Linking wastewater and land use to coral reef health: Integrating monitoring and modeling approaches for conservation planning

Photo by Rob Shallenberger

This report was prepared by The Nature Conservancy under Award NA14NMF4630259 from the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service, as administered by the NOAA Restoration Center's Office of Habitat Conservation. The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of NOAA or the U.S. Department of Commerce.

Linking wastewater and land use to coral reef health: Integrating monitoring and modeling approaches for conservation planning

by

Courtney Couch[1,2], Rebecca Most[3], Kristina Remple[4], Chad Wiggins[3], Julia Rose[3], Kim Falinski[3], Cecile Walsh[3], Craig Nelson[4], Eric Conklin[3], Leilani M Abaya[5], Tracy Wiegner[5]

1. Hawai‘i Institute of Marine Biology, University of Hawai‘i at Mānoa,
46-007 Lilipuna Road
Kāne‘ohe, HI 96744

2. Current address: NOAA/Pacific Islands Fisheries Science Center
Ecosystem Sciences Division
NOAA Inouye Regional Center
1845 Wasp Blvd, Bldg. # 176
Honolulu, HI 96818

3. The Nature Conservancy, Hawai‘i
923 Nu‘uanu Avenue
Honolulu, HI 96817

4. Center for Microbial Oceanography: Research and Education, Department of Oceanography and Sea Grant College Program, School of Ocean and Earth Science and Technology, 1950 East West Road, University of Hawai‘i at Mānoa, Honolulu, Hawai‘i, USA 96822

5. Marine Science Department
University of Hawai‘i at Hilo
200 W. Kawili St., Hilo, HI, USA 96720

July 27, 2019

Suggested Citation:
Couch, C., Most, R., Remple, K., Wiggins, C., Rose, J., Falinski, K. Walsh, C. Nelson, C., Conklin, E., Abaya, L.M., and Wiegner, T. 2019. Linking wastewater and land use to coral reef health: integrating monitoring and modeling approaches for conservation planning. Technical report prepared for NOAA (NA14NMF4630259). 57pp.

Cover Image: Aerial view of the Puakō community and reef looking to the south.

TABLE OF CONENTS

1.0 EXECUTIVE SUMMARY

The scope of stressors on coral reefs is growing as climate change takes corals beyond their thermal tolerance and increased coastal development and suboptimal wastewater treatment exposes reefs to land-based pollution (LBP). While global reductions in carbon emissions are needed for long-term protection of coral reefs, reducing local stressors and promoting coral health by reducing land-based pollution may extend the timescale over which coral reefs can continue to thrive while we combat climate change. Historically known for its crystal clear blue waters, the leeward coast of Hawaiʻi Island has undergone persistent land use change in recent decades that have raised concerns about sources of LBP and their role in documented coral reef decline, and highlight the need to better understand and manage these sources to preserve or restore the resilience of coral reefs to a changing climate.

As part of NOAA's Habitat Blueprint program along West Hawaiʻi, The Nature Conservancy developed a three-year project to expand on our approach for tracking and assessing the impacts of LBP in South Kohala then applied these techniques to the broader West Hawaiʻi coastline. We used a combination of standard benthic coral community surveys, *in situ* water sampling, as well as more advanced molecular and spatial statistical approaches. Our objectives were: **Year 1 (2015):** Improve our understanding of sources of LBP along the Puakō-Mauna Lani reef system and identify hot spots for targeted management. **Year 2 (2016):** Determine whether indicators of sewage input at the reef benthos correlated with coral health across gradients of sewage input in hot spot areas. **Year 3 (2017):** Apply the previous tools and approaches to identify coastal and watershed sources of nutrients along the broader North Kona and South Kohala coastline and assess the relationship between land-based inputs and coral colony and reef health metrics.

Our results provide definitive evidence that sewage pollution is entering the Puakō shoreline and is affecting reef health, and using spatial statistical approaches we identified two hot spots of sewage pollution. Identifying sources of LBP and patterns in water quality across the broader coastline is more complex due to the variety of land use inputs and coastal oceanography. Shoreline dissolved inorganic nutrient concentration is correlated with land use, with higher concentrations near resort developments. Abundance of fecal indicator bacteria was low and varied minimally across the West Hawaiʻi coastline, but was likely underestimated due to low temporal sampling. Consistent with previous studies along West Hawaiʻi, coral health is primarily affected by growth anomalies and direct coral-algal competition, which affected up to 30 to 45% of colonies. In Puakō algal overgrowth was somewhat driven by positive relationship with ammonium and δ^{15} N values, and was more correlated with *in situ* nutrient concentration along the wider coastline. Across the broader coastline, *Porites* growth anomaly prevalence was elevated in areas with higher *in situ* nutrient concentration, watershed nutrients and larger colonies. These results clearly indicate that anthropogenic nutrients negatively affect coral condition at several sites along the West Hawaiʻi coastline, provide evidence that nutrient inputs (both natural and anthropogenic sources) in addition to sewage may play an equally if not more important role in reef health.

2.0 INTRODUCTION & OBJECTIVES

Land-based pollution (LBP) accompanying human population growth and burgeoning coastal development has been implicated as one of the key factors contributing to global coral reef decline alongside coral bleaching, overfishing and coral disease (Bellwood et al., 2004; Harvell et al., 2007; Hughes et al., 2018; Lapointe and Clark, 1992). Understanding the impacts of LBP is challenging with pollution originating from a variety of sources. A wealth of studies have demonstrated that land use changes accompanying agriculture, and residential and resort development can subsidize nutrient inputs into coastal ecosystems through the use of fertilizers and inappropriate development practices (Bowen et al., 2007; Cole et al., 2006; Eckhardt and Stackelberg, 1995; Howarth et al., 2002; Knee et al., 2010a, 2010b). With more than 50% of people living near the coastline and most of the sewage entering coastlines being minimally or completely untreated, sewage pollution is also a growing yet vastly underestimated concern (Islam and Tanaka, 2004; Wear and Thurber, 2015).

Sewage pollution and eutrophication can affect coral disease susceptibility by altering host-pathogen interactions. In field surveys and experimental studies, disease prevalence, severity and progression of certain diseases are often positively correlated with or increase when exposed to elevated nutrients (Bruno et al., 2003; Kaczmarsky and Richardson, 2010; Vega Thurber et al., 2014; Voss and Richardson, 2006). Nutrient enrichment can also alter the community of benefitial and pathogenic microbes that live on and within coral tissues by enhancing growth and virulence of potential bacterial pathogens (Thurber et al., 2009; Zaneveld et al., 2016). In the Caribbean, sewage pollution has also been implicated in the introduction of the coral pathogen *Sercia marsensens* resulting in white pox disease outbreaks on the dominant Acroporid reef building corals (Sutherland et al., 2010).

One of the most well known effects of sewage and nutrient pollution on coral reefs is its role in stimulating algal growth and improving its competitive advantage over coral. In severe cases, the proliferation of algae can result in direct overgrowth of corals, impeding their access to light, nutrients and space (Fabricius, 2005; Smith, 1981; Vermeij et al., 2010). Some of the most dramatic case studies of the impacts of sewage on reefs have occurred in Hawaiʻi. Outfalls of raw sewage into embayments or use of injection wells resulted widespread proliferation of algae and subsequent widespread coral morality in Kāneʻohe Bay, Oʻahu, and Maʻalaea Bay and Kihei, Maui (Hunter and Evans, 1995; Smith, 1981; Smith et al., 2005; van Beukering and Cesar, 2004). While numerous studies have identified a link between algal abundance when exposed to severe point source pollution or during artificial fertilizer experiments, the role of non-point source nutrient pollution, which is more common in regions such as Hawaiʻi, on direct coral-algal interactions is less clear.

In Hawaiʻi, nutrient inputs have been linked to a variety of natural and anthropogenic sources. On volcanic islands with highly porous basaltic rock, submarine groundwater discharge (SGD) provides an important source of nutrients for the coastal ecosystems (Kim et al., 2003; Street et al., 2008). Freshwater quickly percolates through the substrate into underground aquifers, mixes with seawater, and is released into the coastal regions as SGD (Street et al., 2008). Dissolved inorganic nutrient concentrations in groundwater are naturally one to two orders of magnitude higher than nearshore oceanic water, and the large input of groundwater into the coastal ocean creates zones of mixing with strong gradients of nutrients and salinity. (Dollar and Atkinson 1992). Although nutrient levels are naturally elevated in SGD (Johnson et al., 2008; Street et al., 2008; Umezawa et al., 2002), SGD nutrient loads can be subsidized by anthropogenic sources (Amato et al., 2016; Knee et al., 2010b). In West Maui, SGD is an important delivery mechanism for terrestrial fertilizers and wastewater from injection wells and is correlated with elevated macroalgae abundance and lower coral species diversity (Amato et al., 2016). Non-point source pollution from on-site disposal systems (OSDS) are also a major source of nutrients into Hawaii's coastal waters. As of 2014, there were approximately 110,000 OSDSs discharging 69.6 million gallon of effluent per day into coastal ecosystems (Whittier and El-Kadi, 2014). Despite the 2016 statewide ban on new cesspools, cesspools alone represent 80% of OSDSs, more than half of which are located on Hawaiʻi Island (Whittier and El-Kadi, 2014). Cesspools are especially problematic because they directly leach untreated sewage into the surrounding rock and soil. While the impacts of terrestrial runoff on coral reefs has been well studied (Fabricius, 2005), the impacts of SGD on coral health and the degree to which nonpoint source pollution interacts with SGD remains unclear. To help guide and prioritize efforts to mitigate the impacts of non-point source pollution, managers need an understanding their extent and potential sources of LBP.

Nutrient concentrations are commonly used to assess water quality with heavy fertilizer use and sewage pollution resulting in elevated nutrients in coastal waters (Howarth et al., 2002; Lapointe et al., 2004; Lapointe and Clark, 1992). However, given that natural and anthropogenic nutrients can originate from a variety of sources, measuring nutrients alone will not provide an indication of the source of nutrients. Stable nitrogen isotopic composition ($\delta^{15}N$) in macroalgae is a method used for identifying nitrogen sources (e.g. sewage, fertilizers, soils, groundwater and oceanwater) into coastal waters (Abaya et al., 2018b; Savage, 2005; Umezawa et al., 2002; Wiegner et al., 2016). Fecal indicator bacteria (FIB) are widely used as an indicator of sewage pollution and serve as a proxy of risk to human health (Cabelli, 1983; Prüss, 1998). *Enterococcus spp*. is commonly used for marine recreational waters by the United States Environmental Protection Agency (EPA) and Hawaiʻi Department of Health (HDOH). However, *Enterococcu*s is found in the guts of many animal species (Byappanahalli et al., 2012; Layton et al., 2009) and the culture-dependent *Enterococcus* assays used by regulatory agencies do not distinguish between human and other strains of *Enterococcus*. More recently, a technique called microbial source tracking was developed that employs culture-independent molecular techniques to identify the origin of fecal bacteria (Scott et al., 2002). Since most animals have bacteria specific to their guts, microbial source tracking allows us to target bacteria specific to humans, for example, using their genetic signature and then quantifying the abundance of that bacteria (Harwood et al., 2014).

To better understand the potential role of LBP in coral reef health and assist managers and communities concerned about sewage pollution with identifying the extent of this issue, The Nature Conservancy (TNC) began working in the South Kohala District of Hawaiʻi Island in 2013, which was selected as a high priority for management (Hawaiʻi Coral Reef Working Group 2010) and designated a NOAA Habitat Blueprint site. The Puakō-Mauna Lani reef system is one of the most well-developed fringing reefs in the state, yet has experienced a 50% loss in coral cover accompanying substantial changes in land use during the 40 years prior to the 2015 coral bleaching event (Minton et al., 2012). Similar to many regions in Hawaiʻi, Puakō's distance from municipal sewage treatment renders the community reliant on a combination of septic systems, cesspools and one injection well (Schott, 2010). With approximately a third of the Puakō houses using cesspools (Schott, 2010), the community raised concerns about the effects of sewage pollution on coastal ecosystems. Adjacent to Puakō, the Mauna Lani coastline has become one of South Kohala's major resort communities. Unlike Puakō, the Mauna Lani resorts use an aerated lagoon wastewater treatment system, which treats to the R2 level (disinfects sewage to reduce pathogens, but not nutrients) (Schott, 2010). While previous studies have found that coral disease may increase with nutrient input (Couch et al., 2014b) and sewage contamination is leaching into the marine environment (Abaya et al., 2018b; Couch et al., 2014b; Yoshioka et al., 2016), a limited number of fecal indicator bacteria were used and a comprehensive multi-parameter approach for identifying sewage hot spots has not been developed. Beyond the Puakō-Mauna Lani reef system, less is known about the extent of water quality issues along West Hawaiʻi and their impacts on coral reefs. When this work was initiated the general impression was that Puakō's issues with cesspools were the anomaly along a coastline that generally had excellent water quality with minimal impact to reefs. With the State considering legislation that would ban cesspools by 2050 and growing concerns about nutrient enrichments from expanding development along the coast, a better understanding of the sources, extent, and impacts of LBP along this coastline is crucial.

As part of NOAA's Habitat Blueprint program along West Hawaiʻi, TNC developed a three-year project to expand on our approach for tracking and assessing the impacts of LBP in South Kohala then applied these techniques to the broader West Hawaiʻi coastline. Our objectives were:

Year 1 (2015)- Improve our understanding of sources of land-based pollution along the Puakō-Mauna Lani reef system and identify hot spots for targeted management.

Year 2 (2016)- Determine whether indicators of sewage input at the reef benthos correlated with coral health and reef structure across gradients of sewage input in sewage hot spot areas.

Year 3 (2017)- Apply the previous tools and approaches to identify coastal and watershed sources of nutrients along the broader North Kona and South Kohala coastline and assess the relationship between land-based inputs and coral health, coral cover and juvenile coral density.

3.0 METHODS

3.1 Year 1

3.11 Study Sites and Sample Collection

In 2015, we assessed water quality across 12 previously established monitoring sites across the Puakō-Mauna Lani reef system that spanned a range of "low" to "high" SGD/terrestrial input (see Couch et al. 2014 for details on site selection). At each site, we conducted water sampling four times in 2015 (January 19-21st, March 2-5th, May 4-6th, July 15-19th). Samples were collected in the morning during the low tide window (1 hour before to 2 hours after low tide) to minimize UV damage of fecal indictor bacteria and best capture the terrestrial inputs associated with groundwater discharge. During each sampling period, with the exception of January due to high surf conditions, we collected samples from the 12 reef sites (surface and just above the benthos) and 12 corresponding shoreline sites (Figure 1). We measured temperature and salinity (YSI sonde 6920 V2; 2 min sampling period with 5 sec intervals between measurements) at all sampling locations. We collected triplicate 3L water samples from the shoreline, surface and benthic water, and transported the water to our mobile laboratory within 4 hours of collection.

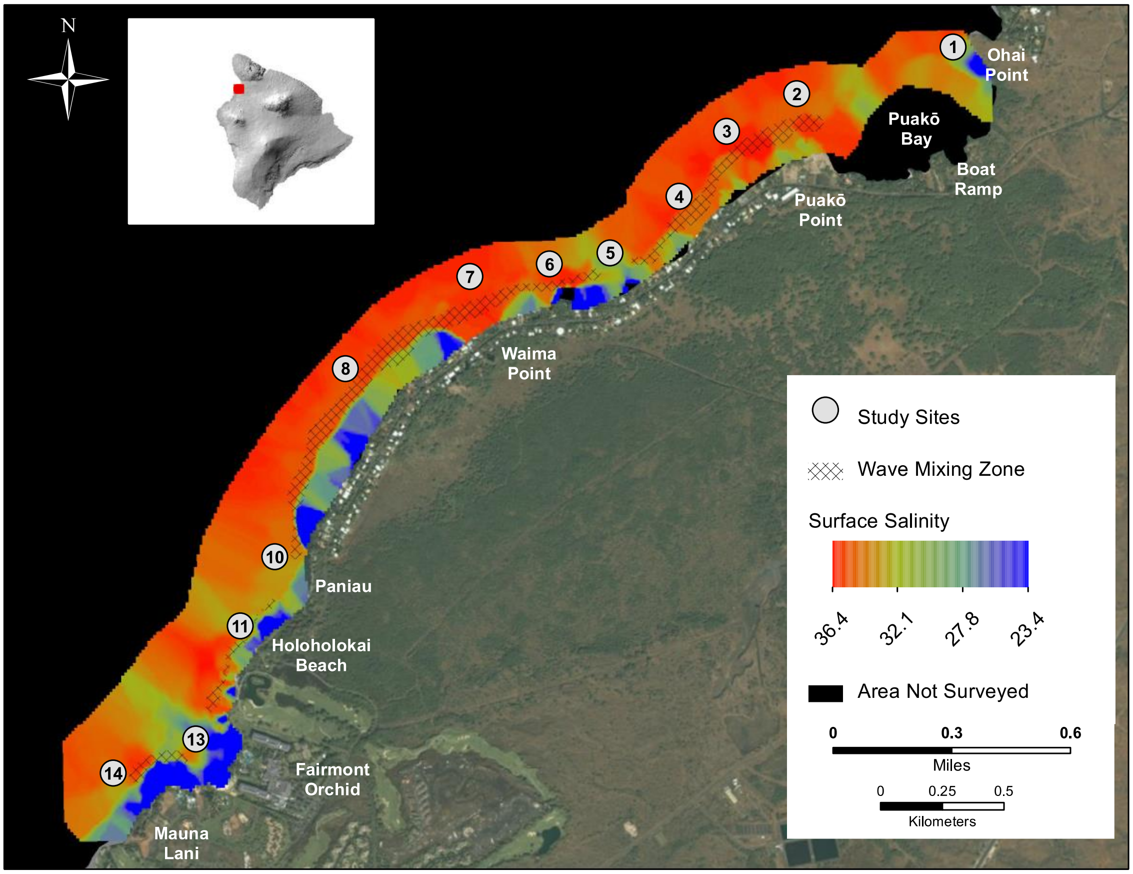

Figure 1. Year 1 map of sampling sites over a surface salinity map created from YSI sonde tows in December 2013 from Couch et al. 2014.

3.12 Sample Processing

To quantify concentration of dissolved inorganic nutrients, duplicate 200ml samples from each site were filtered through GF/F Whatman filters into triple-rinsed acid washed bottles then frozen and processed within 1 week. Dissolved inorganic nutrients (nitrate + nitrite, ammonium, orthophosphate, silica) were analyzed at Natural Energy Laboratory of Hawaiʻi using a flow-injection analysis on an Astoria Pacific Instruments autoanalyzer. To quantify culturable *Enterococcus* abundance, triplicates samples for each site during each sampling period (100ml for shoreline waters and 200ml for reef waters) were filtered through 47mm 0.45 um cellulose nitrate filters onto mEI agar plates without naldixic acid using a modified version of EPA Method 1600. Sterile deionized water was also filtered and run as negative controls. Plates were incubated at 42°C for 24 hours and data were calculated as number of colony forming units/100ml. To quantify other bacterial taxa in the water column using molecular techniques, we also filtered triplicate 1 L samples for each site as well as deionized water negative controls through 47mm 0.2µm PES filters. The filters were stored in eppendorph tubes then immediately frozen at -20°C, shipped on blue ice back to Dr. Craig Nelson's lab at the University of Hawaiʻi at Mānoa and frozen at -80°C until processing.

DNA was extracted using the MoBio Power Soil kits and transported to Dr. Nelson's laboratory where quantitative PCR assays (qPCR) were conducted to quantify the number of gene copies (an indicator of bacterial abundance) of three different gut-associated bacteria. The bacterial group, Bacteroidales was targeted using the GenBac3 assay described in Dick and Field (2004) and serves as a proxy for abundance of mammalian gut bacteria. Human-specific sub-groups of Bacteroidales were targeted using two assays - BacHum (Kildare et al 2007) and HF183 (Haugland et al 2010). For the purpose of this study, human-specific Bacteroidales are presented as the percent of samples that contain at least one gene copy of BacHum or HF183.

The detection of fecal indicator bacteria was performed using four established qPCR assays as outlined in Table 1. Briefly, 25 uL reaction mixtures containing 10uL of either 2.5X 5Prime Real Mastermix Probe (catalogue number: 2200700) or Kapa Probe Force Master mix (catalog number KK4301), 0.1 mg/mL of molecular biology grade Bovine Serum Albumin (New England Biolabs), 400nM of each specific primer, 200nM of each specific probe and 1uL of sample or standard DNA were amplified using an Eppendorf Realplex2 Mastercycler®. A two-minute hot start at 95°C was followed by 45 cycles of 95°C denaturing for 30 seconds, and 60°C elongation for 30 seconds.

Standard curves were constructed from serial dilutions of either genomic DNA or synthetic DNA fragments as outlined in Table 1. For each assay, eight dilution steps ranging from 50,000 - 10 gene copies per well were performed in triplicate on every run plate. R2 values ranged from 0.92 - 0.99 and threshold cycle y-intercepts (a theoretical limit of detection) for these assays ranged from 37.3 -39.6 and 38.4 – 42.4 for general Bacteriodales and human-specific Bacteriodales assays, respectively. Threshold values were controlled manually at 500 RFU.

Table 1. Summary of microbial source tracking assays used.

Assay Target	Assay Name	Gene Target	References	Nucleotide Sequences (Forward, Reverse, 5' Nuclease Probe)	Standard
Bacteroidales	GenBac3	16S rRNA	Dick and Field 2004, Siefring et al. 2008, Method “B” EPA-822-R-10-003	GGGGTTCTGAGAGGAAGGT CCGTCATCCTTCACGCTACT 6-FAM™/CAATATTCC/ZEN™/TCACTGCTGCCTCCCGTA/IB®FQ/	*Bacteroidales thetaiotamicron* strain VPI 5482 (ATCC® 29148™)
Human Bacteroidales	HF183	16S rRNA	Haugland et al. 2010, Green et al. 2014; 3' CAT avoids MGB, OK by ProbeMatch	ATCATGAGTTCACATGTCCG CTTCCTCTCAGAACCCCTATCC 6-FAM™/CTAATGGAA/ZEN™/CGCATCCCCAT/IB®FQ/	IDT gBlocks dsDNA sequence AB242142.1 (Green et al 2014) 16S rRNA sequence for *Bacteroidales dorei* strain DSM 17855
Human Bacteroidales	BacHum	16S rRNA	Kildare et al. 2007	TGAGTTCACATGTCCGCATGA CGTTACCCCGCCTACTATCTAATG 6-FAM™/TCCGGTAGA/ZEN™/CGATGGGGATGCGTT/IB®FQ/	IDT gBlocks dsDNA sequence AB242142.1 (Green et al 2014) 16S rRNA sequence for *Bacteroidales dorei* strain DSM 17855

3.13 Sewage Indicator Score and Mapping

To better assess the extent of sewage pollution and identify potential "hot spots" to be targeted for corrective action, TNC in collaboration with the University of Hawaiʻi at Hilo (UHH) developed a scoring and mapping technique using three sewage indicators (fecal indicator bacteria, $\delta^{15}N$ macroalgae, and nutrients). The data used to create the sewage indicator map in Year 1 includes samples collected from 2013-2015 by TNC, UHH and Cornell University (Abaya et al., 2018b; Couch et al., 2014b; Yoshioka et al., 2016) from shoreline and reef sites spanning 7.3 kilometers (4.5 miles) from Waialea Bay to Paniau. Data were averaged across time at sites that were sampled more than once. The scoring tool had three levels for each indicator: level 1 = low, level 2 = medium, and level 3 = high. Levels for each indicator were based on established standards and previous studies in Puakō and elsewhere and described in detail in Abaya et al. (2018b) (Table 2). Specifically, the scoring tool used HDOH's geometric mean and the single sample maximum for *Enterococcus* spp. concentrations in marine waters, the Fung/Fujioka *C. perfringens* scale for sewage pollution, $\delta^{15}N$ values in macroalgal tissues for different NO_3^- sources (Abaya et al., 2018b), and HDOH's water quality standards for nutrient concentrations in open coastal waters ($NO_3^- + NO_2^-$, $NH4^+$, and total dissolved phosphorus - TDP) (Table 2). Nutrient concentration standards for the wet season criteria were used because the freshwater inputs along the Puakō shoreline ranged from 2083-2730 L/m/h (Paytan et al., 2006), which are an order of magnitude larger than the baseline for the wet season criteria (> 294 L/m/h). TDP was used as the phosphorous water quality indicator since HDOH has no PO_4 water quality standard for open coastal waters (HDOH 2014). It should also be noted that a "medium" level in nutrient concentrations exceeds HDOH standards for open coastal waters wet season criteria. Once each indicator was assigned a level (1-3) based on its measured value and our scoring technique (Table 2), its level was multiplied by a weight factor (1-3), with the most reliable sewage indicators having the greatest weight (Table 2). The greatest weight (weight = 3) was given to *C. perfringens* and δ15N in macroalgal tissue because these indicators are more specific to sewage pollution, more integrative measurements of environmental conditions, and do not fluctuate as much as *Enterococcus* spp. and nutrient concentrations (Dailer et al., 2010; Fung et al., 2007; Viau et al., 2011; Yoshioka et al., 2016). *Enterococcus* spp. concentrations received a medium weight (weight = 2) as HDOH uses this FIB to assess marine recreational water safety specifically for sewage pollution, but not the highest weight because concentrations fluctuate over short time scales (min to h) and have other sources, like soils, in tropical areas (Byappanahalli et al., 2012; Hardina and Fujioka, 1991). Nutrient concentrations received the lowest weight (weight = 1) since sewage pollution is known to increase them, but nutrients can also come from other sources within the watershed and concentrations can vary over short time scales (David et al., 2013; Lapointe and Clark, 1992; Nelson et al., 2015). The equation for calculating the overall sewage pollution score for each site was: (*C. perfringens* level x 3) + ($\delta^{15}N$ macroalgae level x 3) + (*Enterococcus* spp. level x 2) + ($NO_3^-+NO_2^-$ level x 1) + (NH_4^+ level x 1) + (TDP level x 1).

3.14 GIS Interpolation

Raster interpolations of the study area were created with ArcMap 10.2 to visualize the spatial extent of sewage input into nearshore marine waters using the multi-parameter indicator scoring system. To create the raster interpolations, a shapefile of the point data of each shoreline and reef site (surface samples only) with its associated ranking sewage score level (1-3) was created using the Field Calculator in the attribute table. We also created interpolations with benthic sample data, but due to the lower spatial sampling at the benthos, interpolations were not an effective method to visualize sewage input and distribution. A polygon mask was created for the spatial extent of the sites to create interpolations that were representative of the sampling area. For each metric (Table 2), an interpolation of the score was created using the Inverse Distance Weighted (IDW) tool (Spatial Analyst Toolset, IDW settings: Power = 2, Search Radius = Variable, Number of points = 12). The interpolated raster surfaces for each metric were then combined to a multi-metric sewage indicator score using the Weighted Sum Overlay tool (Spatial Analyst). Table 2 lists the metrics used and the weight factor applied.

Table 2. Sewage indicators (fecal indicator bacteria = CFU/100 mL, δ15N in macroalgae = ‰, and nutrients = µmol/L) used in the sewage indicator scoring and mapping. These indicators were ranked (low = 1, medium = 2, and high = 3) based on published water quality standards or previous studies, multiplied by a weight factor, and summed for a final sewage pollution score. * "Medium" nutrient concentration scores exceed HDOH standards (*see* methods for details).

Indicators	**Weight Factor**	**Low (1)**	**Medium (2)**	**High (3)**	**Units**	**Reference**
Enterococcus	*2*	0.0-35	35.1-104.0	104.1+	CFU/100 mL	HDOH 2014
C. perfringens	*3*	0.0-10.0	10.1-100.0	100.1-500.0+	CFU/100 mL	Fung et al. 2007
δ^{15}N macroalgae	3	0.0-5.9	6.0-10.9	11.0+	ppt	Abaya et al. in review
NO_3^-+NO_2^-	1	0.0-0.4	0.5-1.0	1.1-1.8+	umol/L	HDOH 2014
NH_4^+	1	0.0-0.25	0.26-0.61	0.61-1.07+	umol/L	HDOH 2014
TDP	1	0.0-0.7	0.8-1.3	1.4-1.9+	umol/L	HDOH 2014

3.15 Changes in Coral Cover

During Fall of 2015, the state of Hawai‘i experienced the most severe thermal stress event in recorded history. West Hawai‘i was among the most severely affected regions with 18 consecutive weeks of thermal stress and South Kohala among the areas hardest hit. This resulted in widespread coral bleaching with 38-92% of all colonies in South Kohala and North Kona partially or fully bleaching (Maynard et al., 2016). In the shallow reef at Puakō, 71% bleached during this event (Maynard et al., 2016). To assess the impacts of this event across Puakō, we conducted surveys in December 2015 immediately following the mass bleaching event. These surveys were conducted at the same pre-established 12 sites where we collected water quality samples (3.11 Study Sites and Sample Collection); these sites were also imaged in March 2014. To calculate percent cover of coral and macroalgae, photographs of the bottom were taken every meter along the transect line of each of our established 12 survey sites using a Canon Powershot camera mounted on a 0.8-m PVC monopod. This generated 10 images for each site, with each photo covering approximately 0.8 x 0.6 m of the bottom. Photos were analyzed using CoralNet (Beijbom et al., 2015). Thirty random points were overlaid on each photo, and the benthic component under each point was identified to the lowest possible taxonomic level, primarily individual coral species, algae at higher taxonomic resolution (*e.g.*, red, green, brown, turf, and crustose coralline), and abiotic substratum type. All photographs were processed by the same analyst to reduce potential observer variability. Once completed, the raw point data from each photograph was combined to calculate the percent cover of each benthic component for the site.

3.2 Year 2

3.21 Study Sites, Sample Collection and Processing

Using the sewage indicator map developed during Year 1, we tested whether our sewage indicators at the reef benthos were correlated with coral health in the two sewage hot spots identified. To quantify the gradient of benthic water quality across the two hot spots, we sampled 17-19 sites/hot spot = total of 36 reef sites on March 4th to 9th, 2016 (Figure 2). On July 19th and 20th, 2016, we identified 6 of the March 2016 sites from each hotpot with a range in sewage input and resampled those target sites. All water quality assessments were conducted at the benthos. Temperature and salinity were measured at all sites using methods described above, but those data are not included in this report due to erroneous measurements. Triplicate water samples were collected from each site during each sampling period as described above. Duplicate water samples for each site and sampling period were processed for dissolved inorganic nutrients as described above. Triplicate samples were processed as described above for *Enterococcus* abundance and abundance of other fecal indicator bacteria using molecular techniques. Because stable nitrogen isotopes in marine algae have proved to be a useful tool for tracking nitrogen sources, we collected triplicate ~ 5 g samples of marine algae from each of the benthic sites. Multiple taxa were collected at each site because a common macroalgal taxon was not present across all sites. Samples were rinsed with deionized water, identified to lowest possible taxonomic resolution and dried at 60°C for 8 hours. Samples were then transported to UHH where they were ground, homogenized and ~ 2mg of tissue was processed for $\delta^{15}N$ using a Thermo-Finnigan^{TM} Delta V Advantage isotope ratio mass spectrometer (IRMS) with a Conflo III interface and a Costech^{TM} 267 ECS 4010 Elemental Analyzer located at the UHH’s Analytical Laboratory.

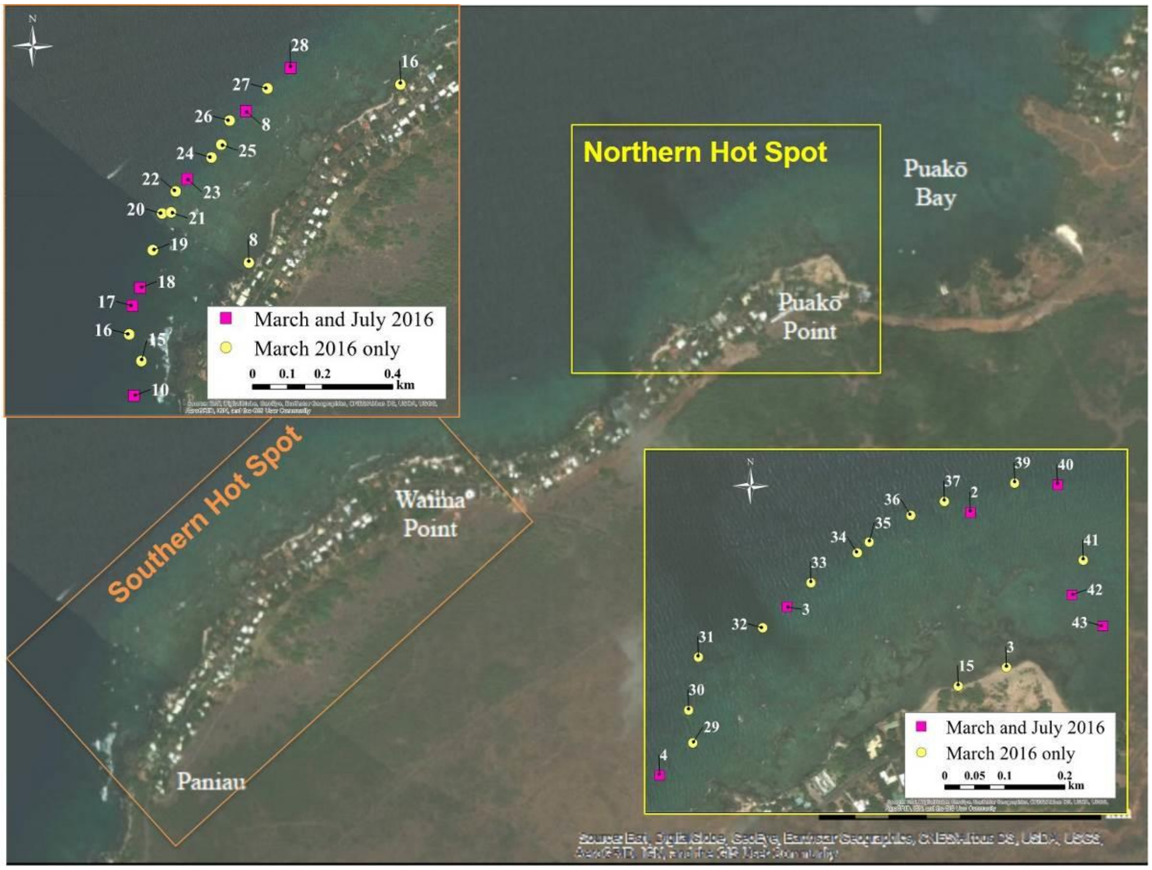

Figure 2. Map of Year 2 sampling sites within the northern and southern sewage hotspot identified in Year 1 (See Fig. 9). Seventeen to nineteen sites were surveyed in each region during March 2016 and then six sites that represented a range of water quality were selected to resample in July 2016.

3.22 Coral Health and Benthic Assessments

Benthic assessments at each site were conducted on July 5th and 6th, 2016 along one 15 m transect line run parallel to the reef drop off (3 to 4m depth). All coral colonies within a 1-m wide belt were identified to species, sized (maximum linear diameter), and enumerated. The condition of each colony was assessed, and any signs of diseases or compromised health (growth anomalies, trematodiasis, and tissue loss syndrome, algal overgrowth, discoloration, bleaching, physical damage, gastropod predation and crown-of-thorns predation) were noted (Table 3 and Figure 3). The percent of each colony affected by each condition was also estimated as a measure of severity.

The overall disease and compromised health prevalence was calculated as follows: number of colonies with at least one disease or compromised health lesion/total number of colonies on a transect. The prevalence of each condition was also calculated as the number of colonies with a given condition/total number of colonies. The prevalence of several genus-specific diseases were also calculated (e.g. *Porites* growth anomalies: number of *Porites* colonies growth anomalies/total number of *Porites.* To calculate average colony size, the maximum colony diameter was recorded and averaged by site. To calculate recruit density, the number of colonies that were ≤ 5m in diameter were enumerated and divided by the total area surveyed at each site.

Table 3. Coral diseases (DZ) and compromised health (COMP) conditions assessed during coral health surveys.

Condition	Type	Description
Growth anomalies (GA)	DZ	Protuberant growths of skeleton accompanied by aberrant calyx formation
Trematodiasis (TRE)	DZ	Multiple small (~5 mm) swollen pink to white nodules on corals in the genus *Porites* (*e.g.*, finger and lobe corals)
Tissue loss syndrome (TL)	DZ	Distinct areas of tissue loss revealing intact white skeleton progressing basally to an algal patina or multiple variably-sized areas of tissue loss
Algal overgrowth (ALOG)	COMP	Areas where macroalgae, turf algae or cyanobacteria actively overgrows, abrades and/or kills underlying coral tissue
Discoloration (DC)	COMP	Areas of discolored and/or swollen tissue not associated with other lesion categories
Bleaching (BL)	COMP	Loss of tissue pigmentation
Physical damage (PHYS)	COMP	Broken branches, abrasion or fishing line damage
Gastropod and COTS predation (PRD)	COMP	Recent predation scars from two gastropods, the horn drupe (*Drupella cornus*) and/or the violet coral shell (*Coralliophilia violacea*), or the recently denuded skeleton caused by crown-of-thorns seastars (*Acanthaster planci*)

To quantify percent cover of coral and macroalgae, photographs of the bottom were taken every meter along the transect line using a Canon Powershot camera mounted on a 0.8-m PVC monopod. This generated 15 images for each site, with each photo covering approximately 0.8 x 0.6 m of the bottom. Photos were analyzed using CoralNet (Beijbom *et al.* 2015). Thirty random points were overlaid on each photo, and the benthic component under each point was identified to the lowest possible taxonomic level, primarily individual coral species, algae at higher taxonomic resolution (*e.g.*, red, green, brown, turf, and crustose coralline), and abiotic substratum type. All photographs were processed by the same analyst to reduce potential observer variability. Once completed, the raw point data from each photograph was combined to calculate the percent cover of each benthic component for the site.

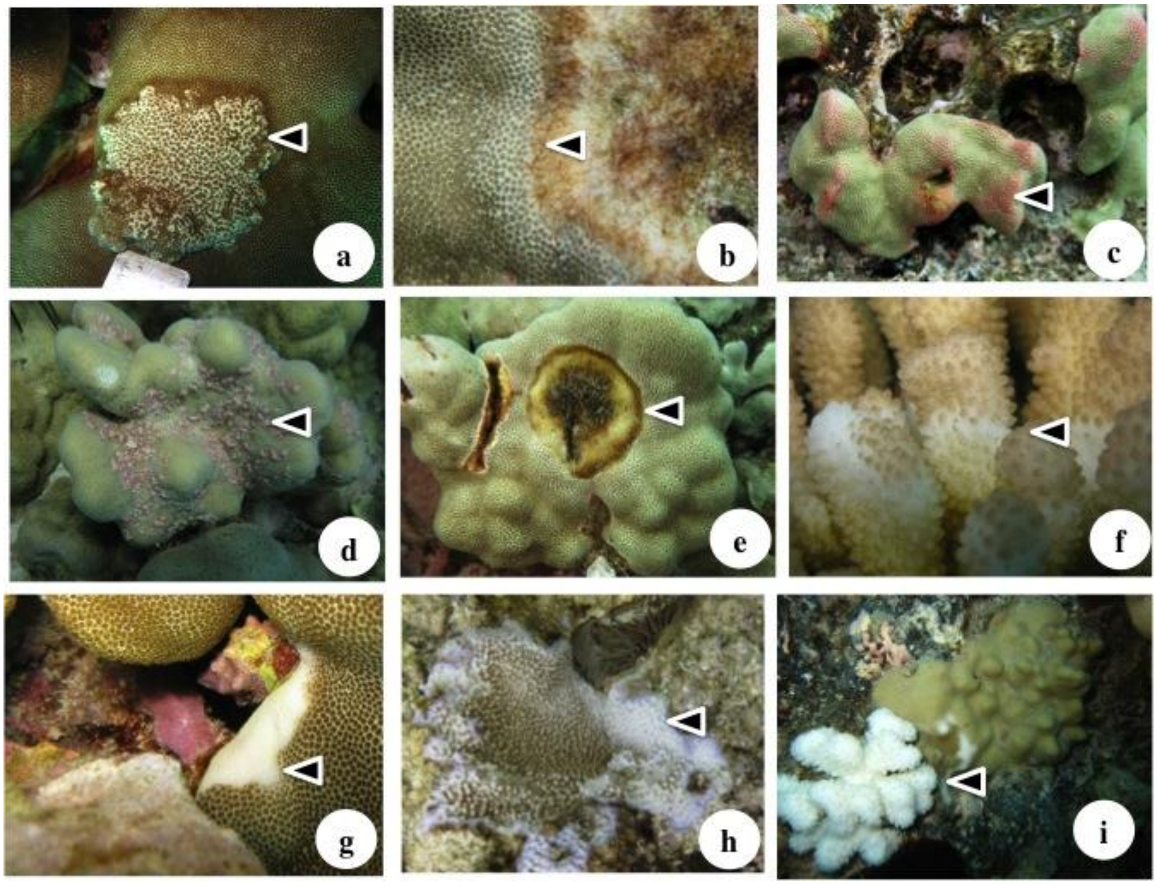

Figure 3. Coral health conditions included in coral health surveys along West Hawai‘i. (a) growth anomalies; (b) algal overgrowth; (c) discoloration; (d) trematodiasis; (e) tissue loss disease on the coral genus *Porites*; (f) tissue loss disease on the coral genus *Pocillopora*; (g) gastropod predation; (h) bleaching; (i) crown-of-thorns predation.

3.23 Data Analyses

To identify drivers of benthic indicators of health, all environmental data were averaged for the sites surveyed in March and July 2016. March-only sites were not used in the subsequent analyses. Benthic data were analyzed in R (version 3.2.4). Separate generalized linear models (GLM) using a binomial distribution were used to determine whether the proportion of healthy colonies, and the two most prevalent conditions (*Porites* growth anomalies and algal overgrowth) were correlated with a combination of demographic (colony density and size) and environmental (dissolved inorganic nutrient concentration, stable nitrogen isotopes in field-collected algae, *Enterococcus* CFUs/100ml, gene copies/ 100 ml of Bacteriodales, proportion of samples that had human-specific Bacteriodales) variables. Coral juvenile density was tested for normality and equal variance. All predictor variables were checked for multicollinearity (correlation coefficient > 0.8), scaled and centered. Due to the high correlation between colony size and *Enterococcus* (correlation coefficient > 0.88), *Enterococcus* was dropped from the analyses. 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 > 4 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). The "best-fit" models were then averaged together and the standardized parameter estimates for each predictor variable were compared to identify the strength and direction of the relationships.

3.3 Year 3

3.31 Study Sites, Sample Collection and Processing

Existing information on land use and nutrient inputs was used to help select sites that represent a spectrum of nutrient inputs along West Hawai'i. Fifteen areas were selected across "low or no impact" (5), "residential communities" (5) and "resort" (5) areas (Figure 4). While it was challenging to find sites that fit neatly into those categories, this effort allowed us to identify a geographic spread of sites that we would expect to have a range of anthropogenic-derived inputs. Thirteen sites were selected in the South Kohala/North Kona Habitat Blueprint focus area and two additional study sites in Kailua Kona, which represented high density residential communities. At each area, we established a reef site at 3-5m depth and an adjacent shoreline site.

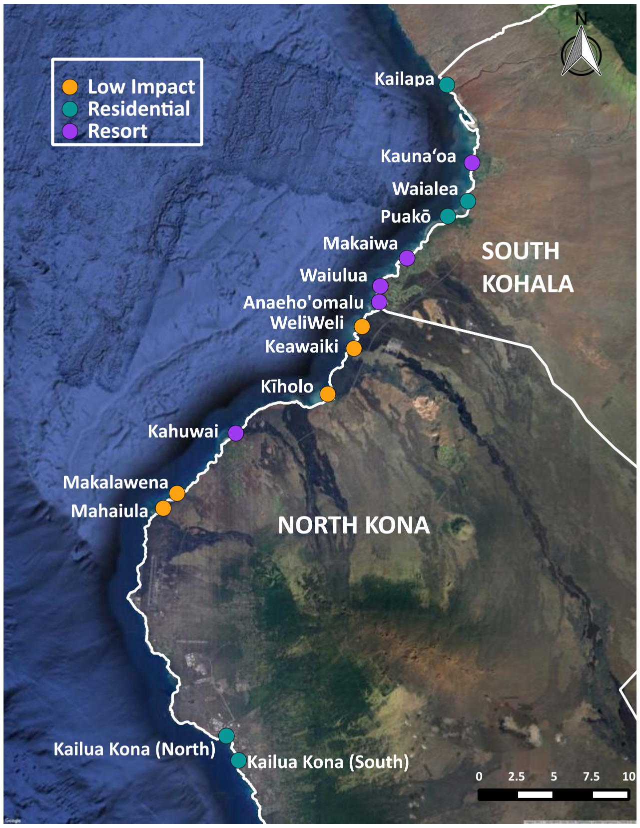

Figure 4. Year 3 (2017) map of low impact, residential and resort sites across West Hawai'i.

3.32 In Situ Water Sampling

Water quality was assessed across the 15 areas twice during April 24-28th and July 7-9th, 2017. All water quality assessments were conducted just above the benthos at the reef sites and in knee-deep water at the shoreline sites. Temperature and salinity were measured in water samples immediately after collection using a Thermo Scientific Orion Star A320 Portable Meter. Triplicate water samples were collected at low tide from each site during each sampling period and transported on ice to our portable laboratory. Single water samples at each site and sampling period were processed for dissolved inorganic nutrients using methods described above. Triplicate samples (100ml for shoreline waters and 200ml for reef waters) were filtered through 47mm 0.45 um cellulose nitrate filters onto mEI agar plates using the EPA Method 1600. Sterile deionized water was also filtered and run as negative controls. Plates were incubated at 42°C for 24 hours and data were calculated as number of colony forming units/100ml. Triplicate water samples per site and sampling period were also processed to quantify other fecal indicator bacteria using molecular techniques as described above. Triplicate resident macroalgal samples were also collected from all sites during each sampling period and processed for stable nitrogen isotopes as described above. All water quality data are presented as means of the sampling periods.

3.33 InVEST Model Methods

The InVEST nutrient delivery model estimates nitrogen retention using the concept of nutrient delivery ratio (NDR). The technique provides quantitative values to a risk-based approach and considers both an estimate of nutrient loading rates and a calculated probability that a nutrient load will reach a stream. Additionally, two transport processes are modeled, nutrient transported by surface flow, the other for subsurface flow (Sharp *et al.*, 2015). For this work in West Hawai‘i, we only considered subsurface flow. The model uses a digital elevation model (DEM) from the Hawai‘i Statewide GIS program to calculate flow paths.. The DEM was additionally processed to remove pits using TauDEM (TauDEM 5.0, http://hydrology.usu.edu/taudem/taudem5/downloads5.0.html).

A key component of the NDR model is the ability to use any land use map and to estimate nitrogen loading at the coast. We used the Coastal and Climate Adaptation Program’s (C-CAP) 2010 high resolution (2.4m) imagery as a base and modified the layer to include other land uses that might be important for nitrogen inputs. Specifically, we delineated kiawe forest, golf courses, hotel landscaping areas and added sites with OSDS. Each resort complex was parameterized separately for golf course and hotel landscaping, although lacking information we used standard golf course export estimates for all hotels. Different golf course practices including irrigation type, fertilizer application method, type of fertilizer, and fertilizer application frequency would affect the capacity of the landscaping to retain nitrogen.

This study developed total nitrogen loading rates and retention parameters (efficiency) for each land use type using literature and local sources (Beaulac & Reckhow, 1982; Cobo, Dercon, & Cadisch, 2010; Johnes, 1996; Lin, 2004; Line, White, Osmond, Jennings, & Mojonnier, 2002; Ling & El-Kadi, 1998; Markewitz, Davidson, Moutinho, & Nepstad, 2004; Young, Marston, & Davis, 1996). The biophysical parameters presented in this study are included in Table 4.

In addition to loading rates by land use, additional parameters for the model include the critical length, defined as the distance after which it is assumed that a patch of land use and land cover (LULC) retains nutrient at its maximum capacity, and the proportion of subsurface N, which is a measure of dissolved nutrients over the total amount of nutrients. Following the work of Kwong *et al.* (2002), we assumed that most (90%) of the nitrogen was exported in the subsurface rather than through surface runoff. We also explored the work of Street *et al*. (2008) to assist in calibration.

Lastly, we wanted to be able to address the question: Are the nutrients concentrations in coastal SGD correlated with inputs close to the coast or from the entire watershed? Or, put another way, does the speed of groundwater affect what the concentrations

Table 4. Biophysical parameters total nitrogen loading rates and retention parameters (efficiency) for each land use type using literature and local sources.

Land Use Description	Land Use Code	Load N (kg/ha)	Efficiency N	Critical Length	Vegetation Present?	Proportion Subsurface N	Comments
Background	0	0	0.75	50	0	1	
Unclassified	1	0	0.75	50	0	1	
Developed High Intensity	2	10	0.1	50	0	1	
Developed Medium Intensity	3	7.5	0.15	50	0	1	
Developed Low Intensity	4	5	0.25	50	0	1	
Developed Open Space	5	50	0.1	1000	1	1	Considering as landscaping.
Cultivated Crops	6	100	0.75	50	1	1	Mostly coffee and mixed vegetables near Captain Cook
Pasture/Hay	7	1	0.9	50	1	1	Considers nutrient load from goats and cattle (although very rocky terrain on the Big Island) (formerly 20 kg/ha)
Grassland/Herbaceous	8	3.1	0.99	50	1	1	Often very sparse in west Hawaiʻi
Deciduous Forest	9	4.7	0.8	50	1	1	n/a
Evergreen Forest	10	11.3	0.99	50	1	1	Citing Ostertag for the native forest nutrient load; Very low erosion because of the type of forest; Predominant forest is south at Napo'opo'o
Kiawe Forest	11	55	0.99	50	1	1	Cited at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC98982/
Scrub/Shrub	12	5.5	0.99	50	1	1	Mostly low grass and shrubs at Puu Waawaa
Palustrine Forested Wetland	13	1.62	0.99	50	1	1	This is how Waipio Valley is classified - which is essentially lo'i and floodplain.
Palustrine Scrub/Shrub Wetland	14	1.62	0.99	50	1	1	
Palustrine Emergent Wetland	15	1.62	0.99	50	1	1	
Estuarine Forested Wetland	16	1.62	0.99	50	1	1	

Land Use Description (cont)	Land Use Code	Load N (kg/ha)	Efficiency N	Critical Length	Vegetation Present?	Proportion Subsurface N	Comments
Estuarine Scrub/Shrub Wetland	17	1.62	0.99	50	1	1	
Estuarine Emergent Wetland	18	1.62	0.99	50	1	1	
Unconsolidated Shore	19	0	0.75	50	0	1	
Bare Land	20	0	0	50	0	1	Here, meaning new volcanic rock
Open Water	21	2.6	0.1	50	0	1	
Palustrine Aquatic Bed	22	1.62	0.75	50	1	1	
Golf courses	23	200	0.1	50	1	1	
Impervious Urban	30	17.1	0.1	50	0	1	
Impervious Ag	31	7.5	0.75	50	0	1	
Cultivated Crops, Coffee	40	140	0.86	50	1	1	Did not separate out Cultivated Crops and Coffee for the Kona coffee farms
Cultivated Crops, Fallow	41	3.1	0.64	50	1	1	
Grazing land	50	47.1	0.6	50	1	1	Same as pasture - left over from Maui
Cultivated Crops, Pineapple	51	424	0.75	50	1	1	
Cultivated Crops, Sugarcane	52	350	0.6	50	1	1	
Ag Subdivision	60	17.1	0.75	50	1	1	n/a for Hawaiʻi
Cesspools	62	2000	0.1	1000	1	1	Buffered by 10m; Approximately 15kg per year for each cesspool. This is an underestimate.
Big Island Country Club	70	100	0.1	50	1	1	Previous versions of the model used golf course numbers by golf course. The highest is approximately 500 kg/ha as documented for West Hawaiʻi hotels.
Hualalai Golf Club	71	100	0.1	50	1	1	
Kūkiʻo Golf Course	72	100	0.1	50	1	1	
Makalei Golf Club	73	100	0.1	50	1	1	

Land Use Description (cont)	Land Use Code	Load N (kg/ha)	Efficiency N	Critical Length	Vegetation Present?	Proportion Subsurface N	Comments
Mauna Lani Golf Course	74	100	0.1	50	1	1	
Waikoloa Beach Course	75	100	0.1	50	1	1	
Waikoloa Kings' Course	76	100	0.1	50	1	1	
Mauna Kea Golf Course	77	100	0.1	50	1	1	
Hapuna Golf Course	78	100	0.1	50	1	1	
Waikoloa Village Golf Course	79	100	0.1	50	1	1	
Lawns and Gardens	80	50	0.1	50	1	1	See lawns citation in Falinski, 2016
Hotel Trees and Shrubs	81	50	0.1	50	1	1	See lawns citation in Falinski, 2016
Agroforestry	112	22.6	0.9	50	1	1	For ulu, etc.
Loi	114	100	0.999	50	1	1	Deenik et al.
Bare Soil	120	3.1	0.9	50	1	1	Areas of bare soil, not volcanic rock associated with Kohala. Giving it the same as grassland because am not sure how much nuts are in soil

are at the coast (groundwater studies from the area have indicated that groundwater flow can be approximately 1 km/yr)? To answer this, we considered three different types of watersheds - watersheds that fed from 2k from the coastline, 10km from the coastline, and the full watershed. We used ArcGIS 10.2 Basins function and eliminated and combined slivers. Using the 3 different sizes of watersheds, we ran the NDR model for the entire West Hawaiʻi coast, and compared the results to coral health data and known nutrient concentrations.

3.34 Wave Power

Wave power (kW/m) was used to determine the degree to which wave action is driving coral health and benthic communities. This metric incorporates both wave period and wave height and therefore represents a more realistic estimate of wave stress on reefs (Gove *et al.*, 2015). These data were obtained using University of Hawaiʻi SWAN (Simulating WAves Nearshore) wave model, available at 1 hr, 0.5 km resolution (Li *et al.*, 2016). Spatial mismatch between model resolution and the high degree of wave refraction, amplification, and dissipation resulted in spurious wave power values in close proximity to shore. Consequently, all model pixels adjacent to shore (≤500 m) were removed prior to analysis. Daily maximum wave power was calculated from the hourly data set. The average of wave power in the 95% percentile for each year was calculated then averaged between 2011-2016 to provide the wave power metric used in the predictive models.

3.35 Coral Health and Benthic Assessments

Benthic assessments at each site were conducted between July 24-27th, 2017 along three 10 m transect lines run parallel to shore (3 to 5m depth). Along each transect, all coral colonies within a 1-m wide belt were identified to species and enumerated. The condition of each colony was assessed using the methods described above. To assess average colony size and coral juvenile density, maximum colony diameter was also recorded within the first and last 2m of each transect (total of $4m^2$ surveyed for colony size/transect). Juvenile density was assessed by quantifying the number of corals < 5cm in diameter per $meter^2$. Condition prevalence and severity was calculated as described above.

To quantify percent cover of coral and macroalgae, photographs of the bottom were taken every meter along the transect line using a Canon Powershot camera mounted on a 0.8-m PVC monopod with image processing and data summarized as described above. To determine whether a given reef was dominated by reef builders vs. non reef builders, the reef builder ratio was calculated as (Coral cover + CCA cover)/(Turf algae cover + Macroalgae cover) (Smith *et al.*, 2016).

The topographic complexity of the bottom at each site was estimated using an index of rugosity calculated along the 10 m transect by dividing the length of brass chain required to contour the bottom by the 10-m transect length (McCormick, 1994). For this index, a value of one represents a flat surface with no topographic relief, and increasing values represent more topographically complex substratum.

3.36 Data Analyses

Benthic data were analyzed in R (version 3.2.4). Generalized linear models (GLMs-binomial errors with logit link) were used to determine whether *Porites* growth anomalies (PorGA) prevalence varied between land use type for each condition. Generalized linear hypothesis tests were used to identify significant differences between land use types within each condition and a Bonferroni multiple tests correction was used. Coral juvenile density and rugosity were log transformed and reef builder ratio was power transformed to meet assumptions of normality and equal variance and then the effect of land use type was tested using an ANOVA with Tukey *post hoc* tests for each response variable with Bonferroni corrections.

Initial analyses testing for correlations between coral reef metrics and benthic water quality revealed little to no relationship, which may be due in part to the limited amount of temporal sampling we were able to do of highly-variable benthic waters. To better capture the maximum watershed inputs into adjacent coral reefs, average shoreline water quality data from each site were used in subsequent analyses. Separate GLMs using a binomial distribution were used to determine whether the proportion of healthy colonies, and the two most prevalent conditions (*Porites* growth anomalies and algal overgrowth) were correlated with a combination of demographic (average maximum colony diameter) and environmental (dissolved inorganic nutrient concentration, stable nitrogen isotopes in field-collected algae, *Enterococcus* CFUs/100ml, gene copies /100ml Bacteriodales, proportion of samples that had human-specific Bacteriodales, modeled 2km nitrogen export, and wave power) variables. Separate linear models were run on the transformed coral juvenile density and rugosity with the predictor variables listed above. All predictor variables were checked for multicollinearity (correlation coefficient > 0.8), scaled and centered. Due to the high correlation between *Enterococcus* CFUs/100ml and the proportion of samples with human-specific Bacteriodales (correlation coefficient > 0.8), *Enterococcus* was dropped from the analyses. Due to the high correlation between the *in situ* nutrient parameters, a Principle Components Analysis (PCA) was used to condense nutrient concentration onto one principle component. This PC was characterized by increasing concentration of all nutrient parameters and accounted for 76% of the variance in nutrients (data not shown). 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 > 4 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). The "best-fit" models were then averaged together and the standardized parameter estimates for each predictor variable were compared to identify the strength and direction of the relationships.

4.0 RESULTS & DISCUSSION

4.1 Year 1

4.11 Nutrients

Dissolved inorganic nutrient levels were 2.5-31 times higher in shoreline waters compared to surface and benthic water over the reef with the most striking spatial patterns observed in silica and nitrite + nitrate concentrations (Figure 5). Most notably, nitrate + nitrite were as high as 107 umol/L at Pauʻoa Bay (site 13) and 125 umol/L at Paniau (site 10) compared to the other sites (Figure 5). Nitrite + nitrate concentration was also elevated in surface waters at sites 6 and 8-14 compared to the other study sites. Comparably high nitrite + nitrate concentrations have also been observed elsewhere along this coastline with values as high 196 umol/L near site 8 (Abaya et al., 2018b). While the relative spatial variation in nutrient levels was subtler at the benthos, similar trends persisted with the highest levels at sites 1 and 10-13 (Fig. 5). These spatial patterns are consistent with previous studies in Puakō, which found the highest nutrient levels around sites 1, 5, 10 and 13 (Abaya et al., 2018b; Couch et al., 2014b). The temporal patterns in nutrient concentration suggest that dissolved inorganic nutrient concentration increased from spring into summer likely due to an increase in SGD as indicated by lower salinity values and higher silica values in the summer (Figure 6).

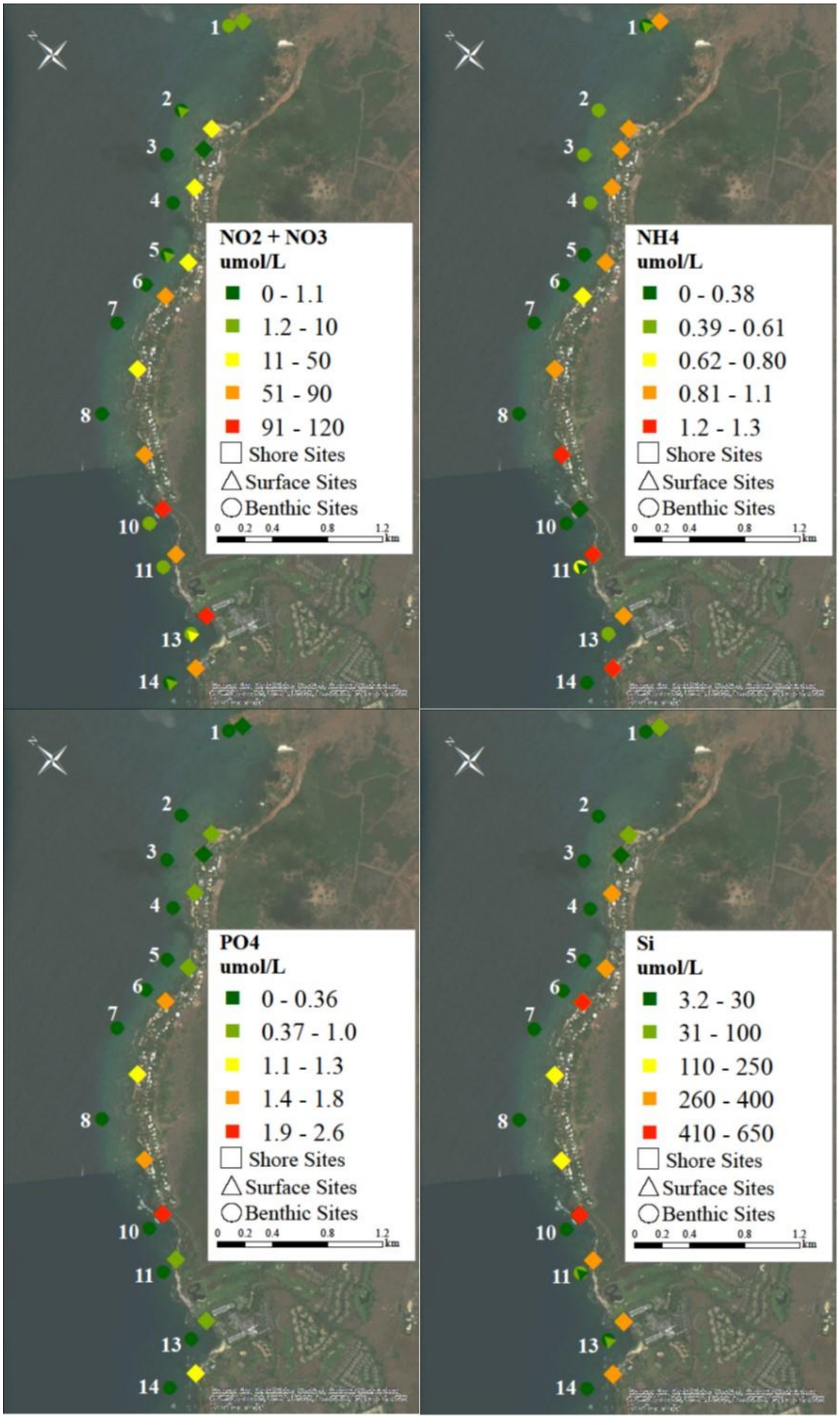

Figure 5. Map of dissolved inorganic nutrient concentration in shoreline and reef sites (surface and benthic water). Data represent mean of sampling periods during Year 1 (2015).

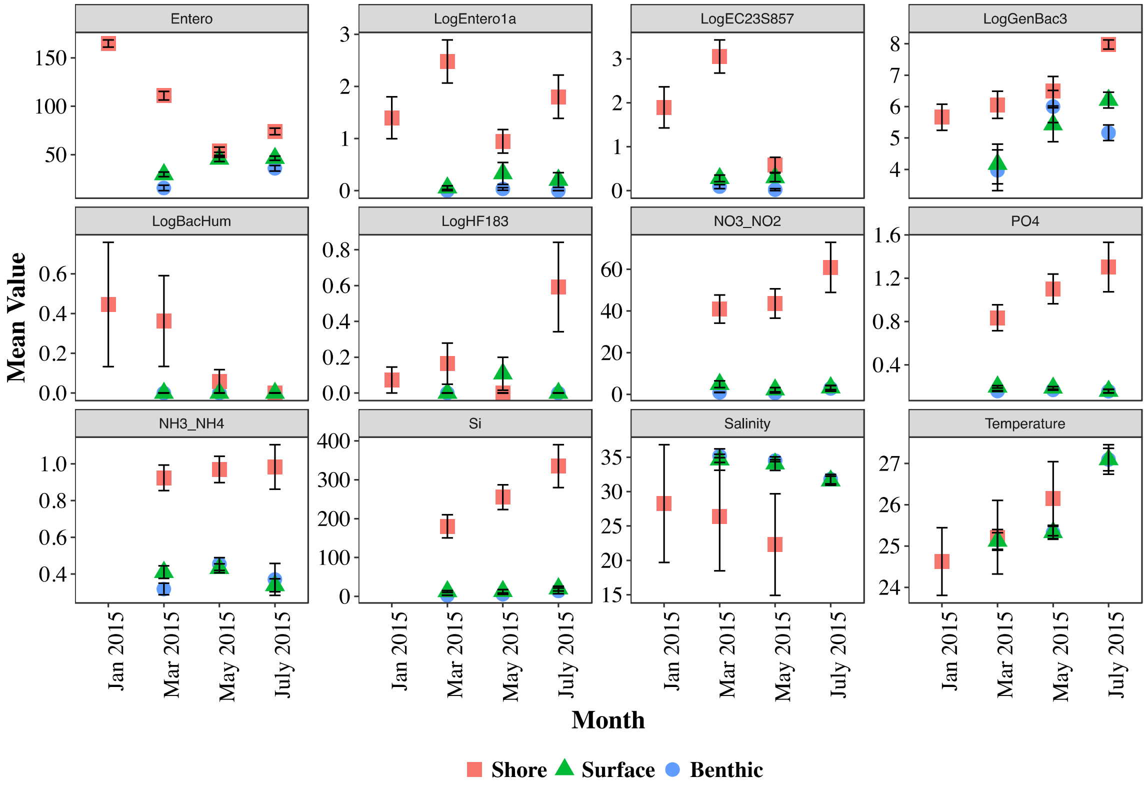

Figure 6. Mean (±SE) levels of different water quality indicators including dissolved inorganic nutrients and fecal indicator bacteria in shoreline and reef sites (surface and benthic water) during 4 sampling periods (n=2 samples/metric/sampling location/month). Note, no surface or benthic samples were collected in January 2015 due to weather.

4.12 Fecal Indicator Bacteria

Mean enterococci abundance was three times higher in knee-deep shoreline (35.20 – 407.14 CFUs/100ml) waters compared to surface (12.70-107.17 CFUs/100ml) and benthic (15.97-95.04 CFUs/100ml) water at the study sites, which is consistent with other studies in this region (Abaya et al., 2018a; Couch et al., 2014b). *Enterococcus* at shoreline sites 3, 5 and 10 remain hot spots compared to the previous year (Couch et al. 2014). Interestingly, the sites within and just south of Pau‘oa Bay (sites 13 and 14) continue to have low enterococci abundance despite high recreational use (Couch et al., 2014b). The patterns in the general Bacteriodales group were highly variable across the coastline with mean abundances ranging from ~ 10^1 -10^4 gene copies per 100 mL across the shoreline (Figure 7), and average values increasing into the summer months (Figure 6). An apparent hot spot of this group was observed in the region between sites 10 and 14 (Figure 7). While patterns in the general Bacteroidales population were unclear, the human-specific Bacteroidales assays indicated that similar to the *Enterococcus,* there is detectable human sewage entering the shoreline at sites 5 and 10. Temporal patterns in fecal indicator bacteria were not as clear as patterns in nutrients and were not consistent between bacterial indicators

(Figure 6). Generally speaking, fecal indicator values varied most in the shoreline sites, with *Enterococcus* and human-specific Bacteriodales declining considerably from January to July 2015.

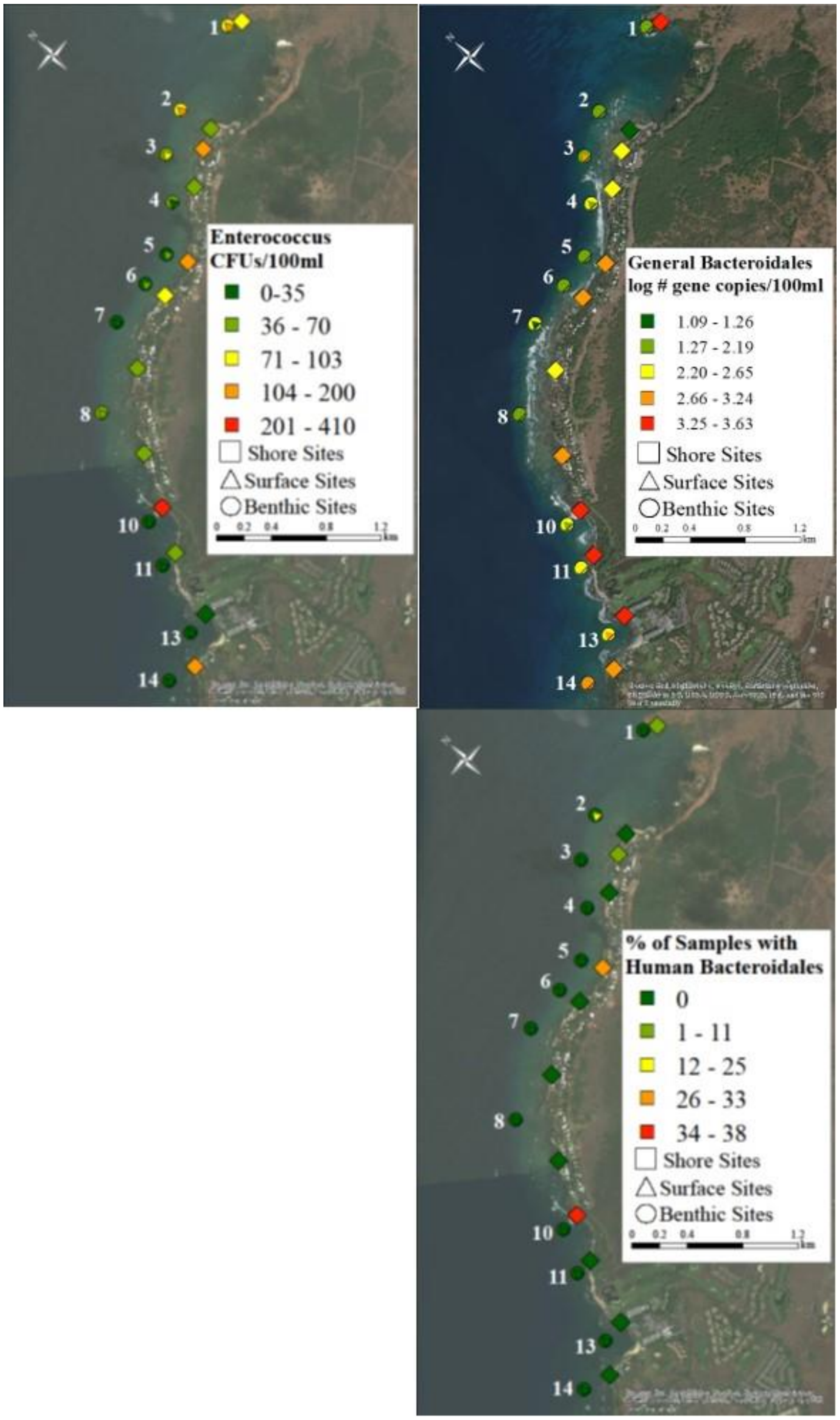

Figure 7. Map of fecal indicator bacteria abundance in shoreline and reef sites (surface and benthic water). Data represent mean of sampling periods during Year 1 (2015).

4.13 Sewage Indicator Score

As our study and many others have demonstrated, sewage indicators are highly spatially and temporally variable and often provide conflicting data of the presence and severity of sewage pollution (Abaya et al., 2018a; Couch et al., 2014b; Shibata et al., 2004; Yoshioka et al., 2016). To address this challenge, monitoring programs and costal managers often use indices to condense data from multiple indicators (Pesce and Wunderlin, 2000; Simsek and Gunduz, 2007). Using several years of water quality data collected from multiple agencies and a scoring system developed from HDOH standards and the literature as well as the weighted sum overlay tool in ArcGIS, we generated a map of sewage pollution using a sewage indicator score. As discussed in the methods, the indicators included in this analysis were chosen based on their link with sewage. When each indicator was mapped individually, water quality varied considerably along the coastline. In the case of *Enterococcus* and *Clostridium perfringens*, our combined datasets highlight the severity of the water quality issue in portions of the coastline with values exceeding EPA and HDOH standards by several orders of magnitude (Figure 8). Taken together, the sewage indicator map highlights sewage is entering the shoreline and dispersing to the reef and suggests that there are two sewage pollution hot spots along this coastline. These areas are located in the southern end of Puakō near Paniau and the northern area near Puakō Point (**Figure 9**).

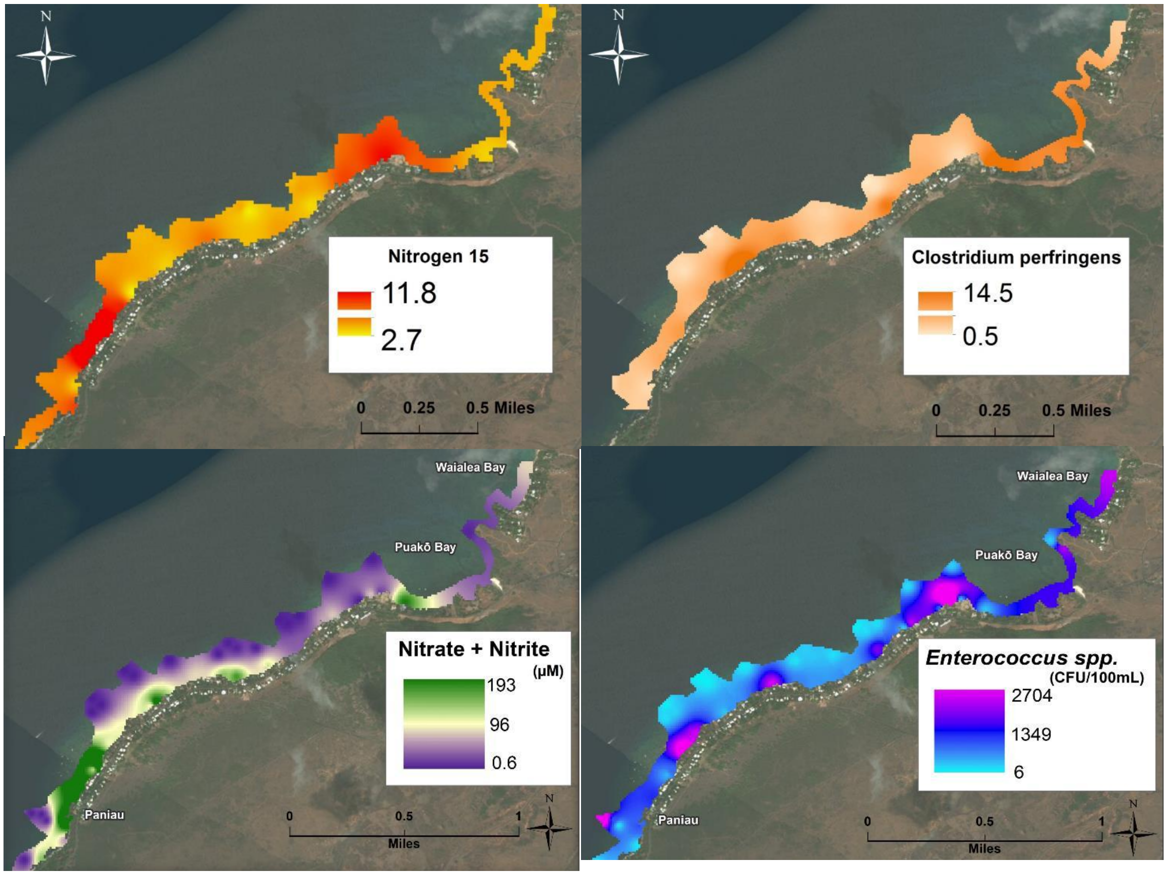

Figure 8. Interpolated maps of four sewage indicators used for creating the sewage indicator map.

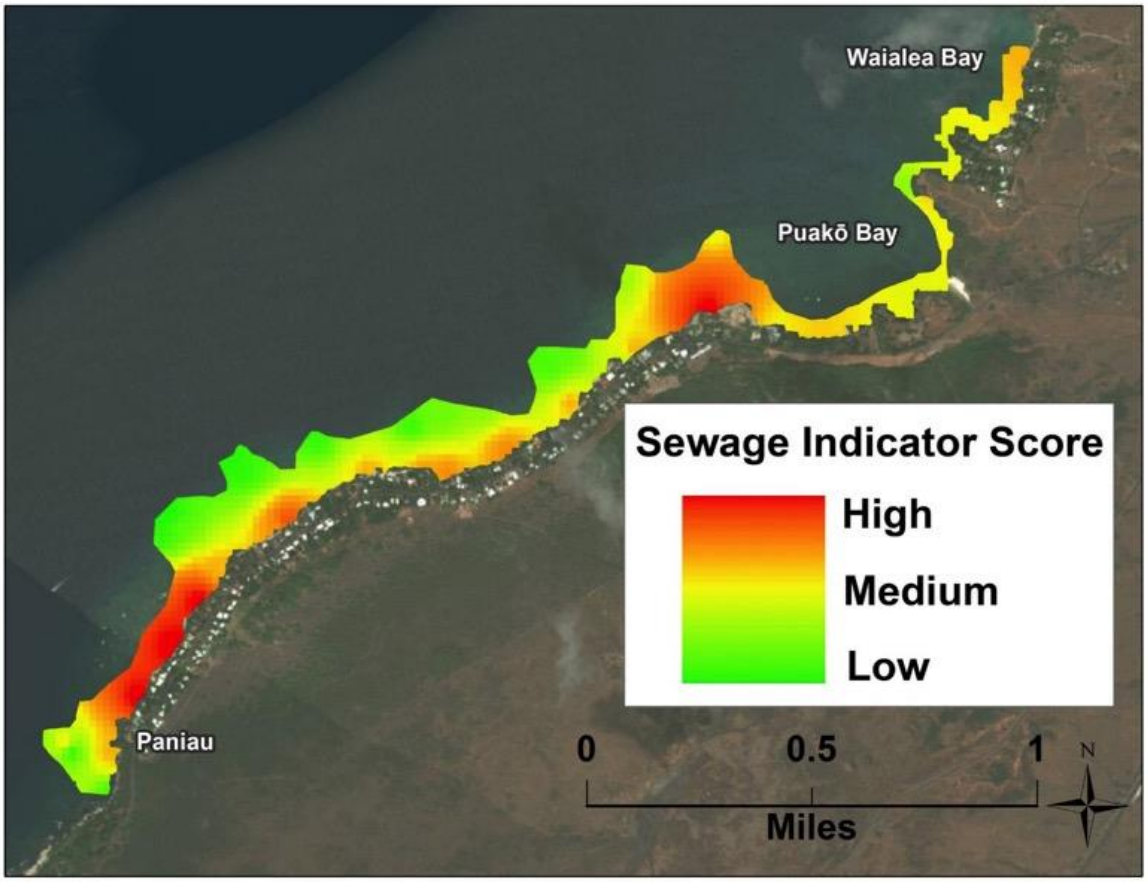

Figure 9. Sewage indicator map used to identify hot spots of sewage pollution by weighting and combining four sewage indicators. See methods section for details.

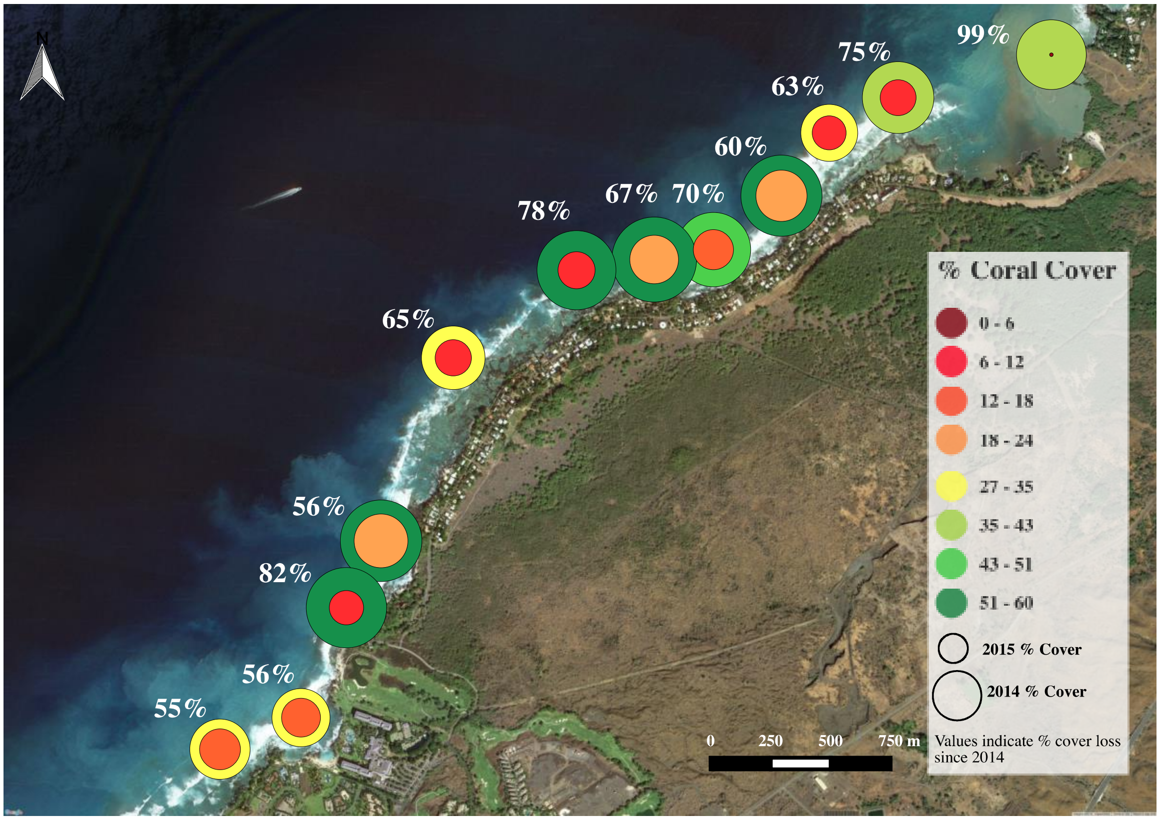

Figure 10. Percent change in coral cover at 12 permanent sites between March 2014 and December 2015. Colored circles represent absolute coral cover for each year and numbers represent percent change between years following the mass coral bleaching event. The size of the circles is proportional to % coral cover.

4.14 Bleaching-related Mortality

Overall, coral cover at the 12 sites surveys at Puakō pre- and post-2015 mass coral bleaching event declined from 42.7 ± 2.3% in 2014 to 12.9 ± 1.3% in 2015 (Figure 10). This is consistent with mortality reported across West Hawaiʻi with the Hawaiʻi Division of Aquatic Resources reporting 50% mortality across 29 West Hawaiʻi sites (Kramer et al. 2016). Coral cover decreased across all sites but varied considerably, with the lowest mortality observed near the Fairmont Orchid (56% decline) and the highest mortality at the northern end of Puakō Bay (99% decline).

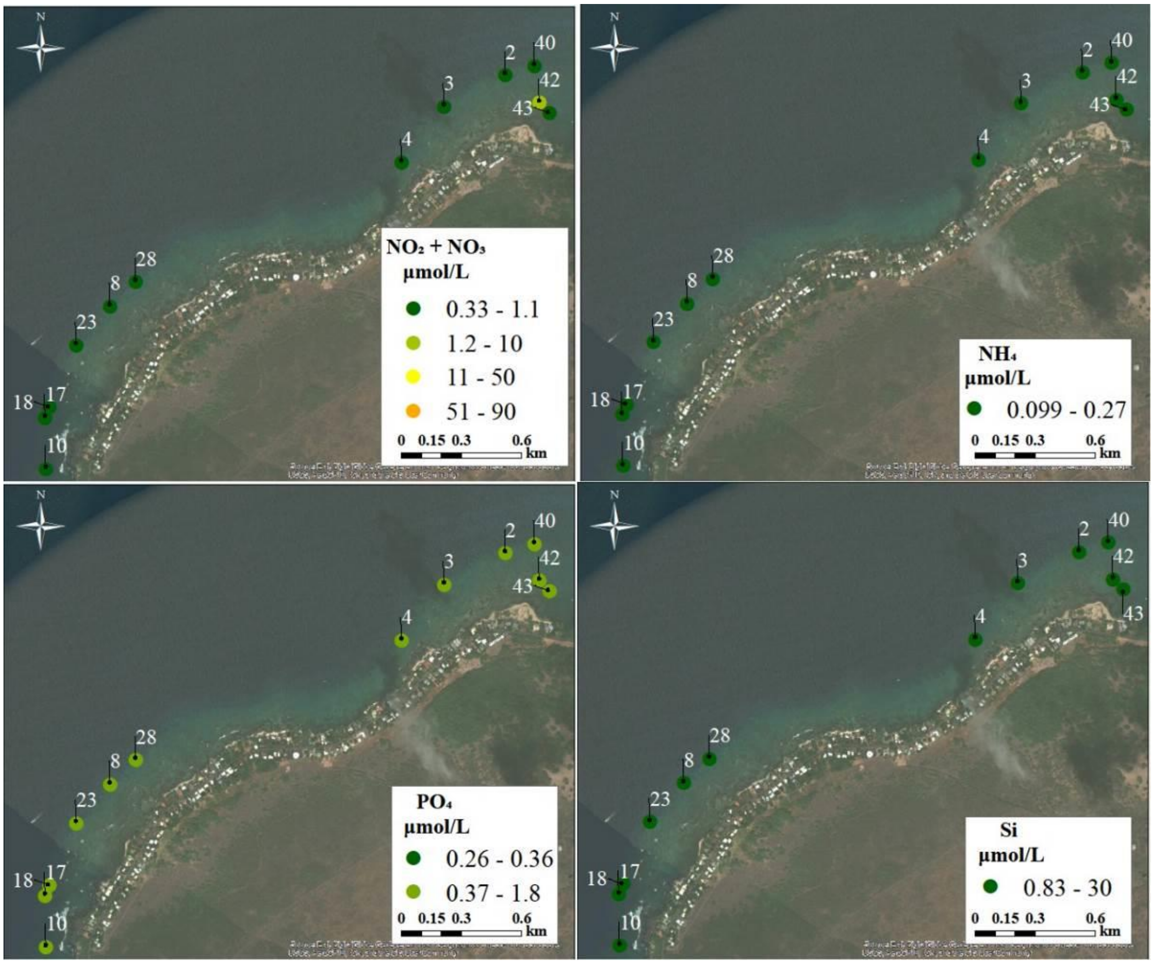

Figure 11. Map of dissolved inorganic nutrient concentration in benthic sites sampled in March and July 2016. Data represent mean of sampling periods.

4.2 Year 2

4.21 Nutrients

Dissolved inorganic nutrient concentration was low at the benthic sampling sites in Year 2 (**Error! Reference source not found.**). Nutrient concentrations varied minimally for the four metrics included in this study (nitrite + nitrate: 0.33 – 1.32 umol/L, ammonium 0.10 – 0.26 umol/L, phosphate 0.73 – 1.73 umol/L, silica 0.82 – 6.90 umol/L). These results are consistent with previous studies that found a high degree of mixing as coastal waters mix with offshore water, rapidly diluting nutrients delivered from land (Abaya et al., 2018a; Couch et al., 2014b). Given the focus on benthic processes in Year 2, we only sampled 1 shoreline site in each region to confirm the persistence of impaired water quality. Consistent with previous years, shoreline samples in these 2 hotspots continued to have elevated dissolved inorganic concentration (data not shown).

4.22 Stable Nitrogen Isotopes and Fecal Indicator Bacteria

δ^{15} N in macroalgae show distinct spatial patterns with the highest values in the northern- and southern-most extents of the 2 hot spots (**Figure 12**). The range of values in macroalgae samples (2-5‰) recorded at a majority of benthic sites is generally consistent with most bench (i.e., shallow water) sites in a recent Puakō study (Abaya et al., 2018a), with the exception of average elevated values >5‰ observed at sites 18 and 43. These patterns highlight that δ^{15} N in macroalgae is sensitive enough to detect distinct spatial patterns in nutrient sources even at the benthos along the reef crest. While the abundance of General Bacteriodales did not show any distinct spatial patterns, *Enterococcus* CFUs/100ml and the percent of samples with human-specific Bacteroidales were highest near Puakō Point. Taken together, these data provide yet further support for the persistent presence of sewage and suggest that areas near Puakō Point and just north of Paniau would benefit most from remediation efforts.

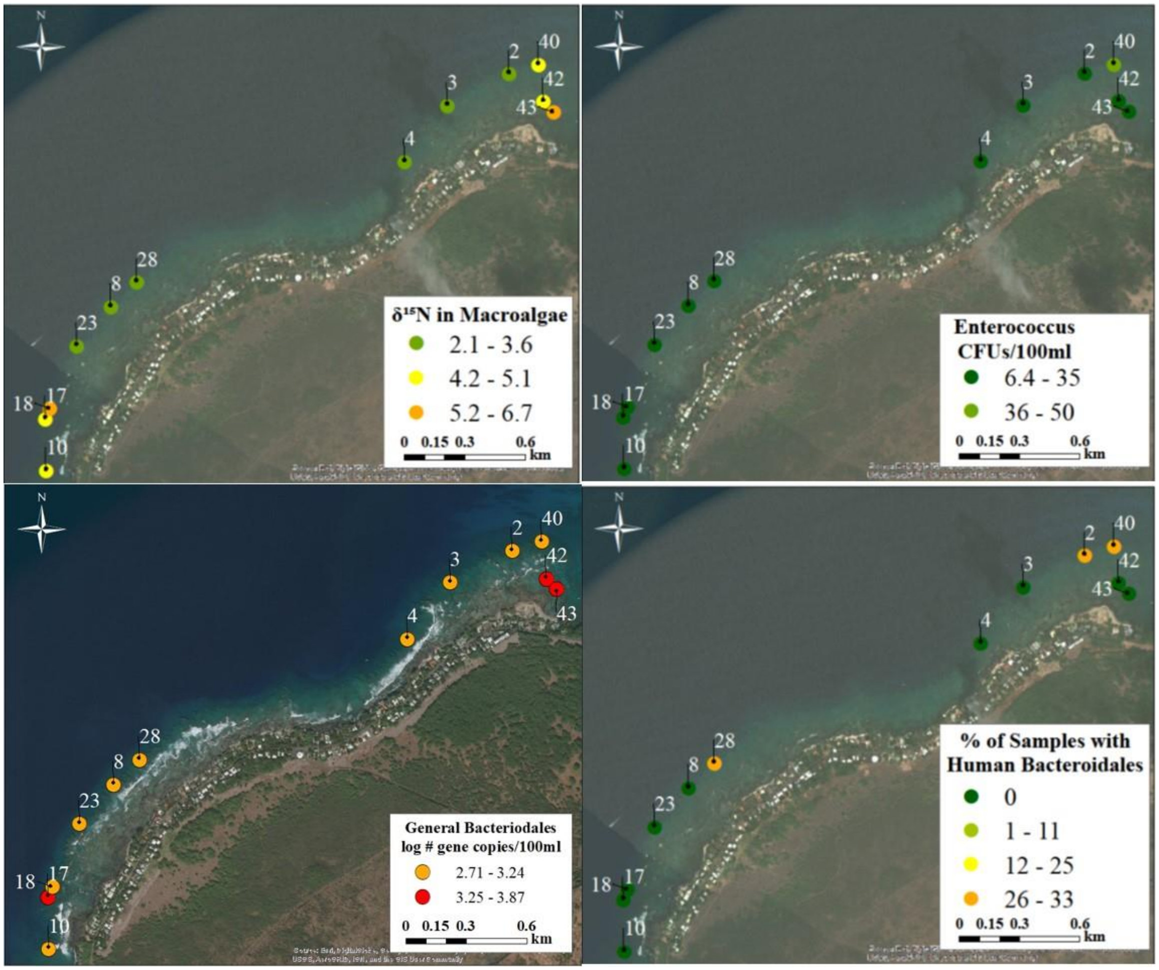

Figure 12. Map of δ^{15}N values in macroalgae and fecal indicator bacteria abundance at benthic sites sampled in March and July 2016. Data represent mean of sampling periods.

4.23 Coral Health

Overall, 34.76 ± 2.7% of Puakō's coral assemblage showed signs of compromised health and 14.29 ± 2.21% was affected by at least one type of disease (Figure 13 and Figure 14). The most prevalent sign of compromised health was algal overgrowth with 27% of all colonies affected, followed by non-bleaching discoloration and predation. Similar to other regions of West Hawaiʻi, filamentous red turf algae was the primary taxon involved in direct coral-algal competition, often resulting in discoloration or mortality of the coral tissue. Interestingly, the prevalence of colonies experiencing direct algal overgrowth is among the highest recorded values for West Hawaiʻi, and although we did not survey the same sites in 2016 as previous years, regionally, the mean prevalence changed from 20% in 2014 to 27% in 2016 (Couch et al., 2014a, 2014b; Minton et al. 2017). Similar to other West Hawaiʻi reefs, we observed three types of diseases, all of which are classified as slow progressing chronic or sub-acute diseases (Couch et al., 2014a). *Porites* growth anomalies were the most prevalent disease with 14% of colonies affected, followed by tissue loss syndrome affecting 0.5% of colonies (Figure 13). These disease levels are lower than previously reported for this region in 2014 (PORGA: 20%, TL: 3.5%) (Couch et al., 2014a).

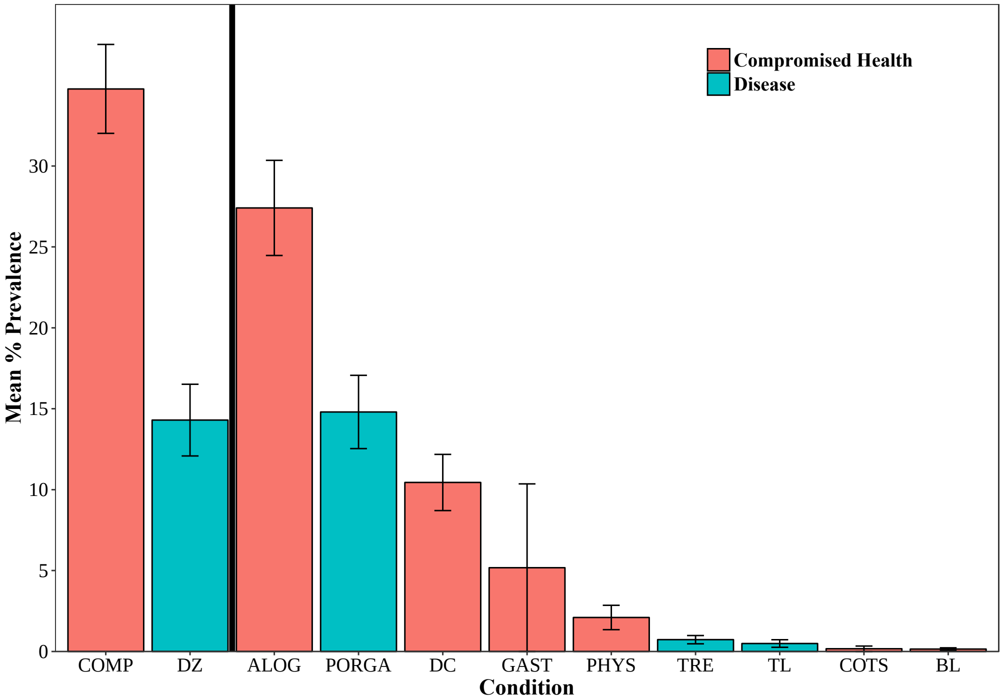

Figure 13. Mean (± SE) prevalence (# of colonies with condition/total # of colonies) of coral health and disease conditions across all sites (n=22 sites). Pink bars indicate colonies with compromised health. Teal bars indicate colonies with disease. Vertical line separates COMP (colonies with at least one compromised health lesion) and DZ (colonies with at least one disease lesion) from the individual conditions: ALOG= algal overgrowth, PORGA = Porites growth anomaly , PRD=invertebrate predation, DC= discoloration, PHYS= physical damage, PALE= paling, TRE= trematodiasis, TL= tissue loss syndrome, and BL= bleaching. Note: single colonies often had multiple conditions, thus overall compromised health and disease prevalence do not equal the sum of individual conditions. See Table 3 for condition descriptions.

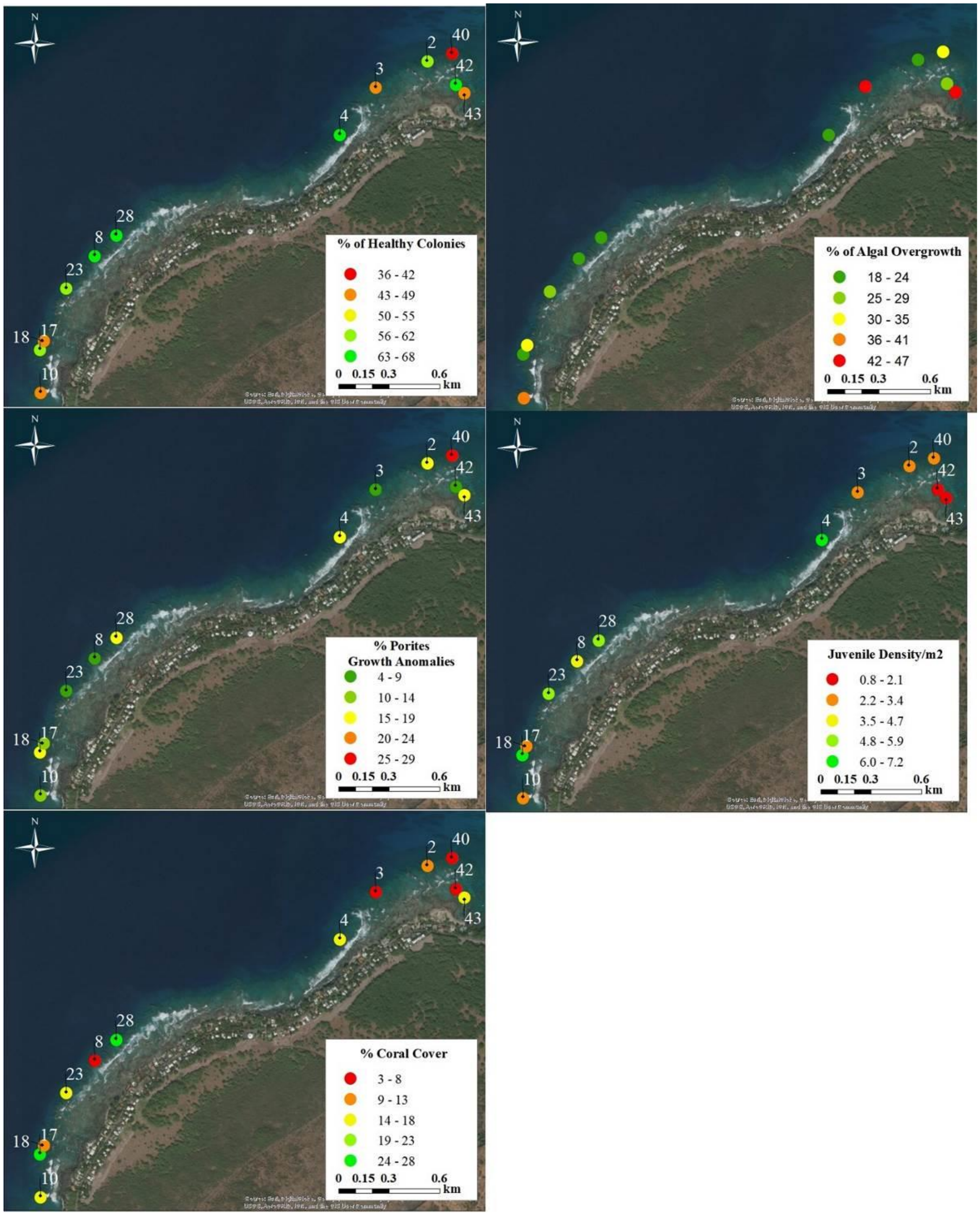

Figure 14. Map of % of healthy colonies, % of colonies with algal overgrowth and % of *Porites* with growth anomalies, juvenile density and % coral cover across Puakō sites surveyed in 2016.

4.24 Drivers of Coral Health

After selecting and averaging the “best-fit” models (delta AIC <4), we compared the relative importance of the top drivers of each benthic factor by comparing the standardized predictor estimates. Overall, this approach suggests that each response variable is driven by a combination of factors with some factors being more important than others (Figure 15). Results suggest that the proportion of healthy colonies increased when exposed to lower δ^{15} N values, which was the only factor significantly influencing overall proportion of healthy colonies (Figure 15). The proportion of colonies experiencing direct coral-algal competition increased with NH_4 concentration and δ^{15} N values, which had 2-3 times more influence on ALOG than other predicator variables. The proportion of *Porites* colonies with growth anomalies increased on reef with larger colonies, higher groundwater input (Si) and a higher presence of human-specific bacteria, which were 1-2 times more influential than other predictors (Figure 15). It is important to note that *Enterococcus* levels (not included in model selection) were highly correlated with colony size and are therefore also positively correlated with *Porites* growth anomalies. Juvenile density increased when exposed to lower NO_2 + NO_3 concentration, while coral cover was not significantly affected by any predictor variable included in this model. These results show some similarities as well as differences with regards to underlying drivers of coral health

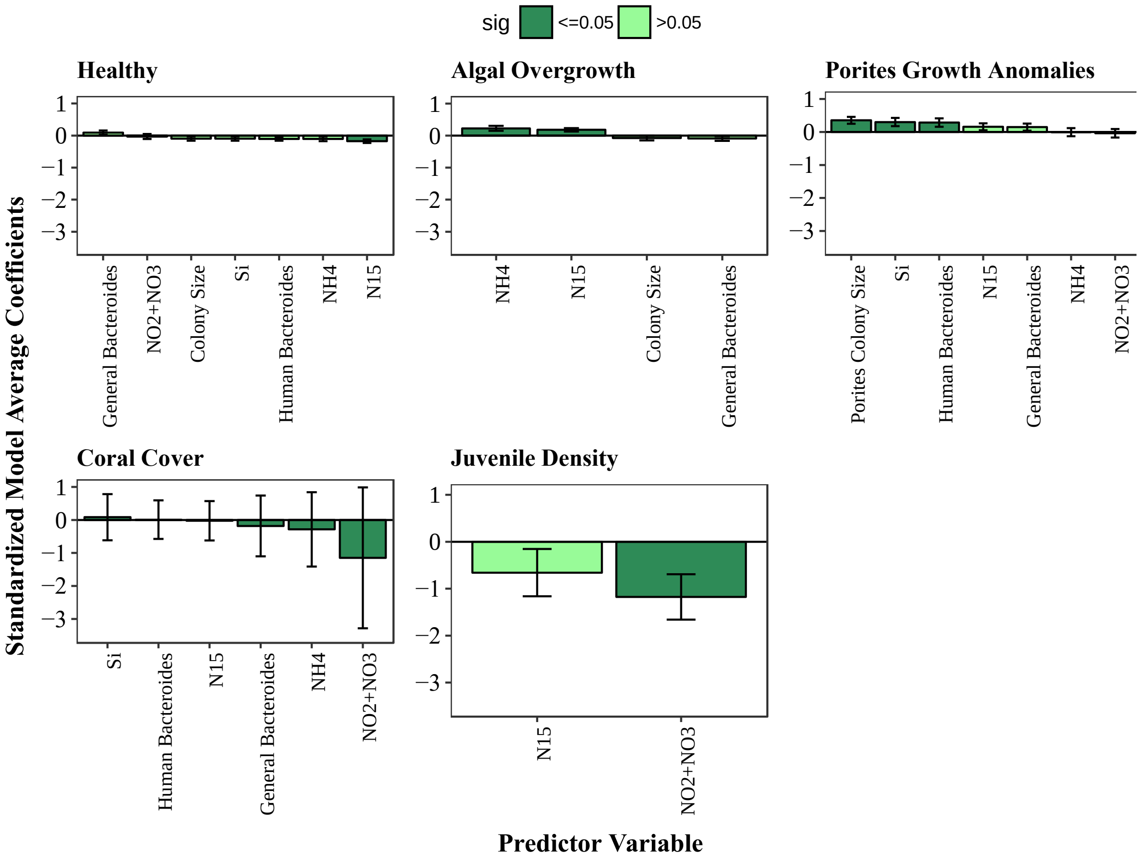

Figure 15. Explanatory variables of proportion of: health colonies, direct coral-algal competition, and *Porites* colonies with growth anomalies, as well as coral juvenile density and coral cover and their standardized parameter estimates (±SE) from the average of the “best-fit” models ΔAIC_C <4. Only variables included in the “best-fit” models were included in model averaging.

when compared to previous studies conducted in this region prior to the 2015 bleaching event. Similar to the 2014 study as well as previous research along West Hawai‘i, PorGAs are more prevalent on reefs with larger colonies and higher submarine groundwater discharge (Couch et al., 2014b). Unlike previous years, PorGA were also more prevalent on reef with higher fecal indicator bacteria. Unlike the present study, we found no drivers of coral-algal competition in 2014.

4.3 Year 3

4.31 Spatial Patterns in Water Quality

Consistent with our Puakō studies and others along West Hawai‘i (Couch et al., 2014b; Knee et al., 2010b, this study) dissolved inorganic nutrient concentration was markedly higher along the shoreline compared to the reef benthos (Figure 16 and Figure 17). This highlights that submarine groundwater discharge and other sources of nutrient inputs from coastal sources are mixed and diluted with oceanic water before being measured on the reef. Overall, nutrient concentration in shoreline locations was low to moderate compared to previous coastwide studies of nutrient concentrations (Knee et al., 2010b; Street et al., 2008). Given the minimal temporal sampling conducted during this study, the nutrient concentrations observed in this study likely do not represent the full range of nutrient inputs that a given site experiences. However, the relative spatial patterns observed suggest that land use affects nutrient concentrations in the adjacent coastal waters (Figure 17). More specifically, nitrite + nitrate was elevated in waters surrounding resorts compared to low impact and residential sites. Ammonium was elevated near resorts and residential areas compared to low impact areas. Phosphate was higher near resorts compared to residential areas, but not different from low impact areas.

Although there was no difference among land use types, the abundance of the Bacteroidales group varied between $10^{1.5}$-10^{4} copies per 100 mL along the coastline during the sampling period. As found in previous years, samples collected at the shore varied minimally between sites (typically $10^{2.5}$ -$10^{3.5}$ gene copies/100mL), and were typically an order of magnitude higher than corresponding benthic samples (Figure 16 and Figure 18). The geometric mean *Enterococcus* abundance did not exceed HDOH standards at any sites and was far lower than previously reported for Puakō. This indicator also did not vary as a function of sampling location or land use type. The proportion of samples with human-specific gut bacteria was extremely low with only one sample testing positive at three different sites (Figure 18).

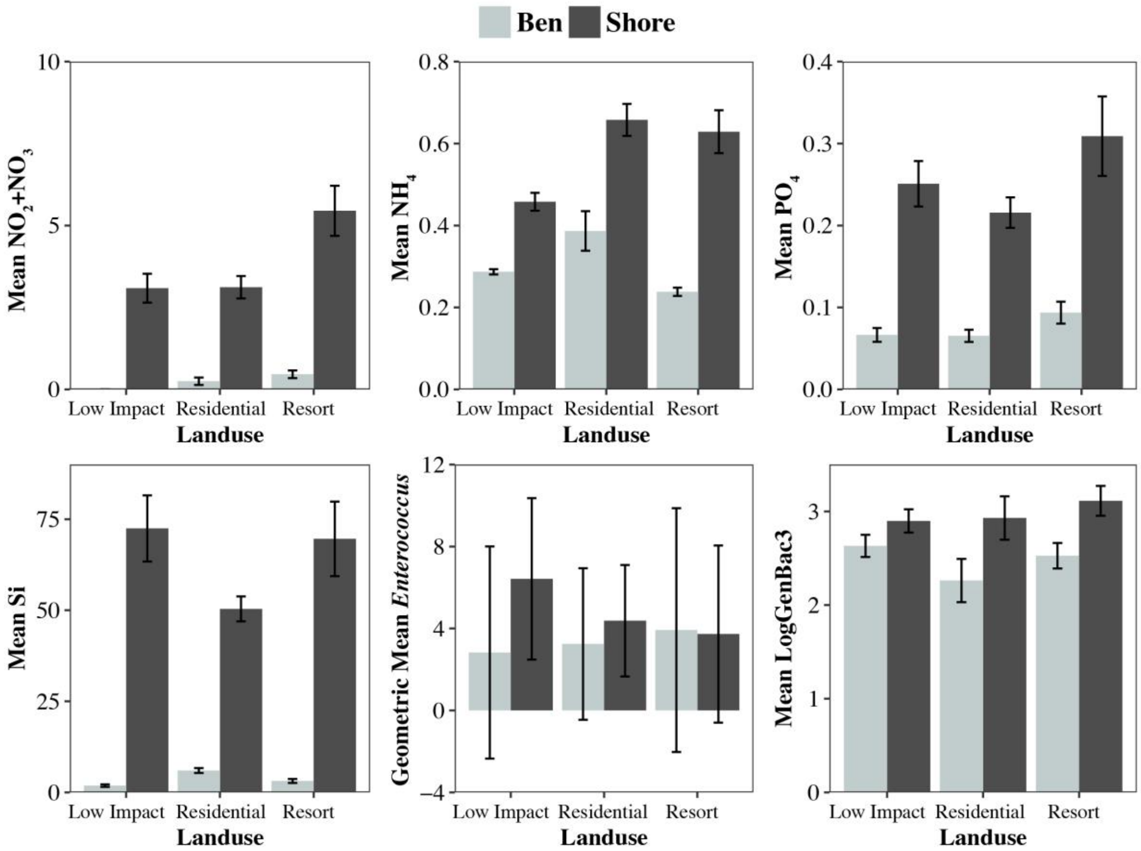

Figure 16. Mean dissolved inorganic nutrient concentration and fecal indicator bacteria abundance at benthic and shoreline sites collected during 2017 across different land use types. Values are averaged across low impact, residential and resort sites (n = 2-3/sampling point/site x 5 sites/land use type).

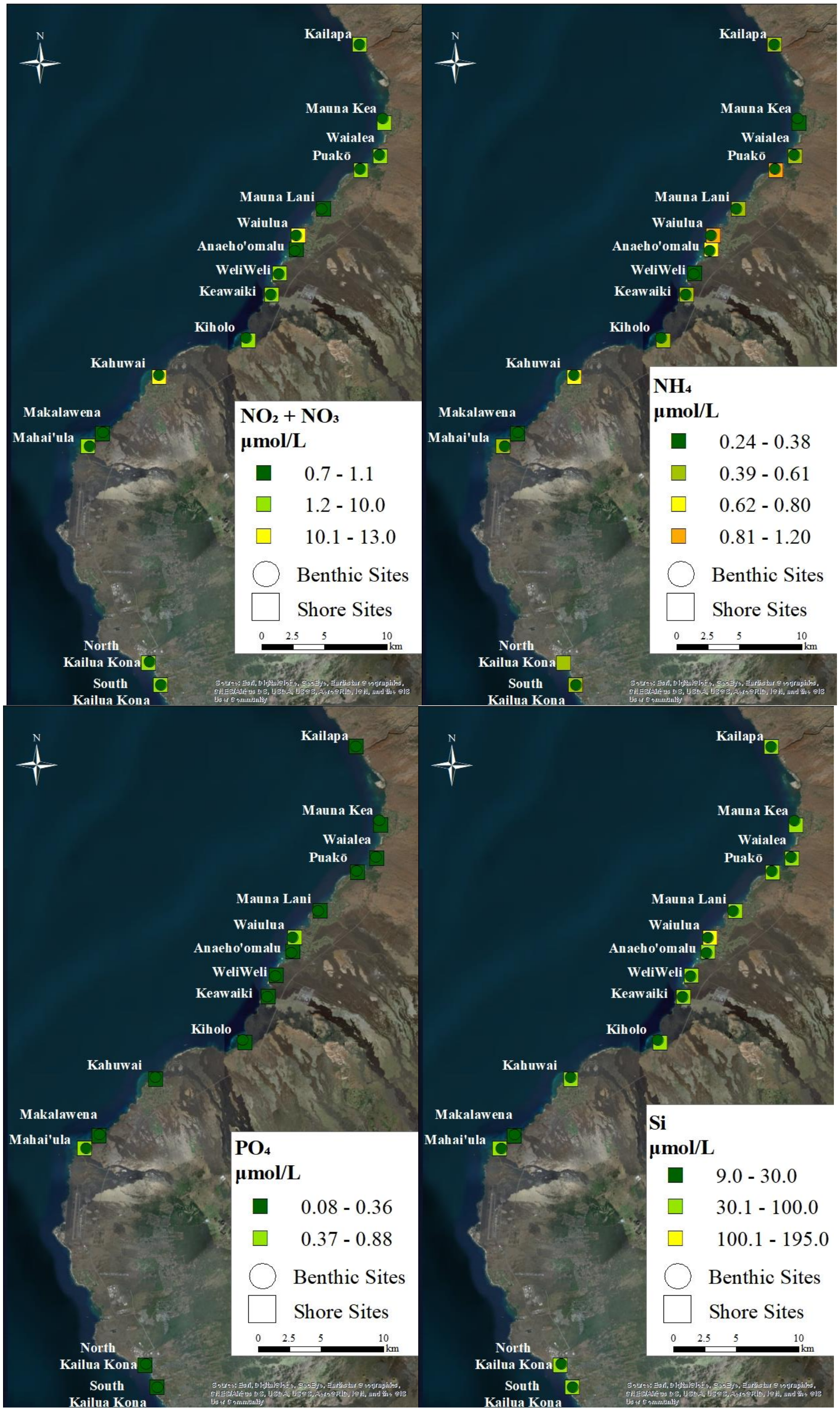

Figure 17. Map of dissolved inorganic nutrient concentrations in benthic and shoreline sites sampled in 2017. Data represent mean of sampling periods.

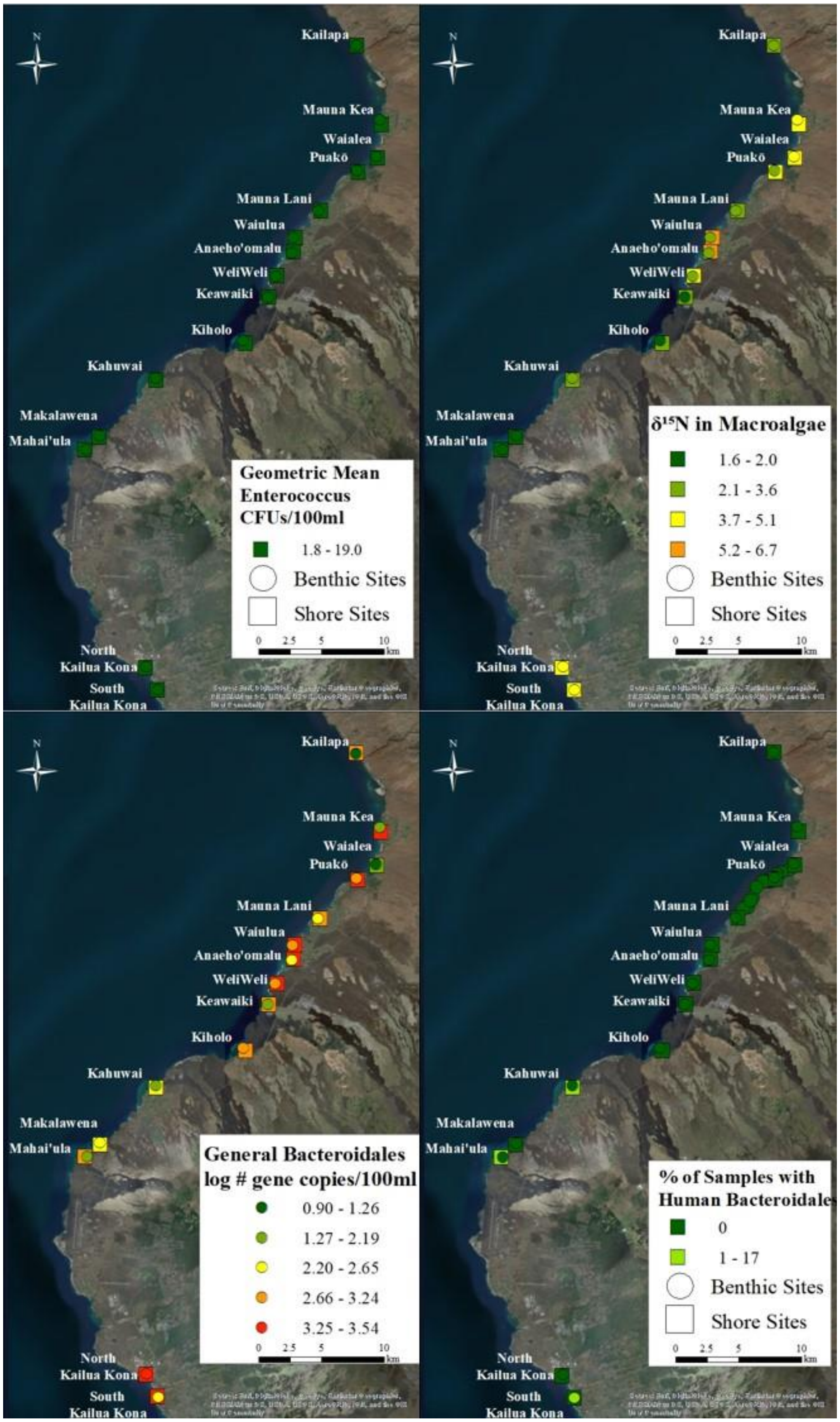

Figure 18. Map of $\delta^{15}N$ values in macroalgae and fecal indicator bacteria abundance at benthic and shoreline sites sampled in 2017. Data represent mean of sampling periods.

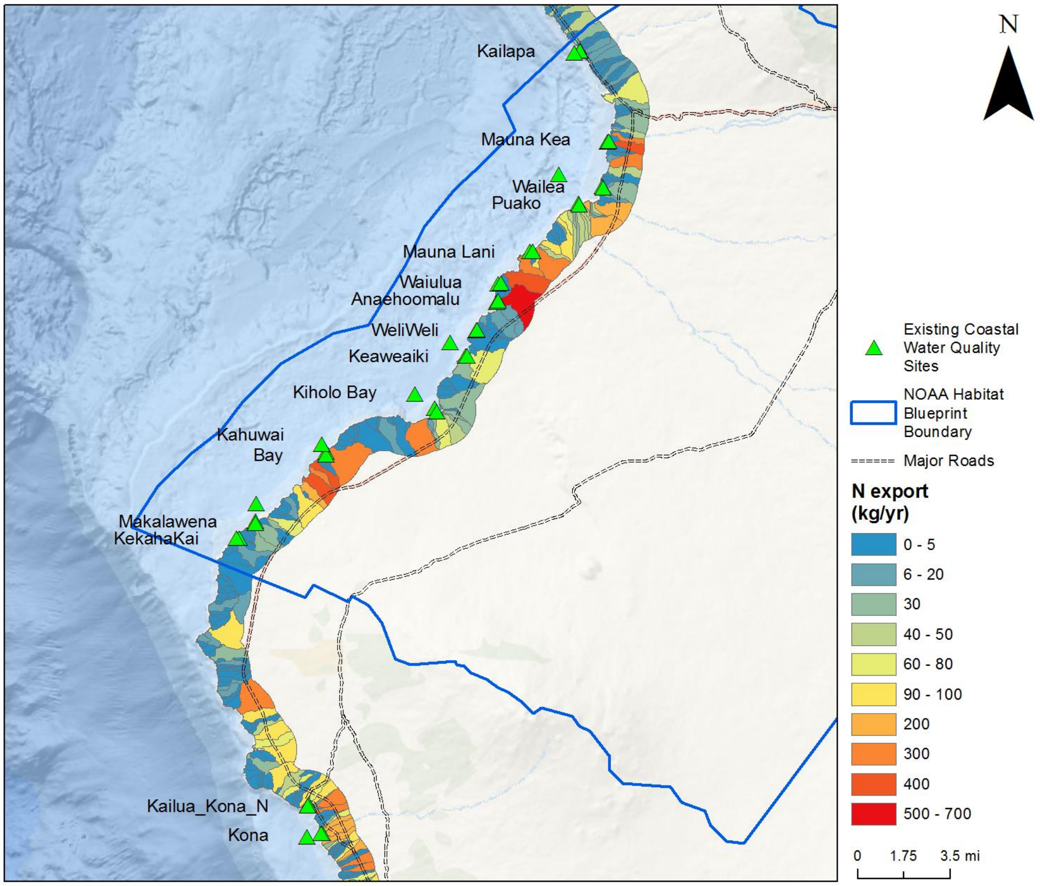

Figure 19. Nitrogen export from 2km of watershed as modeled using InVEST.

4.32 Watershed Nutrient Inputs

Watershed nutrient inputs were modeled using InVEST and showed considerable variation along the coast in the predicted delivery of nitrogen (**Error! Reference source not found.**). However, the predicted nitrogen export modeled from within 2km of the shoreline was not correlated to the dissolved inorganic nitrogen concentrations recorded from field samples (**Error! Reference source not found.**). Si was only weakly negatively correlated with modeled results (**Error! Reference source not found.**). Model results estimating nitrogen export from within 10km of the shoreline were also compared against measure values, with no relationship found (data not shown).

There are several possible explanations for the lack of correspondence between modeled and actual results. As discussed above, the limited amount of water quality sampling we were able to conduct is likely insufficient to generate accuate estimates nutrient concentrations at all sites, and it may be that modeled export rates represent a more integrated measure of export than our sampling quanitified. And while the paramaterization of the model was based on the best available data (see above), it is possible that further refinement of the model itself and the pramaterization of the model is necessary to generate reliable and accurate predictions of nitrogen export along this coastline. Developing an accurate model that would both allow sites of concern for water quality to be identified and be able to predict the likely impacts of land use changes on water quality (and its impact on reef condition) would be a major contribution to our ability to identify and manage coastal inputs to marine systems. Modeled watershed nutrients did add explanatory power to the assessment of the environmental factors driving coral reef condition, and will be discussed in more detail below.

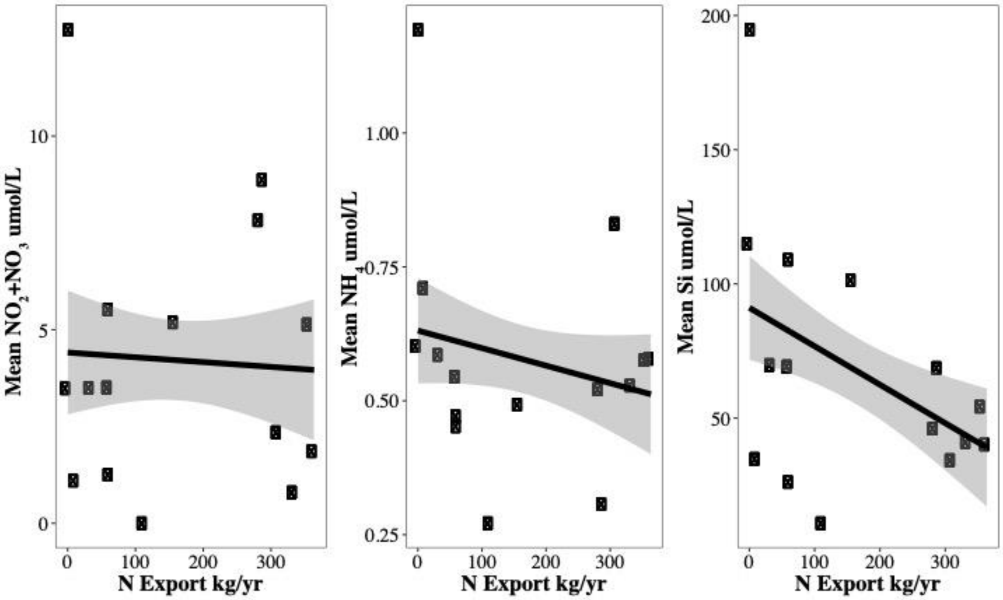

Figure 20. Linear regressions of dissolved inorganic nutrient concentration collected during 2017 along the West Hawai‘i shoreline sites vs. modeled watershed nitrogen export within 2km of each shoreline site. Grey area represents standard error.

4.33 Spatial Patterns in Benthic Communities

Consistent with previous studies along West Hawai‘i, we observed considerable spatial variability in coral health across the study region (Figure 21). Furthermore, the prevalence of coral health conditions varied significantly between land use types for overall colony health, *Porites* growth anomalies, algal overgrowth and discoloration (Figure 22). More specifically, overall colony health and *Porites* growth anomalies were significantly higher on reefs adjacent to resorts than residential and low impact areas. Algal overgrowth was significantly higher near resorts compared to residential areas, but not significantly different than low impact areas. Discoloration near resorts was significantly higher than low impact areas, but not different from residential areas. The other recorded coral health conditions did not vary significantly between land use types (Figure 22).

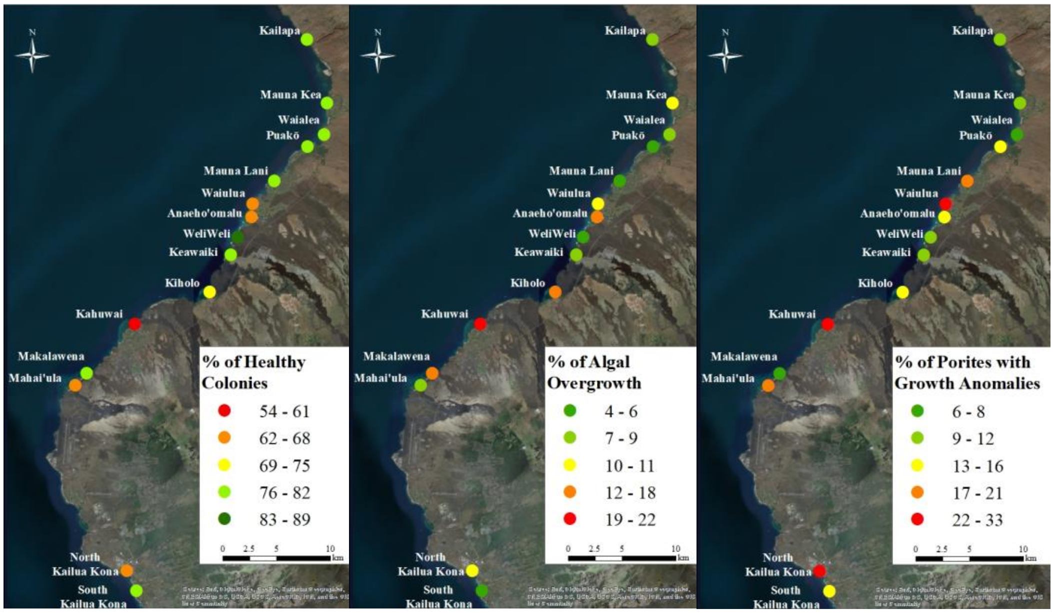

Figure 21. Map of prevalence of healthy colonies, algal overgrowth and *Porites* growth anomalies across sites surveyed in 2017.

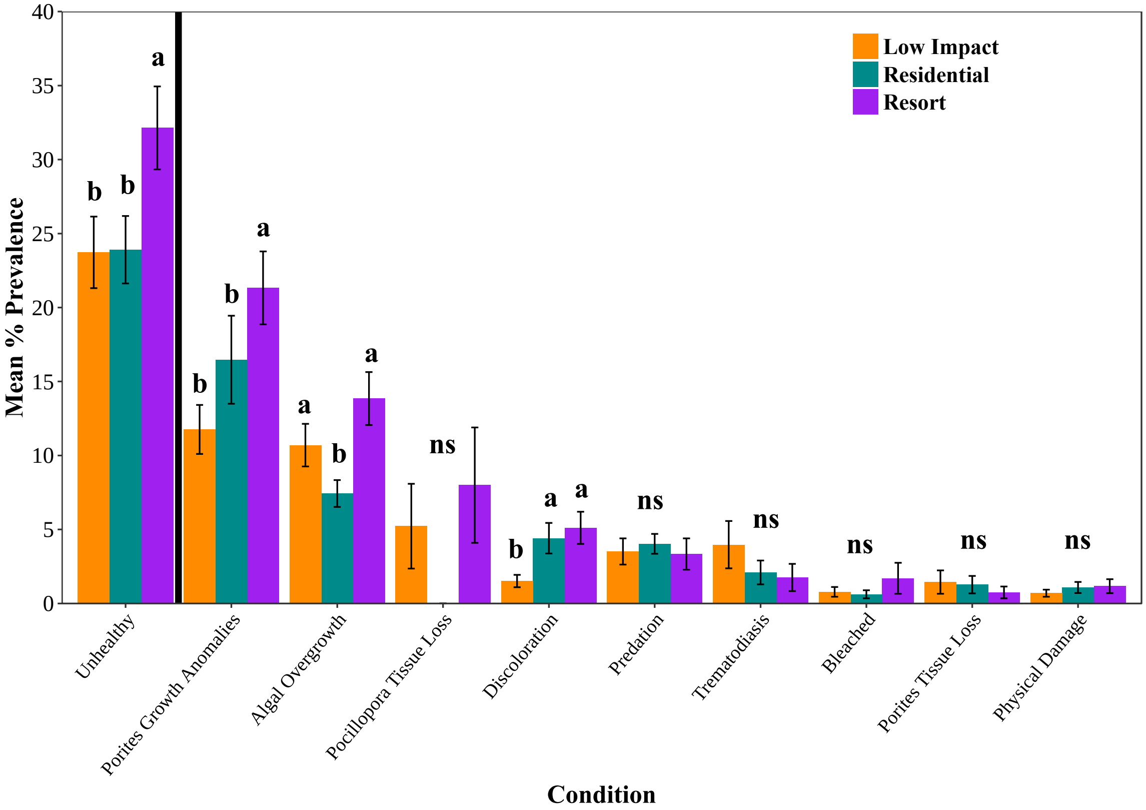

Figure 22. Mean (±SE) prevalence of coral health and disease conditions by land use type. Unhealthy = prevalence of colonies with at least one type of lesion. Letters represent significance between land use types within each condition using glht post hoc tests with a Bonferonni correction α = 0.001 (ns = no significant difference between groups). N= 15 transects/land use type.

In contrast, while the other metrics of the benthic community measured varied spatially along the coast (Figure 23), they showed differing patterns as it related to land use. Juvenile coral density was significantly higher near residential areas compared to low impact and resort areas (Figure 24). Reef builder ratio and rugosity varied noticeably along the coastline, but with no significant effect of land use type (Figure 24).

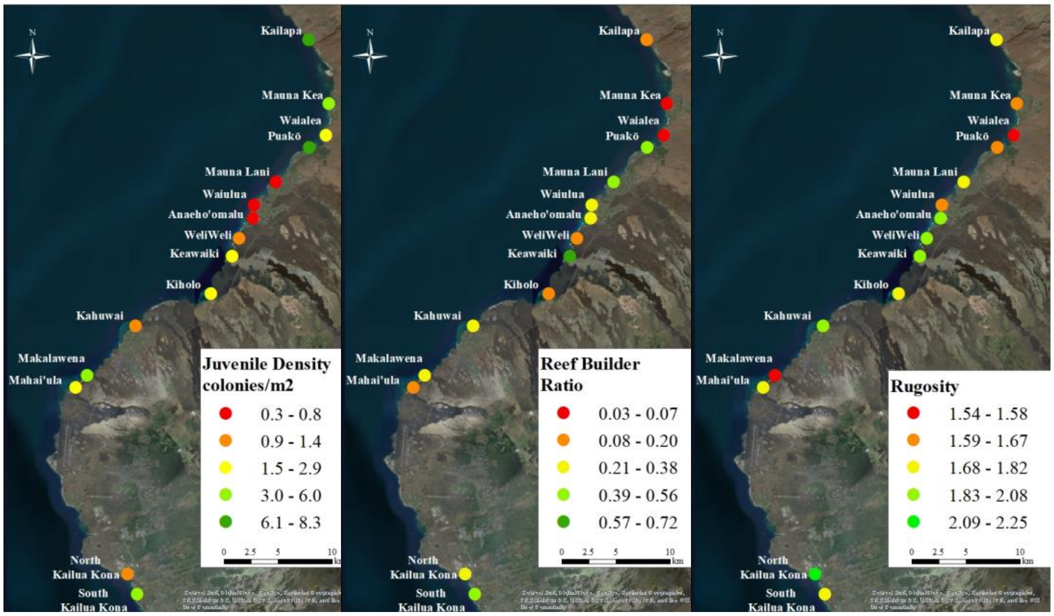

Figure 23. Map of coral juvenile density (colonies/m^2), reef builder ratio and rugosity across sites surveyed in 2017.

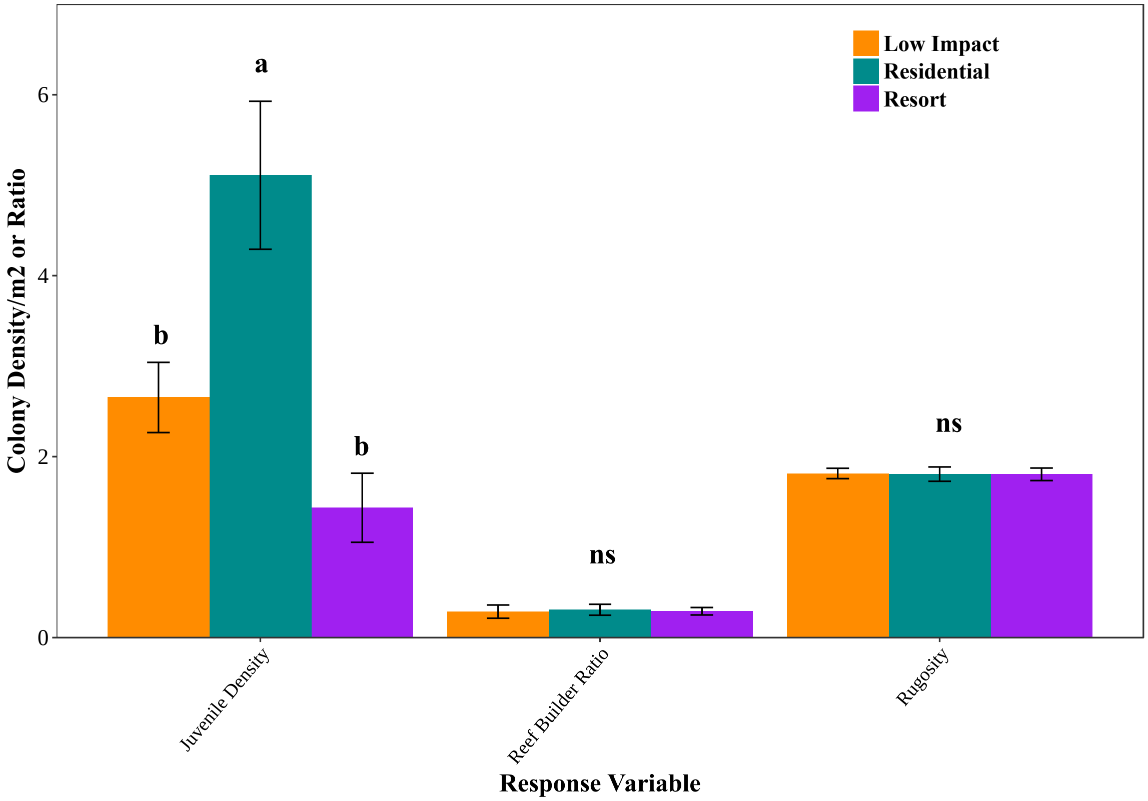

Figure 24. Mean (±SE) juvenile density, reef builder ratio and rugosity by land use type. Letters represent significance between land use types within each response variable using TukeyHSD post hoc tests with a Bonferonni correction α = 0.005 (ns = no significant difference between groups). N= 15 transects/land use type.

4.34 Drivers of Coral Health

Similar to results from our Puakō studies, coral health was driven by a combination of environmental and colony-level factors (

Figure 25). Similar to our Year 2 study in Puakō, our results suggest that the proportion of healthy colonies increased when exposed to lower δ^{15}N values as well as lower watershed nutrients and *in situ* shoreline nutrient concentration. In fact, *in situ* nutrients and δ^{15}N levels had 2-3 times the influence on the proportion of healthy colonies than the bacterial indicators, wave power and colony size. The proportion of colonies experiencing direct coral-algal competition increased with *in situ* nutrient concentration and the proportion of samples with human-specific bacteria, as well as lower δ^{15}N values. These factors played a stronger role in driving patterns in algal overgrowth than factors such as colony size, watershed nutrients, abundance of general Bacteriodes and wave power. The proportion of *Porites* colonies with growth anomalies increased on reefs with higher *in situ* nutrient concentration, watershed nutrients and larger colonies. While fecal indicators were not a strong predicator of PorGA prevalence, the role of inorganic nutrients and larger colony size increasing disease risk is consistent with our previous research in Puakō as well as other studies (Couch et al., 2014a, 2014b). Juvenile density decreased when exposed to higher wave energy and lower δ^{15}N values with nutrient and bacterial indicators included in the best fit models, but much less important than wave energy and δ^{15}N. However, other benthic indicators such as reef builder ratio and rugosity were not strongly predicted by environmental factors included in this study.

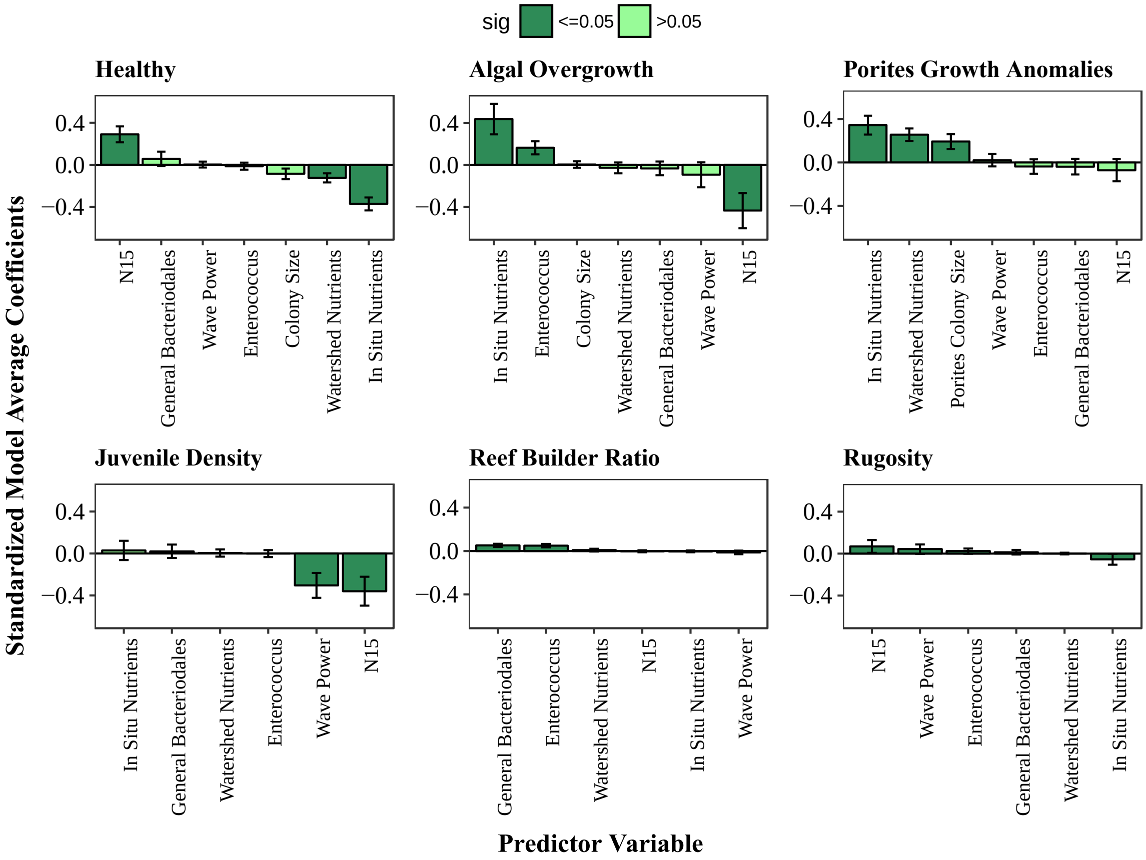

Figure 25. Explanatory variables of proportion of: health colonies, direct coral-algal competition, and *Porites* colonies with growth anomalies, as well as coral juvenile density, reef builder ratio and rugosity and their standardized parameter estimates (±SE) from the average of the "best-fit" models ΔAIC$_C$ <4. Only variables included in the "best-fit" models were included in model averaging.

5.0 INTEGRATION

The scope of stressors on coral reefs is growing as climate change takes corals beyond their thermal tolerance and increased coastal development and suboptimal wastewater treatment exposes reefs to land-based pollution. While global reductions in carbon emissions are needed for long-term protection of coral reefs, reducing local stressors and promoting coral health by reducing land-based pollution may extend the timescale over which coral reefs can continue to thrive while we combat climate change. The leeward coast of Hawaiʻi Island has undergone persistent land use change in recent decades. These changes raise concerns about sources of land-based pollution and their role in documented coral reef decline (Dollar and Grigg, 2004; Walsh et al., 2010, 2018). In this three-year study we used standard and advanced monitoring and modeling techniques to provide definitive evidence of hot spots of sewage pollution in Puakō. While recent coral bleaching has reshaped much of West Hawaiʻi, our results suggest that sewage inputs may be linked with impaired reef health in Puakō. Beyond Puakō, we also demonstrated that land use is influencing coastal nutrient inputs, with several indications of higher inputs near resorts, and those inputs are correlated with coral health across the broader West Hawaiʻi coastline. While it was previously thought that degraded water quality and its impacts on coral reefs was a rare occurrence along the West Hawaiʻi coastline, we have demonstrated here that the concerns are broader and the need for working together to address issues greater.

This study adds to the growing body of literature indicating that sewage pollution into coastal waters is a persistent issue in Puakō. Microbial source tracking has become a widely used tool for identifying and tracking sources of animal waste into riparian and coastal waters. The presence of human-specific gut bacteria in Puakō study support the presence of sewage, which was suggested in previous studies following *Enterococcus* and *Clostridium profringens* levels that far exceeded HDOH and EPA standards (Abaya et al., 2018a, 2018b; Couch et al., 2014b).

Consistent with our previous research, SGD is still an important delivery mechanism of nutrients into the coastal waters (Couch et al., 2014b; Knee et al., 2010b; Street et al., 2008). However, the level of dissolved inorganic nutrients entering the shoreline at some sites in the Puakō-Mauna Lani reef system documented in this study and other Puakō studies (Abaya et al., 2018a, 2018b) are among the highest reported values for shoreline coastal waters along West Hawaiʻi (Johnson et al., 2008; Knee et al., 2010b; Street et al., 2008). The degree to which SGD is a delivery mechanism for sewage contamination is varied. While previous research in Puakō found a negative correlation between fecal indicator bacteria and salinity (i.e., sewage is associated with freshwater inputs, or SGD) (Abaya et al., 2018a; Couch et al., 2014b), Abaya et al. (2018b) did not find a strong relationship between sewage indicators and salinity. Using mixing plot models, Abaya et al. (2018b) suggest that higher elevation groundwater is a significant source of SGD into the coastline, but that a majority of the nutrients in the shoreline originate from sources directly along the shoreline. These results were further supported by dye tracer studies that demonstrated that on site disposal systems are able to deliver pollutants into the coastal ecosystem within 9 hours to 3 days after flushing. When tracking non-point sources of pollution it is often impossible to identify where pollution is entering the shoreline and from which sources without employing a multi-parameter approach with high spatial sampling. Using spatial statistical approaches in ArcGIS and a sewage indicator score, we identified two hot spots of sewage pollution. Over the course of this project TNC and UHH have presented these results to the Puakō Community Association, which has been actively assessing sewage treatment remediation solutions. This information has both helped demonstrate the real need for remediation as well as helped identify which sections of coastline could benefit most from immediate management.

Beyond Puakō, identifying sources of land-based pollution and patterns in water quality is more complex and driven by the variety of land use inputs and coastal oceanography. Dissolved inorganic nutrient concentrations along the shoreline were low to moderate compared to previous studies along the leeward Hawai‘i Island coastline (Knee et al., 2010b; Street et al., 2008) and lower than our previous Puakō studies. Nitrate and nitrite concentrations from shoreline sampling were higher adjacent to resorts compared to residential and low impact areas. Ammonium concentration was also higher near resorts and residential areas than low impact areas. In a previous West Hawai‘i study, Knee et al. (2010b) assessed the relationship between coastal nutrient concentrations and land use. The authors discovered a negative correlation between population density and Si, a positive correlation between bare land and nitrate + nitrite, and a positive correlation between bare land and Si. While only two of their locations had golf courses, their results also suggested that dissolved inorganic nitrogen is higher near golf courses. The potential role of golf courses and other large-scale landscaping activities in subsidizing nitrogen inputs into coastal areas is further supported by our study given that golf courses were present at all resort sites and none of the other sites. While golf courses and landscaping may provide a significant source of nutrients, coastal waters surrounding resorts are also exposed to considerable recreational use, which may contribute to nutrient inputs.

Identifying nutrient sources can be challenging, and δ^{15}N isotope in algae have been used as one tool to identify nitrogen sources. However in many instances there are multiple sources of nitrogen to the coastal waters that all contribute to the δ^{15}N of the algal tissue, causing it to have δ^{15}N values in between source values and difficult to interpret. In our study, δ^{15}N values were lower adjacent to low impacted (1.0-5.1 ‰) areas compared to residential (3.1-5.2‰) and resort areas (2.2-7.2‰). Based on previous studies, the values along the shoreline at low impact areas reflect nitrogen sources from soil, ocean, and high elevation groundwater, whereas values in residential areas reflect ocean to high elevation groundwater nitrogen sources, and resort values suggest soil, ocean, and high and low elevation ground water (Abaya et al., 2018b).

While chemical contamination was not measured in this study, it is also important to note that groundwater surrounding residential and resort areas may have also exposed coastal ecosystems to chemicals such as pesticides and oil and gasoline from roadways. It is possible that if present, these pollutants may have contributed to the decreased coral condition seen in these areas.

In Puakō, fecal indicator bacteria have been an important indicator of sewage pollution. However, in the present study abundance of *Enterococcus* and human-specific

Bacteroidales was low and varied minimally across the West Hawaiʻi coastline. While this does suggest that sewage pollution is minimal along the broader coastline, given the often high temporal and spatial heterogeneity of fecal indicator bacteria (Abaya et al., 2018b; Couch et al., 2014b), it is likely that sewage pollution was underestimated in this study. For example, the Kailua-Kona watersheds have some of the highest density of OSDSs on the West Hawaiʻi coastline (HDOH, 2017). Additionally, eddies are common in this area due to the island's steep topology and prevailing northeasterly tradewinds (Seki, Lumpkin, and Flament 2002). The frequency and intensity of these features is likely to influence the concentration and distribution of fecal indicator bacteria, especially those that are anaerobic (i.e. Bacteriodales). Given passage of Act 125, which mandates the upgrade of all cesspools by 2050, and the HDOH's efforts to educate the public about this mandate, more in-depth monitoring of sewage input outside Puakō could more efficiently guide sewage treatment remediation.

Coral health was primarily affected by direct coral-algal competition and growth anomalies, which affected up to 30 to 45% of colonies, a results in line with previous studies along West Hawaiʻi. Algal overgrowth was largely the result of red turf algae, morphologically similar to *Corallophila huysmansii*, and filamentous cyanobacteria (Figure 3). *C. huysmansii* is commonly associated with tissue mortality across the Pacific (Couch et al., 2014b; Jompa and McCook, 2003; Myers and Raymundo, 2009; Willis et al., 2004). 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. This study represents the first attempt to understand the underlying drivers of this widespread condition. During 2016, Puakō algal overgrowth was somewhat driven by positive relationship with ammonium and δ^{15}N values, but the relative effect of these predictors was fairly low when compared to other benthic metrics.

One factor that potentially played a role in promoting algal competition but was not included in our analyses was the spatial variation in bleaching stress and mortality. As documented in this study, Puakō experienced a 55-99% loss in coral cover following the 2015 bleaching event. During and after this event, a noticeable proliferation of filamentous algae occurred in Puakō and elsewhere along the coast (TNC unpubl. data). Along the broader coastline, the proportion of colonies affected by algal overgrowth increased with shoreline *in situ* nutrient concentration, which is consistent with predictions, as inorganic nutrients are known to stimulate algal growth. The negative correlation with δ^{15}N suggests that naturally derived nitrogen may also exacerbate coral-algal competition, or perhaps that fertilizers, which also have a low δ^{15}N, are contributing to algal growth.

This study also contributes to growing evidence of the role that terrestrial inputs play in increasing PorGA levels. Although growth anomalies are not typically associated with widespread mortality of corals, they do have a number of deleterious effects on corals such as reduced growth, reproductive output and increased partial mortality (Domart-Coulon et al., 2006; Work et al., 2008). In Puakō, PorGA increased with Si concentration, exposure to human gut bacteria and colony size. The relationship with Si and colony size is consistent with our previous study (Couch et al., 2014b). Along West Hawaiʻi, PorGA prevalence was higher when exposed to higher *in situ* nutrient concentration, watershed nutrients and larger colonies. These results are consistent with previous studies that found a positive correlation between growth anomaly prevalence and nitrogen concentrations (Couch et al., 2014b; Kaczmarsky and Richardson, 2010; Kuta and Richardson, 2002; 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 dissolved inorganic nitrogen as well as silica in this study also suggests that there may be other components of the groundwater, such as chemical contaminants or pathogens, that we did not account for that are driving coral health.

In addition to coral health, we also assessed several other indicators of reef health. High coral recruitment and juvenile density is commonly used an indicator of a community's capacity to recover and persist (Gilmour et al., 2013; Miller et al., 2000; Pearson, 1981). In Puakō, we documented lower juvenile density in areas with higher dissolved inorganic nitrogen concentration. Eutrophication and sedimentation has previously been associated with lower coral recruitment in other regions (Fabricius et al., 2005; Hodgson, 1990). This pattern may be the result of lower fecundity and larval survival or higher post settlement mortality due to physiological stress and enhanced benthic algal growth in regions with poorer water quality(reviewed by Ritson-Williams et al., 2009). Contrary to our findings in Puakō, *in situ* nutrients were not a strong predictor of juvenile density along the broader coastline. Alternatively, juvenile density was correlated with wave power and δ^{15}N values, whereby we observed higher density on reefs with low wave energy and low δ^{15}N values. With higher wave action coral larvae are likely to have lower settlement success as well as potentially higher post settlement mortality due to scouring, particularly in areas with mixed sand and hard substrate. High coral cover, reef builder ratio and rugosity have also been used as metrics of reef health, but in the present study these metrics were not correlated with any environmental metric in either the Puakō or broader coastwide study and did not vary as a function of land use. These results highlight that colony-level health are potentially more valuable and sensitive indicators of changing reef health compared widely used metrics such as coral cover and rugosity, which are coarse and may take years or decades to detect a reef-level response.

6.0 KEY FINDINGS

- The presence of human-specific gut bacteria in Puakō provides definitive evidence of sewage pollution and adds to the growing number of studies indicating that sewage is a persistent issue in Puakō.
- Dissolved inorganic nutrients entering the shoreline at some sites in Puakō and the Mauna Lani in this study and other Puakō studies are among the highest reported values for shoreline coastal waters along West Hawai‘i.
- Using spatial statistical approaches in ArcGIS and a sewage indicator score, we identified two hot spots of sewage pollution in Puakō.

- Beyond Puakō, identifying sources of land-based pollution and patterns in water quality is more complex and driven by the variety of land use inputs.
- Dissolved inorganic nutrient concentrations along the shoreline were low to moderate compared to previous studies along the leeward Hawaiʻi Island coastline.
- Shoreline dissolved inorganic nutrient concentration is correlated with land use, with nitrate and nitrite concentrations higher adjacent to resorts compared to residential and low impact areas.
- Abundance of fecal indicator bacteria was low and varied across the West Hawaiʻi coastline, but was likely underestimated due to low temporal sampling.
- More in-depth monitoring of sewage input outside Puakō is recommended to guide ongoing sewage treatment remediation.
- Puakō experienced 55-99% coral mortality following the 2015 bleaching event.
- Consistent with previous studies along West Hawaiʻi, coral health is primarily affected by growth anomalies and direct coral-algal competition, which affected up to 30 to 45% of colonies.
- In Puakō algal overgrowth was somewhat driven by positive relationship with ammonium and δ^{15}N values, and was more strongly correlated with *in situ* nutrient concentration along the wider coastline.
- Similar to previous studies, *Porites* growth anomaly prevalence was elevated in areas with higher Si and colony size in Puakō, and higher *in situ* nutrient concentration, watershed nutrients and larger colonies across the broader coastline.
- In Puakō, we documented lower juvenile density in areas with higher dissolved inorganic nitrogen concentration.
- Along West Hawaiʻi, juvenile colony density was higher on reefs with low wave energy and low δ^{15}N values.
- Taken together, these results clearly show that increased nutrient inputs along the West Hawaiʻi coastline are negatively affecting coral health through effects on recruitment and the condition of adult colonies, and those inputs are associated with land use such as sewage disposal and land management practices.
- Coral cover, the proportional cover of reef builders and rugosity were not correlated with land use or any metric of water quality, demonstrating that coral colony-level health metrics are more sensitive indicators of change.

7.0 ACKNOWLEDGEMENTS

This project was made possible by a wonderful team of volunteers from KUPU YCC, University of Hawaiʻi at Hilo, Cornell University, Hui Aloha Kīholo, and the Ala Kahakai Community Association and include Ethan Souza, Anna Mikkelsen, Courtney Hurt, Jenna Budke, Cecily Tilley, Amy Olsen, Joseph Ziak, Rose Hart, Amber Datta, Rebecca Cope, Nic Vanderzyl, Allison Webster, Sierra Helmann, Phoebe Dawkins, Yin Zeng, Lehua Kamaka, Pua'ala Subica, and Chris Hawkins. Special thanks to Megan Donahue and the members of the Donahue lab for field assistance and feedback on experimental design and data analysis.

8.0 LITERATURE CITED

Abaya, L. M., Wiegner, T. N., Beets, J. P., Colbert, S. L., Kaile'a, M. C., and Kramer, K. L. (2018a). Spatial distribution of sewage pollution on a Hawaiian coral reef. *Mar. Pollut. Bull.* 130, 335–347.

Abaya, L. M., Wiegner, T. N., Colbert, S. L., Beets, J. P., Kaile'a, M. C., Kramer, K. L., et al. (2018b). A multi-indicator approach for identifying shoreline sewage pollution hotspots adjacent to coral reefs. *Mar. Pollut. Bull.* 129, 70–80.

Amato, D. W., Bishop, J. M., Glenn, C. R., Dulai, H., and Smith, C. M. (2016). Impact of submarine groundwater discharge on marine water quality and reef biota of Maui. *PLoS One* 11, e0165825.

Beaulac, Michael N, & Reckhow, Kenneth H. (1982). An examination of land use: nutrient export relationships. Journal of the American Water Resources Association, 18(6), 1013-1024.

Beijbom, O., Edmunds, P. J., Roelfsema, C., Smith, J., Kline, D. I., Neal, B. P., et al. (2015). Towards automated annotation of benthic survey images: Variability of human experts and operational modes of automation. *PLoS One* 10, 1–22. doi:10.1371/journal.pone.0130312.

Bellwood, D. R., Hughes, T. P., Folke, C., and Nystrom, M. (2004). Confronting the coral reef crisis. *Nature* 429, 827–833.

Bowen, J. L., Kroeger, K. D., Tomasky, G., Pabich, W. J., Cole, M. L., Carmichael, R. H., et al. (2007). A review of land–sea coupling by groundwater discharge of nitrogen to New England estuaries: Mechanisms and effects. *Appl. geochemistry* 22, 175–191.

Bruno, J. F., Petes, L. E., Harvell, C. D., and Hettinger, A. (2003). Nutrient enrichment can increase the severity of coral diseases. *Ecol. Lett.* 6, 1056–1061.

Burnham, K. P., and Anderson, D. R. (2002). *Model selection and multi-model inference: a practical information-theoretic approach*. New York: Springer-Verlag.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R., and Harwood, V. J. (2012). Enterococci in the Environment. *Microbiol. Mol. Biol. Rev.* 76, 685–706. doi:10.1128/mmbr.00023-12.

Cabelli, V. J. (1983). *Health effects criteria for marine recreational waters*. Health Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency.

Cobo, Juan Guillermo, Dercon, Gerd, & Cadisch, Georg. (2010). Nutrient balances in African land use systems across different spatial scales: a review of approaches, challenges and progress. *Agriculture, ecosystems & environment, 136*(1), 1-15

Cole, M. L., Kroeger, K. D., McClelland, J. W., and Valiela, I. (2006). Effects of watershed land use on nitrogen concentrations and δ 15 nitrogen in groundwater. *Biogeochemistry* 77, 199–215.

Couch, C. S., Garriques, J. D., Barnett, C., Preskitt, L., Cotton, S., Giddens, J., et al. (2014a). Spatial and Temporal Patterns of Coral Health and Disease along Leeward Hawaiʻi Island. *Coral Reefs*.

Couch, C. S., Most, R., Wiggins, C., Minton, D., Conklin, E., Sziklay, J., et al. (2014b). Understanding the consequences of land-based pollutants on coral health in South Kohala Final Report, 2014 NOAA CRCP, Award #NA11NOS4820006. 35pp.

Dailer, M. L., Knox, R. S., Smith, J. E., Napier, M., and Smith, C. M. (2010). Using δ15N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. *Mar. Pollut. Bull.* 60, 655–671. doi:https://doi.org/10.1016/j.marpolbul.2009.12.021.

David, A., Tournoud, M., Perrin, J., Rosain, D., Rodier, C., Salles, C., et al. (2013). Spatial and temporal trends in water quality in a Mediterranean temporary river impacted by sewage effluents. *Environ. Monit. Assess.* 185, 2517–2534. doi:http://dx.doi.org/10.1007/s10661-012-2728-4.

Dick, L.K. and Field, K.G. (2004). Rapid estimation of numbers of fecal Bacteroidetes by use of a quantitative PCR assay for 16S rRNA genes. Appl Environ Microbiol. 70, pp. 5695–5697.

Dollar, S.J., Atkinson M.J. (1992) Effects of nutrient subsidies from groundwater to nearshore marine ecosystem off the Island of Hawai'i. Estuar Coast Shelf Sci 35: 409-424.

Dollar, S. J., and Grigg, R. W. (2004). Anthropogenic and natural stresses on selected coral reefs in Hawai'i: a multidecade synthesis of impact and recovery. *Pacific Sci.* 58, 281–304.

Domart-Coulon, I. J., Traylor-Knowles, N., Peters, E., Elbert, D., Downs, C. A., Price, K., et al. (2006). Comprehensive characterization of skeletal tissue growth anomalies of the finger coral Porites compressa. *Coral Reefs* 25, 531–543.

Eckhardt, D. A. V, and Stackelberg, P. E. (1995). Relation of ground-water quality to land use on Long Island, New York. *Groundwater* 33, 1019–1033.

Fabricius, K., De'ath, G., McCook, L., Turak, E., and Williams, D. M. (2005). Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. *Mar. Pollut. Bull.* 51, 384–398. doi:https://doi.org/10.1016/j.marpolbul.2004.10.041.

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Mar. Pollut. Bull.* 50, 125–146.

Fung, D. Y. C., Fujioka, R., Vijayavel, K., Sato, D., and Bishop, D. (2007). Evaluation of Fung Double Tube test for Clostridium perfringens and Easyphage test for F-specific RNA coliphages as rapid screening tests for fecal contamination in recreational waters of Hawaii. *J. Rapid Methods Autom. Microbiol.* 15, 217–229.

Gilmour, J. P., Smith, L. D., Heyward, A. J., Baird, A. H., and Pratchett, M. S. (2013). Recovery of an Isolated Coral Reef System Following Severe Disturbance. *Science (80-.).* 340, 69 LP-71. doi:10.1126/science.1232310.

Gove, J. M., Williams G. J., McManus, M. A., Clark, S. J., Ehses, J. S., and Wedding, L. (2015). Coral reef benthic regimes exhibit non-linear threshold responses to natural physical drivers. *Mar. Ecol. Prog. Ser.* 522, 33–48. Available at: https://www.int-res.com/abstracts/meps/v522/p33-48/.

Green, HC, Haugland, RA, Varma, M, Millen, HT, Borchardt, MA, Field, KG et al. (2014). Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. Appl Environ Microbiol. 80, pp. 3086–3094.

Hardina, C. M., and Fujioka, R. S. (1991). Soil: The environmental source of Escherichia coli and enterococci in Hawaii's streams. *Environ. Toxicol. Water Qual.* 6, 185–195. doi:10.1002/tox.2530060208.

Harvell, C. D., Jordán-Dahlgren, E., Merkel, S., Rosenberg, E., Raymundo, L., Smith, G. W., et al. (2007). Coral disease, environmental drivers and the balance between coral and microbial associates. *OCEANOGRAPHY* 20, 58–81.

Harwood, V. J., Staley, C., Badgley, B. D., Borges, K., and Korajkic, A. (2014). Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS Microbiol. Rev.* 38, 1–40. doi:10.1111/1574-6976.12031.

Haugland, R.A., Varma, M., Sivaganesan, M., Kelty, C., Peed, L. and Shanks, O.C. (2010). Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. Systematic and applied microbiology. 33, pp. 348–357.

HDOH (Hawaiʻi Department of Health). 2017 *Regular session relating to cesspools and prioritization for replacement.* REPORT TO THE TWENTY-NINTH LEGISLATURE STATE OF HAWAIʻI 2018 REGULAR SESSION, Report in response to Act 125, 2017 Regular Session (House Bill 1244, HD1, SD2, CD1).

Hodgson, G. (1990). Sediment and the settlement of larvae of the reef coral Pocillopora damicornis. *Coral Reefs* 9, 41–43.

Howarth, R. W., Sharpley, A., and Walker, D. (2002). Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25, 656–676.

Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., et al. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science (80-.).* 359, 80–83. doi:10.1126/science.aan8048.

Hunter, C. L., and Evans, C. W. (1995). Coral reefs in Kaneohe Bay, Hawaii: two centuries of western influence and two decades of data. *Bull. Mar. Sci.* 57, 501–515.

Islam, M. S., and Tanaka, M. (2004). Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Mar. Pollut. Bull.* 48, 624–649.

Johnes, P. J. (1996). Evaluation and management of the impact of land use change on the nitrogen and phosphorus load delivered to surface waters: The export coefficient modelling approach. Journal of Hydrology, 183(3-4). doi: 10.1016/0022-1694(95)02951-6

Johnson, A. G., Glenn, C. R., Burnett, W. C., Peterson, R. N., and Lucey, P. G. (2008). Aerial infrared imaging reveals large nutrient-rich groundwater inputs to the ocean. *Geophys. Res. Lett.* 35, L15606.

Jompa, J., and McCook, L. J. (2003). Contrasting effects of turf algae on corals: massive Porites spp. are unaffected by mixed-species turfs, but killed by the red alga Anotrichium tenue. *Mar. Ecol. Prog. Ser.* 258, 79–86.

Kaczmarsky, L., and Richardson, L. L. (2010). Do elevated nutrients and organic carbon on Philippine reefs increase the prevalence of coral disease? *Coral Reefs* 30, 253–257.

Kildare, B.J., Leutenegger, C.M., McSwain, B.S., Bambic, D.G., Rajal, V.B. and Wuertz, S. (2007). 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: a Bayesian approach. Water Research. 41, pp. 3701–3715.

Kim, G., Lee, K., Park, K., Hwang, D., and Yang, H. (2003). Large submarine groundwater discharge (SGD) from a volcanic island. *Geophys. Res. Lett.* 30.

Knee, K. L., Gossett, R., Boehm, A. B., and Paytan, A. (2010a). Caffeine and agricultural pesticide concentrations in surface water and groundwater on the north shore of Kauai (Hawaii, USA). *Mar. Pollut. Bull.* 60, 1376–1382.

Knee, K. L., Street, J. H., Grossman, E. E., Boehm, A. B., and Paytan, A. (2010b). Nutrient inputs to the coastal ocean from submarine groundwater discharge in a groundwater-dominated system: Relation to land use (Kona coast, Hawaii, USA). *Limnol. Oceanogr.* 55, 1105–1122.

Kramer, K. L., Cotton, S. P., Lamson, M. R., and Walsh, W. J. Bleaching and catastrophic mortality of reef-building corals along West Hawai'i Island: Findings and Future Directions. . *Proc. 13th Int Coral Reef Symp.*

Kuta, K. G., and Richardson, L. L. (2002). Ecological aspects of black band disease of corals: relationships between disease incidence and environmental factors. *Coral Reefs* 21, 393–398.

Kwong, Kfnk, Bholah, A., Volcy, L., & Pynee, K. (2002). Nitrogen and phosphorus transport by surface runoff from a silty clay loam soil under sugarcane in the humid tropical environment of Mauritius. Agriculture Ecosystems & Environment, 91(1-3), 147-157.

Lapointe, B., and Clark, M. (1992). Nutrient inputs from the watershed and coastal eutrophication in the Florida keys. *Estuaries* 15, 465–476. doi:10.2307/1352391.

Lapointe, B. E., Barile, P. J., and Matzie, W. R. (2004). Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. *J. Exp. Mar. Bio. Ecol.* 308, 23–58.

Layton, B. A., Walters, S. P., and Boehm, A. B. (2009). Distribution and diversity of the enterococcal surface protein (esp) gene in animal hosts and the Pacific coast environment. *J. Appl. Microbiol.* 106, 1521–1531. doi:10.1111/j.1365-2672.2008.04113.x.

Li, N., Cheung, K. F., Stopa, J. E., Hsiao, F., Chen, Y.-L., Vega, L., et al. (2016). Thirty-four years of Hawaii wave hindcast from downscaling of climate forecast system reanalysis. *Ocean Model.* 100, 78–95. doi:https://doi.org/10.1016/j.ocemod.2016.02.001.

Lin, Jeff P. (2004). Review of published export coefficient and event mean concentration (EMC) data. (ERDC-TN-WRAP-04-3). Vicksburg, MS: Engineer Research and Development Center.

Line, Daniel E, White, Nancy M, Osmond, Deanna L, Jennings, Gregory D, & Mojonnier, Carolyn B. (2002). Pollutant export from various land uses in the Upper Neuse River Basin. Water Environment Research, 74(1), 100-108.

Ling, Ge, & El-Kadi, Aly I. (1998). A lumped parameter model for nitrogen transformation in the unsaturated zone. Water Resources Research, 34(2), 203-212.

Markewitz, Daniel, Davidson, Eric, Moutinho, Paulo, & Nepstad, Daniel. (2004). Nutrient loss and redistribution after forest clearing on a highly weathered soil in Amazonia. Ecological Applications, 14(sp4), 177-199.

Maynard, J., Conklin, E. J., Minton, D., Most, R., Couch, C. S., Williams, G. J., et al. (2016). Relative resilience potential and bleaching severity in the West Hawai'i Habitat Focus Area in 2015.

McCormick, M. I. (1994). Comparison of field methods for measuring surface topography and their associations with a tropical reef fish assemblage. *Mar. Ecol. Prog. Ser. Oldend.* 112, 87–96.

Miller, M. W., Weil, E., and Szmant, A. M. (2000). Coral recruitment and juvenile mortality as structuring factors for reef benthic communities in Biscayne National Park, USA. *Coral Reefs* 19, 115–123. doi:10.1007/s003380000079.

Minton, D., Conklin, E., Weiant, P., and Wiggins, C. (2012). 40 Years of Decline on Puako's Coral Reefs: A review of Historical and Current Data (1970-2010), The Nature Conservancy. 140pp.

Minton, D., Couch, C., Lynch, H., Most, R., Rose, J., Stephenson, M., Walsh, C., Wiggins, C., and Conklin, E. (2017). Baseline biological surveys of the coral reefs of Makalawena, Hawaiʻi. Tech. Report prepared for Kamehameha Schools. 66 pp.

Myers, R. L., and Raymundo, L. J. (2009). Coral disease in Micronesian reefs: a link between disease prevalence and host abundance. *Dis. Aquat. Organ.* 87, 97–104.

Nelson, C. E., Donahue, M. J., Dulaiova, H., Goldberg, S. J., La Valle, F. F., Lubarsky, K., et al. (2015). Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. *Mar. Chem.* 177, 232–243. doi:https://doi.org/10.1016/j.marchem.2015.06.026.

Paytan, A., Shellenbarger, G. G., Street, J. H., Gonneea, M. E., Davis, K., Young, M. B., et al. (2006). Submarine groundwater discharge: an important source of new inorganic nitrogen to coral reef ecosystems. *Limnol. Oceanogr.* 51, 343–348.

Pearson, R. G. (1981). Recovery and Recolonization of Coral Reefs. *Mar. Ecol. Prog. Ser.* 4, 105–122. Available at: http://www.jstor.org/stable/24812966.

Pesce, S. F., and Wunderlin, D. A. (2000). Use of water quality indices to verify the impact of Córdoba City (Argentina) on Suquía River. *Water Res.* 34, 2915–2926.

Prüss, A. (1998). Review of epidemiological studies on health effects from exposure to recreational water. *Int. J. Epidemiol.* 27, 1–9.

Ritson-Williams, R., Arnold, S. N., Fogarty, N. D., Steneck, R., Vermeij, M. J. A., and Paul, V. J. (2009). New perspectives on ecological mechanisms affecting coral recruitment on reefs. *Smithson. Contrib. Mar. Sci.* 38, 437–457.

Savage, C. (2005). Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. *AMBIO A J. Hum. Environ.* 34, 145–150.

Schott, E. (2010). Mauna Lani, DW Aina Leʻa and Puakō/Wailea Bay Waste Water Assessment (Phase 1 and 2).

Scott, T. M., Rose, J. B., Jenkins, T. M., Farrah, S. R., and Lukasik, J. (2002). Microbial source tracking: current methodology and future directions. *Appl. Environ. Microbiol.* 68, 5796–5803.

Seki, M., Lumpkin, R., and Flament, P. (2002). Hawaii Cyclonic Eddies and Blue Marlin Catches: The Case Study of the 1995 Hawaiian International Billfish Tournament. Journal of Oceanography. 58. 739-745. 10.1023/A:1022854609312.

Sharp, R. , Tallis, H.T., Ricketts, T, Guerry, A.D., Wood, S.A., Chaplin-Kramer, R, . . . Bierbower, W. . (2015). InVEST 3.2 User's Guide. The Natural Capital Project: Stanford University, University of Minnesota, The Nature Conservancy, and World Wildlife Fund.

Shibata, T., Solo-Gabriele, H. M., Fleming, L. E., and Elmir, S. (2004). Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. *Water Res.* 38, 3119–3131.

Siefring S.C., Varman M., Atikovic, E., Wymer, L.J., Haugland, R.A. (2008). Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. J. Water Health 6:225-237.

Simsek, C., and Gunduz, O. (2007). IWQ index: a GIS-integrated technique to assess irrigation water quality. *Environ. Monit. Assess.* 128, 277–300.

Smith, J. E., Runcie, J. W., and Smith, C. M. (2005). Characterization of a large-scale ephemeral bloom of the green alga Cladophora sericea on the coral reefs of West Maui, Hawai'i. *Mar. Ecol. Prog. Ser.* 302, 77–91.

Smith, S. V (1981). "Responses of Kaneohe Bay, Hawaii, to relaxation of sewage stress," in *Estuaries and nutrients* (Springer), 391–410.

Smith, J. E., Brainard, R., Carter, A., Grillo, S., Edwards, C., Harris, J., ... & Vroom, P. S. (2016). Re-evaluating the health of coral reef communities: baselines and evidence for human impacts across the central Pacific. Proc. R. Soc. B, 283(1822), 20151985.

Street, J. H., Knee, K. L., Grossman, E. E., and Paytan, A. (2008). Submarine groundwater discharge and nutrient addition to the coastal zone and coral reefs of leeward Hawai'i. *Mar. Chem.* 109, 355–376.

Sutherland, K. P., Porter, J. W., Turner, J. W., Thomas, B. J., Looney, E. E., Luna, T. P., et al. (2010). Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, Acropora palmata. *Environ. Microbiol.* 12, 1122–1131. doi:Doi 10.1111/J.1462-2920.2010.02152.X.

Thurber, R. V, Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Angly, F., et al. (2009). Metagenomic analysis of stressed coral holobionts. *Environ. Microbiol.* 11, 2148–2163.

Umezawa, Y., Miyajima, T., Kayanne, H., and Koike, I. (2002). Significance of groundwater nitrogen discharge into coral reefs at Ishigaki Island, southwest of Japan. *Coral Reefs* 21, 346–356.

van Beukering, P., and Cesar, H. S. J. (2004). Ecological economic modeling of coral reefs: Evaluating tourist overuse at Hanauma Bay and algae blooms at the Kihei Coast, Hawai'i. *Pacific Sci.* 58, 243–260.

Vega Thurber, R. L., Burkepile, D. E., Fuchs, C., Shantz, A. A., McMinds, R., and Zaneveld, J. R. (2014). Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Glob. Chang. Biol.* 20, 544–554.

Vermeij, M. J. A., Van Moorselaar, I., Engelhard, S., Hörnlein, C., Vonk, S. M., and Visser, P. M. (2010). The effects of nutrient enrichment and herbivore abundance on the ability of turf algae to overgrow coral in the Caribbean. *PLoS One* 5, e14312.

Viau, E. J., Goodwin, K. D., Yamahara, K. M., Layton, B. A., Sassoubre, L. M., Burns, S. L., et al. (2011). Bacterial pathogens in Hawaiian coastal streams—Associations with fecal indicators, land cover, and water quality. *Water Res.* 45, 3279–3290. doi:https://doi.org/10.1016/j.watres.2011.03.033.

Voss, J. D., and Richardson, L. L. (2006). Nutrient enrichment enhances black band disease progression in corals. *Coral Reefs* 25, 569–576.

Walsh, W. J., Zamzow, J., and Kramer, K. L. (2018). Continued long-term decline of the coral reef biota at Puakō and Pauoa, West Hawai′ i (1979–2008). *Hawai'i Div. Aquat. Resour. Rep.*

Walsh, W., Russell, S., Barnett, C., Couch, C. S., Cotton, S., White, D., et al. (2010). Long-Term Monitoring of Coral Reefs of the Main Hawaiian Islands.

Wear, S. L., and Thurber, R. V. (2015). Sewage pollution: mitigation is key for coral reef stewardship. *Ann. N. Y. Acad. Sci.* 1355, 15–30.

Whittier, R., and El-Kadi, A. (2014). Human Health and Environmental Risk Ranking of On-site Sewage Disposal Systems for the Hawaiian Islands of Kaua'i, Moloka'i, Maui and Hawai'i. Available at: https://health.hawaii.gov/wastewater/files/2015/09/OSDS_NI.pdf.

Wiegner, T. N., Mokiao-Lee, A. U., and Johnson, E. E. (2016). Identifying nitrogen sources to thermal tide pools in Kapoho, Hawai'i, USA, using a multi-stable isotope approach. *Mar. Pollut. Bull.* 103, 63–71.

Williams, G. J., Aeby, G. S., Cowie, R. O. M., and Davy, S. K. (2010). Predictive Modeling of Coral Disease Distribution within a Reef System. *PLoS One* 5, e9264. doi:e926410.1371/journal.pone.0009264.

Willis, B. L., Page, C. A., and Dinsdale, E. A. (2004). "Coral Disease on the Great Barrier Reef," in *Coral Health and Disease*, eds. E. Rosenberg and Y. Loya (Berlin: Springer-Verlag), 69–104.

Work, T. M., Aeby, G. S., and Coles, S. L. (2008). Distribution and morphology of growth anomalies in Acropora from the Indo-Pacific. *Dis. Aquat. Organ.* 78, 255–264.

Yoshioka, R. M., Kim, C. J. S., Tracy, A. M., Most, R., and Harvell, C. D. (2016). Linking sewage pollution and water quality to spatial patterns of Porites lobata growth anomalies in Puako, Hawaii. *Mar. Pollut. Bull.* 104, 313–321.

Young, William J, Marston, Frances M, & Davis, Richard J. (1996). Nutrient exports and land use in Australian catchments. Journal of Environmental Management, 47(2), 165-183.

Zaneveld, J. R., Burkepile, D. E., Shantz, A. A., Pritchard, C. E., McMinds, R., Payet, J. P., et al. (2016). Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nat. Commun.* 7, 11833.