### NOAA Coral Reef Conservation Program Project Progress Report

I. Recipient: Marine Science Department, University of Hawai'i at Hilo

II. Project Title: Spatial distribution and effects of sewage on Puakō's (Hawai'i) coral reefs

III. Award Number: NA14NOS4820087

IV. Award Period: July 1, 2014 - December 31, 2016 (approved no cost extension)

V. Period Covered by this Report: August 1, 2016 – January 31, 2017

### VI. Report

**A.** *Introduction.* Hawai'i's coral reefs contribute ~\$800 million dollars annually to the state's economy. Unfortunately, these coral reefs are declining as a result of multiple stressors. Sewage from cesspools is one of most devastating stressors in rural areas where reefs are still relatively healthy. Cesspools are used more widely in Hawai'i than any other state in the U.S., and their discharge of pathogens, nutrients, cleaning chemicals, and hydrocarbons pose a threat to coral reef and human health. Hence, Hawai'i State's Coral Reef Strategy, Objective 1, is to reduce key anthropogenic threats to near-shore reefs. Puakō, a coastal community on Hawai'i Island, is located within one of the two priority sites in the state identified for site-based actions.

While Puakō's coral reefs are some of the richest in Hawai'i State, there has been increasing concern about sewage pollution since the 1960s. Hawai'i's Division of Aquatic Resources (HDAR) found Puako's reefs to be in 'dire straits', with coral cover decreasing 35% and turf and macroalgae cover increasing 38% over the last 30 years. The Puakō Community Association (PCA) contacted the University of Hawai'i at Hilo (UH Hilo) and requested a study to determine whether sewage was entering their coastal waters and impacting their reef. To do this, dye tracer tests,  $\delta^{15}$ N macroalgal and fecal indicator bacteria (FIB) measurements, as well as water quality and benthic sampling, surface and benthic water quality mapping, and coral pathogen testing were conducted. With data from UH Hilo's study, PCA will have scientifically-defensible results that will demonstrate to Hawai'i County and State the urgency to remove cesspools from their community and to replace them with an improved sewage treatment system. Options under consideration include: 1) building an on-site sewage treatment plant, 2) connecting homes within their community to an existing sewage treatment plant at the Mauna Lani through construction of a sewer line, or 3) replacing their cesspools with aerobic treatment units (ATU). Removal of cesspools will improve water quality at Puakō and help mitigate coral disease, future coral cover loss, and reduce human health hazards.

**B.** *Purpose.* In November 2013, PCA contacted UH Hilo's Marine Science Department and requested that they conduct a study to determine whether sewage was entering their coastal waters and impacting their reefs. They wanted to document the presence of sewage in their near-shore waters to convince Hawai'i County and State of the urgency to improve sewage collection and treatment in their community. Data collected by UH Hilo, as part of this study, is providing PCA with baseline data to compare to following any sewage collection and treatment upgrade efforts, and allowing them to evaluate whether those upgrades were effective. PCA would like to be a model community for Hawai'i Island and State with regards to a community-based initiative to improve near-shore water quality and coral reef health. Hawai'i State needs

examples like Puakō to help convince the public that a cesspool ban is necessary to improve coastal water quality and decrease the health risks to recreational water users. In 2015, Hawai'i's Department of Health (HDOH) revised its proposed 2014 cesspool ban and it was signed into legislation. It bans construction of new cesspools and provides a tax credit to homeowners near waterbodies who <u>voluntarily</u> remove their cesspools and replace them with septic tanks, ATU, or connect to an existing sewer line.

In collaboration with PCA, goals and objectives to address their sewage pollution issue were derived. The <u>Project's Goals were to</u>: (1) use chemical and biological approaches to determine if sewage pollution was entering near-shore waters with coral reefs, (2) determine whether the sewage pollution was impacting water quality, and (3) assess whether the sewage pollution was eliciting a community-level response on the reef. The <u>Project's</u> <u>Objectives were to:</u> (1) determine the connectivity between domestic onsite sewage disposal systems (OSDS) and adjacent coastal waters through dye tracer tests, (2) evaluate the presence of sewage in near-shore waters through  $\delta^{15}$ N measurements in macroalgal tissues and FIB, (3) determine if state water quality standards were exceeded in Puakō waters through FIB measurements, and (4) assess whether there was coral reef community response to sewage through measurements of benthic cover.

### **D.** Accomplishments and Results to Date. The UH Hilo Marine Science research team has

Checks ( $$ ) indicate complete extension was awarded until 1 2017.													ry	
20171							Year							
	2014 -	2015										2	2016	2017
Task	J - J	F	M	A 1	M	J	J	Α	S	O	N	D	J-J	A-J
1. Community/outreach			<b>V</b>					1				√	√	<b>√</b>
events/advisory board														
2. Planning/preparation														
-Hire personnel						$\checkmark$								
-Order equipment/supplies						$\checkmark$								
-Draft work plan/schedule														
-Permit applications														
-GIS site maps														
-Database preparation														
3. Personnel training														
-Equipment use														
-Water sampling														
-δ 15N macroalgal assay														
4. Initial sampling														
-Water sampling/mapping	$\sqrt{}$													
-Macroalgal sampling														
-δ <sup>15</sup> N macroalgal assay														
-Final site selection														
5. Project Sampling														
-Dye trace studies														
-Water sampling/mapping														
-δ 15N macroalgal assay														
-Benthic community														
structure														
6. Data Analyses						,	,	,	,	,	,	,	,	
-Sample processing			V				√.		$\sqrt{}$	$\sqrt{}$	V	V	√.	
-Statistical analysis														
7. Reporting														
-Progress reports	$\sqrt{}$						√							$\checkmark$
-Presentations														
-Final report														X

successfully accomplished all, but one of the tasks outlined in the proposal (Table 1). The remaining task is the final report due March 31, 2017. Additionally, findings have been presented at meetings and conferences, 1-page project summaries for the general public have been generated and circulated, community outreach events have been attended, undergraduate and graduate students have been trained, and a conference session was organized. Below, accomplishments and results for each objective are described.

Objective 1: In order to determine the connectivity of OSDS with near-shore coastal

waters at Puakō, groundwater seeps that may be transporting sewage were identified during low tide when groundwater influence is greatest and easiest to detect through measurements of surface water salinity. These data were then used to make a near-shore surface salinity map. This map was used to identify ideal locations for dye tracer tests and sampling stations for Objectives 2-4 (Fig. 1).

Based on the location of the groundwater seeps, as well as cooperating homeowners, dye tracer tests were completed at four oceanfront homes' OSDS, three were cesspools in the southern portion of Puakō, and one

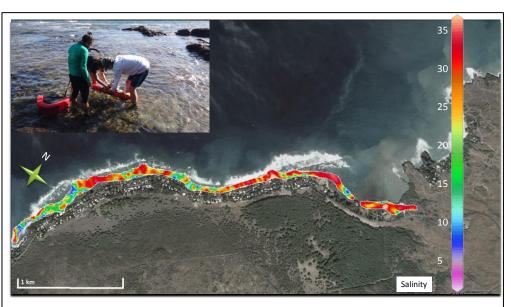
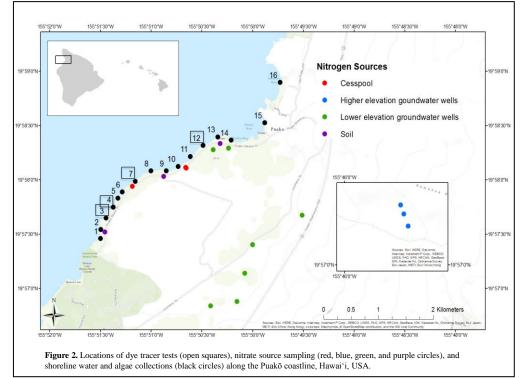


Figure 1. Map of surface salinity along the Puakö shoreline (June 2014). The map was created using a YSI 6600 sondes and GPS. Shown in the picture is Dr. Steve Colbert with two of the three summer interns (NSF REU program at UH-Hilo) at the beginning of the surface salinity mapping effort.



was a fractured ATU (not in use) in the central portion of the community (Fig. 2, black squares). Five stations along the shoreline in front of each home were sampled before and after the dye was added to the OSDS. Samples were analyzed for salinity and fluorescein (a non-toxic fluorescent dye). Fluorescein concentration vs. time data were used to calculate dye travel time, flow rate, and dilution before entering the near-shore waters. Dye was visually observed at the shoreline in front of all four homes. For each test, there was only one spring with dye, which was located on the beach in front of the home, suggesting that the groundwater flow between the

OSDS was restricted to specific fractures in the aquifer. At three homes, dye was only observed during low tide and was highly diluted (max. observed dye concentration =0.02% initial concentration). At the third home, while

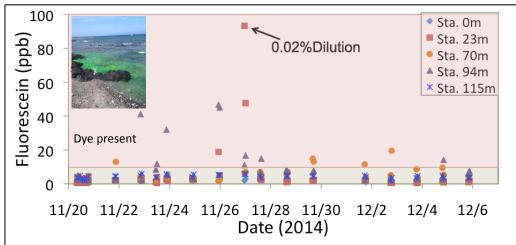


Figure 3. Time series of fluorescein dye concentration in near-shore waters of Puakō following dye injection into a cesspool (20 Nov 2014). Background fluorescence levels are indicated by the gray-shaded area. The concentration of the dye injected was 500 ppm. Dye was detected within three days of the initial release and continued to be detected for five more days (pink-shaded area). The dye was only detected at two sampling locations in front of the home and only observed during low tides. Inset picture is from dye tracer study conducted in November 2015. Here, the dye reached the shoreline in nine hours and persisted in nearshore waters for several days, unlike what was observed during the other three dye tracer tests.

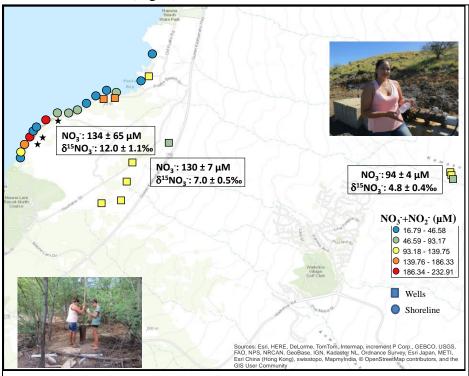
the same amount of dye was added to the OSDS, the discharge was much less diluted, and dye was visible during low and high tides for several days, as it was trapped in an area with little water circulation (Fig. 3, inset). The dye from these springs dispersed over an area between 0.25 to 4 m². Initial detection of fluorescein at the shoreline ranged from 0.4 to 9.3 days after release, and it continued to flow out during low tide over the next several days (Fig. 3). Three homes had comparable flow rates between 4 to 14 m/day; the OSDS at one home had a remarkably faster flow rate, where dye in the groundwater traveled 76 m/day. Based on dilution of the dye, the maximum fraction of sewage in the freshwater at the shoreline varied from <0.02 to 0.14%, depending on how much mixing occurred before shoreline discharge.

Objective 2: Three different approaches were used to evaluate the presence of sewage in near-shore surface and benthic waters. First, groundwater and shoreline waters were sampled and analyzed for nutrient concentrations and  $\delta^{15}N$  -NO<sub>3</sub><sup>-</sup> (*Upland well measurements* section). Second, macroalgal tissues and nearshore waters were collected along the shoreline for  $\delta^{15}N$  and FIB analyses, respectively (*Shoreline measurements* section); FIB data are discussed in Objective 3's results. Finally, macroalgal tissues were deployed in surface and benthic cages and analyzed for  $\delta^{15}N$ , with concurrent nutrient and FIB water measurements at cage stations (*Cage deployment* section).

Upland well measurements—During January 2015, upland groundwater samples were collected from drinking (high elevation, n=3) and irrigation (low elevation, n=7) wells within the Puakō watershed (Fig. 2, blue and green circles). Samples were analyzed for nutrient concentrations and  $\delta^{15}$ N-NO<sub>3</sub>. These samples were taken as part of the N source  $\delta^{15}$ N-NO<sub>3</sub>.

determination effort (*see Shoreline measurements* below). Water samples were also collected at 16 shoreline stations for nutrient analyses as part of the *Shoreline measurements* described below.  $\delta^{15}\text{N-NO}_3^-$  was quantified only once at three shoreline stations (3, 4, and 7), as they were suspected of being contaminated with sewage pollution.

 $NO_3^- + NO_2^-$  concentrations were ~ 40 µM lower in high elevation wells compared to the low elevation wells (Fig. 4). In contrast,  $PO_4^{3-}$  and  $NH_4^+$  concentrations were similar between



**Figure 4.** Nitrate + nitrite (NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub>) concentrations ( $\mu$ M) and  $\delta$ <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> (‰) in up-mountain groundwater and shoreline coastal waters. Shoreline waters at some locations have concentrations ~70- 120  $\mu$ M higher than up-mountain groundwater

high and low elevation wells (Table 2).  $NO_3 + NO_2$ concentrations increased ~70 to 120 µM from the high elevation groundwater wells to the shoreline stations. Comparable increases in PO<sub>4</sub><sup>3-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were not observed.  $\delta^{15}$ N-NO<sub>3</sub> became increasing enriched downslope from the high elevation groundwater wells to the shoreline stations (Table 2). Additionally, nutrient concentrations (NO<sub>3</sub>

<b>Table 2.</b> Average $\pm$ SE of $\delta$ <sup>15</sup> N - NO <sub>3</sub> (‰) and NO <sub>3</sub> + NO <sub>2</sub> , PO <sub>4</sub> <sup>3</sup> -, and NH <sub>4</sub> + concentrations ( $\mu$ M) of N sour	ces collected in the
Puakō watershed. (n = sample size)	

N Source	n	$\delta$ <sup>15</sup> N in NO <sub>3</sub> -	$NO_3^- + NO_2^-$	$\mathrm{NH_4}^+$	PO <sub>4</sub> <sup>3-</sup>
Cesspools	3	$10.45 \pm 0.58$	$20.76 \pm 10.50$	6370.00 ± 806.16	378.58 ± 16.59
Soil	3	$2.13 \pm 2.37$	6366.67 ± 3682.45	594.52 ± 93.24	193.56 ± 141.56
Ocean	2	$3.02 \pm 0.79$	1.43 ± 0.07	$2.53 \pm 0.55$	0.11 ± 0.05
High elevation groundwater wells	3	$4.76 \pm 0.43$	93.87 ± 4.35	4.84 ± 1.43	2.48 ± 0.19
Low elevation groundwater wells	7	$7.03 \pm 0.50$	130.09 ± 6.69	4.82 ± 1.19	2.47 ± 0.54
Shoreline	3	11.95 ± 1.13	133.93 ± 64.68	n/a	n/a

+ NO<sub>2</sub><sup>-</sup>, TDN, PO<sub>4</sub><sup>3-</sup>, TDP, and H<sub>4</sub>SiO<sub>4</sub>) significantly differed among shoreline stations (p <0.001; Table 3). NH<sub>4</sub><sup>+</sup> concentrations were similar across all shoreline stations.

Comparison of NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> concentration data from high and low elevation groundwater wells with nearshore coastal waters indicate that there is some source between these two locations adding NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> to the water (Fig. 4). The observation that NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> concentrations increased from low elevation wells (Mauna Lani Resort just above Puakō and

Puakō on the mountain-side of the street) to the nearshore waters suggests that leakage from OSDS is a likely source. Enrichment of  $\delta^{15}$ N-NO<sub>3</sub> from the low elevation groundwater wells to the shoreline further suggest OSDS leakage is the source, as shoreline values were within range reported for sewage (Table 2). Results from our dye tracer tests confirm that OSDS are the source, as dye was detected at in front of the homes with the highest NO<sub>3</sub>-NO<sub>2</sub> concentrations

**Table 3.** Average  $\pm$  SE and [range] of NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, TDN, PO<sub>4</sub><sup>3-</sup>, TDP, H<sub>4</sub>SiO<sub>4</sub> concentrations ( $\mu$ M), and salinity for shoreline stations at Puakō. Superscript letters indicate significant groupings from One-way ANOVA and post-hoc Tukey's test.  $\alpha$  = 0.05; n = 4.

Station	$NO_3^- + NO_2^-$	NH <sub>4</sub> <sup>+</sup>	TDN	PO <sub>4</sub> 3-	TDP	H <sub>4</sub> SiO <sub>4</sub>	Salinity
	27.87 ± 4.09 <sup>b-e</sup>	20.83 ± 0.15	41.4 ± 6.8 <sup>c-f</sup>	$0.44 \pm 0.04^{fg}$	$0.70 \pm 0.12^{fg}$	132.61 ± 22.80 <sup>a-c</sup>	27.58 ± 1.44 <sup>a-c</sup>
1	[18.10-36.79]	[0.78-1.23]	[24.6-57.5]	[0.33-0.51]	[0.51-1.04]	[86.85-195.35]	[23.63-30.37]
	149.94 ± 12.79ab	$0.49 \pm 0.11$	158.7 ± 12.8ab	$2.24 \pm 0.24^{a-d}$	$2.86 \pm 0.26^{a-e}$	580.91 ± 154.78ab	$7.12 \pm 0.61^{e}$
2	[129.62-187.09]	[0.18 - 0.72]	[139.2-194.6]	[1.62-2.73]	[2.21-3.45]	[187.35-875.96]	[5.77-8.70]
	137.12 ± 35.39 <sup>a-c</sup>	$1.95 \pm 0.30$	153.6 ± 39.4 <sup>a-c</sup>	$3.81 \pm 0.92^{ab}$	$4.28 \pm 0.72^{ab}$	376.56 ± 124.15a-c	16.26 ± 3.96 <sup>b-e</sup>
3	[36.22-190.37]	[1.04-2.29]	[41.2-217.1]	[1.34-5.37]	[2.42-5.09]	[112.21-646.18]	[9.50-25.73]
	196.05 ± 28.14a	$1.34 \pm 0.05$	221.3 ± 26.0a	$7.42 \pm 1.11^{a}$	$8.25 \pm 1.36^{a}$	501.07 ± 113.17ab	15.25 ± 2.30 <sup>c-e</sup>
4	[125.66-263.07]	[1.24-1.47]	[153.2-267.1]	[4.12-9.0]	[4.45-10.84]	[172.26-683.13]	[9.10-20.20]
	46.92 ± 8.73 <sup>a-e</sup>	$1.32 \pm 0.16$	70.2 ± 11.8 <sup>a-f</sup>	$1.34 \pm 0.17^{b-f}$	$1.74 \pm 0.28$ <sup>b-f</sup>	179.13 ± 40.75a-c	24.98 ± 2.35a-d
5	[23.44-65.52]	[0.86-1.57]	[41.5-86.7]	[0.90-1.71]	[0.90-2.13]	[85.38-278.15]	[19.70-31.07]
	$26.78 \pm 11.48^{de}$	$1.22 \pm 0.10$	$43.7 \pm 15.9^{d-f}$	$0.66 \pm 0.21^{e-g}$	$0.85 \pm 0.22^{fg}$	95.35 ± 42.89°	30.77 ± 2.31a
6	[2.50-54.16]	[1.03-1.46]	[22.5-86.4]	[0.25-1.17]	[0.25-1.26]	[21.60-219.16]	[24.53-35.53]
	134.56 ± 54.94a-d	$1.69 \pm 0.65$	130.5 ± 42.7a-d	$3.08 \pm 0.44^{a-c}$	$3.41 \pm 0.50^{a-c}$	446.70 ± 132.37ab	$21.98 \pm 0.97^{a-d}$
7	[42.27-285.74]	[0.46-2.90]	[52.5-240.8]	[2.12-3.83]	[2.19-4.51]	[164.00-803.60]	[19.87-24.03]
	39.15 ± 14.53 <sup>c-e</sup>	$2.40 \pm 0.97$	$59.0 \pm 18.5^{b-f}$	$0.70 \pm 0.23^{e-g}$	$1.01 \pm 0.21^{e-g}$	252.83 ± 83.24 <sup>a-c</sup>	$20.60 \pm 4.90^{a-d}$
8	[0.99-67.10]	[0.53-5.07]	[12.3-98.5]	[0.52-1.07]	[0.56-1.55]	[31.05-416.30]	[14.10-35.17]
	69.74 ± 9.06 <sup>a-e</sup>	$1.00 \pm 0.33$	$85.2 \pm 7.3^{a-e}$	$1.37 \pm 0.13^{b-f}$	$1.80 \pm 0.17^{b-f}$	341.87 ± 89.74 <sup>a-c</sup>	15.28 ± 2.31 <sup>cd</sup>
9	[47.81-91.92]	[0.89-1.77]	[73.6-105.4]	[1.15-1.73]	[1.48-2.30]	[219.17-608.54]	[8.53-18.53]
	56.72 ± 17.48 <sup>a-e</sup>	$0.95 \pm 0.27$	$73.1 \pm 19.0^{\text{b-f}}$	1.14 ± 0.31 <sup>c-g</sup>	$1.48 \pm 0.16^{b-f}$	354.04 ± 75.56a-c	$15.03 \pm 3.60^{de}$
10	[11.59-94.94]	[0.47-1.51]	[19.7-106.1]	[0.34-1.84]	[1.18-1.84]	[129.10-444.74]	[4.90-21.90]
	16.52 ± 1.21 <sup>de</sup>	$0.96 \pm 0.30$	29 ± 3.9ef	$0.49 \pm 0.04^{e-g}$	$0.76 \pm 0.22^{fg}$	108.26 ± 26.71bc	$28.30 \pm 0.93^{ab}$
11	[14.08-18.73]	[0.18-1.45]	[23.2-40.5]	[0.40-0.58]	[0.25-1.33]	[52.94-172.90]	[26.07-30.60]
	$35.80 \pm 4.37^{a-e}$	$1.34 \pm 0.25$	$46.4 \pm 4.7^{b-f}$	$0.99 \pm 0.11^{c-g}$	$1.26 \pm 0.29^{c-g}$	259.66 ± 104.79a-c	$24.50 \pm 0.96^{a-d}$
12	[25.62-46.59]	[0.78-1.88]	[34.2-55.6]	[0.40-1.31]	[0.91-2.11]	[111.52-567.91]	[22.57-27.13]
	34.89 ± 4.73 <sup>a-e</sup>	1.21 ± 0.19	$48.5 \pm 6.7^{b-f}$	$1.64 \pm 0.28^{b-e}$	$1.89 \pm 0.17^{b-f}$	207.44 ± 23.43 <sup>a-c</sup>	$23.96 \pm 2.00^{a-d}$
13	[22.54-44.18]	[0.73-1.56]	[34.5-66.9]	[0.91-2.29]	[1.66-2.38]	[166.70-267.48]	[19.90-28.27]
	89.08 ± 5.48 <sup>a-d</sup>	$1.15 \pm 0.29$	100.9 ± 6.9a-d	$2.61 \pm 0.17^{a-c}$	$2.91 \pm 0.27^{a-d}$	651.66 ± 173.89a	$6.43 \pm 0.63^{e}$
14	[75.93-101.22]	[0.64-1.54]	[83.7-117.1]	[2.22-2.98]	[2.35-3.61]	[358.62-1017.63]	[5.33-8.07]
	13.37 ± 2.80e	$1.07 \pm 0.17$	$21.6 \pm 2.6^{f}$	$0.39 \pm 0.09^{g}$	$0.57 \pm 0.21^{g}$	120.33 ± 24.28a-c	$29.94 \pm 0.70^{a}$
15	[5.73-19.24]	[0.75-1.44]	[14.8-27.4]	[0.16-0.55]	[0.25-1.12]	[52.40-157.86]	[28.67-31.27]
	38.53 ± 7.17 <sup>a-e</sup>	$0.63 \pm 0.31$	$45.8 \pm 4.1^{c-f}$	$0.81 \pm 0.13^{d-g}$	$1.14 \pm 0.30^{d-g}$	322.79 ± 86.47a-c	17.13 ± 3.44 <sup>b-e</sup>
16	[17.35-47.44]	[0.18-1.51]	[33.8-51.7]	[0.45-1.09]	[0.60-1.99]	[141.63-552.47]	[7.94-24.53]

and most enriched  $\delta^{15}$ N-NO<sub>3</sub> values.

Additionally, the change in the  $\delta^{15}$ N-NO<sub>3</sub> from the high to low elevation groundwater wells suggests a change in NO<sub>3</sub> source from forest soil to sewage (Table 2). It is possible that sewage is contaminating the low elevation groundwater as an upslope development (Waikoloa Village) has over 4,800 people whose homes have OSDS (U.S. Census Bureau 2000). Additionally, NO<sub>3</sub> concentrations increased ~40  $\mu$ M from the high to low elevation groundwater wells (Table 2).

Shoreline measurements  $-\delta^{15}N$  measurements in near-shore macroalgal tissues were used to identify locations with sewage pollution along the Puakō coastline. Sixteen stations were identified as sampling locations based on the surface salinity map (Figs. 1 and 2, black circles). At each station, the macroalgal community was characterized, and the most predominant species were collected and analyzed for  $\delta^{15}N$  (species included: *Ulva fasciata*, *Cladophora* spp., and *Gelidiella acerosa*). For this study, a pilot collection at six stations occurred during July 2014, four full sampling efforts occurred in November 2014, and March, June, and July 2015, and

sampling at five stations (algal cage deployment shoreline stations) continued monthly from September 2015 through February 2016. In September 2015, several new stations south and north of Puakō were sampled to address concerns of residents that resorts in these areas might be contributing to their local pollution problem.

In January, February, and June 2015, potential N sources (sewage, fertilizers, upmountain groundwater, soil under Kiawe trees, ocean water) were sampled and analyzed for  $\delta^{15}$ N-NO<sub>3</sub><sup>-</sup> (Fig. 2, blue, green, red, purple circles).  $\delta^{15}$ N fertilizer values from another study on Hawai'i Island were used in our study (Wiegner et al. 2016). Additionally, in September 2015, shoreline water samples were collected and analyzed at three of the 16 stations (stations 3, 4, and 7) where sewage was thought to be most concentrated for  $\delta^{15}$ N-NO<sub>3</sub><sup>-</sup> analyses. N source values were compared to those in the macroalgal tissues and at water at the three shoreline stations to help identify sources of N pollution at Puakō.

The  $\delta^{15}$ N macroalgal tissue values ranged from 4.23 to 11.88% across all 16 shoreline stations and significantly differed among them (p<0.0001), with stations 3 and 4 being the most

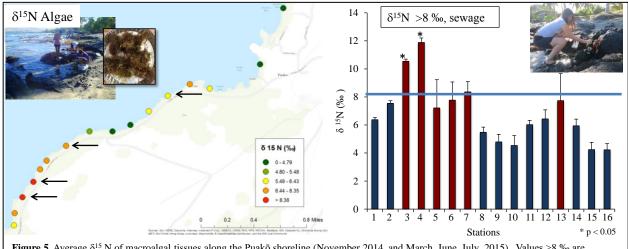
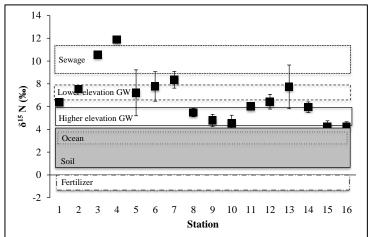


Figure 5. Average  $\delta^{15}$  N of macroalgal tissues along the Puakō shoreline (November 2014, and March, June, July 2015). Values >8 ‰ are indicative of sewage pollution (light blue line). Arrows indicate location of dye tracer tests.

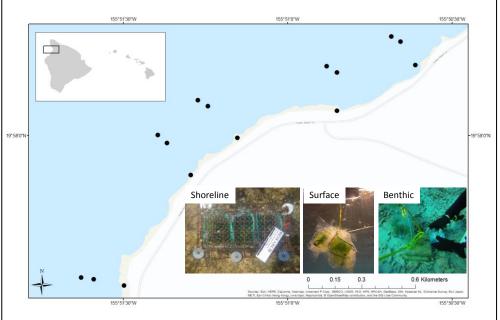


**Figure 6.** Average  $\pm$  SE  $\delta^{15}$  N (‰) of macroalgae found at 16 stations in Puakō. Background areas represent (average  $\pm$  SE)  $\delta^{15}$  NO<sub>3</sub><sup>-</sup> of the N sources (fertilizer, soil, ocean, high elevation groundwater wells, low elevation groundwater wells, and sewage; Table ) measured as part of this study. Fertilizer values are from previous sewage study on Hawai'i Island (Wiegner et al. 2016).

enriched (Fig. 5). Overall, six of the 16 stations fell within the sewage  $\delta^{15}$ N-NO<sub>3</sub> range, including stations 3 and 4, as well as 5, 6, 7, and 13 (Fig. 6, encompassing SE of source averages). The remaining stations fell within the high and low elevation groundwater ranges (Fig. 6). These results suggest that Stations 3 and 4 are two sewage pollution hotspots. However, past studies have found that macroalgae assimilate N more rapidly under low NO<sub>3</sub> concentrations (Fujita 1985), and that  $\delta^{15}$ N in macroalgal tissue can be underestimated by up to 6% in waters with high NO<sub>3</sub> concentrations (>10 μM) (Swart et al. 2014). All of the

stations had  $NO_3^- + NO_2^-$  concentrations exceeding 10  $\mu$ M, suggesting that the  $\delta^{15}N$  macroalgal values may be underestimated. If this is the case, then all 16 stations fall within the sewage range. From these measurements, sewage pollution appears to be widespread along the Puakō shoreline with some areas having more concentrated pollution (Fig. 5). Similar patterns were not observed in front of the resorts;  $\delta^{15}N$  macroalgal ranged from  $\delta^{15}N$  -1.0% to +0.1%, the range reported for fertilizers (shown on Fig. 6).

Cage deployments— To determine the spatial extent of sewage pollution offshore, as well as possible inputs from benthic seeps that could directly impact the coral reefs, water was sampled for FIB and nutrients. Additionally, the native green macroalga, *Ulva fasciata*, was



**Figure 7.** Location of water sample collection (for FIB and nutrients) and algal cage deployments (for  $\delta$  <sup>15</sup>N in *U. fasciata*). Water and macroalgal samples were taken at three zones (shoreline, bench, deep) in Puakō to determine the spatial extent of sewage pollution in surface and benthic waters offshore. Pictures of algal cage deployment design are shown in lower right corner of figure.

deployed during bioassays for  $\delta^{15}N$ analysis at five stations (Fig. 7). These stations encompassed three zones (shoreline, bench, and slope) and two depths (surface and benthic) (Fig. 7). Benthic zones were chosen based on physiography features. The bench zone was ~7 m deep, and ~196 m from the shoreline. The slope one was ~15 m in depth, and

~267 m from the shoreline. The bench and slope zones were ~65 m apart. Collection of water samples and algal cage deployments were conducted in June and July 2015. There was one sample collection and cage deployment per month. Additionally, wild algae from the benthos were also collected for  $\delta^{15}N$  analyses at all algal cage deployment stations.

*Enterococcus* counts were similar among surface water zones, but significantly differed among benthic zones (p =0.04; Fig. 8A,D). The greatest differences in the benthos were detected between shoreline and slope zones, which were almost an order of magnitude different. In contrast, *C. perfringens* significantly differed among surface (p =0.01) and benthic (p <0.01) zones (Fig. 8 B,E). In surface waters, the largest differences were detected between shoreline and slope zones (Fig. 8B). Shoreline *C. perfringens* counts were also significantly higher compared to benthic bench and slope waters (Fig. 8E). Nutrient concentrations (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, TDN, PO<sub>4</sub><sup>3-</sup>, TDP, and H<sub>4</sub>SiO<sub>4</sub>) were highest on the shoreline in both surface (p <0.02) and benthic (p <0.01) waters (Table 4). Nutrient concentrations among zones in surface and benthic waters were similar between bench and slope zones. Salinity also varied among zones in both surface (p<0.01) and benthic waters (p<0.01), with the shoreline having the freshest (lowest) values (Table 4). δ<sup>15</sup>N in *U. fasciata* significantly varied in surface (p =0.01) and benthic zones

(p<0.01) (Fig. 8C,F). Shoreline values were the highest, followed by slope, and bench. Both  $\delta^{15}N$  for surface and benthic *U. fasciata* samples fell within the  $\delta^{15}N$  -  $NO_3^-$  range for soil, seawater, and low elevation groundwater at all zones (Fig. 9).

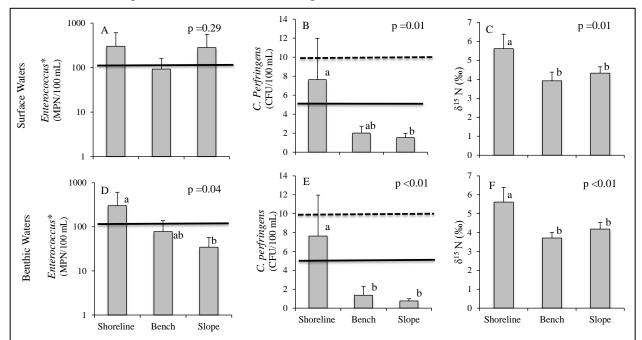


Figure 8. Average  $\pm$  SE of sewage parameters (A, D) *Enterococcus* (\*log scale), (B, E) *C. perfringens, and* (C, F)  $\delta^{15}$ N in *U. fasciata* collected within three zones (shoreline, bench, slope) in both surface and benthic waters in Puakō. Black lines represent the HDOH single sample maximum for *Enterococcus* (104 CFU/100 mL) and Fujioka's recommendation (1997) for *C. perfringens* in marine recreational waters (5 CFU/100mL). Dashed lines represent non-point source sewage contamination level of 10 CFU/100 mL for *C. perfringens* (Fung et al. 2007) Results from GLM and Tukey's test are shown, with different letters indicating significant differences (α =0.05). FIB n =10. Sample size varied for  $\delta^{15}$ N in *U. fasciata* in both surface waters (shoreline, n =9; bench, n =6; slope, n =10) and benthic waters (shoreline, n =9; bench, n =8; slope, n =10).

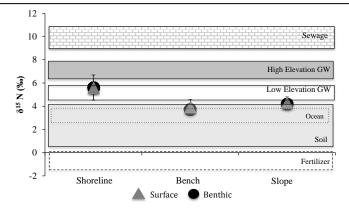
Averages of sewage indicators: *Enterococcus*, *C. perfringens*, nutrient concentrations  $(NO_3^- + NO_2^-, NH_4^+, TDN, PO_4^{3-}, and TDP)$ , and  $\delta^{15}N$  in *U. fasciata* were similar among water depths.  $H_4SiO_4$  concentrations did vary with the greatest differences detected between surface waters at the bench and benthic waters at the slope (p < 0.01). Salinity was similar between surface and benthic waters.

Pre- and post-deployment  $\delta^{15}N$  *U. fasciata* values differed (p <0.01), with the greatest differences occurring at the shoreline (Fig. 10). Within the slope zone, surface and benthic waters showed smaller differences in pre- and post-deployment  $\delta^{15}N$ , followed by the bench zone in surface and benthic waters.

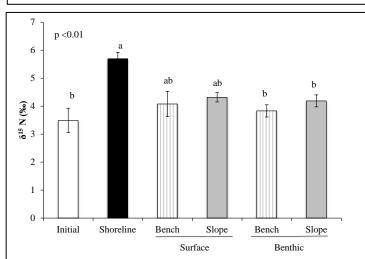
 $\delta^{15}$ N in benthic wild macroalgae and deployed cages were similar to one another, but differed from both wild and caged at the shoreline. Bench zone  $\delta^{15}$ N in wild algae ranged from -0.57 to +4.02% (average  $\pm$ SE; +2.90%  $\pm$  1.96), whereas caged bench zone *U. fasciata* ranged from +3.23 to +4.27%, (+3.83%  $\pm$  0.49). In the slope zone,  $\delta^{15}$ N in wild algae ranged from +3.48 to +8.92% (+6.09%  $\pm$  2.31) and deployed *U. fasciata* ranged from +3.50 to +4.78% (+4.19%  $\pm$  0.48). Wild shoreline algae ranged from +5.07 to +10.18% (+7.75%  $\pm$  1.25) and caged *U. fasciata* ranged from +3.37 to +7.27% (+5.61%  $\pm$  1.08). The highest shoreline  $\delta^{15}$ N values in both wild and caged macroalgae were observed at station 2.

**Table 4.** Average  $\pm$  SE and [range] of nutrient concentrations ( $\mu$ M) and salinity for surface and benthic water samples among zones (shoreline, bench, slope) in Puakō. A GLM was used and superscript letters indicate grouping from post hoc Tukey's test.  $\alpha = 0.05$ ; n = 10.

Zone	$NO_3^- + NO_2^-$	$NH_4^+$	TDN	PO <sub>4</sub> <sup>3-</sup>	TDP	$H_4SiO_4$	Salinity
Shoreline	$66.87 \pm 11.47^{a}$	$1.52 \pm 0.16^{a}$	$72.9 \pm 11.4^{a}$	$1.67 \pm 0.22^{a}$	$1.98 \pm 0.22^{a}$	$439.18 \pm 74.06^{a}$	$18.52 \pm 3.08^{a}$
	[11.59 - 139.72]	[0.18 - 3.05]	[21.1 - 120.6]	[0.47 - 2.56]	[0.70 - 3.25]	[153.57 - 616.73]	[3.78 - 29.63]
Surface							
Bench	$1.43 \pm 0.26^{b}$	$0.57 \pm 0.14^{b}$	$9.8 \pm 0.5^{b}$	$0.14 \pm 0.03^{b}$	$0.64 \pm 0.13^{b}$	$7.34 \pm 3.07^{b}$	$33.26 \pm 1.11^{b}$
	[0.83 - 1.84]	[0.18 - 1.56]	[7.9 - 11.7]	[0.02 - 0.27]	[0.25 - 1.23]	[1.31 - 20.92]	[29.95 - 34.47]
Slope	$1.23 \pm 0.18^{b}$	$0.38 \pm 0.11^{b}$	$9.4 \pm 0.6^{b}$	$0.12 \pm 0.02^{b}$	$0.59 \pm 0.11^{b}$	$5.00 \pm 1.42^{b}$	$34.24 \pm 0.41^{b}$
	[0.40 - 2.14]	[0.18 - 1.06]	[6.5 - 13.0]	[0.02 - 0.24]	[0.25 - 0.96]	[1.21 - 11.10]	[33.75 - 34.62]
<b>Benthic</b>							
Bench	$1.10 \pm 0.13^{b}$	$0.50 \pm 0.12^{b}$	$9.5 \pm 0.6^{b}$	$0.18 \pm 0.05^{b}$	$0.58 \pm 0.11^{b}$	$2.16 \pm 0.78^{b}$	$33.55 \pm 0.95^{b}$
	[0.53 - 2.06]	[0.18 - 1.23]	[7.2 - 12.9]	[0.02 - 0.49]	[0.25 - 0.94]	[0.83 - 5.49]	[31.03 - 35.0]
Slope	$1.57 \pm 0.51^{b}$	$1.10 \pm 0.53^{ab}$	$8.8 \pm 0.7^{b}$	$0.24 \pm 0.11^{b}$	$0.94 \pm 0.29^{b}$	$0.65 \pm 0.11^{b}$	$34.46 \pm 0.30^{b}$
	[1.10 - 6.09]	[0.18 - 5.58]	[7.0 - 13.3]	[0.02 - 1.13]	[0.25 - 3.25]	[0.55 - 0.99]	[34.22 - 34.85]



**Figure 9.** Average  $\pm$  SE  $\delta^{15}$  N (‰) of *U. fasciata* deployed within three benthic zones (shoreline, bench, slope) in Puakō. Background areas represent average  $\pm$  SE of  $\delta^{15}$  N – NO $_3$  of the N sources and fertilizer from another study on Hawai'i Island (Wiegner et al. 2016). Surface samples are represented by grey triangles and benthic samples by black circles.



**Figure 10.** Average  $\pm$  SE  $\delta^{15}$  N (‰) of *U. fasciata* pre-(initial) and post-deployments within three benthic zones (shoreline, bench, slope) and two depths (surface and benthic) in Puakō. GLM was used and shared lettering indicates no significant differences in Tukey's post hoc test. Sample size varied (initial, n =11; shoreline, n =5; surface bench, n =4; surface slope, n =5; benthic bench, n =5; benthic slope, n =50.05.

Sewage indicators (FIB,  $\delta^{15}$ N macroalgae, nutrients) were highest along the shoreline compared to values offshore in surface and benthic waters in both the bench and slope zones. These results suggest that sewage pollution is concentrated along the shoreline, and that low offshore values reflect smaller direct sewage inputs through benthic seeps or dilution of nearshore inputs.

Objective 3: To determine if state water quality standards are exceeded in Puakō's near-shore environment for FIB (*Enterococcus* and

C. perfringens), water samples were collected at 16 shoreline stations (Fig. 2, black circles). Values for these parameters were compared to state water quality standards to determine if state benchmarks were exceeded. Pilot sampling occurred at six stations during July 2014, four full shoreline samplings occurred November 2014, March, June, and July 2015, and five stations from September 2015 to February 2016. During November 2014, July 2015, and July 2016 samples were also collected for Bacteroides analysis. Bacteriodes are the most numerous bacteria in the human gut and there are molecular probes to identify those specifically from humans. Dr. Craig Nelson from

UH Mānoa, Center for Microbial Oceanography (C-MORE), School of Ocean and Environmental Sciences and Technology (SOEST) analyzed these samples using the BacHum-UCD and HF183 markers.

Our results indicate that FIB levels are quite variable and often higher than the HDOH standards at several stations (Fig. 11). For Enterococcus, 14 of the 16 stations had average values that were higher than the HDOH single sample maximum recreational water quality standard (no single sample shall exceed 104 MPN/100 mL; Fig. 11a). Eleven of the 16 stations also had C. perfringens values higher than the recommended standard to HDOH of 5 CFU/100 mL (Fig. 11b; Fujioka et al. 1997). Four of the stations also had values of 10 CFU/100 mL or higher which is indicative of non-point source sewage pollution (Fung et al. 2007). Overall, 11 of the 16 stations had Enterococcus and C. perfringens values that were both higher than established or recommended HDOH standards (Fig. 11). Lastly, one of the stations with high C. perfringens values was also one of the locations where a dye tracer test was conducted (Station 7); these results confirm that the high bacteria levels were from sewage pollution (Figs. 2 and 11). Eight stations (3, 4, 6, 8, 10, 11, 14, and 15) had positive hits for human Bacteriodes markers, two of which were dye tracer test locations (Fig. 12).

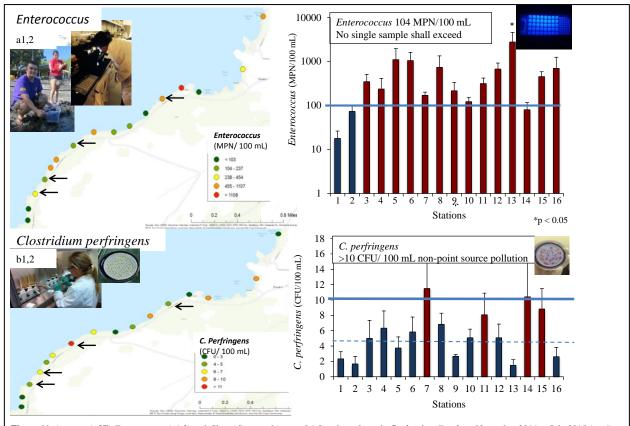


Figure 11. Average (±SE) Enterococcus (a1,2) and Clostridium perfringens (b1,2) values along the Puakō shoreline from November 2014 to July 2015 (n= 4). Red bars indicate values that are above established or recommended standards to HDOH (light blue lines). For Enterococcus, no single sample shall exceed 104 MPN/100 mL. For C. perfringens, the recommended standard for recreational water is 5 CFU/100 mL (solid line; Fujioka et al. 1997) and 10-100 CFU/100 mL is considered to be indicative of non-point sewage pollution (dashed line; Fung et al. 2007). Arrows are indicative of dye tracer tests.

In June 2015, shoreline water samples were also collected for *Staphylococcus aureus* analysis at the 16 stations (Fig. 13); sampling at five of these stations continued from September 2015 to February 2016. *S. aureus* is a human pathogen that can be found in sewage. It often causes skin infections that are thought to be acquired during recreational water use. Two stations

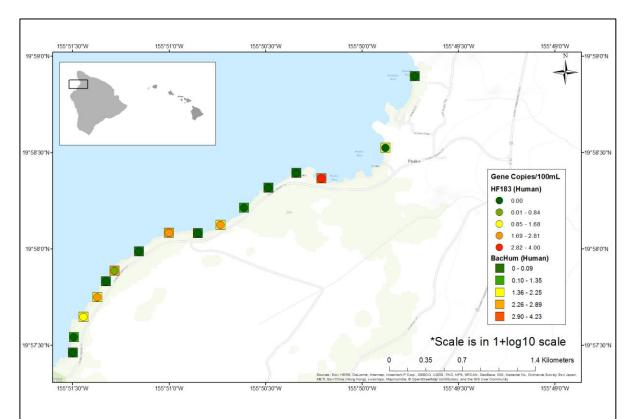
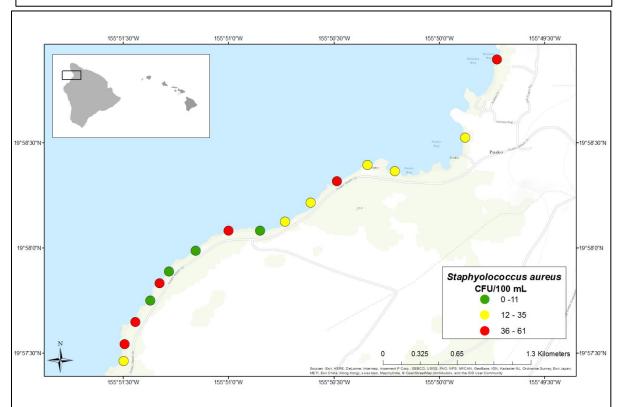


Figure 12. Human-associated *Bacteroides* in nearshore waters along the Puakō coastline (November 2014, July 2015, and July 2016). Two molecular markers were used to detect these bacteria (HF183 and BacHum). Data were log transformed ( $\log 10 (x+1)$ ).



**Figure 13.** *Staphylococcus aureus* counts in nearshore waters along the Puakō coastline (June 2015). There are no HDOH standards for *S. aureus* in recreational waters; however, it has been recommended that counts be lower than 100 CFU/ 100 mL in recreational waters (Shenawy 2005).

recreational waters (Shenawy 2005). Presently, there are no HDOH *S. aureus* water quality standards.

Objective 4: To assess the benthic community responses to sewage inputs at Puakō,

shoreline stations and the two primary coastal benthic environments (basalt bench and coraldominated fore-reef slope) were surveyed using standardized techniques during the two algal cage deployments in June and July 2015. Data from these surveys have been summarized (Tables 5 and 6). The majority of the shoreline stations were dominated with turf and basalt (Table 5). Benthic cover at the bench and slope stations consisted of turf, coral, and crustose coralline algae, with turf comprising the greatest percentage at the bench and coral at the slope (Table 6). Sampling for coral pathogens (Serratia marcescens and *Vibrio* spp.) occurred from

September 2015 to February 2016 at five

<b>Table 5</b> . Summary of benthic cover at 16 shoreline stations along the Puakō shoreline. Values are
presented as (%) cover. Eight major categories were summarized: basalt, coral, crustose coralline
algae (CCA), turf, macroalgae, limestone, sand, and invertebrates.

Station	Basalt	Coral	CCA	Turf	Macroalgae	Limestone	Sand	Invertebrates
1	51.5%	0.0%	0.0%	39.5%	0.0%	0.0%	9.0%	0.0%
2	10.7%	26.8%	2.8%	52.2%	0.0%	2.0%	5.0%	0.5%
3	87.5%	0.0%	0.0%	7.0%	0.0%	4.0%	1.5%	0.0%
4	38.0%	0.0%	0.0%	52.5%	0.0%	0.0%	9.5%	0.0%
5	11.0%	0.0%	0.0%	72.0%	0.0%	1.5%	15.5%	0.0%
6	12.7%	7.8%	11.0%	64.8%	0.0%	1.0%	2.7%	0.0%
7	18.2%	23.3%	10.5%	40.8%	0.0%	0.5%	6.5%	0.2%
8	27.0%	0.0%	0.0%	41.0%	0.0%	11.0%	21.0%	0.0%
9	8.3%	19.7%	8.3%	61.5%	0.0%	2.2%	0.0%	0.0%
10	23.5%	0.0%	0.0%	70.5%	0.0%	6.0%	0.0%	0.0%
11	4.8%	16.3%	18.7%	59.5%	0.0%	0.5%	0.0%	0.2%
12	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%
13	23.0%	0.0%	0.0%	77.0%	0.0%	0.0%	0.0%	0.0%
14	16.5%	0.0%	0.0%	79.0%	0.0%	0.0%	4.5%	0.0%
15	26.5%	0.0%	0.0%	73.5%	0.0%	0.0%	0.0%	0.0%
16	21.0%	0.0%	0.0%	78.0%	0.0%	0.0%	1.0%	0.0%

**Table 6**. Summary of benthic cover at deployments stations onshore at the two primary coastal benthic environments (bench and slope) in Puakō. Values are presented as (%) cover. Eight major categories were summarized: basalt, coral, crustose coralline algae (CCA), turf, macroalgae, limestone, sand, and invertebrates.

Station	Basalt	Coral	CCA	Turf	Macroalgae	Limestone	Sand	Invertebrate
2								
Shoreline	32.0%	0.0%	0.0%	61.5%	0.0%	6.0%	0.5%	0.0%
Bench	0.0%	35.5%	0.0%	63.0%	0.0%	0.0%	0.0%	1.5%
Slope	0.0%	45.0%	8.5%	32.0%	0.0%	0.0%	14.5%	0.0%
5								
Shoreline	38.0%	0.0%	0.0%	54.0%	0.0%	3.0%	5.0%	0.0%
Bench	0.0%	1.0%	20.0%	79.0%	0.0%	0.0%	0.0%	0.0%
Slope	0.0%	22.5%	13.0%	61.5%	0.0%	0.0%	3.0%	0.0%
7								
Shoreline	54.5%	0.0%	0.0%	44.5%	0.0%	0.5%	0.5%	0.0%
Bench	0.0%	26.0%	16.5%	37.5%	0.0%	1.0%	19.0%	0.0%
Slope	0.0%	44.0%	15.0%	40.5%	0.0%	0.0%	0.0%	0.5%
9								
Shoreline	25.0%	0.0%	0.0%	75.0%	0.0%	0.0%	0.0%	0.0%
Bench	0.0%	16.0%	13.0%	64.5%	0.0%	6.5%	0.0%	0.0%
Slope	0.0%	43.0%	12.0%	45.0%	0.0%	0.0%	0.0%	0.0%
11								
Shoreline	14.5%	0.0%	6.5%	77.5%	0.0%	1.5%	0.0%	0.0%
Bench	0.0%	12.0%	20.0%	67.5%	0.0%	0.0%	0.0%	0.5%
Slope	0.0%	37.0%	29.5%	33.5%	0.0%	0.0%	0.0%	0.0%

shoreline locations, and coincided with  $\delta^{15}N$  macroalgal tissue, FIB, and nutrient sample collection. Both pathogens were detected in the nearshore waters of Puakō.

<u>Development of a novel "Sewage Pollution Score":</u> As this study and others have shown, sewage indicators can provide conflicting information on the intensity and location of sewage pollution. In this study, for example, *Enterococcus* concentrations were highly variable among shoreline stations, with some exceeding HDOH standards, and station 13 having the

highest concentrations (Fig. 11a). In contrast, *C. perfringens* concentrations were similar among shoreline stations, but averages for stations 7, 11, 14, and 15 were in the non-point source sewage pollution range (Fig. 11b; Fung et al. 2007). Additionally, δ <sup>15</sup>N in macroalgal tissue were found to be highly variable along the shoreline, with six stations (3, 4, 5, 6, and 13) falling within the range of our sewage source value (Figs. 5 and 6, Table 2). Previous studies have confronted similar issues with their sewage indicator data (Shibata et al. 2004; Yoshioka et al. 2016). Hence, we developed a sewage pollution score using sewage indicators to more holistically assess sewage pollution in coastal waters. This score was developed in collaboration with The Nature Conservancy (TNC). Water quality scores and indices have been used successfully in the past to assess water quality conditions for both humans and ecosystems (Zambrano et al. 2009; Wang et al 2015).

Our scoring system used sewage indicators (FIB,  $\delta^{15}$ N macroalgae, and nutrients) and was applied to shoreline and offshore surface and benthic waters at Puakō. The scoring system 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 or literature information (Table 7). Specifically, the scoring system used HDOH's single sample maximum for

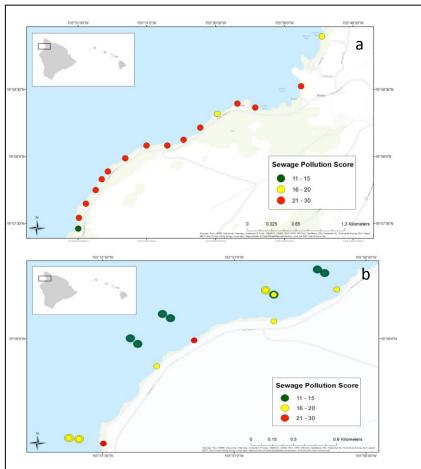
**Table 7.** Parameters (FIB = CFU/100 mL,  $\delta$   $^{15}N$  = ‰, and nutrients =  $\mu$ M) used to evaluate water quality along the Puakō coastline, as well as offshore surface and benthic waters. Sewage parameters were ranked (low = 1, medium = 2, high = 3), multiplied by a weight factor, and summed for a final sewage pollution score. \* "Medium" nutrient concentration ranks exceed HDOH standards for open coastal waters wet criteria.

	Weight	Low	Medium	High	
Sewage Parameter	Factor	(1)	(2)*	(3)	Reference
C. perfringens	3	0 - 10	11 - 100	101 - 505+	Fung et al. 2007
$\delta$ <sup>15</sup> N in	3	+2 - +7	-5 - +1.9	+7 - +20	Wiegner et al. 2016
macroalgae					
Enterococcus	2	0 - 35	36 - 104	105+	HDOH 2014
$NO_3^- + NO_2^-$	1	0 - 0.4	0.5 - 1	1.1 - 1.8 +	HDOH 2014
NH <sub>4</sub> <sup>+</sup>	1	0 - 0.25	0.26 - 0.61	0.61 - 1.07 +	HDOH 2014
TDP	1	0 - 0.7	0.8 - 1.3	1.4 - 1.9 +	HDOH 2014

Enterococcus concentrations in marine waters (HDOH 2014), the Fung/Fujioka *C. perfringens* scale for sewage pollution (Fung et al. 2007), δ <sup>15</sup>N values in macroalgal tissue for different N sources (*reviewed in* Wiegner et al. 2016), and HDOH's water quality standards for nutrient concentrations in open coastal waters (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, TDP) (HDOH 2014) (Table 7). Nutrient concentration standards for the wet criteria were used because the freshwater inputs along the Puakō shoreline ranged from 2083-2730 L m<sup>-1</sup> h<sup>-1</sup> (Paytan et al. 2006), an order of magnitude larger than the baseline for the wet criteria (>294 L m<sup>-1</sup> h<sup>-1</sup>). Two dissolved inorganic forms of N were chosen for the score system rather than TDN because the latter contains DON and there are no well-established patterns with this constituent for sewage pollution. TDP was used as the phosphorous water quality parameter since HDOH has no PO<sub>4</sub><sup>3-</sup> water quality standard for open coastal waters (HDOH 2014). It should also be noted that a 'medium' score in nutrient concentrations exceeds HDOH standards for open coastal waters wet criteria.

Once each indicator was assigned a level (1-3) based on its measured value and our scoring system (Table 7), its level was multiplied by a weight factor (1-3), with the most reliable sewage indicators having the greatest weight. The greatest weight (weight = 3) was given to C. perfringens and  $\delta^{15}N$  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* and nutrient concentrations (Fung et al 2007; Dailer et al. 2010; Viau et al. 2011; Yoshioka et al. 2016). *Enterococcus* 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 (Hardina & Fujioka 1991; Byappanahalli & Fujioka 1998; Byappanahalli & Fujioka 2004). Nutrient concentrations received the lowest weight (weight = 1) since sewage pollution is known to increase nutrient concentrations, but nutrients



**Figure 14.** Sewage pollution scores for the (a) shoreline and (b) algal cage deployment studies at Puakō. The score is based on standards and literature values for sewage indicators (FIB,  $\delta^{15}$  N in macroalgae, and nutrients). Sewage pollution score represents the following catergories: Low = 11 - 15; Medium = 16 - 20; High = 21 - 30.

can also come from other sources within the watershed and concentrations can vary over short time scales (Lapointe et al. 1990; David et al. 2013; Nelson et al. 2015). The equation for deriving the overall sewage pollution score for each station was: (C. perfringens level x 3) +  $(\delta^{15}N)$  macroalgae level x 3) + (Enterococcus level x)2) +  $(NO_3^- + NO_2^- \text{ level x 1})$ + (NH<sub>4</sub><sup>+</sup> level x 1) + (TDP)level x 1). Sewage pollution score categories were: 'low' = 11-15, 'medium' = 16-20, and 'high' = 21-30.

The shoreline stations with highest pollution sewage scores were station 7 (score =30) and 4 (30) (Fig. 14a). Note, that based on dye tracer tests, these two stations are known locations of OSDS

leakage. Station 3 (score = 27), another location of known OSDS leakage, had the third highest pollution score. Overall, 13 stations fell in the high category, two were medium, and one was low (Fig. 14a). These results confirm of the effectiveness of our score in identifying sewage pollution hotspots.

During the algal cage deployments, shoreline stations had the overall highest scores (medium and high), with stations 2 and 7 being the highest (Fig. 14b). As noted above, station 7 was a dye tracer test location (Fig. 2). Offshore transport or direct sewage discharge onto the reef through benthic seeps was localized, as stations 2 and 9 offshore surface and benthic waters only had medium sewage pollution scores (Fig. 14b). Most offshore stations fell in the low sewage pollution score category (Fig. 14b).

The sewage pollution score is an integrated approach that accurately identified sewage hotspots along the Puakō coastline. At these locations, it is critical for homes to remove their cesspools and employ better sewage treatment technology. These maps also provide information to the community on areas where community members may want to limit water exposure during recreational activities until sewage treatment is improved.

## E. Outreach. The UH Hilo Marine Science research team was involved in 25 outreach and advisory board events from July 2014 to January 2017 (Table 8). They met with PCA 10 times.

In June 2014, UH Hilo met

PCA to inform them of the funding of the proposal, review the objectives of the project, and introduce the research team. In August 2014, the team met with them during a NOAA CRCP site visit. UH Hilo also attended seven community association meetings: November 2014, January, April, August 2015, and January, April, and October 2016. At the November 2014 meeting, Dr. Wiegner gave a presentation and handed out a 1-page informational sheet on this project and its results to date (Fig. 15, see Appendix 1). In January 2015, UH Hilo attended PCA's meeting to answer any questions regarding this project, and how its results support the 'Puakō Sewage Disposal Upgrade Project' led by the Coral Reef Alliance. An

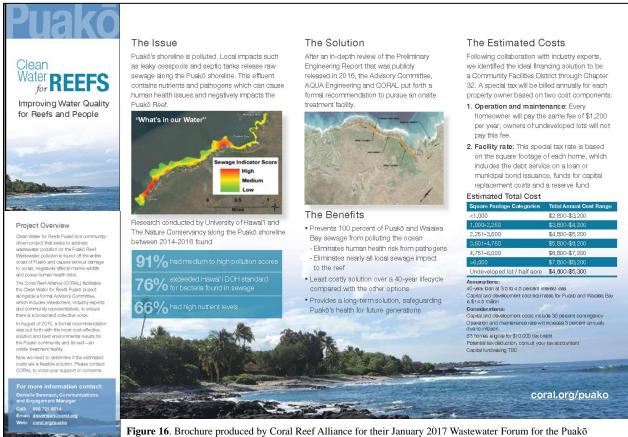
updated 1-page information sheet was circulated at this meeting. In April 2015, Drs. Wiegner and Beets attended a community meeting where the engineering firm (Aqua Engineering) contracted by Coral Reef Alliance for a sewage treatment upgrade feasibility study was introduced to the community. In August 2015, Dr. Wiegner attended a community meeting where Aqua Engineering presented results and recommendations from their preliminary feasibility study. In January 2016, Dr. Colbert gave a presentation at the annual PCA meeting summarizing results from UH Hilo's and TNC's efforts at Puakō; this presentation, as well as a 1-page handout that was distributed, were a joint effort between the two

Table 8. Outreach during UH Hilo's NOAA Coral Reef						
Conservation Program project from July 2014 to January 2017.						
Organization	Number of events					
	(year)					
Puakō Community Association	10(2014 = 3; 2015 = 3;					
	2016 = 3; 2017 =1)					
Coral Reef Alliance's 'Puakō	7 (2014 = 1; 2015 = 2;					
Sewage Disposal Upgrade Project'	2016 = 3; 2017 = 1)					
Advisory Board						
South Kohala Conservation Action	4 (2016 = 4)					
Plan Advisory Board						
Hawai'i Theatre for Youth "The	1 (2016)					
Story of Water and Hawai'i"						
performance –Water Hero						
appearance						
NOAA BWET water quality	2 (2015 = 1; 2016 = 1)					
lectures						
"Flushing Our Future" workshop	1 (2017)					
panelist – ASLO 2017 Conference						



Figure 15. Meeting with the Puakō Community Association (PCA) in November 2014. From left to right, (front row): Sierra Tobiason (UH Sea Grant), Tracy Wiegner (UH-Hilo), Erica Perez (Coral Reef Alliance), Kaile`a Carlson (UH-Hilo), Leilani Abaya (UH-Hilo), Wes Crile (Coral Reef Alliance), (back row) Steve Colbert (UH-Hilo), and Jim Beets (UH-Hilo). Photo is from the Coral Reef Alliance letter included in the PCA January 2015 newsletter.

research groups (*see* Appendix 2). In April 2016, Dr. Wiegner attended a PCA meeting with NOAA officials to discuss research in NOAA's Habitat Blue Print area (which includes Puakō).



**Figure 16.** Brochure produced by Coral Reef Alliance for their January 2017 Wastewater Forum for the Puakō community. UH Hilo and TNC provided input to brochure regarding their scientific findings at Puakō. Drs. Tracy Wiegner and Steve Colbert served as scientific experts on their panel.

In October 2016, Dr. Wiegner attended a PCA meeting with the new director of HDAR to discuss ways in which HDAR could support the 'Puakō Sewage Disposal Upgrade Project' led by the Coral Reef Alliance. Additionally, Drs. Wiegner, Colbert, and Beets are members of the Coral Reef Alliance's Advisory Board for the 'Puakō

# HTY Embarks on Statewide Tour with H20: THE STORY OF WATER AND HAWAII

by BWW News Desk Sep. 20, 2016

Honolulu Theatre for Youth will tour its 2015-16 season finale *H20*, THE STORY OF WATER AND HAWAII to Maui County, Kauai and the Big Island in October and November. This sweeping musical by the HTY company will immerse both school and public audiences in a celebration of our islands' most precious resource. Show dates, times and locations are:

The extraordinary musical floods the senses as HTY's cast of "Water Warriors" (alternately scientists, activists and rock stars) explores the cultural, historical, environmental and physical properties of water through song, humor and interactive story telling. At the close of each performance, different "Water Heroes" from the surrounding community will take the stage and share their knowledge on an aspect of water in the islands.

Big Island - Hilo
UH Hilo Performing Arts Center
Public Performance Friday, October 21, 7 p.m.
Trickets \$10 all seats all ages, available via <a href="https://www.htyweb.org">www.htyweb.org</a>, (808) 8399885 ext. 720, or at the door.
(School performances Oct. 19, 20, 21)



Figure 17. October 2016, Hawai'i Theatre for Youth performed at the UH Hilo Performing Arts Center and Dr. Tracy Wiegner was their "Water Hero" during one of their Hawai'i Island school group performances. She talked about sewage pollution on Hawai'i Island.

Sewage Disposal Upgrade Project'; they met with the board in October 2014, August and December 2015, November 2016, and January 2017. Dr. Wiegner also attended a two-day workshop in August 2016 held by the Coral Reef Alliance to develop a 10-year monitoring plan for Puakō. Drs. Wiegner and Colbert also served as panelist at a recent forum held by the Coral Reef Alliance to address PCA's questions regarding options for cesspool removal (Fig. 16). At this meeting, a 1-page handout summarizing results from UH Hilo and TNC was distributed (*see* Appendix 3). Data from UH Hilo's CRCP project were also submitted in written testimony to the HDOH in support of their proposed cesspool ban in September 2014 and included in a letter to Hawai'i's Governor encouraging him to sign the ban on new cesspool construction in the state (March 11, 2016).

Drs. Wiegner and Colbert are also members of the South Kohala Conservation Action Plan Marine Advisory Board, and attended four meetings in 2016 (March, June, August, and December). In October 2016, Dr. Wiegner was also a "Water Hero" in the Hawai'i Theatre for Youth's performance of "The Story of Water and Hawai'i" at the UH Hilo Performing Arts Center where she spoke about sewage pollution on Hawai'i Island to local K-12 students (Fig. 17). Dr. Wiegner has also given two online lectures (January and November 2016) to Hawai'i State public school teachers (6-12 grade) regarding water pollution in Hawai'i State as part of the NOAA BWET "OPIHI" project at UH Mānoa led by Dr. Kanesa Seraphin Duncan, Education Director for University of Hawai'i Sea Grant College. In February 2017, Dr. Wiegner will be a panelist for a town hall event entitled "Flushing Our Future" at the Association for the Sciences of Limnology and Oceanography (ASLO) Conference in Honolulu, HI. This event is being organized by Dr. Craig Nelson from UH Mānoa's C-MORE program.

### **F.** Student Training. This project has trained 12 undergraduates and one graduate student to

<b>Table 9.</b> Organizations that have provided student (undergraduate and graduate) support during UH Hilo's NOAA Coral Reef Conservation Program project from July 2014 to January 2017.						
Organization	Number of students supported					
Puakō Community Association	1 graduate student					
UH Hilo PIPES (NSF REU)	8 undergraduate summer interns					
UH Mānoa C-MORE (NSF)	3 undergraduate trainees					
USEPA GRO	1 undergraduate fellow					
UH Hilo STEM Honors Program	1 undergraduate senior					
(NSF)						
Sigma Xi	1 undergraduate					
'Ike Wai (NSF EPSCoR)	1 undergraduate					
ASLO Minority Program	3 student travel grants (2 graduate, 1					
(ASLOMP)	undergraduate)					
UH Hilo Marine Science	3 undergraduate senior theses; 12					
Department	undergraduate interns					
Ecological Society of America	1 undergraduate travel grant					
(ESA)						

date with a variety of funding sources (Figs. 18 and 20, Table 9). Between summer 2014 and 2016, eight interns (2014: Evelyn Braun, Maile Aiwohi, Ricky Tabandera; 2015: Bryan Tonga, Devon Aguiar, Jazmine Panelo; 2016 Saria Sultan and Christopher Thompson) from the UH Hilo Pacific Internship Program for Exploring Science (PIPES, funded by the National Science Foundation [NSF]) worked with Drs.

Wiegner and Colbert. Both years, the students conducted field and laboratory work, wrote final reports, and presented their findings at a student symposium. In 2014, their results served as pilot data for this project. They helped identify groundwater seep locations (Fig. 1), work out the logistics for macroalgal and water quality sampling, processing, and analyses, as well as conduct

the first dye tracer test. In 2015, the interns' projects were designed to collect data for portions

of the larger project. During the 2014-2015 academic year, two undergraduates (Cherie Kauahi and Devon Aguiar), supported by UH Mānoa's C-MORE program (NSF funded), assisted Dr. Colbert on his dye tracer tests and Dr. Wiegner on her *Enterococcus* sampling. Another undergraduate (Carrie Soo Hoo) completed her senior thesis with Dr. Wiegner examining the  $\delta^{15}N$  distribution in coastline macroalgae. She received funding for her project from UH Hilo's Science, Technology, Engineering, and Math (STEM) Honor's program (NSF funded) and Sigma Xi. Another undergraduate (Serina Kiili) received a U.S. Environmental Protection Agency (USEPA) Greater Research Opportunities (GRO)



**Figure 18.** UH-Hilo PIPES 2014 summer interns. From left to right: Ricky Tabandera (UH-Hilo), Maile Aiwohi (UH-Hilo), and Evelyn Braun (UH-Mānoa).

fellowship to examine sewage pathogens affecting coral health. During the 2015-2016 academic

<b>Table 10.</b> Products from UH Hilo's NOAA Coral Reef Conservation Program						
project from July 2014 to January 2017.						
Product	Number produced					
Reports	6 (NOAA, biannual = 5 [2015-2017];					
	HDAR = 1 [2016])					
Manuscript (submitted/in prep)	2 (Marine Pollution Bulletin)					
M.S. thesis	1 (UH Hilo, TCBES, August 2016)					
B.S. senior theses	3 (UH Hilo, Marine Science Department,					
	2015 = 1; 2016 = 2)					
Presentations	32 (12 PI, 5 graduate student, 15					
	undergraduate student)					
Posters	5 (1 PI, 1 graduate student, 3					
	undergraduate student)					
Community handouts	4 (PCA, 1 per year from 2014 -2017)					
Newspaper/magazine/newsletter	3 (UH System News [2015, Fig. 20],					
articles	Hawai'i Tribune Herald [2016, Fig. 21],					
	Hawai'i Business [2017, http://www.					
	hawaiibusiness.com/water-warning/])					
Videos	1 (Coral Reef Alliance [2017,					
	http://coral.org/puako/])					
Testimony regarding Hawai'i state	2 (1 [2015], 1 [2016])					
cesspool ban						
HCC Land-based pollution	1 (2015)					
conference session						

year, two undergraduates (Devon Aguiar and Jazmine Panelo), supported by UH Mānoa's C-MORE program, assisted Dr. Wiegner on her *Enterococcus* and *S*. aureus sampling. Ms. Panelo's and Kiili's senior thesis projects focused on S. aureus and coral pathogens, respectively. Fall 2016, Carey Demapan joined the research team as an 'Ike Wai scholar supported through the **UH** system NSF EPSCoR grant. Lastly, Leilani Abaya, a graduate student enrolled in the Tropical

Conservation Biology and Environmental Science (TCBES) Master's program at UH Hilo, defended her research proposal in February 2015 and thesis in April 2016. Her thesis was submitted to UH Hilo Library August 2016.

**G. Products.** Sixty products have resulted from this project. These include: reports, student theses, manuscripts, presentations, posters, 1-page information sheets, newspaper/ magazine/ online articles, videos, testimony, and a conference session (Table 10). Reports have been



submitted to NOAA's CRCP (biannual) and HDAR (algal cage deployment permit report). Dr. Wiegner has given eight presentations on this project to date – The Hawai'i Ecosystem Meeting (July 2014, Hilo, HI), HDOH, Clean Drinking Water Branch, Inter-government Water Conference (INVITED, August 2014, Kona, HI), PCA meeting (November 2014), NOAA CRCP/HDAR meeting (April 2015, Honolulu, HI), NOAA Mokupāpapa Discovery Center (INVITED, May 2015, Hilo, HI), UH Hilo (Public lecture, September 2015, jointly with Dr. Colbert; Fig. 19), International Coral Reef Symposium (ICRS, June 2016, Honolulu, HI), and at the 2017 ASLO Conference (Honolulu, HI). Dr. Colbert has presented twice on this project – a poster at the Hawai'i Conservation Conference (HCC, Hilo, HI, August 2015) and a presentation at the annual PCA meeting (January 2016). Rebecca Most from TNC also presented results from this project in a joint talk at the ICRS. Dr. Courtney Couch from TNC and UH Mānoa's Hawai'i Institute of Marine Biology (HIMB) will be



### Pollution and coral reef health focus of UH Hilo research



Figure 20. University of Hawai'i System News story highlighting UH-Hilo's NOAA CRCP project

June 10, 2015. From left to right: graduate student Leilani Abaya (UHH TCBES), and 2015 PIPES summer interns Devon Aguiar, Bryan Tonga, and Jazmine Panelo (UH-Hilo), and Belytza Velez-Gamez (U. of Puerto Rico). Article by Jaysen Niedermeyer.

presenting results from this project in a joint talk at HCC in July 2017. Fifteen undergraduate student presentations have been given at the **UH Hilo PIPES Summer Internship** Symposium, the UH Hilo Marine Science Department Senior Thesis Symposium, and the UH Hilo STEM Honors Program Symposium. Three undergraduate posters and one oral presentation were given at the annual C-MORE symposium (2 posters May 2015, one poster and one presentation May 2016). August 2016, Ms. Panelo presented findings from her undergraduate senior thesis at the **Ecological Society of America** (ESA) Annual Meeting (Fort Lauderdale, FL). Ms. Panelo received a travel grant through this

### Big Island lawmakers lobbied against cesspool ban

ublished March 15, 2016 - 1:30am



By COLIN M. STEWART Hawaii Tribune-Herald

The state has taken an important step toward addressing water pollution, according to some isle scientists.

A statewide ban on new cesspool construction approved Friday by Gov. David Ige came despite protests from seven Hawaii Island legislators, who claimed the ban would place undue financial burdens on local homeowners who might

The new rules also implement a 2015 law providing a tax credit of up to \$10,000 for cesspools upgraded to sever or septic system during the north levy lears, limited to \$5 million or about \$50 cesspool upgrades a year. When the law, owners of cesspools located within 200 feet of the ocean, streams or marsh areas, or near drinking water sources, can outsift for the credit.

In announcing the ban, Ige said Hawaii had been the only state in the union that allowed the construction of cesspools

"Today's action banning new cesspools statewide would stop the addition of pollution from approximately 800 new cesspools per year." he said.

Cesspools, which are effectively "just holes in the ground," according to University of Hawaii at Hilo marine scient Tracy Wiegner, inject about 55 million gallons of raw, untreated sewage into Hawaii's groundwater every day, potentially spreading diseases and harming the quality of drinking water supplies and recreational waters.

Wiegner applauded the ban on Monday, calling it "a good first step towards reducing sewage pollution in our near shore waters."

**Figure 21.** Hawaii Tribune Herald article highlighting results from UH-Hilo's NOAA CRCP project March 15, 2016. Picture taken by Steven Colbert.

society. She and Ms. Sultan will also be presenting their results at the 2017 ASLO Conference (Honolulu, HI). Ms. Sultan received a travel grant through this society's minority students' program. Additionally, five graduate student presentations and one poster were given – ASLO in Granada, Spain (February 2015), UH Hilo TCBES Symposium (April 2015), HCC (August 2015), Ocean Sciences Meeting (OSM) in New Orleans (February 2016), M.S. Thesis defense (April 2016), and Hawai'i Ecosystems Meeting in Hilo (July 2016). Leilani Abaya won best student presentation at the ASLO conference and was also awarded a travel grant through this society's program for minority students. Ms. Abaya also received a travel grant to OSM through their minority students' program. The UH Hilo Marine Science research team organized a session for the HCC (August 2015) on land-based pollution effects on coral reefs and near-shore waters. This project was also highlighted in the UH system-wide news (June 2015; Fig. 20) and in the Hawai'i Tribune Herald (March 2016; Fig. 21).

### H. Related UH Hilo Funded Projects.

- 1. NOAA/HDAR Coral Reef Working Group. 2016. Sewage pollution source tracking on Puakō's coral reefs. Tracy Wiegner (PI), Steve Colbert, Jim Beets, Courtney Couch, and Craig Nelson. \$83,918. Recommended for funding. (2018-2019).
- 2, NOAA. West Hawaii Habitat Focus Area. 2016. Water quality and coral reef health. Stuart Goldberg (PI), Lani Watson, Jamie Gove, Jonathan Martinez, Tracy Wiegner, Steve Colbert, Eric Conklin, Courtney Couch, Chad Wiggins, Kim Falinski. Rebecca Most, and Julia Rose. \$99,955. (2016-2017).
- 3. NOAA/HDAR Coral Reef Working Group. 2016. Sewage pollution source tracking at Puakō and comparison of onsite waste disposal systems for management actions. Tracy Wiegner (PI), Steve Colbert, and Jim Beets. \$80,555. (2016-2017)

#### I. Collaborators.

<b>Table 11.</b> Collaborators on UH Hilo's NOAA Coral Reef Conservation Program project from July 2014	
to January 2017.	
Organization	Collaborators
UH Mānoa, Hawai'i Institute of Marine Biology	Courtney Couch
(HIMB)	
The Nature Conservancy (TNC)	Chad Wiggins, Rebecca Most, Amy Bruno, Eric
	Conklin, Kim Falinski
UH Mānoa, School of Oceanography and	Craig Nelson, Kristina Remple, Barbara Bruno
Environmental Science and Technology (SOEST),	
Center for Microbial Oceanography Research and	
Education (C-MORE)	
Puakō Community Association (PCA)	Peter Hackstedde, George Fry, Robby Robertson,
	Mike O'Toole
Coral Reef Alliance	Erica Perez, Jos Hill, Cherrie Kauahi, Danielle
	Swanson, Wes Crile, Michael Webster
South Kohala Conservation Partnerships (SKCP)	Julia Rose, Sierra Tobiason
UH Hilo PIPES	Sharon Ziegler-Chong, Noe Puniwai, Rebecca
	Ostertag, Ulu Ching, Erika Perry, Rita Miller,
	Linnea Heu
NOAA Habitat Blue Print	Lani Watson, Stuart Goldberg
Aqua Engineering	Justin Logan
Cornell University	C. Drew Harvell
NOAA Coral Reef Conservation Program	Paulo Maurin
Seattle Aquarium	Shawn Larson, Amy Green

#### J. Cited Literature.

- Byappanahalli, M., R. Fujioka. (1998). Evidence that tropical soil environment can support the growth of *Escherichia coli*. Water Science and Technology 38:171-174.
- Byappanahalli, M., and R. Fujioka. (2004). Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. Water Science and Technology 50:27-32.
- Dailer, M.L., R.S. Knox, J.E. Smith, M. Napier, and C.M. Smith. (2010). Using  $\delta^{15}N$  values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Marine Pollution Bulletin 60:655-671.
- David, A., M. Tournoud, J. Perrin, D. Rosain, C. Rodier, C. Salles, C. Bancon-Montigny, and B. Picot. (2013). Spatial and temporal trends in water quality in a Mediterranean temporary river impacted by sewage effluents. Environmental Monitoring and Assessment 185:2517-2534.
- Fujioka, R.S., B. Roll, and M. Byappanahalli. (1997). Appropriate recreational water quality standards for Hawaii and other tropical regions based on concentrations of *Clostridium perfringens*. Proceedings of the Water Environment Federation 4: 405-411.
- Fujita, R.M. (1985). The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. Journal of Experimental Marine Biology and Ecology 92:283-301.
- Fung, D.Y.C., R. Fujioka, K. Vijayavel, D. Sato, and D. Bishop. 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. Journal of Rapid Methods & Automation in Microbiology 15: 217-229.
- Hardina, C., and R. Fujioka. (1991). Soil: The environmental source of *Escherichia coli* and *Enterococci* in Hawaii's streams. Environal Toxicology and Water Quality 6:185-195.
- HDOH (2014) State of Hawaii water quality monitoring and assessment report. HDOH CWA §305(b)
- Lapointe, B., J. O'Connell, and G. Garrett. (1990). Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry 10:289-307.
- Nelson, C., M. Donahue, H. Dulaiova, S. Goldberg, F. La Valle, K. Lubarsky, J. Miyano, C. Richardson, N. Silbiger, and F. Thomas. (2015). Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. Marine Chemistry 177:232-243.
- Paytan, A., G.G. Shellenbarger, J.H. Street, M.E. Gonneea, K. Davis, M.B. Young, and W.S. Moore. (2006). Submarine groundwater discharge: An important source of new inorganic nitrogen to coral reef ecosystems. Limnology and Oceanography 51:343-348.
- Shenawy, M.E. 2005. *Staphylococcus aureus* and fecal indicators in Egyptian coastal waters of Aqaba Gulf, Suez Gulf, and Red Sea. Egyptian Journal of Aquatic Resources 31: 113-124.
- Shibata, T., H. Solo-Gabriele, C.D. Sinigalliano, M.L. Gidley, L.R.W. Plano, J.M. Fleisher, J.D. Wang, S.M. Elmir, G. He, M.E. Wright, A.M. Abdelzacher, C. Ortega, D. Wanless, A.C. Garza, J. Kish, T. Scott, J. Hollenback, L.C. Backer, and L.E. Fleming. (2010). Evaluation of conventional and alternative monitoring methods for a recreational marine beach with nonpoint source of fecal contamination. Environmental Science and Technology 44:8175-8181.
- Swart, P.K., S. Evans, T. Capo, and M.A. Altabet. (2014). The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate. Biogeosciences 11:6147–6157.
- Viau, E., K. Goodwin, K. Yamahara, B. Layton, L. Sassoubre, S. Burns, H. Tong, S. Wong, Y. Lu, and A. Boehm. (2011). Bacterial pathogens in Hawaiian coastal streams associations with fecal indicators, land cover, and water quality. Water Research 45:3279-3290.
- Wang, Y., J.M. Wilson, and J.M. Vanbriesen. (2015). The effect of sampling strategies on assessment of water quality criteria attainment. Journal of Environmental Management 154:33-39.
- Wiegner, T.N., A. Mokiao-Lee, and E.E. Johnson. (2016). Identifying nitrogen sources to thermal tide pools in Kapoho, Hawai'i, U.S.A, using a multi- stable isotope approach. Marine Pollution Bulletin 103:63-71.
- Yoshioka, R.M., C.J.S. Kim, A.M. Tracy, R. Most, and C.D. Harvell. (2016). Linking sewage pollution and water quality to spatial patterns of *Porites lobata* growth anomalies in Puako, Hawaii. Marine Pollution Bulletin 104:313-321.
- Zambrano, L., V. Contreras, M. Mazari-Hiriart, and A. Zarco-Arista. (2009). Spatial heterogeneity of water quality in a highly degraded tropical freshwater ecosystem. *Environmental Management* 43:249-263.

### K. Appendices

- 1. UH Hilo handout for Puakō Community Association meetings. November 2014 and January 2015.
- 2. UH Hilo and TNC joint handout for the Puakō Community Association annual meeting. January 2016.
- 3. Joint UH Hilo and TNC handout for Coral Reef Alliance's Wastewater Forum for the Puakō community. January 2017.

*Appendix 1.* UH Hilo handout for Puakō Community Association meetings. November 2014 and January 2015.

### Spatial Distribution and Effects of Sewage on Puakō's Coral Reefs

### Goals

- Use chemical and biological tools to determine if sewage is entering coastal waters
- Determine if sewage is impacting water quality
- Assess coral reef community-level response to sewage





### **Objectives**

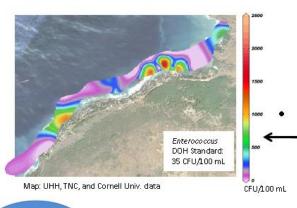
- Dye Tracer Studies: Use dye to document connection between cesspools and ocean
- 2.  $\underline{\delta^{15}N}$  Seaweed Measurements: Evaluate presence and spatial extent of sewage near- and offshore
- 3. <u>Fecal Indicator Bacteria & Nutrient Measurements</u>: Determine if DOH water quality standards are exceeded
- 4. <u>Benthic Community Responses</u>: Assess responses of corals, fishes, and macroinvertebrates to wastewater

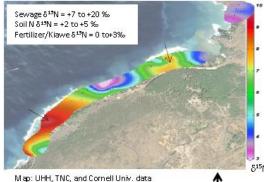


Appendix 1. Continued. Page 2 of UH Hilo handout. November 2014 and January 2015.

### **Findings**

 Dye travel time was 3 days from cesspool to ocean





Fecal indicator bacteria

- (Enterococcus) and δ<sup>15</sup>N seaweed

values indicate sewage presence

at 2 locations

### Remaining Work

- 3 more dye tracer studies
- Nutrient, bacteria, and δ<sup>15</sup>N seaweed measurements including offshore seaweed cage experiments
- · Coral and fish sampling



Caged seaweed experiments

### **UHH Faculty**

Dr. Tracy Wiegner (PI, water quality) wiegner@hawaii.edu Dr. Jim Beets (Coral & fish) beets@hawaii.edu Dr. Steve Colbert (Dye studies) colberts@hawaii.edu Dr. Jason Adolf jadolf@hawaii.edu

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#### Undergraduates:

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200 West Kawili St Hilo, HI 96720

Funded by NOAA Coral Reef Conservation Program and Puakō Community Association













*Appendix 2.* UH Hilo and TNC joint handout for the Puakō Community Association annual meeting. January 2016.



### WHAT'S IN OUR WATER?

Meandering underground streams flowing beneath Puakō and entering the ocean through springs and seeps once nourished an abundant fishery and vibrant coral reefs. So, when residents began noticing declines in fish and corals, they enlisted partners to help them understand why these changes were occurring.

Today, Cornell University, the University of Hawai'i at Hilo Marine Science Department (UH Hilo), The Nature Conservancy (TNC), and the Hawai'i Institute of Marine Biology (HIMB) are working with the Puakō Community Association to identify causes of the declines and solutions for restoring coral reef health at Puakō.

Domestic wastewater (sewage) was suspected as one of the threats to the reef. Research found outdated cesspools leaching untreated sewage through permeable rock to beaches, tide pools, and the reef, impacting nearshore water quality.

How far offshore does the sewage travel from the nearshore seeps? How quickly does sewage from cesspools enter nearshore waters? What are the impacts of sewage to the reef ecosystem? These are the questions currently being addressed by research groups.

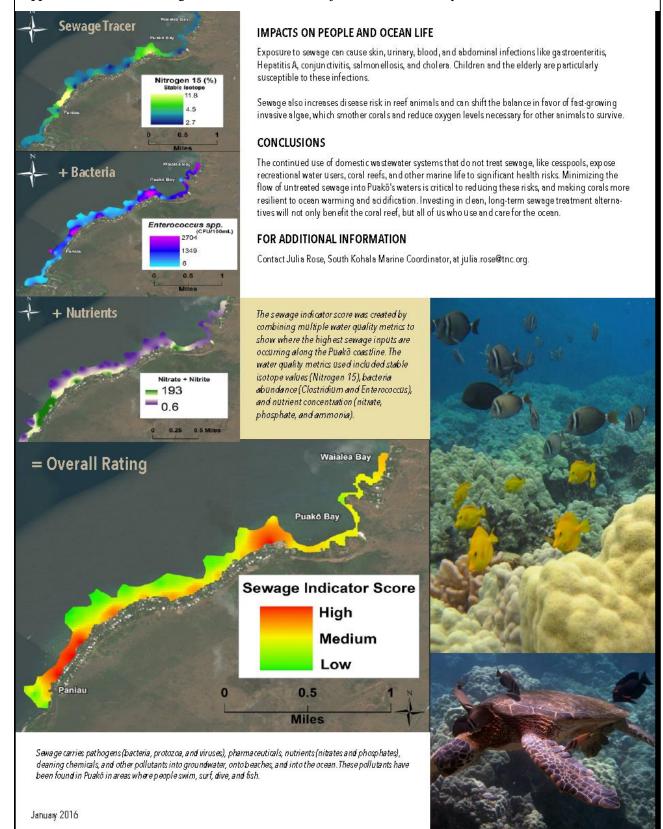
#### **KEY FINDINGS**

Indicators of domestic wastewater have been found in coastal and marine areas where they are likely impacting people, coral reefs, and other marine life:

- Dye tracer studies found that sewage from cesspools reached seeps along the Puakö coast within six hours to three days.
- At some shoreline locations, often corresponding to those of the dye tracer studies:
  - Levels of two bacteria associated with sewage often exceeded Hawai'i Department of Health standards.
  - Nitrate levels were two times higher than those in mauka groundwater from Waikoloa and Mauna Lani.
  - Nitrogen isotope measurements in seaweed were indicative of sewage pollution.
- Coral growth anomalies—tumor-like growths on coral skeletons—were highest on reefs with evidence of groundwater input and elevated nutrients.
- Studies conducted across the region show Puakō's reefs have especially high levels of red filamentous algae, which overgrow and can kill corals.



Appendix 2. Continued. Page 2 of UH Hilo and TNC joint handout. January 2016.



*Appendix 3.* Joint UH Hilo and TNC handout for Coral Reef Alliance's Wastewater Forum for the Puakō community. January 2017.



### WHAT'S IN OUR WATER?

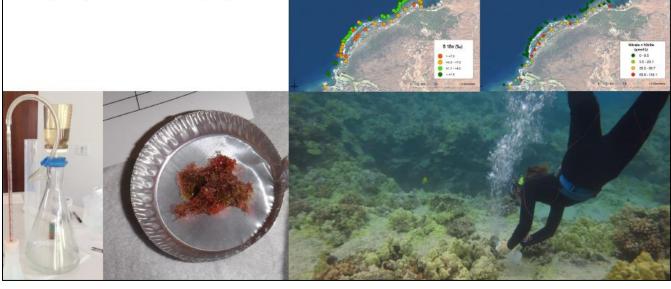
Meandering underground streams flowing beneath Puakō and entering the ocean through springs and seeps once nourished an abundant fishery and vibrant coral reefs. So, when residents began noticing declines in fish and corals, they enlisted partners to help them understand why these changes were occurring.

Today, scientists from The Nature Conservancy, University of Hawai'i at Hilo Marine Science Department, Hawai'i Institute of Marine Biology, University of Hawai'i at Mānoa, and Cornell University are working with the Puakō Community Association to identify causes of the declines and solutions for reviving coral reef health at Puakō.

Using a combination of tools, including stable nitrogen isotopes and DNA-based tools which are able to identify the presence of human waste, the research confirms what has long been suspected: cesspools are leaching untreated sewage underground to Puakō's beaches, tide pools, and reef.

#### **KEY FINDINGS**

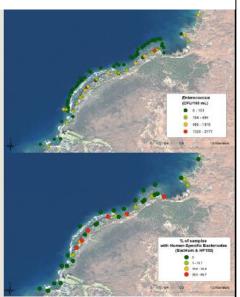
- Nutrients are elevated at coastal springs and seeps, as evidenced by high nitrates across 66% of the sites. Measurements were especially high in areas with high levels of submarine groundwater.
- Stable nitrogen isotope measurements in seaweed (> + 7 %) are indicative of sewage pollution at several locations, with the highest values observed at the southern and northern end of Puako's shoreline.
- Elevated levels of stable nitrogen isotopes at several reef stations are indicative of sewage reaching the reef.
- Stable nitrogen isotope measurements showed that groundwater became
  increasingly polluted with sewage moving down slope to the shoreline, with the
  highest values being measured within Puakō and lower values found at Waikaloa
  Village.



### Appendix 3. Continued. Page 2 of joint UHH and TNC handout. January 2017.

#### KEY FINDINGS (continued)

- Between 2014 and 2016, measurements at 76% of shoreline sites exceeded Hawai'i
  Department of Health standard (single sample maximum, 104 CFUs/100ml) for Enterococcus
  concentrations in coastal waters.
- Like the stable nitrogen isotope data, Enterococcus concentrations were lower over the reef
  compared to the shoreline but were relatively high (36-91 CFUs/100ml) at 20% of the reef
  stations, also suggesting that sewage pollution is reaching some locations along the reef.
- Using DNA-based tools, researchers found that 36-67% of the samples collected during 2015, contained bacteria only found in the human gut, suggesting frequent exposure to sewage pollution.
- Similar to the stable nitrogen isotope seaweed data, the highest values were found in the northern and southern portions of Puakō.





#### CONCLUSIONS

Ongoing research provides strong evidence of sewage pollution along Puakō's shoreline and reef. Minimizing the flow of untreated sewage into Puakō's waters by investing in clean, long-term sewage treatment alternatives will reduce risks to human health and to marine life. Our research constitutes a baseline against which reductions in pollution levels can be measured if wastewater treatment improves.

### IMPACTS ON PEOPLE AND OCEAN LIFE

Exposure to sewage can cause skin, urinary, blood, and abdominal infections like gastroenteritis, Hepatitis A, conjunctivitis, salmonellosis, and cholera. Children and the elderly are particularly susceptible to these infections. At Enterococcus concentrations of 35 CFU/100ml, like those documented at Puakō, recreational water users have a 3.6% chance of contracting gastroenteritis. Sewage pollution also increases disease risk in reef marine animals and can shift the balance in favor of fast-growing invasive algae, which smother corals and reduce oxygen levels necessary for other animals to survive.

### FOR ADDITIONAL INFORMATION

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