutrients

Dissolved inorganic nutrient levels were elevated at the surface compared to the benthos with the most striking spatial patterns observed in silica and nitrite + nitrate concentrations (Fig. 7). Most notably, silica in surface waters was 4 to 20 times higher at site 13 (Pauʻoa Bay) compared to the other sites. Similarly, nitrite and nitrate concentration was 7 to 75 times higher at site 13. Silica and nitrite/nitrate concentration was also elevated in surface waters at sites 1 and 5 compared to the other study sites. While the relative spatial variation in nutrient levels was subtler at the benthos, similar trends persisted with the highest levels at sites 1, 5 and 13 (Fig. 7 &12).
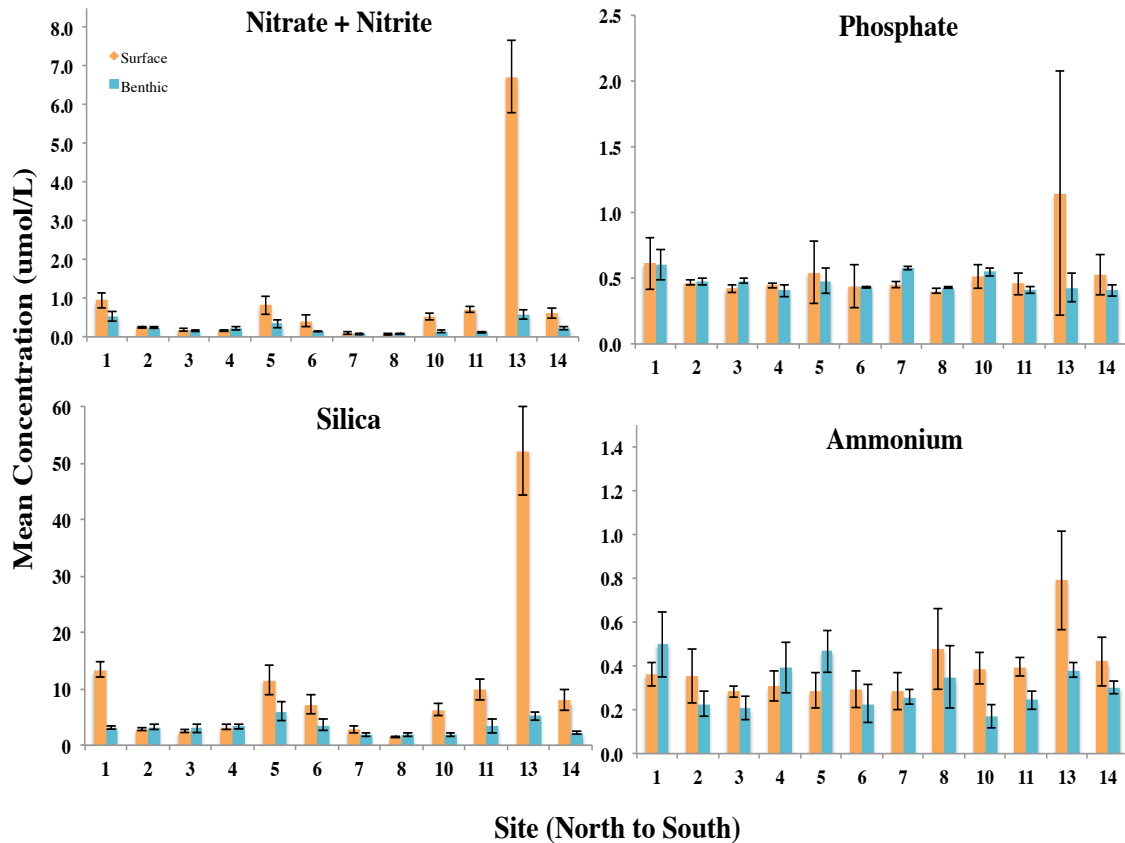
Figure 7. Mean values (± SE) in surface and benthic nitrate + nitrite, phosphate, silica, ammonium across 12 shallow study sites. n= 2 replicate samples/depth/month (n=4 months).

Enterococcus

Mean enterococci abundance was significantly elevated and 7 times higher in knee-deep shoreline (25.75-167 CFUs/100ml) waters compared to surface (2.50-27.58 CFUs/100ml) and benthic (4.17-30.20 CFUs/100ml) water at the study sites (ANOVA, $F_{2,27}$=34.162, p<0.00001, *post hoc* tests p<0.05, Fig. 8). Our results indicate that average enterococci abundance exceeded the U.S. Environmental Protection Agency standard for marine recreational waters (35 CFU/100mL) for 9 of our 12 sites in shoreline waters (Fig. 9 & 13). Overall enterococci abundance was highest in both shoreline and reef waters at sites 6, 10 and shoreline samples at site 11 compared to other sites (Fig. 9 & 13). Interestingly, the sites within and just south of Pauʻoa Bay (sites 13 and 14) have relatively low enterococci abundance despite high recreational use. Enterococci abundance was significantly and negatively correlated with salinity, which explained 40% of the variation and was primarily driven by the shoreline sites (Fig. 10). This suggests that SGD is contributing to the delivery of fecal indicator bacteria into coastal regions. Enterococci abundance was not correlated with dissolved inorganic nutrient level (Linear regression, R^2 = 0.079, $F_{1,30}$ = 2.581, p = 0.119).
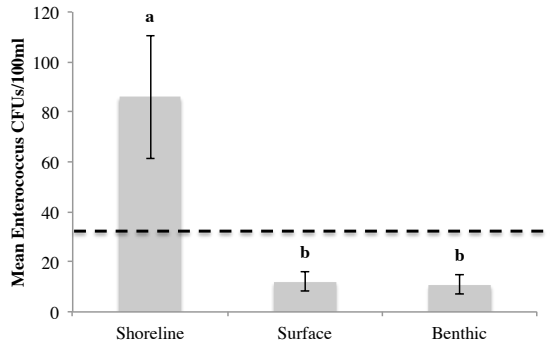
Figure 8. Mean (± SE) *Enterococcus* CFUs/100ml in shoreline (knee-deep), and reef water at the surface (just below surface) and benthos (just above substrate). Shoreline samples were collected in March and June (n=3/sampling period/site), reef water samples were collected in March, June and September (n=3/sampling period/site at surface and benthos). Dotted line indicates U.S. Environmental Protection Agency standard for recreational waters (35 CFU/100mL). Letters indicate significant difference (α = 0.5).
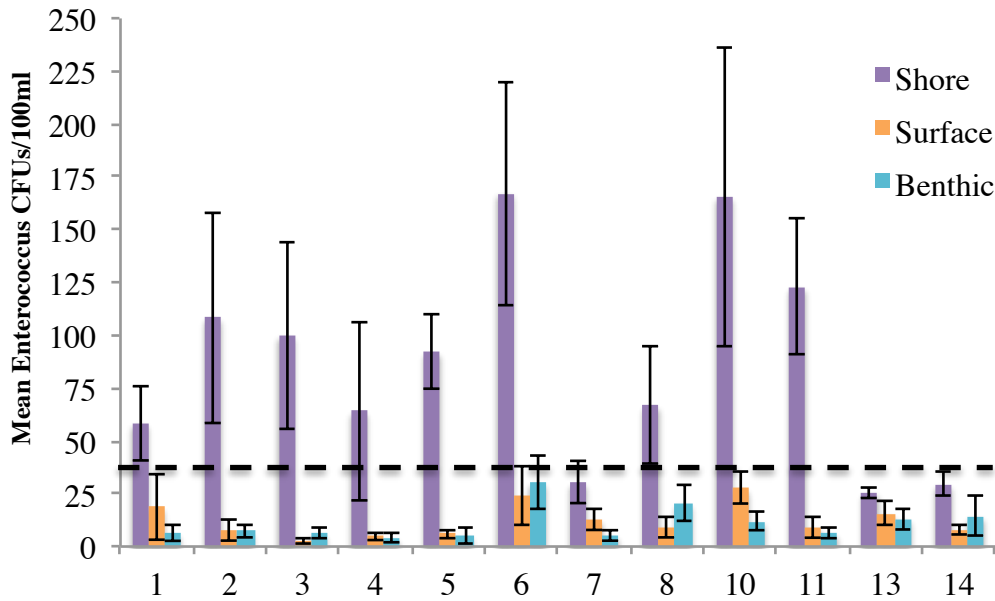

Figure 9. Mean (± SE) *Enterococcus* (colony forming units/100ml) in shoreline waters (knee-deep) and reef waters (surface and just above the substrate at each site in 2-3m in depth) across 12 sites. Shoreline samples were collected in March and June (n=3/sampling period/site), reef water samples were collected in March, June and September (n=3/sampling period/site at surface and benthos). Dotted line indicates EPA standard for recreational waters (35 CFU/100mL).
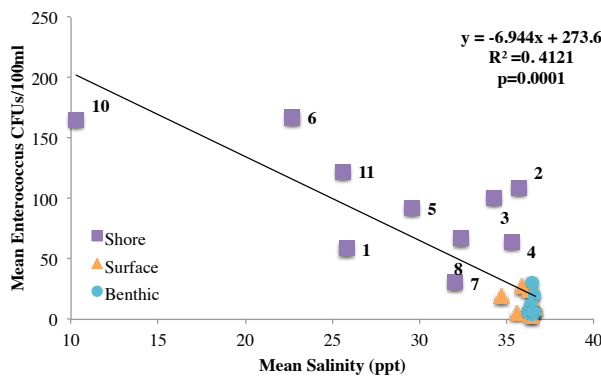

Figure 10. Relationship between enterococci abundance and salinity in shoreline waters (knee-deep) and reef waters (surface and just above the substrate at 2-3m) at sites 1 through 11. Samples collected in March and June (n=3/month/location/site); salinity = 2 min. average during each sampling period. *Note:* Sites 13 and 14 were excluded from analysis due to their self-contained wastewater treatment system that removes fecal bacteria. Numbers indicate shoreline sites. *Enterococcus* data were box-cox transformed.
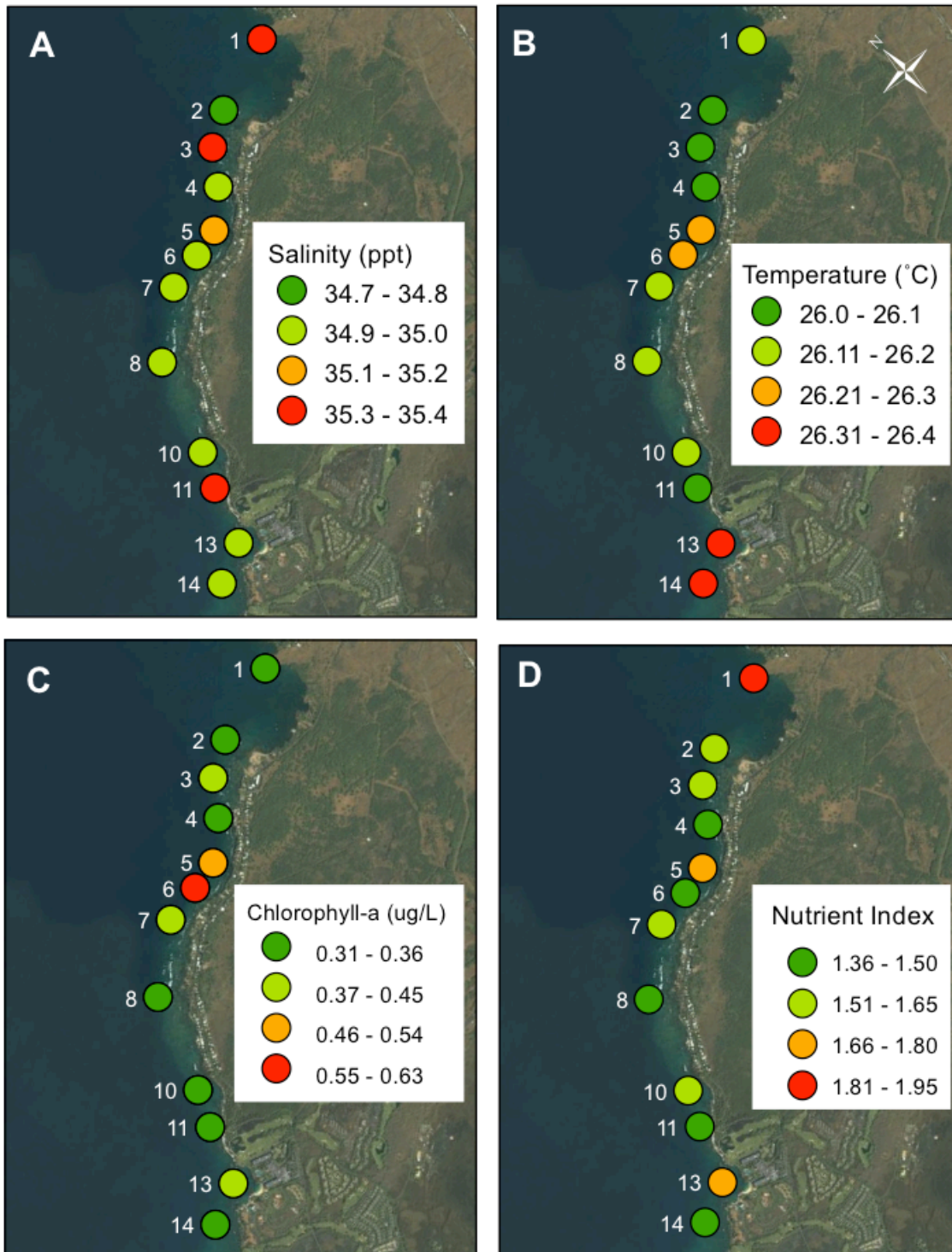
Figure 11. Mean benthic (A) salinity, (B) temperature, (C) corrected chlorophyll-*a* (D) nutrient index (1st principle component of dissolved inorganic nutrient concentration, which explains 80% of the variance in nutrients across space) in benthic waters across 12 sites.
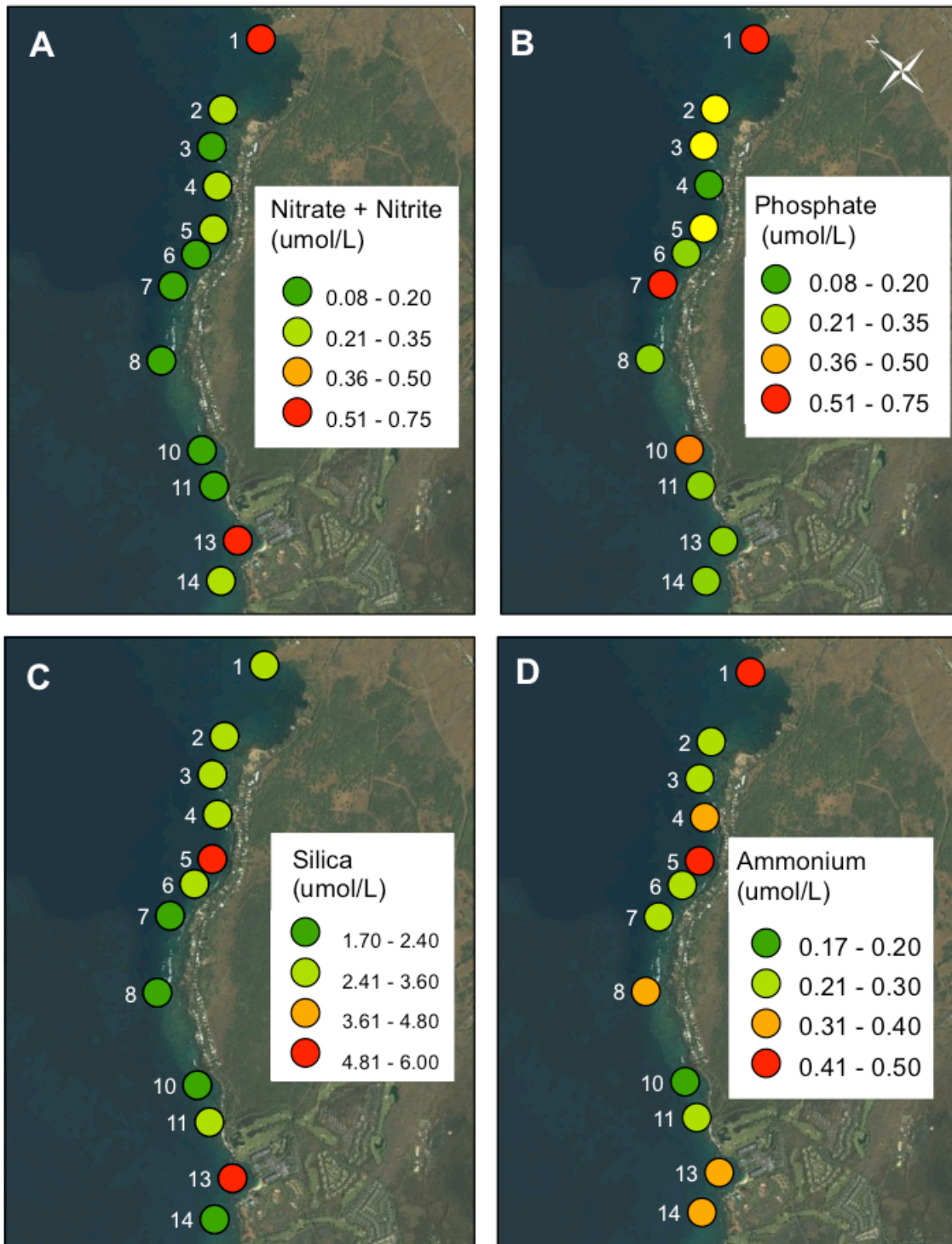
Figure 12. Mean (A) nitrate + nitrite, (B) phosphate, (C) silica (D) ammonium in benthic waters across 12 sites (n=2 samples/month/site). Samples collected in December 2013, March 2014, June 2014 and September 2014.

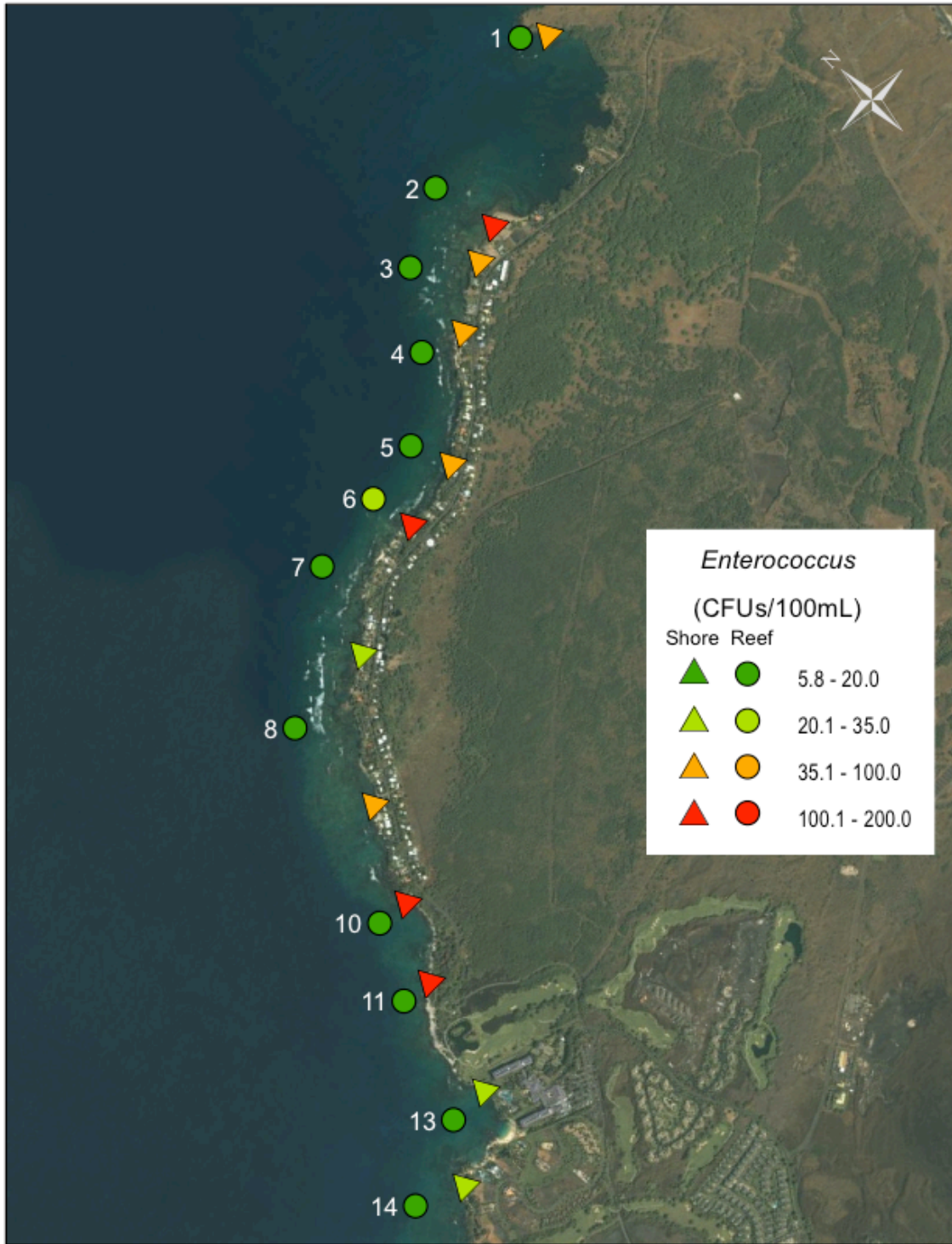**Figure 13.** Mean *Enterococcus* (colony forming units/100ml) in shoreline waters (knee-deep) and reef waters (average of samples taken at the surface and just above the substrate at each site at 2-3m in depth) across 12 sites. Shoreline samples were collected in March and June (n=3/sampling period/site), reef water samples were collected in March, June and September (n=3/sampling period/site at surface and benthos).

Coral Health

Overall, 24% of the Puakō-Mauna Lani coral community was affected by at least one type of disease, including trematodiasis and 21% excluding trematodiasis (Fig. 14). Twenty seven percent of all corals also had signs of compromised health (algal overgrowth, discoloration, bleaching, and physical damage; Fig. 14). We observed three types of diseases, all of which are classified as slow progressing chronic or sub-acute diseases. Growth anomalies were the most prevalent disease with 20% of colonies affected, followed by trematodiasis and tissue loss diseases (Fig. 14). While growth anomalies were observed in *Porites, Montipora and Pocillopora,* growth anomalies levels were primarily driven by *Porites* growth anomalies (data not shown). The most prevalent sign of compromised health was algal overgrowth with 19.74% of all colonies affect, followed by discoloration, physical damage (e.g. breakage or abrasion) and bleaching (Fig. 14). *Porites* was most affected by disease, while compromised health states were widespread and prevalent across a majority of the coral species (Fig. 15).
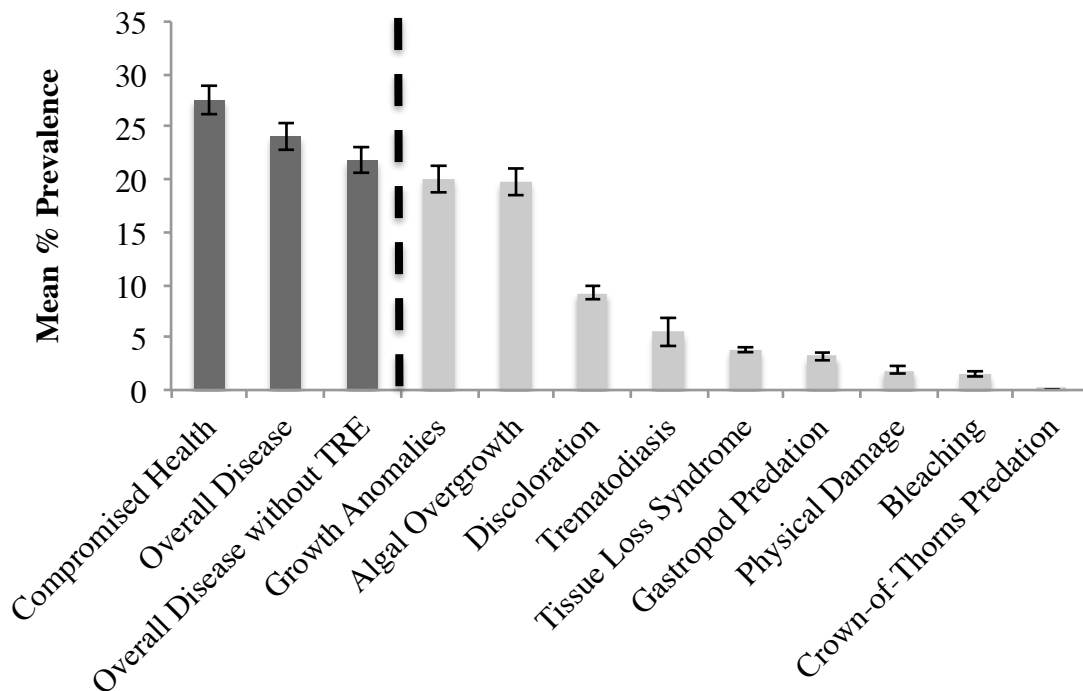
Figure 14. Mean (± SE) prevalence (# of colonies with condition/total # of colonies) of coral health and disease conditions across all sites (n=3 transects/site). Dark grey bars indicate colonies with at least one sign of compromised or disease lesion, while light grey bars indicate the prevalence of the specific disease or compromised health conditions. Note: single colonies often had multiple conditions, thus overall compromised health and disease prevalence do not equal the sum of individual conditions. See figure 3 for condition descriptions.

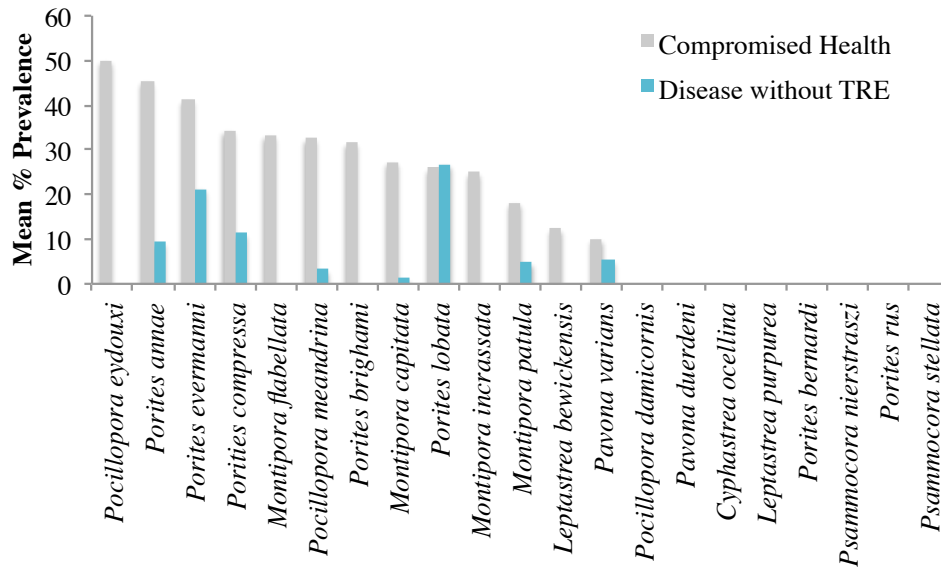**Figure 15.** Mean % of compromised health and overall disease prevalence (excluding trematodiasis) by species summarized over all transects.

Average prevalence and severity of disease conditions, excluding trematodiasis, and compromised health states were highly variable across the study sites (Fig. 16 & 17). When combined, disease pressure (prevalence x severity) was highest at sites 5, 4 and 6, while compromised health pressure was highest at sites 14, 13 and 6 (Fig. 16 & 17). While there are several exceptions, these spatial patterns in disease and compromised health pressure appear to be largely driven by the most prevalent conditions (growth anomalies and algal overgrowth). Overall, sites 4, 5, 6, 13 and 14 are of particular concern given the especially high prevalence and elevated severity disease and compromised health, namely growth anomalies and algal overgrowth (Fig. 16 & 17).
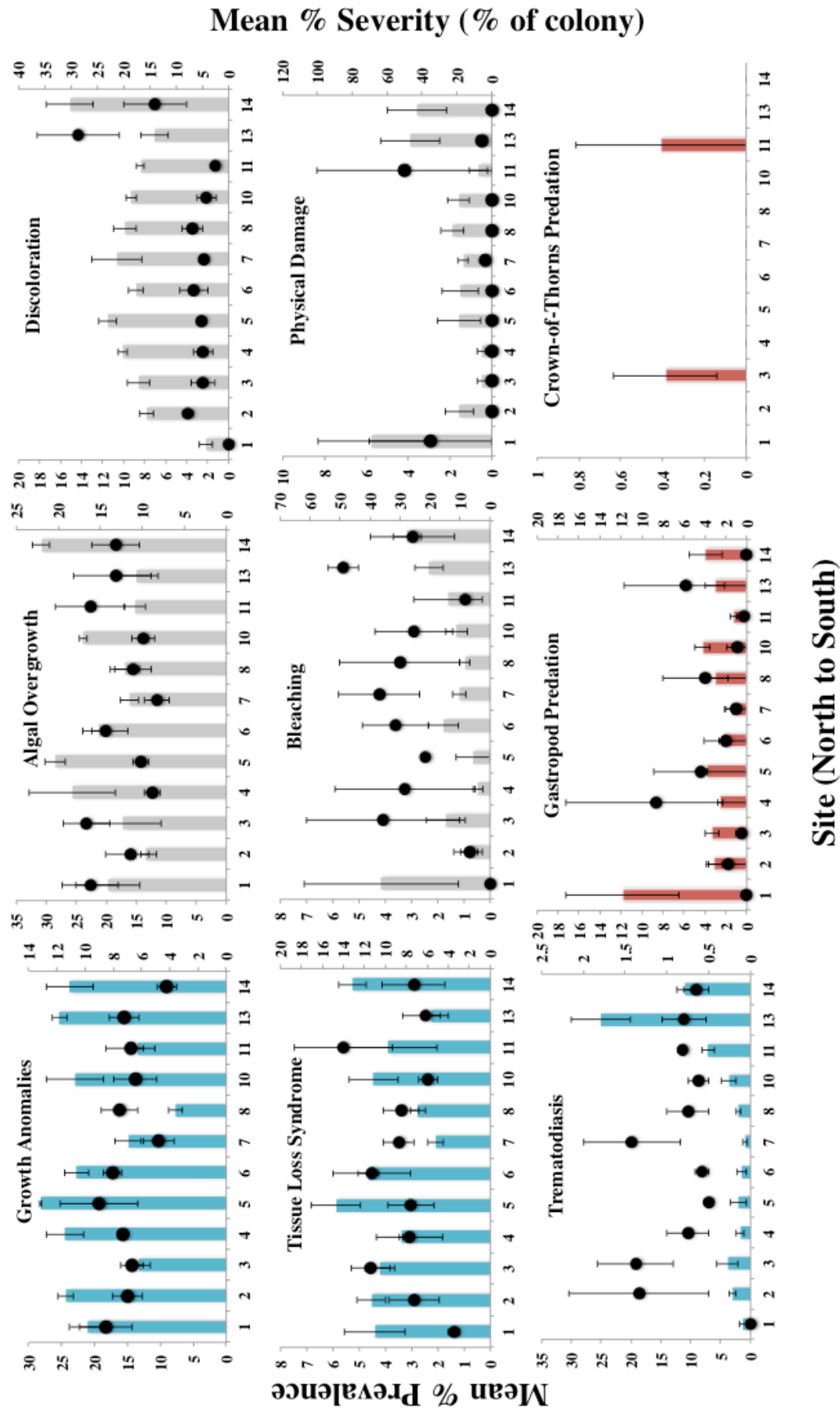
Figure 16. Mean (± SE) prevalence (bars) and severity (black dots) of each coral health condition by site. Blue/green bars = disease conditions, grey bars = compromised health conditions, Red bars = Predation.
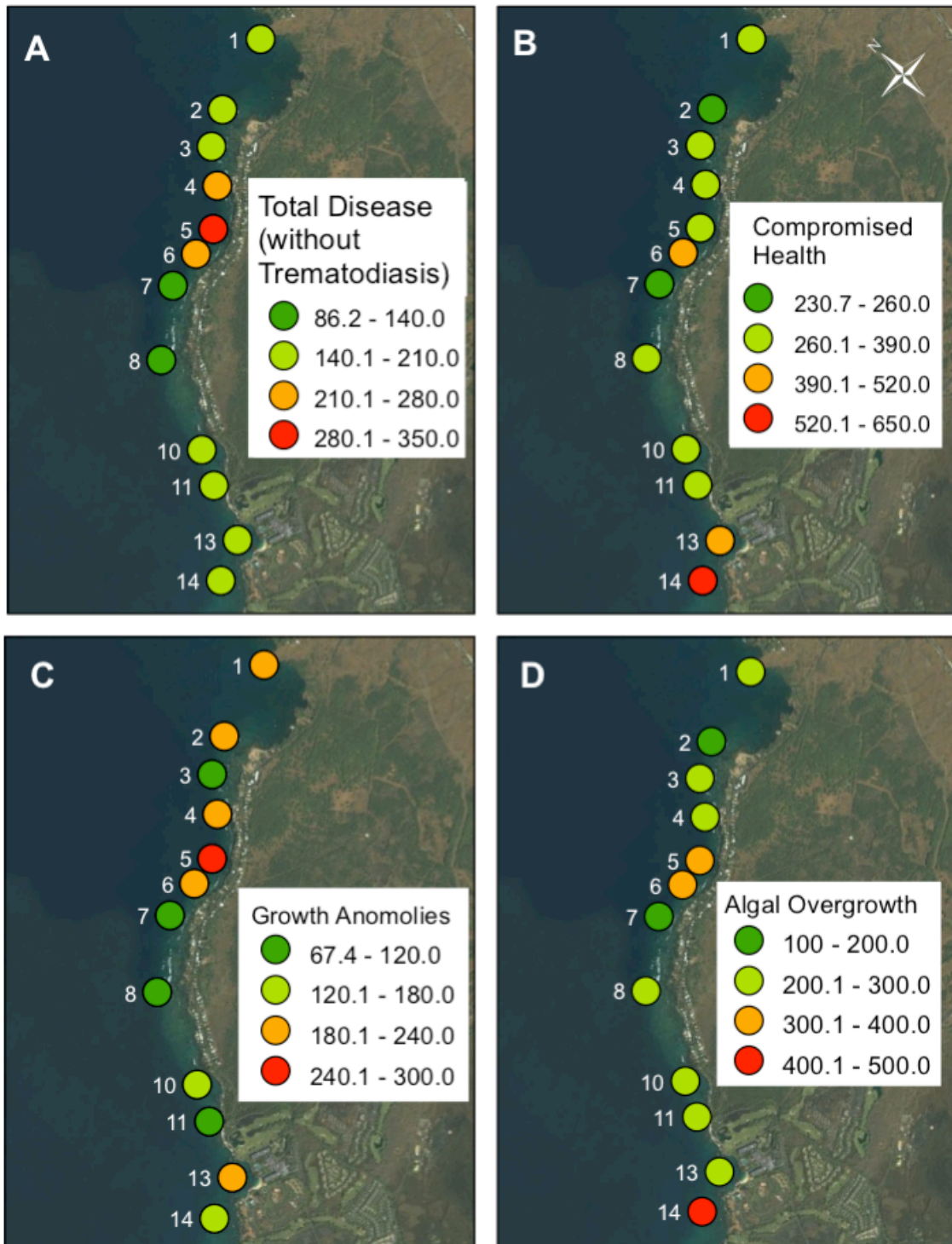
Figure 17. Mean pressure (prevalence x severity) of overall disease (without trematodiasis), compromised health, growth anomalies and algal overgrowth across sites.

Environmental Drivers of Coral Health

A combination of linear mixed models and linear regressions was used to determine which biological and environmental factors might be driving patterns in coral health (condition pressure). Total disease pressure, excluding trematodiasis, was best predicted by and positively correlated with chlorophyll-*a* concentration, which accounted for 22% of the AIC weight alone and was included in the top two models (Table 1, Fig. 18A), and only minimally by *Enterococcus*, colony size and nutrient level (Table 1). The importance of chlorophyll-*a* was, however, strongly driven by high disease pressure and chlorophyll-*a* at sites 5 and 6 (Fig. 18A). Compromised health pressure was best predicted by a combination of temperature and colony density, which together accounted for 30% of the AIC weight and were included in all of the best-fit models (Table 1, Fig. 18B). Compromised health increased with temperature and was primarily driven by a strong correlation between discoloration and temperature (Spearman correlation coefficient = 0.685, p= 0.01731; data not shown). Growth anomaly pressure was best explained by nutrient level (1st nutrient principal component), which was included in all of the best-fit models and only minimally by colony size and chlorophyll-*a* (Table 1). The positive correlation between growth anomaly pressure and nutrient level appears to be strongly driven by silica and nitrate/nitrite concentration, which accounted for 44% and 60% of the variation in pressure, respectively, with the highest pressure and concentration at sites 1, 5 and 13 (Fig. 18 C & D). Algal overgrowth was not correlated with any environmental variable and only weakly predicted by colony density and colony size with only 18% and 16% of the AIC weight explained by each factor, respectively (Table 1, Fig. 18 E & F). Despite their weak explanatory power, the negative correlation with colony density and positive correlation with average colony size suggests that reefs dominated by larger but few colonies such as site 14 have more algal overgrowth.

Table 1. "Best-fit" linear mixed effects models for total disease (excluding trematodiasis), compromised health, growth anomaly and algal overgrowth pressure (prevalence x severity) with biotic (colony density and colony size) and environmental predictors as fixed effects and site as a random effect. "Best-fit" models indicated by ΔAIC.< 2.

Total Disease (no TRE) Pressure Best Fit Models	K	LL	AICc	ΔAICc	w_i
Chlorophyll*a*	4	-9.35	28.19	0.00	0.22
Chlorophyll*a* + *Enterocuccus*	5	-8.74	29.78	1.59	0.10
Colony Size	4	-10.20	29.89	1.70	0.09
Nutrient Level	4	-10.36	30.21	2.02	0.08

Compromised Health Pressure Best Fit Models	K	LL	AICc	ΔAICc	w_i
Temperature + Colony Density	5	-8.75	29.82	0.00	0.29
Temperature	4	-10.60	30.68	0.86	0.19
Colony Density	4	-11.25	31.98	2.16	0.10

GA Pressure Best Fit Models	K	LL	AICc	ΔAICc	w_i
Nutrient Level + Colony Size	5	-175.81	363.93	0.00	0.27
Nutrient Level	4	-177.62	364.73	0.80	0.18
Nutrient Level + Chlorophyll*a*	5	-176.46	365.23	1.30	0.14

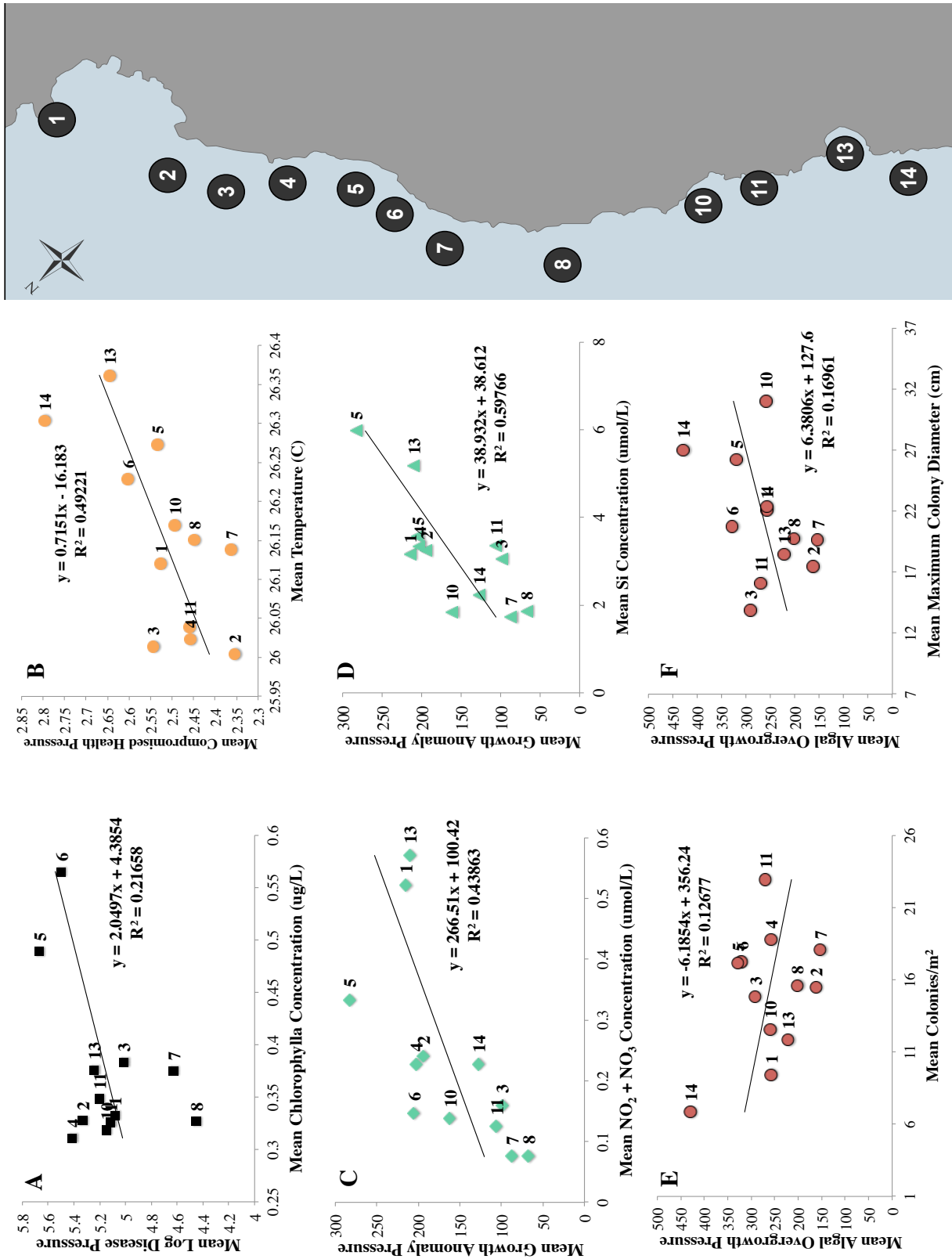| Algal Overgrowth Pressure Best Fit Models	K	LL	AICc	ΔAICc	w_i
Colony Density	4	-195.92	401.31	0.00	0.18
Colony Size	4	-196.00	401.47	0.16	0.16
Colony Density + Colony Size	5	-195.06	402.42	1.11	0.10
Temperature	5	-196.81	403.10	1.78	0.07

Figure 18. Linear regressions of coral health as a function of the best predictor variables. Numbers indicate site numbers.

Benthic Communities

To determine whether the spatial patterns of coral disease across the larger study region were consistent with the 12 shallow sites, coral disease surveys were conducted at 24 of the 37 random sites, focusing on growth anomalies.

The pattern of growth anomaly prevalence at the broad-scale survey sites confirmed the coral health hot spots between 4 and 6 and Pauʻoa Bay/Mauna Lani, and to a lesser degree sites between 2 and 3 as well as Paniau (Fig. 18). Overall, 16.74 ± 1.67% of coral colonies across the 24 random sites were affected by growth anomalies. At one site within Puakō Bay, 52% of the colonies were affected by growth anomalies. However, this site was removed from the dataset due to the effects of chronic sedimentation and very low colony density (25 colonies/$8m^2$) compared to the other sites this. Growth anomaly prevalence were not correlated with % coral cover (R^2=0.0189, p=0.5411), confirming that other site-level factors are playing a stronger role in coral health than differences in coral abundance. Growth anomaly prevalence was not correlated with biomass of fish known to cause injury to coral tissue (parrotfish, barred filefish and spotted pufferfish; R^2= 0.0224, p=0.397).
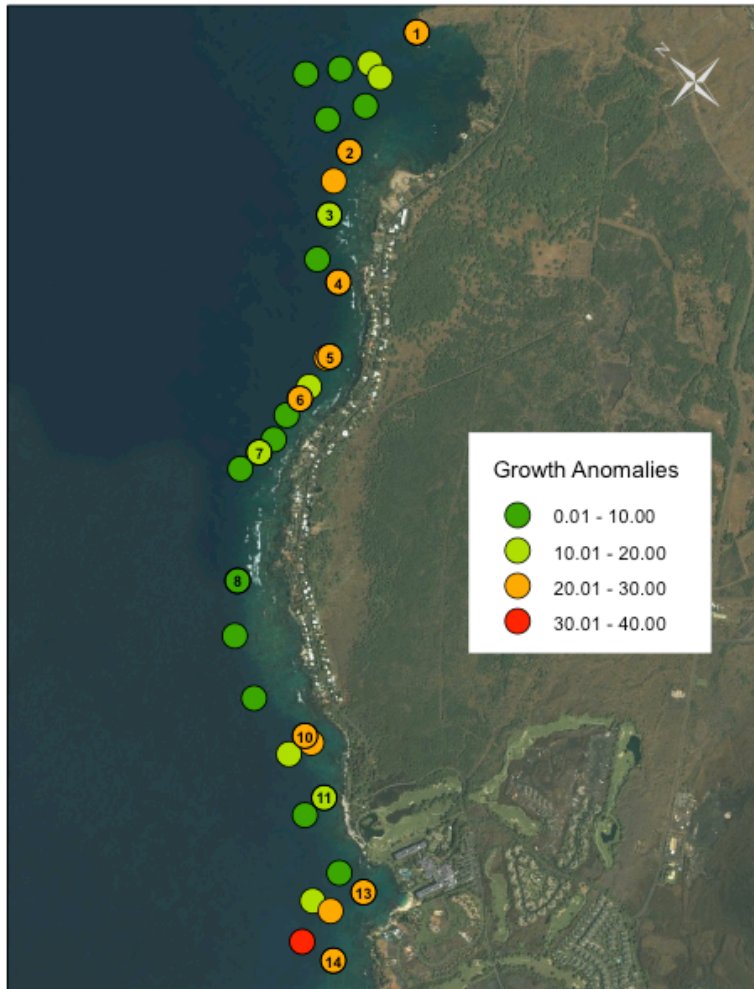
Figure 19. Mean % prevalence of growth anomalies across 24 randomly-selected sites (n=1 transect/site) and 12 shallow sites (with sites numbers 1-14; n=3 transects site).

Overall, coral cover was 34.3 ± 2.5% across all randomly-selected study sites, and was dominated by *Porites lobata* and *P. compressa* (Table 2). While most sites had similar benthic communities, as evidenced by minimal clustering within the nMDS plot (Fig. 20), depth weakly, but significantly explained some of the difference among sites (ANOSIM, R=0.091, p=0.04), with *P. compressa* and turf as the indicators (both more prevalent on reefs deeper than 10 m. However, *P. lobata*, crustose coralline algae, and sand were also important contributors to the observed difference (SIMPER Analysis).

Table 2. Mean (± SE) percent cover of the bottom by organism type found at across 37 randomly-selected sites. Only groups that comprised >1% of the bottom cover are shown. All coral species observed have been included.

Organism	**2014**
Coral	34.3 ± 2.5
Porites lobata	24.5 ± 2.7
Porites compressa	7.2 ± 1.2

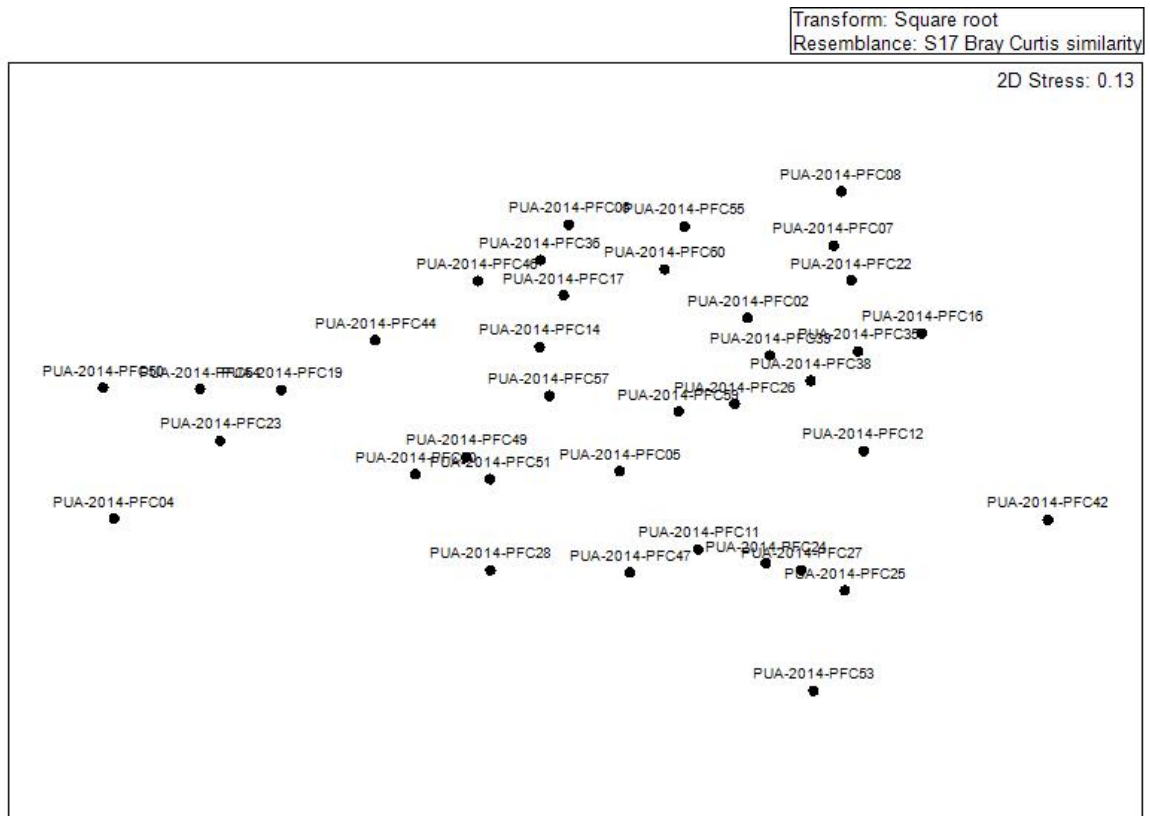|	
Pocillopora meandrina	0.9 ± 0.2
Porites lutea	0.7 ± 0.4
Montipora capitata	0.6 ± 0.1
Pavona varians	0.2 ± 0.1
Montipora patula	0.1 ± 0.1
Porites rus	<0.1
Pavona duedeni	<0.1
Lepastrea purpurea	<0.1
Pocillipora damicornis	<0.1
Cyphastrea ocellina	<0.1
Psammocora stellata	<0.1
Porites bernardi	<0.1
Coral sp.	-
Turf Algae	40.3 ± 2.4
CCA	16.4 ± 3.0
Bare Substratum	7.2 ± 1.5
Macroalgae	0.6 ±0.2

Figure 20. nMDS of benthic percent cover at 37 randomly-selected sites.

Fish Communities

Overall, total fish biomass averaged 41.7 ± 6.4 g/m^2, with 58% of the fish biomass comprised of "resource species" and 27.5 % comprised of "prime spawners" (Table 3). Ninety-five fish species were observed during this study with the most common fish family being surgeonfish, followed by parrotfish, wrasses and triggerfish (Table 4). These four families comprised almost 75% of the total fish biomass. The only observed invasive fish was roi (*Cephalopholis argus*). While it was still relatively abundant compared to other areas in Hawai'i, the biomass of roi at Puakō was only 2.13 ± 0.65 g/m^2.

Table 3. Mean (±SE) total richness, site richness, total density (individuals/m^2), and biomass (g/m^2) of "Prime Spawners," "Resource Species" (reef species targeted or regularly harvested by fishermen), and "Total" fish at Puakō.

Sites	Puakō Richness	Site Richness	Fish Density	Biomass Prime Spawners	Resource Species	Total
37	95	21.2±1.7	0.7±0.1	11.5±4.7	24.2±5.5	41.7±6.4

Table 4. Mean biomass (±SE) of fish families observed at Puakō in 2014.

Fish Family	Species Richness	Biomass (g/m^2)
Acanthuridae	16	16.0 ± 4.0
Scaridae	6	8.4 ± 2.4
Labridae	14	3.4 ± 0.5
Balistidae	6	3.2 ± 0.6
Chaetodontidae	12	2.2 ± 0.3
Serranidae	1	2.1 ± 0.6
Lethrinidae	1	1.3 ± 1.0
Mullidae	5	1.3 ± 0.6
Pomacentridae	11	1.1 ± 0.4
Holocentridae	3	0.8 ± 0.6
Monacanthidae	3	0.6 ± 0.3
Diodontidae	2	0.5 ± 0.3
Cirrhitidae	3	0.2 ± 0.1
Carangidae	1	0.1 ± 0.1
Zanclidae	1	0.1 ± 0.1
Aulostomidae	1	0.1 ± 0.1
Pomacanthidae	3	0.1 ± 0.1
Lutjanidae	1	<0.1
Tetraodontidae	3	<0.1
Ostraciidae	1	<0.1
Fistulariidae	1	<0.1

Blenniidae	1	<0.1
TOTAL	**96**	**41.7±6.4**

Discussion

With the overall decline in South Kohala's reef health, the primary goals of this study were to target regions of concern for coral health and determine whether that health is correlated with LBP. Our study suggests that coral health and water quality are highly variable across the Puakō-Mauna Lani reef system with distinct "hot spots" of terrestrial input and impaired coral health. This study also suggests that certain coral health conditions may be driven by differences in nutrient/SGD input.

Water Quality

While a variety of previous studies have assessed SGD input and water quality at several Puakō-Mauna Lani sites as part of coast-wide assessments (Knee et al. 2010, Street et al. 2008, Dailer et al. 2011, Kim et al. 2014), our study represents the first water quality study with high spatial and temporal resolution across this study region. Our research supports previous studies (Street et al. 2008, Knee et al. 2010) suggesting the Puakō-Mauna Lani coastal ecosystem is exposed to pronounced SGD as evidenced by the lower salinity and elevated silica (enriched in groundwater due to weathering of basaltic rock) (Dollar and Atkinson 1992). Salinity ranges are consistent with other regions along West Hawai'i, spanning moderate SGD input to well-mixed sites (Knee et al. 2010, Street et al. 2008). While the SGD signal was most pronounced in surface waters due to groundwater's lower density, groundwater was also transported onto the reefs through visible benthic seeps on the reef. The highest chronic SGD was observed at site 13, which not only had the lowest average salinity values and highest silica, but also consistently had the thickest brackish water lens (~1- 2.5m) of all sites. Persistent SGD was also observed at sites 1, 5 and 6 with ephemeral seeps at 2. While SGD seeps were observed at other shoreline regions (Fig. 3), wave action played an important role in mixing and should be quantified in future studies to determine how quickly groundwater is diluted across the reef system. This study also highlights that SGD input can be highly dynamic even when sampling solely at low tide. These data suggest that the extensive infrared imagery of SGD plumes along leeward Hawai'i Island (Johnson 2008) does not capture the temporal variability of these plumes or the warmer brackish groundwater plumes. Adequately capturing this variability is essential to accurately assess vulnerability to LBP and prioritize regions needing improved management.

Our results are also consistent with previous studies (Street et al. 2008) indicating that the submarine groundwater discharge is an important source of new nutrients into West Hawai'i's coastal waters, as evidenced by elevated dissolved inorganic nutrients at sites such as 1, 5 and 13. Despite these spatial patterns, it is important to note that dissolved inorganic nutrient levels were still low across all study sites compared to reef waters in previous West Hawai'i studies (Knee et al. 2008; Street et al. 2008; Knee et al. 2010).

Determining the degree to which LBP affects naturally occurring SGD in this region is difficult for a variety of reasons. First, nutrient levels are naturally elevated in SGD (Street et al. 2008). "Natural" nutrient levels are also likely dependent on the community composition of coastal plants and marine organisms, and their ability to uptake nutrients (Lapointe and Clark 1992; Koop et al. 2001). Nutrients can also be subsidized naturally from the benthic communities themselves (Larned 1998) or nitrogen-fixing plants in coastal regions (Street et al. 2008). However, previous studies along the Kona coast suggest that fertilizer additions to golf courses and treated wastewater may augment nitrogen and phosphorus levels in groundwater (Street et al. 2008, Johnson et al. 2008, Dollar and Atkinson et al. 1992). Sewage pollution is also widely known to subsidize nutrient levels of reefs (e.g. Pastorok and Bilyard 1985; Baker et al. 2010) and may be a potential source on Puakō's reefs.

In this study, we found no correlation between nutrient level and *Enterococcus*, a commonly used fecal indicator bacteria, in the present study. This would suggest that sewage is not a major contributor to nutrient levels measured here. However, nutrient levels along the shoreline were not measured, and the rapid biological uptake of dissolved inorganic nutrients may have obscured a sewage signal, or it may have been obfuscated by nutrient additions from sources such as irrigation or fertilizers. Nutrient levels were consistently higher around the Mauna Lani resort, but for the reasons stated above, it is unclear whether these elevated nutrient levels are a natural feature of the area or the result of LBP. $\delta^{15}N$ can provide a valuable tool for more accurately determining the relative contribution of different nitrogen sources into coral reef ecosystems (Lapointe and Clark 1992; Baker et al. 2010). While Dailer et al. (2011) detected $\delta^{15}N$ values in Puakō indicative of sewage pollution, work currently underway by the University of Hawai'i at Hilo using $\delta^{15}N$ at high spatial and temporal resolution should provide valuable new insight into the relative contribution of different nutrient sources.

Our study does provide clear evidence that sewage contamination is a source of LBP into at least portions of Puakō's nearshore ecosystems, as evidenced by the patterns of enterococci abundance in nearshore waters. Most notably, shoreline *Enterococcus* CFUs/100ml was seven times higher in shoreline (knee-deep) water compared to samples collected on the reef (50-150m from shore) and 2.5 times higher than the EPA standard for recreational marine waters (35 CFU/100mL, EPA 2011). These results support previous studies that also found *Enterococcus* levels exceeding EPA suggesting that sewage contamination may also be a threat to human health. Based the current study, there appears to be several *Enterococcus* "hot spots" in shoreline and reef waters, near sites 6 (east of Waima Point) and 10 (Paniau), as well as elevated levels in shoreline waters near sites 2 (Condos) and 11 (Hololokai Beach Park). The high spatial variability in shoreline and reef waters is consistent with findings by Kim et al. (2014), and may be explained by a complex suite of factors. The level of mixing with oceanic water could play an important role in nearshore waters by decreasing residence time of land-based inputs in well-flushed regions. For example, shoreline samples from sites 2, 6, 8, 10, 11 and 14 were collected in tidepools or regions with reduced water circulation. *Enterococcus* can be trapped in sand and sediments and then resuspended, thus confounding new sources of LBP (e.g., Yamahara et al. 2007, Oshiro and Fujioka 1995). However, the negative relationship of *Enterococcus* with salinity in this study (Fig. 10)

suggests that SGD may deliver sewage contamination and new enteric bacteria into Puakō's coastal waters. This hypothesis is supported by extremely high *Enterococcus* levels (25 to greater than 1000 CFUs/100ml) in anchialine ponds surveyed in 2013 (Kim et al. 2014). While the degree to which brackish water from the ponds mix with Puakō's SGD and contribute to LBP is unclear, studies in Kaloko-Honokohau indicate that brackish water from these ponds mix with coastal waters (Knee et al. 2008). Enterococci levels are also likely influenced by proximity to poor sewage treatment. For example, preliminary results from a dye tracer study showed that domestic wastewater injected into a cesspool was transported to the nearshore waters within three days (Colbert et al. unpubl.). Likewise, the present study provides convincing evidence that Mauna Lani's self-contained sewage treatment system is effective in reducing fecal indicator bacteria load into coastal waters as evidenced by the lower levels at sites 13 and 14 despite its high recreational use. Importantly, spatial patterns may also be influenced by confounding signals from other animal fecal sources. *Enterococcus* is found in the guts of most animal species including turtles, birds and rodents (Layton et al. 2009; Byappanahalli et al. 2012) and despite its widespread use, EPA's culture-dependent *Enterococcus* assay does not distinguish between human and other strains of *Enterococcus*. Given the potential importance of SGD (lower salinity water) and proximity to potential pollution sources, more sensitive microbial source tracking approach, targeting human-specific microbes in addition to $\delta^{15}N$ is needed to accurately detect sewage contamination.

Coral Health

We observed considerable spatial heterogeneity in coral health assemblages across the Puakō-Mauna Lani reef system with coral health primarily driven by growth anomalies and algal overgrowth, which each affected 20% of colonies (Fig. 13). As a disease with broad host range (Sutherland et al. 2004), growth anomalies were observed on *Montipora* and *Pocillopora*, but primarily affected the dominant reef-builder, *Porites*. The causative agent of this disease is still unknown. This pattern is consistent with previous studies in Hawai'i (Aeby et al. 2011b; Couch et al. 2014). While other diseases such as trematodiasis and tissue loss diseases were recorded, these conditions were neither prevalent nor severe (Fig. 16), with the exception of 25% trematodiasis prevalence at site 13. Algal overgrowth was primarily the result of red turf algae, morphologically similar to *Corallophila huysmansii* and filamentous cyanobacteria (Fig. 3). *C. huysmansii* is commonly associated with tissue mortality across the Pacific (Jompa and McCook 2003; Willis et al. 2004; Myers and Raymundo 2009; Couch et al. 2014). While *C. huysmansii* is hypothesized to excrete allelotoxic compounds used to overgrow coral tissue (Jompa and McCook 2003)the processes governing their growth and distribution are unknown. Our results highlight that the role of native algal species in colony-level health should not be underestimated. Spatial patterns in compromised health were also mediated to a lesser degree by discoloration. This condition is a common sign of compromised health in scleractinians and is often attributed to a variety of localized stressors such as physical damage and algal overgrowth (Willis et al. 2004; Raymundo et al. 2005). Using a combination of the 12 shallow and 24 randomly-selected sites, we identified four areas of concern for coral health including: the reef between sites 4 and 6, as well as Pau'oa Bay/Mauna Lani (near 13 and 14), and to a lesser degree sites between 2 and 3 as well as

Paniau (Fig. 17 & 19). This high spatial heterogeneity in coral health along just 3.5km suggests that small scale biotic or environmental factors drive coral health.

Along the Puakō-Mauna Lani reef system, disease pressure (prevalence x severity) excluding trematodiasis, was most strongly correlated with nutrient level and exposure to SGD. Chlorophyll-a concentration alone explained 22% of the variance in total disease pressure, but this pattern was driven by high disease and chlorophyll-*a* at sites 5 and 6 (Fig. 18A). The lack of a strong driver of overall disease patterns is not surprising given that growth anomalies and tissue loss diseases likely have differing underlying causes with varying sensitivity to environmental change. This relationship is primarily driven by a correlation between growth anomalies and dissolved inorganic nutrients. Nitrate + nitrite concentration, alone explained 44% of the variance in growth anomaly pressure and is consistent with previous studies that found a positive correlation between growth anomaly prevalence and nitrogen concentrations (Kuta and Richardson 2002; Kaczmarsky and Richardson 2010; Williams et al. 2010). While the underlying mechanisms behind this relationship and the causative agent of this disease are still unknown, eutrophication has been hypothesized to indirectly compromise coral physiology by altering host-pathogen interactions and/or the symbiosis (Harvell et al. 2007). The strong relationship between growth anomalies and silica in the present study also suggests that there may be other components of the groundwater, such as chemical contaminants or pathogens that we did not account for driving coral health. While the lack of a correlation between coral health and fecal indicator bacteria suggests that sewage contamination is not as important as other watershed nutrient inputs, sewage pollution should not underestimated. Finally, despite the lack of a relationship between growth anomaly prevalence and fish associated coral injury, these surveys represented one snapshot in time and the link between coral disease and fish assemblages warrants further investigation.

Coral disease was only minimally driven by coral abundance (% cover and colony density) or size and generally only when combined with environmental factors. This is contrary to many previous studies that found that coral disease increased with coral abundance (e.g. Bruno et al. 2007; Aeby et al. 2011a) and colony size (Burns et al. 2011; Roff et al. 2011). This is not surprising given that we chose sites with similar coral community structure to minimize confounding host effects. Consistent with previous West Hawai‘i research (Couch et al. 2014), coral health was not strongly correlated with depth at the 24 randomly-selected sites, which together with the minimal host effects, further emphasizes the importance of local environmental factors.

Compromised health was driven by a combination of temperature, colony density, with higher compromised health on reefs with warmer water and fewer colonies. Within the compromised health category, temperature appeared to be most important in predicting discoloration pressure and algal overgrowth to a lesser degree. One explanation is that elevated temperature, commonly associated with physiological stress in corals, compromises the immune function leading to increased discoloration. However, given the minimal temperature fluctuation there may be another factor correlated with temperature that is more strongly modulating compromised health. Algal overgrowth was positively correlated with temperature, which may be the result of stimulated growth in warmer

water. Algal overgrowth also increased with colony size, which may be due to increased partial mortality in large colonies. However, neither temperature nor size were especially strong predictors, suggesting that additional research is needed to identify what is driving the distribution of algal overgrowth. Herbivory may be one mechanism driving patterns of algal overgrowth, given the importance of herbivores in regulating algal growth on coral reefs. While herbivorous fish were present across all of the sites, their ability to graze red filamentous turf was not addressed in this study.

Local hydrology may also explain patterns in coral health across the Puakō-Mauna Lani region. Reduced water motion can increase residence time of microbes and nutrients in the water as well as cause physiological stress in corals by impairing gas exchange, waste and sediment removal, nutrient exchange and photosynthesis (Dennison and Barnes 1988; Lesser et al. 1994; Finelli et al. 2006). On Hawai'i Island, higher growth anomalies have been found on reefs with lower water circulation (Burns et al. 2011, Couch 2014) and may promote settling and growth of turf algae and cyanobacteria thus increasing algal overgrowth.

Key Findings

- SGD plumes were pronounced across the study region and may serve as a delivery mechanism of LBP.
- Water quality was highly variable across the year, emphasizing the importance of incorporating temporal variability when assessing vulnerability to LBP.
- Dissolved inorganic concentration was generally low compared to other West Hawai'i studies, but highly variable between sites.
- Silica and nitrite + nitrate concentration was elevated in surface waters at sites 1 (near Ohai Point), 5 (east of Waima Point), and most notably at site 13 (Pau'oa Bay) compared to the other study sites.
- Enterococci abundance CFUs/100ml were 7 times higher in knee-deep shoreline waters compared to reef waters. Levels exceeded the EPA standard for recreational waters (35 CFU/100mL) for 9 of the 12 shoreline sites and was particularly high at sites 2 (Condos), 6 (east of Waima Point) and 10 & 11 (near Paniau).
- Overall, 24% of corals were diseased and 27% showed signs of compromised health, with coral health primarily driven by growth anomalies and algal overgrowth, each affecting 20% of colonies. We also recorded signs of trematodiasis and tissue loss syndrome, discoloration, bleaching, physical damage, gastropod predation and crown-of-thorns predation.
- Overall, coral cover was 34%. Porites dominated the coral communities and was also the most affected by disease.
- We observed considerable spatial heterogeneity in coral health along just 3.5km suggesting that small scale biotic or environmental factors drive coral health.
- We identified four areas of concern for coral health including: the reef between sites 4 and 6, as well as Pau'oa Bay/Mauna Lani (near 13 and 14), and to a lesser degree sites between 2 and 3 as well as Paniau.
- Overall, environmental variability played a stronger role in coral health patterns than coral demography (% cover, colony density and colony size).

- Nutrient concentration was the strongest environmental diver of disease, namely growth anomalies, with higher growth anomaly pressure (prevalence x severity) on reefs with elevated silica and nitrate + nitrate concentration. This suggests that nutrients or another component of the groundwater (e.g. chemical pollutants) are increasing disease levels.
- While *Enterococcus* was not a strong predictor of coral health, additional indicators of human sewage pollution are needed to more accurately detect sewage contamination and assess its affects on coral health.
- These results also indicate that other watershed nutrient inputs in addition to sewage may be equally if not more important.

Future directions:

- Determine the role of other factors such as water motion/mixing and chemical contamination in coral health along this reef system.
- Determine the relative contribution of different nutrient sources (e.g. sewage, fertilizing, and natural) to nutrient levels.
- Use microbial source tracking techniques and human-specific gut microbes to better identify regions affected by sewage contamination.
- Conduct experimental studies to test the role of nutrient on growth anomaly onset and progression.
- Gain a better understanding of the role of algal overgrowth in colony-level mortality and the processes driving *Corallophila huysmansii* growth and interactions with coral.

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