NOAA Coral Reef Conservation Program
Final Report

A. Award Number: (NA15NOS4820037)

B. PO #: C70643

C. Amount of Award: $80,555

D. Recipient: (PI's name): Tracy Wiegner and Steven Colbert

E. Project Title: Sewage pollution source tracking along Puakō's shoreline and comparison of on-site sewage disposal systems for management actions

F. Award Period: September 1, 2016 – September 30, 2018

G. Period Covered by this Report: September 1, 2016 – September 30, 2018

H. Summary of Progress and Expenditures to Date: *When describing the progress of projects, please evaluate projects against the scope of work described in the final application submitted to NOAA CRCP*

Project:

1. Project Title: Sewage pollution source tracking along Puakō's shoreline and comparison of on-site sewage disposal systems for management actions

2. Project Status (please x): No activities to date ____ Planning ____ In progress_____ Completed: X

3. Summary of Project Accomplishments (by each objective):

Objective 1: *Determine the source of nitrogen (N) and fecal indicator bacteria (FIB) pollution through δ^{15}Nand δ^{18}O measurements of nitrate (NO_3^-) in nearshore waters, mixing models, and microbial source tracking using Bacteroides.*

Accomplishments and ongoing efforts: Groundwater samples from wells in Waikoloa Village (high elevation), Mauna Lani Resort (mid elevation), and Puakō (low elevation) were collected from June – August 2016, and they have been analyzed for nutrients, FIB (including *Bacteroides*), and δ^{15}N- and δ^{18}O-NO_3^-. Shoreline water and algal tissue samples from Puakō were also collected during this time, and analyzed for nutrients, FIB, δ^{15}N- and δ^{18}O-NO_3^-, and δ^{15}N algal tissue content. Later (January – March 2017), the same sample types were collected at four resorts adjacent to Puakō (Mauna Kea, Hapuna Prince, Fairmont Orchid, and Mauna Lani). Analyses of Puakō and resort samples are complete. We also collected additional samples from watershed NO_3^- sources including fertilized soils (n = 2), additional Onsite Sewage Disposal Systems (OSDS, type, septic tanks n= 3, Aerobic Treatment Units (ATU) n = 3), and ocean water (n = 3) in 2017. Analysis of all of our δ^{15}N- and δ^{18}O-NO_3^- samples is now complete, and these data have been analyzed for NO_3^- source partitioning for groundwater wells, anchialine ponds, and shoreline waters. Analysis of *Bacteroides* samples collected after 2016 for the Puakō shoreline and adjacent resorts are still pending.

Results from these measurements were presented at 6 professional venues: 1) the Association for the Sciences of Limnology and Oceanography (ASLO) Aquatic Sciences conference in Honolulu, HI (February 2017), 2) the ASLO Aquatic Sciences conference in Victoria, Canada (June 2018), 3) Hawaiʻi Department of Health (HDOH) Cesspool Update (June 2018), 4) Hawaiʻi Conservation Conference, Honolulu, HI (July 2018), 5) HDOH Joint Government Water Conference, Hilo, HI (August 2018), and 6) HDOH Joint Government Water Conference, Kailua Kona, HI (August 2018). Findings were also shared with the Puakō community at three community meetings (January and May 2017, January 2018). Another community meeting is scheduled for October 2018 in Waimea, HI, where final results from this project will be presented.

<u>Findings to date:</u> *Enterococcus* spp. concentrations were an order of magnitude higher at the Puakō anchialine ponds compared to the other watershed locations (average ±SE: 8165 ± 2935 MPN/100

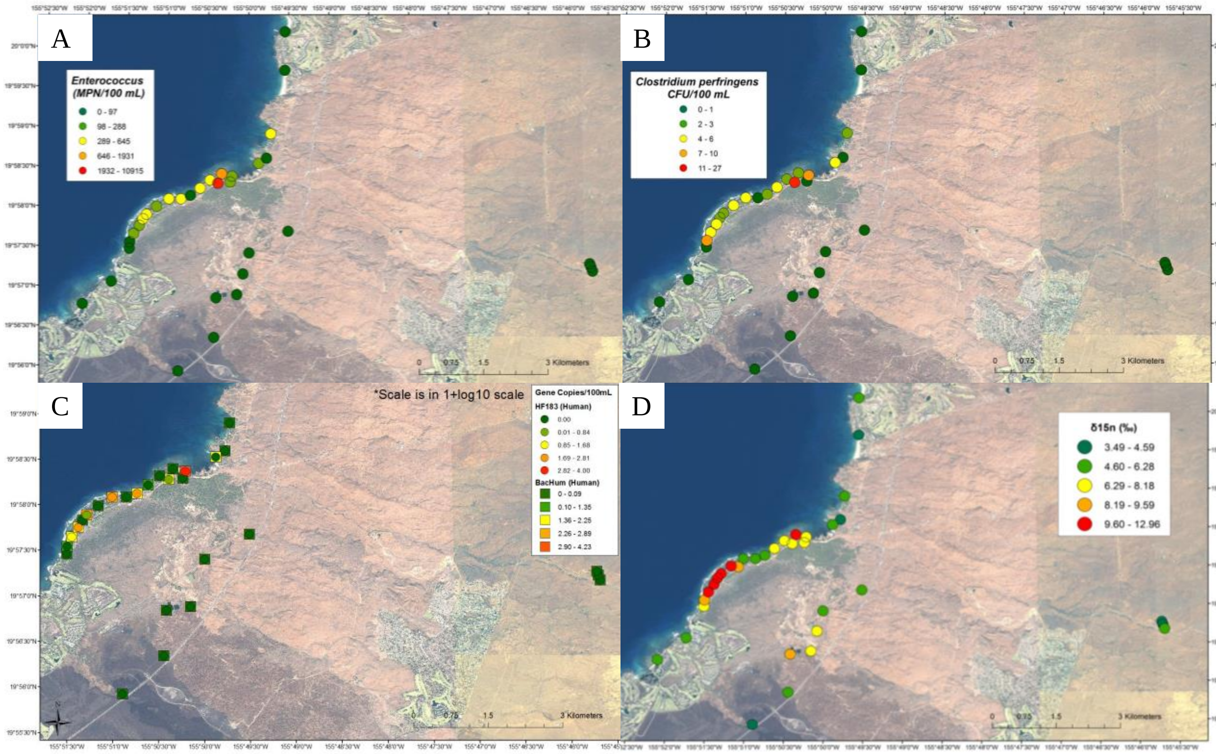

Figure 1. Spatial distribution of sewage indicators in the Puakō watershed, including shoreline stations at adjacent resorts north (Mauna Kea, Hapuna Prince) and south (Fairmont Orchid, Mauna Lani) of the community on Hawai`i Island. A) *Enterococcus* spp.,B) *Clostridium perfringens*, C) Human-associated *Bacteroides*, and D) $\delta^{15}NO_3^-$. Puakō watershed samples were collected from June – August 2016; resort samples were collected from January – March 2017. Sample analysis for *Bacteroides* at the resorts is pending.

Table 1. Average ± SE of *Enterococcus* spp. *Clostridium perfringens*, human-associated *Bacteroides* (markers HF183 and BacHum) and $\delta^{15}N$ –NO_3- and $\delta^{18}O$ –NO_3- in water samples for high elevation wells (Waikoloa Village), mid elevation wells (Mauna Lani), low elevation wells (Puakō wells), resort shoreline stations (Mauna Kea, Hapuna Prince, Fairmont Orchid, and Mauna Lani), Puakō shoreline stations (low and high salinity stations), and Puakō ponds (anchialine ponds). Number of stations sampled within each watershed category varied between 3-16 (station n) and the total number of observations per location per parameter are footnoted below. Water samples collected at the resorts for human-associated *Bacteroides* analyses have not yet been completed (n/a).

Watershed Location	Station (n)	*Enterococcus* spp. (MPN/100mL)	*C. perfringens* (CFU/100mL)	HF183 (Human) (Copies/100mL)	BacHum (Human) (Copies/100mL)	$\delta^{15}N$ –NO_3- (‰)	$\delta^{18}O$ –NO_3- (‰)
High Elevation Wells	3	15 ± 9	0 ± 0	0.00 ± 0.00	0.00 ± 0.00	4.47 ± 0.21	1.98 ± 0.43
Mid Elevation Wells	6	5 ± 0	0 ± 0	0.00 ± 0.00	0.00 ± 0.00	6.37 ± 0.40	1.23 ± 0.32
Low Elevation Wells	2	119 ± 67	0 ± 0	0.00 ± 0.00	0.05 ± 0.04	6.29 ± 0.70	2.85 ± 0.60
Resort Shoreline	4	32 ± 14	0 ± 0	n/a	n/a	5.16 ± 0.31	2.61 ± 0.70
Puakō Shoreline	16	410 ± 83	4 ± 1	0.58 ± 0.16	0.71 ± 0.17	8.64 ± 0.25	7.92 ± 0.02
Puakō Ponds	2	8165 ± 2935	18 ± 11	0.47 ± 0.28	1.74 ± 0.55	8.59 ± 0.41	4.19 ± 0.53

Number of observations per location, per parameter:
High Elevation Wells: *Enterococcus* spp. = 6, *C. perfringens* = 6, HF183 (Human) = 6, BacHum (Human) = 6, $\delta^{15}N$ –NO_3- = 9, $\delta^{18}O$ –NO_3- = 9
Mid Elevation Wells: *Enterococcus* spp.= 16, *C. perfringens* = 16, HF183 (Human) = 11, BacHum (Human) = 11, $\delta^{15}N$ –NO_3- = 21, $\delta^{18}O$ –NO_3- = 21
Low Elevation Wells: *Enterococcus* spp. = 6, *C. perfringens* = 6, HF183 (Human) = 4, BacHum (Human) = 4, $\delta^{15}N$ –NO_3- = 7, $\delta^{18}O$ –NO_3- = 7
Resort Shoreline: *Enterococcus* spp. = 12, *C. perfringens* = 12,
Puakō Shoreline: *Enterococcus* spp. = 137, *C. perfringens* = 137, HF183 (Human) = 48, BacHum (Human) = 48, $\delta^{15}N$ –NO_3- = 89, $\delta^{18}O$ –NO_3- = 89
Puakō Ponds: *Enterococcus* spp. = 7, *C. perfringens* = 5, HF183 (Human) = 4, BacHum (Human) = 4, $\delta^{15}N$ –NO_3- = 7, $\delta^{18}O$ –NO_3- = 7

mL; range: 324 - 19,018 MPN/100 mL) (Fig. 1A, Table 1). These ponds had values that consistently exceeded the HDOH statistical threshold standard of 130 MPN/100 mL. The second highest *Enterococcus* spp. concentrations were found along Puakō's shoreline (410 ± 83 MPN/100 mL; 0-7985 MPN/100 mL), with concentrations on average exceeding the HDOH statistical threshold standard. *Enterococcus* spp. concentrations were an order of magnitude lower at the resorts than those measured along the Puakō shoreline and anchialine ponds, and lower than state standards (Table 1). Epidemiological studies have shown that a swimmer's chance of contracting gastroenteritis is 3.6% at *Enterococcus* spp. concentrations of 35 MPN/100 mL (*reviewed in* Fujioka et al 2015). *Clostridium perfringens* concentrations were similar among high elevation wells (0 ± 0 CFU/100 mL), mid elevation wells (0 ± 0 CFU/100 mL), low elevation wells (0 ± 0 CFU/100 mL), and at the resorts shoreline (0 ± 0 CFU/100 mL) (Fig. 1B, Table 1). The average *C. perfringens* concentration along the Puakō shoreline (4 ± 1 CFU/100 mL) was similar to the groundwater wells and resorts shoreline averages. However, the range for *C. perfringens* concentrations along Puakō's shoreline was greater (0 - 90 CFU/100mL) compared to ranges for groundwater wells and resorts' shoreline waters. *C. perfringens* concentrations in Puakō's anchialine ponds (18 ± 11 CFU/100 mL; 0 - 52 CFU/100 mL) exceeded the recommended standard to HDOH of 5 CFU/ 100 mL for marine recreational waters (Fujiokoa et al. 1997) and were within the range reported for non-point source sewage pollution (Fung et al. 2007). Human-associated *Bacteroides* (using two different molecular markers HF183 and BacHum) were the highest along Puakō's shoreline and within the Puakō's anchialine ponds (Fig. 1C, Table 1). A recent study found that a swimmer had 1.2 – 3% chance of contracting gastroenteritis at BacHum levels between 1.7 – 3.6 copies/ 100 mL (Boehm et al. 2015) – the levels observed within Puakō's anchialine ponds and shoreline waters (Fig. 1C).

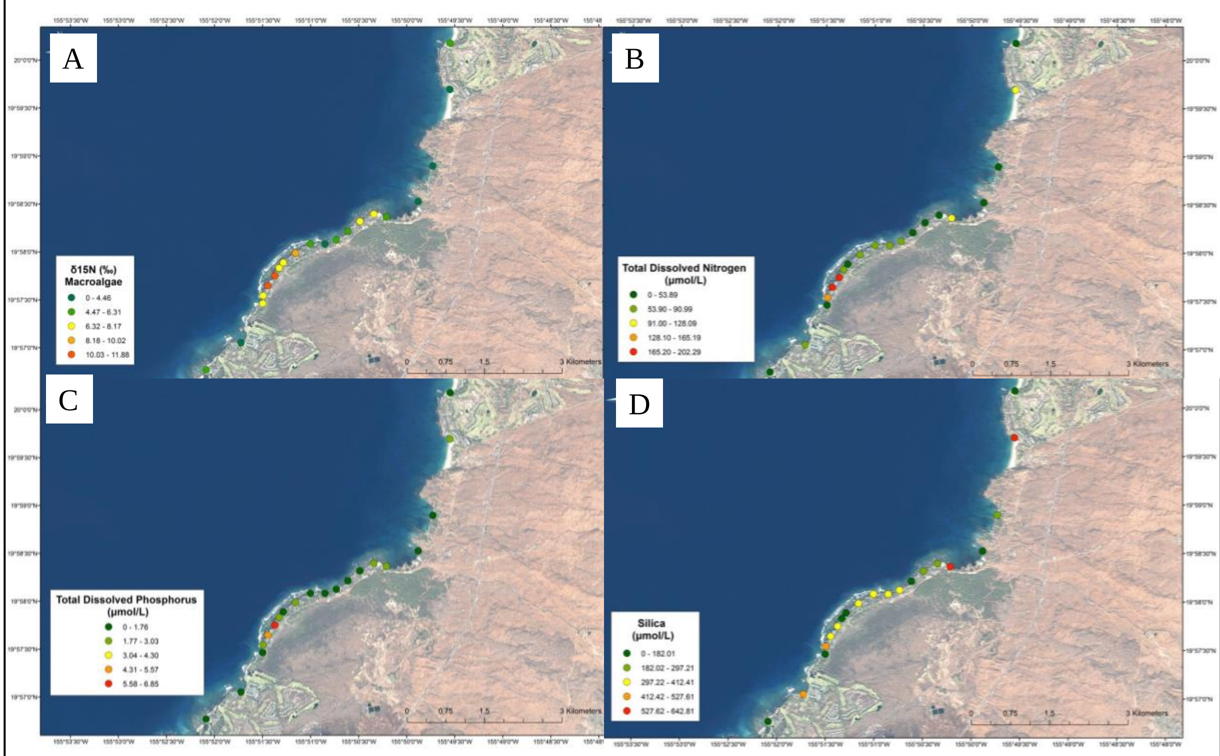

Figure 2. Spatial distribution of $\delta^{15}N$ macroalgae values and nutrient concentrations along the Puakō shoreline, and at four adjacent resorts north (Mauna Kea, Hapuna Prince) and south (Fairmont Orchid, Mauna Lani) of the community on Hawai`i Island. A) $\delta^{15}N$ macroalgae, B) Total Dissolved Nitrogen (TDN), C) Total Dissolved Phosphorus (TDP), and D) dissolved silica (H_4SiO_4). Samples were collected from June – August 2016, and January to March 2017. Averages (±SE) for parameters can be found in Figure 3 and Table 2.

Seven stations (station #: 2, 3, 4, 5, 6, 7, and 14) along the Puakō shoreline had $\delta^{15}N$ macroalgal values +7‰ or greater, which is considered to be within the range of values measured for sewage (*reviewed in* Wiegner et al. 2016). At the resorts, $\delta^{15}N$ macroalgal values were lower than those observed at many of the Puakō shoreline stations (Fig. 2A), with values indicative of NO_3^- from upper and lower elevational wells, well fertilized soils and ones under Kiawe trees, and ocean water (Fig. 3). However, it is important to note that past studies have found that macroalgae assimilate N more rapidly under low NO_3^- concentrations (Fujita 1985), and that $\delta^{15}N$ in macroalgal tissues can be underestimated by up to 6‰ in waters with high NO_3^- concentrations (> 10 µmol/L) (Swart et al. 2014). All shoreline stations had NO_3^-+NO_2^- concentrations that exceeded this value. If the $\delta^{15}N$ macroalgal values are adjusted for possible increased N isotope discrimination at higher NO_3^- concentrations, then algal shoreline values fall within the range reported for sewage (> +7‰, *reviewed in* Wiegner et al. 2016).

There were no consistent nutrient concentration patterns across sampling locations (Table 2). However, the greatest variability in nutrient concentrations (NO_3^-+NO_2^-, TDN, and PO_4^{3-}) was generally observed along Puakō's shoreline and

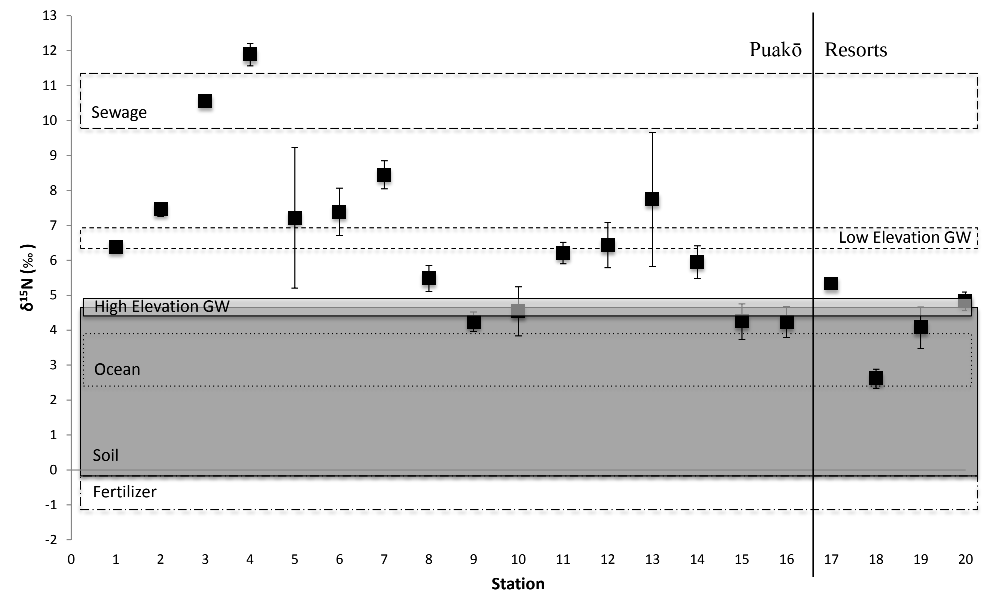

Figure 3. Average (± SE) δ^{15} N (‰) of wild shoreline macroalgae collected at 16 stations (south to north: 1-16) along the Puakō shoreline, and at four adjacent resorts north (17: Mauna Kea, 18: Hapuna Prince) and south (19: Fairmont Orchid, 20: Mauna Lani) of the community on Hawai`i Island. Shaded and outlined background areas represent (average ± SE) $\delta^{15}NO_3^-$ of N sources (fertilizer, soil, ocean, high elevation groundwater (GW), low elevation GW, and sewage) measured as part of this and an earlier study at Puakō (Abaya et al. 2018). Fertilizer values are from a previous study (Wiegner et al. 2016) on Hawai'i Island.

Table 2. Average ± SE of salinity, turbidity (NTU), and nutrient concentrations (µmol/L) in water samples for high elevation wells (Waikoloa Village), mid elevation wells (Mauna Lani), low elevation wells (Puakō wells), resort shoreline stations (Mauna Kea, Hapuna Prince, Fairmont Orchid, and Mauna Lani), Puakō shoreline stations (low and high salinity stations), and Puakō ponds (anchialine ponds). Number of stations sampled within each watershed category varied between 3-16 (station n) and the total number of observations per location per parameter are footnoted below.

Watershed Location	Station (n)	Salinity	Turbidity	NO_3- + NO_2-	NH_4+	TDN	PO_4^{3-}	H_4SiO_4
High Elevation Well	3	0.18 ± 0.01	0.39 ± 0.07	95.27 ± 2.33	2.29 ± 0.89	102 ± 1	2.35 ± 0.06	775 ± 53
Mid Elevation Well	6	1.76 ± 0.09	0.84 ± 0.15	120.35 ± 4.62	1.46 ± 0.36	133 ± 8	1.54 ± 0.05	760 ± 28
Low Elevation Well	2	1.91 ± 0.27	6.88 ± 2.74	114.55 ± 8.93	3.31 ± 1.12	127 ± 15	1.94 ± 0.57	657 ± 76
Resorts Shoreline	4	22.37 ± 2.93	1.11 ± 0.18	47.01 ± 9.68	0.80 ± 0.18	57 ± 11	1.01 ± 0.19	328 ± 77
Puakō Shoreline	16	21.53 ± 0.76	0.23 ± 0.02	65.51 ± 5.21	1.64 ± 0.10	77 ± 5	1.60 ± 0.15	447 ± 88
Puakō Pond	2	5.29 ± 0.61	10.62 ± 2.33	110.32 ± 13.48	3.94 ± 1.24	120 ± 17	1.79 ± 0.50	655 ± 90

Number of observations per location, per parameter:
High Elevation Wells: Salinity = 8, Turbidity = 6, NO_3- + NO_2- = 11, NH_4+ = 11, TDN = 6, PO_4^{3-} = 11, H_4SiO_4 = 8
Mid Elevation Wells: Salinity = 20, Turbidity = 16, NO_3- + NO_2- = 25, NH_4+ = 25, TDN = 16, PO_4^{3-} = 25, H_4SiO_4 = 20
Low Elevation Wells: Salinity = 6, Turbidity = 6, NO_3- + NO_2- = 8, NH_4+ = 8, TDN = 6, PO_4^{3-} = 8, H_4SiO_4 = 6
Resort Shoreline: Salinity = 12, Turbidity = 12, NO_3- + NO_2- = 12, NH_4+ = 12, TDN = 12, PO_4^{3-} = 12, H_4SiO_4 = 12
Puakō Shoreline: Salinity = 137, Turbidity = 137, NO_3- + NO_2- = 135, NH_4+ = 135, TDN = 135, PO_4^{3-} = 135, H_4SiO_4 = 135
Puakō Ponds: Salinity = 6, Turbidity = 2, NO_3- + NO_2- = 7, NH_4+ = 7, TDN = 6, PO_4^{3-} = 7, H_4SiO_4 = 6

anchialine ponds (Table 2). Nutrient concentrations in waters fronting the resorts were comparable to those observed along the Puakō shoreline (Table 2), except that Puakō had more stations with higher concentrations of TDN and TDP (Fig. 2B, C). H_4SiO_4 concentrations varied along the shoreline, with high concentrations indicative of areas of submarine groundwater discharge (SGD) that were both located within Puakō and in front of the resorts (Fig. 2D).

To further examine sources of NO_3^- to mid-elevation groundwater wells, low elevation groundwater wells, anchialine ponds, and shoreline waters, bi-plots of δ^{15}N- and δ^{18}O-NO_3^- were created, plotting averages for each station relative to averages for all potential NO_3^- sources (Fig. 4). This step allowed for the stable isotope data for samples to be visualized relative to values for the sources. These figures were used to decide on which sources to include in the Stable Isotope Analysis in R (SIAR, v. 4.0) mixing models. They also allowed us to determine if different sources have overlapping stable isotope values. This is important because if there is overlap, it is harder to distinguish each source's contribution to a sample from one another in the modeling effort. Based on this analysis, we decided to include sewage, groundwater, and kiawe/fertilized soil. Note that ocean water was not included as a NO_3^- source as its NO_3^- concentrations were below detection limits for stable isotope analysis (> 2 µmol/L, Coplen et al. 2012).

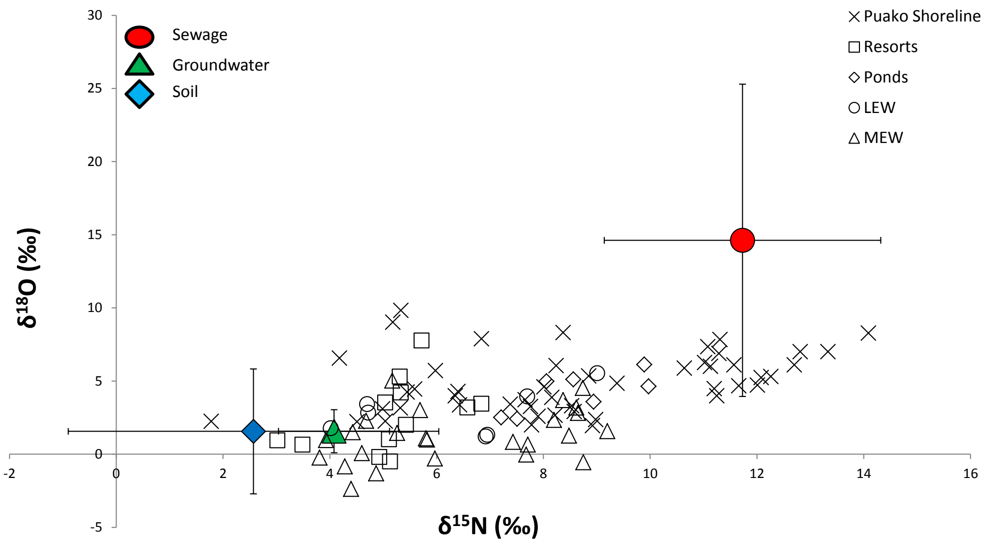

Figure 4. Mean δ^{15}N- and δ^{18}O- NO_3- values of NO_3^- sources (SD, sewage, groundwater, and kiawe/fertilized soil) and water samples from Puakō's and the adjacent resorts' shoreline, Puakō's anchialine ponds, low elevation groundwater wells (LEW), and mid elevation groundwater wells (MEW).

Table 3. Mean ± SE of δ^{15}N- and δ^{18}O- NO_3^- (‰) and nutrient concentrations (µmol/L) of potential NO_3- sources (high elevational groundwater, sewage, and kiawe/fertilized soil) collected in Puakō as part of this study and a previous one (Abaya et al. 2018). Sources and nutrients without SE indicate single sample (n=1).

Source	δ^{15}N NO_3^-	δ^{18}O NO_3^-	NO_2^- + NO_3^-	NH_4^+	TDN	PO_4^{3-}	H_4SiO_4
Groundwater	4.08 ± 0.31	1.57 ± 0.44	95.27 ± 2.33	2.29 ± 0.89	102 ± 1	2.35 ± 0.06	775 ± 53
Sewage	11.73 ± 1.16	14.62 ± 4.77	49.06 ± 33.90	4803.99 ± 1160.31	2787± 1602	307.21 ± 47.29	467 ± 97
Soil	2.57 ± 1.74	1.56 ± 2.13	4775.26 ± 3051.69	446.41 ± 162.12	3	145.33± 111.11	1

To determine the percent contribution of potential NO_3^- sources to the various water types examined in this project, we used SIAR. Both δ^{15}N- and δ^{18}O- NO_3 were used in the models, and the potential NO_3- sources examined were sewage, groundwater, and kiawe/fertilized soil (Table 3). SIAR was first used to examine potential NO_3^- sources to mid-elevation groundwater wells (MEW), low-elevation groundwater wells (LEW), Puakō's anchialine ponds (Ponds), the resorts' shoreline waters (Resorts), and Puakō's shoreline waters (Fig. 5, Table 4). Note, in this model, data from all stations within a water type were averaged and station differences within water types were not examined. The second SIAR model then examined potential NO_3^- sources to Puakō's and the resorts' shoreline stations (Fig. 6, Table 5). This analysis was done to identify any shoreline sewage pollution hot spots. The Shapiro-Wilk test for normality was used to verify that isotope data for the three NO_3^- sources were normally distributed. δ^{15}N- and $\delta^{18}O$-NO_3^- data were normally distributed for all sources. Relative percent contributions of each NO_3^- source to each water type or station are reported as 50% Bayesian credibility interval. This credibility interval allows a wider range of natural variability and uncertainty to be reported within a system with multiple sources (Parnell et al. 2010).

Sewage contributions to the NO_3^- pool varied among water types (Fig. 5, Table 4). Puakō's anchialine ponds had the largest sewage contribution to the NO_3^- pool (36 – 55%), followed by Puakō's shoreline waters (23 – 26%), resort shoreline waters (12 – 17%), low elevational groundwater wells (9 – 20%), and mid-elevational groundwater wells (1 – 4%). Along Puakō's shoreline, ten stations (station #: 1, 2, 3, 4, 5, 6, 11, 12, 13, and 14) had sewage contributing up to 40% or more (high end of the range) to the shoreline NO_3^- (Fig. 6, Table 5). Several of these stations have been identified as sewage pollution hotspots either from δ^{15}N macroalgal values, sewage pollution scores, or dye tracer tests (Abaya et al. 2018, Wiegner et al. unpubl. data)

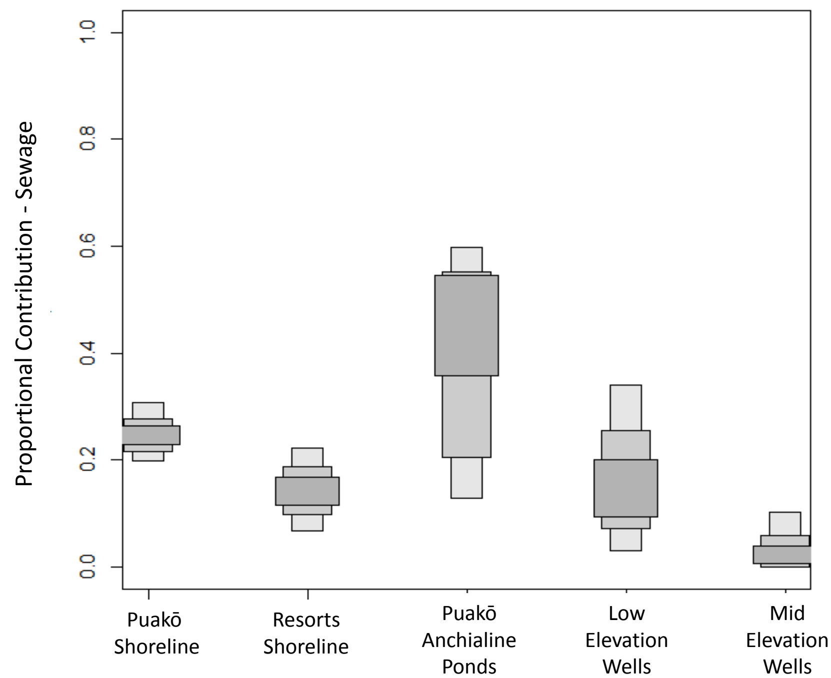

Figure. 5. Average proportional contribution of sewage to the NO_3^- pool in different water types measured in Puakō's watershed, Hawaiʻi. Proportions were estimated using SIAR (v. 4.0). Boxplots illustrate the 50th, 75th, and 95th, percentiles from light to dark.

Table 4. The range of proportional contributions (%) of potential NO_3- sources (sewage, high groundwater, kiawe/fertilized soil) within five different water types: mid elevation wells (MEW), low elevation wells (LEW), anchialine ponds within Puakō (Ponds), shoreline waters at resorts adjacent to Puakō (Resorts) , and shoreline waters at Puakō. Percent contributions are reported as the 50% Bayesian Credibility interval and analyses were run in SIAR (v. 4.0).

Water Type	Sewage	Groundwater	Soil
MEW	1 – 4	84 - 97	0 – 10
LEW	9 - 20	38 - 64	20 - 43
Ponds	36 - 55	31 - 51	1 - 20
Resorts	12 - 17	59 - 81	3 - 24
Puakō Shoreline	23 - 26	66 - 75	0 – 7

Objective 2: *Quantify water quality impairment caused by homes with septic tanks and aerobic treatment units (ATU) through dye tracer studies, and measurements of macroalgal tissue δ^{15}N, FIB (including Bacteroides), and nutrients (including δ^{15}N – and $\delta^{18}O$-NO_3^-).*

Accomplishments and ongoing efforts: In late 2016, we informed homeowners of our interest in doing dye tracer studies through a community email newsletter (Clean Water for Reefs - Puakō newsletter), an announcement at a community meeting, and through direct interaction with homeowners. Homeowners interested in participating in the dye tracer studies contacted us, and we discussed the experimental process with them. We completed all six dye tracer tests. They were conducted at homes with septic tanks (n = 3) and ATUs (n = 3) (February - May 2017).This included two homes on the landward (mauka) side of the street. Note, dye tracer tests were conducted for cesspools in an earlier project (NOAA CRCP). Analyses of field samples from the dye tracer tests have been completed. Macroalgae and water quality samples were collected in front of all of the dye tracer locations from June – August 2017. FIB, δ^{15}N macroalgae tissue, and nutrient samples have all been analyzed. Analysis of δ^{15}N- and δ^{18}O- of NO_3^- in water samples is complete and a SIAR model was

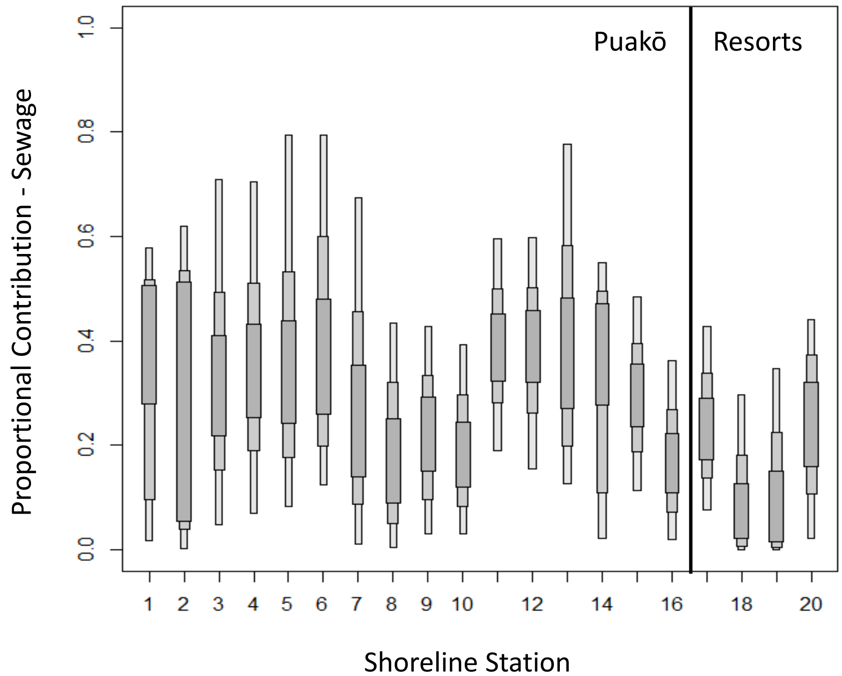

Figure. 6. Average proportional contribution of sewage to the NO_3^- pool in shoreline waters at Puakō and adjacent resorts, Hawaiʻi. Proportions were estimated using SIAR (v. 4.0). Boxplots illustrate the 50th, 75th, and 95th, percentiles from light to dark.

Table 5. The range of proportional contributions (%) of potential NO_3- sources (sewage, high elevation groundwater, and kiawe/fertilized soils) at shoreline stations in Puakō (Stations: 1-16) and adjacent resorts (Stations: 17-20) in Hawaiʻi. Percent contributions are reported as the 50% Bayesian credibility interval. Analysis was conducted in SIAR (v. 4.0)

Location	Station #	Sewage	Groundwater	Soil
Puakō	1	28 - 50	27 - 50	16 – 40
	2	5 - 52	26 - 51	16 – 41
	3	22 - 41	25 - 48	20 – 43
	4	24 - 43	23 - 46	19 – 43
	5	24 - 44	23 - 47	19 – 43
	6	26 - 47	21 - 45	14 - 39
	7	13 - 35	27 - 52	21 – 45
	8	9 - 25	29 - 53	27 – 49
	9	14 - 28	33 - 57	20 – 43
	10	12 - 24	35 - 60	19 – 42
	11	33 - 45	24 - 45	33 – 45
	12	31 - 45	24 - 45	20 – 41
	13	27 - 48	21 - 44	14 – 38
	14	27 - 47	28 - 52	15 – 40
	15	24 - 36	27 - 48	23 – 42
	16	11 - 22	35 - 60	21 – 43
Mauna Kea Beach	17	17 - 29	32 - 55	21 – 42
Hapuna Beach Hotel	18	2 - 12	35 - 58	30 – 51
Fairmont Orchid	19	2 - 16	35 - 63	23 – 47
Mauna Lani Beach	20	17 - 33	33 - 56	20 - 42

utilized to determine the relative percent contributions of NO_3^- sources in front of homes with different

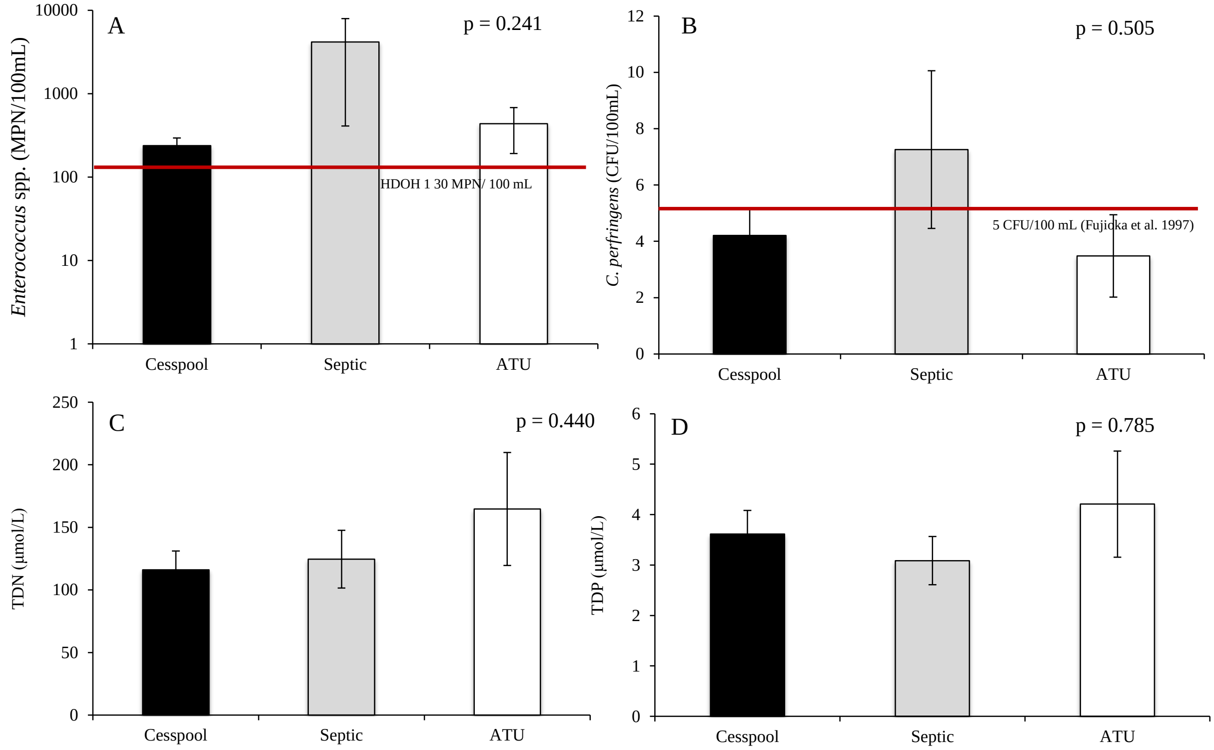

Figure 7. Comparison of average (±SE) water quality parameters in front of homes with differing onsite sewage disposal systems (OSDS, cesspools n = 4, septic tanks n = 3, Aerobic Treatment Units (ATU) n = 3) in Puakō, Hawai`i. A) *Enterococcus* spp., B) *Clostridium perfringens*, C) Total Dissolved Nitrogen (TDN), and D) Total Dissolved Phosphorus (TDP). Samples were collected three times from June – August 2017. Cesspool data were collected as part of an earlier project, three cesspools were sampled seven times, and one was sampled 12 times from 2014-2016 (Abaya et al. 2018). Red lines on figure indicate state and recommended FIB standards for marine recreational waters. Results from one-way ANOVA shown on figure $\alpha = 0.05$.

OSDS. Analysis of *Bacteroides* samples is still pending.

<u>Findings</u>: Dye was observed at the shoreline in five of the six tests. The time it took the dye to travel from the septic tank or ATU to the shoreline varied from 5 hours to 11 days, with flow rates from 3 to 137 m/d. In general, dye appeared at the shoreline sooner in front of homes with drainage fields closer to the shoreline. The type of system (septic tank vs. ATU) did not affect how fast dye reached

Table 6. Average (±SE) water quality condition in front of homes with different on-site sewage disposal systems (OSDS: cesspool, septic tanks, aerobic treatment units (ATU)) at Puakō, Hawaiʿi. Measurements were made from November 2014 - July 2017. OSDS n = number of OSDS within a type sampled. Samples n = number of samples collected in front of each home.

OSDS type	OSDS n	Sample n	*Enterococcus* spp. (MPN/100 mL)	*Clostridium perfringens* (CFU/100 mL)	$\delta^{15}N$ Algae (‰)	$\delta^{15}N$-NO_3^- (‰)	NO_3^-+NO_2^- (µmol/L)	NH_4^+ (µmol/L)	TDN (µmol/L)	PO_4^{3-} (µmol/L)	TDP (µmol/L)	H_4SiO_4 (µmol/L)
Cesspool	4	4 - 10	239 ± 56	4 ± 1	9.12 ± 0.37	11.07 ± 0.43	105.88 ± 14.98	2.01 ± 0.22	116 ± 15	3.18 ± 0.43	3.62 ± 0.47	325 ± 34
Septic tank	3	3	4182 ± 3772	7 ± 3	7.04 ± 0.45	7.55 ± 0.41	117.14 ± 20.22	3.00 ± 1.28	125 ± 23	2.62 ± 0.40	3.09 ± 0.48	439 ± 44
ATU	3	3	439 ± 244	3 ± 1	8.19 ± 1.18	9.03 ± 1.09	157.51 ± 41.27	3.42 ± 0.71	165 ± 45	3.86 ± 0.93	4.21 ± 1.05	588 ± 86

the shoreline, with ATUs having both the slowest and fastest flow rates. These results suggest that the underlying geology (e.g., presence or absence of large fractures in the basalt) likely controls how fast sewage flows from the OSDS to the shoreline.

The only test where dye was not observed at the shoreline was from the home farthest landward (mauka). Here, the drainage field was 122 m from the shoreline; at the other five homes, this averaged 40 m. Additionally, this home was located in the northern part of the neighborhood, where fewer groundwater springs have been observed. It is possible that we either missed the spring where the dye emerged, or that we did not sample long enough to capture the dye emerging from the spring (after 16 days, flow rate of <8 m/d).

Concentrations of FIB and nutrients were similar in front of homes with different OSDS (Fig. 7). The average concentration of *Enterococcus* spp. in front of homes with differing OSDS all exceeded the HDOH statistical threshold value of 130 MPN/100 mL (Fig. 7A). *Enterococcus* spp. concentrations ranged from 0 – 34,330 MPN/100 mL. The average concentration of *C. perfringens* in front of the homes with differing OSDS encompassed the standard recommended to HDOH for marine recreational waters of 5 CFU/100 mL (Fujioka et al. 1997), but they were not higher than the range reported for non-point sewage pollution of 10 -100 CFU/100 mL (Fig. 7B, Fung et al. 2007). *C. perfringens* concentrations ranged from 0 – 27 CFU/100 mL. δ^{15}N in macroalgae was similar in front of homes with the different OSDS types (Fig. 8). The average δ^{15}N value in front of homes with cesspools was 9.12 (± 0.37, 4.90 – 12.78), septic tanks 7.04 (± 0.45, 4.84 – 8.70), and ATUs 8.19 (± 1.18, 2.75 – 10.88). Average nutrient concentrations in front of all homes were high. TDN average concentrations in front of homes with different OSDS were all greater than100 µmol/L, with individual measurements ranging from 13 – 415 µmol/L (Fig 7C). NO_3^-+NO_2^- comprised most of the TDN. TDP averages in front of the homes with differing OSDS were all greater than 3 µmol/L, with individual measurements ranging from 0.25 – 10.84 µmol/L (Fig. 7C). PO_4^{3-} comprised most of the TDP. H_4SiO_4 concentrations did differ in front of the homes with different OSDS, but this difference was most likely from SGD and not from sewage as $H_4SiO_4$is an indicator for groundwater (data not shown).

SIAR modeling was used to partition potential NO_3^- sources in front of homes with different OSDS types. Details on model effort are described in the results section for Objective 1. Proportional contributions of sewage to the NO_3^- pool in front of homes with different OSDS types varied (Fig. 9). Sewage comprised 25 – 35% of the NO_3^- in front of homes with cesspools, 41 – 49% in front of homes with septic tanks, and 12 – 23% in front of homes with ATUs (Table 7).

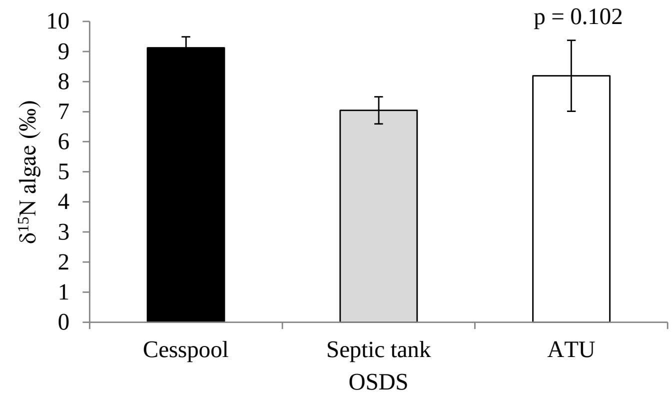

Figure 8. Average (±SE) δ^{15} N macroalgae in front of homes with different onsite sewage disposal systems (OSDS: cesspools, septic tanks, aerobic treatment units (ATU)) in Puakō, Hawaiʻi. Results from one-way ANOVA are shown on figure.

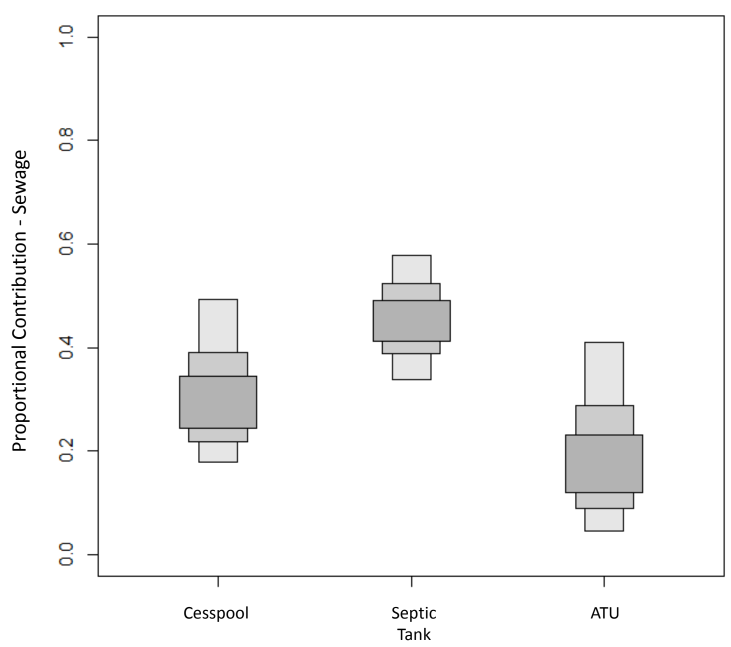

Figure 9. Proportional contributions of sewage as a NO_3^- source for three OSDS (cesspool, septic tanks, ATUs) estimated using SIAR (v. 4.0). Boxplots illustrate the 50th, 75th, and 95th, percentiles from light to dark.

Objective 3: *Assess whether the proposed sewage treatment upgrades are sufficient for meeting water quality standards.*

Table 7.. The range of proportional contributions (%) of potential NO_3- sources (sewage, high elevation groundwater, and kiawe/fertilized soils) at three different on-site disposal systems (OSDS) in Puakō: cesspools, septic tanks, and ATUs. Percent contributions are reported as the 50% Bayesian credibility interval. Analysis was conducted in SIAR (v. 4.0)

OSDS Type	Sewage	Groundwater	Soil
Cesspools	25 – 35	33 – 53	14 – 35
Septic Tanks	41 – 49	30 – 49	3 – 20
ATUs	12 - 23	36 - 58	23 - 44

Accomplishments and ongoing efforts: Data from objectives 1 and 2 have been finalized. A three end-member mixing model was used to determine if sewage treatment upgrades are sufficient for meeting water quality standards for FIB.

Findings: A three end-member mixing model (sewage, groundwater, seawater) was used to estimate the fraction of FIB (*Enterococcus* spp. and *C. perfringens*) from sewage in groundwater spring samples along Puakō's shoreline, and to evaluate potential shoreline water quality improvements that will occur by switching from the current distribution of OSDS to all septic systems, all ATUs, or a sewage treatment plant. FIB concentrations measured in groundwater, sewage, and ocean water from this study and our previous NOAA CRCP grant were used in these calculations. The sewage end-member was assumed to be a mixture of water from different OSDS, with 44% cesspool, 48% septic systems and 8% ATUs (Aqua Engineering 2015). FIB concentrations in cesspools were based on measurements of raw sewage at Puakō, which are similar to raw sewage values measured elsewhere (Ahmed et al. 2008, Kay et al. 2008). Septic tanks were assumed to decrease *Enterococcus* spp. and *C. perfringens* concentrations by 50% and 68%, respectively, based on published studies (Chauret et al. 1999, Withers et al. 2011). We found no published information regarding the effectiveness of ATUs in reducing FIB concentrations in their effluent, so we assumed the same reduction as septic systems. This assumption may underestimate the FIB concentration in the ATU effluent since the residence

Table 8. Calculated concentration of fecal indicator bacteria (*Enterococcus* spp. and *Clostridium perfringens*) at shoreline under different scenarios of neighborhood-wide OSDS conversion (*see* Objective 3 for details).

	Enterococcus spp. (MPN/100 mL)				*C. perfringens* (CFU/100 mL			
Station	Today	Septic	ATU*	Treatment*	Today	Septic	ATU	Treatment
1	14	10	10	1.3	1.38	0.72	0.72	0.01
2	97	68	68	3.9	9.72	5.03	5.03	0.00
3	272	190	190	2.7	3.71	1.92	1.92	0.00
4	155	108	108	2.3	3.67	1.90	1.90	0.00
5	645	448	448	0.5	2.14	1.11	1.11	0.01
6	408	283	283	0.5	2.58	1.34	1.34	0.01
7	177	123	123	1.2	5.47	2.83	2.83	0.01
8	426	296	296	2.1	4.10	2.12	2.12	0.00
9	360	251	251	2.0	1.17	0.61	0.61	0.00
10	77	54	54	2.7	2.90	1.50	1.50	0.00
11	485	337	337	0.5	5.11	2.65	2.65	0.01
12	396	275	275	0.8	3.10	1.60	1.60	0.01
13	1931	1341	1341	0.5	1.86	0.96	0.96	0.01
14	248	173	173	3.6	6.81	3.52	3.52	0.00
15	288	200	200	0.1	6.23	3.23	3.23	0.01
16	420	292	292	2.0	1.54	0.80	0.80	0.00

* ATU = Aerobic Treatment Unit; Treatment = Sewage Treatment Facility

time of wastewater in ATUs tends to be less than septic tanks, and there may be differences in how

Figure10. Three summer interns (Saria Adnan Sultan, Christopher Thompson, and Adel Sharif) were supported by the National Science Foundation (NSF) Research Experiences for Undergraduates (REU) program at University of Hawai'i at Hilo [UH Hilo; Pacific Internship Programs for Exploring Science (PIPES) –https://hilo.hawaii.edu/uhintern/). Interns assisted with collecting water samples from A) wells at the Mauna Lani Resort (Saria and Christopher in black, summer 2016), B) at the Puakō shoreline (same as panel A), and C) processing FIB samples collected in front of homes where dye tracer studies were conducted (Adel, summer 2017). Note, these were the primary students working on this project; other interns or student assistants also participated.

the system affects *Enterococcus* spp. and *C. perfringens*, as *Enterococcus* spp. can survive in aerobic conditions, while *C. perfringens* is an obligate anaerobe.

Conversion of the entire Puakō neighborhood to septic tanks or ATUs would reduce average *Enterococcus* spp. and *C. perfringens* concentrations at the shoreline by 30% and 48%, respectively (Table 8). For *Enterococcus* spp., this transition would reduce the percent of stations that exceed the HDOH statistical threshold value (130 CFU/100 mL) from 81% to 69%. For *C. perfringens*, the reduction is greater with septic and ATUs, with only one station exceeding the recommended marine standard of 5 CFU/100 ml (Fujioka et al. 1997). A wastewater treatment plant would reduce FIB concentrations well below the HDOH standards by specifically targeting the removal of FIB with chlorine, ozone, or ultraviolet (UV) treatment. With complete

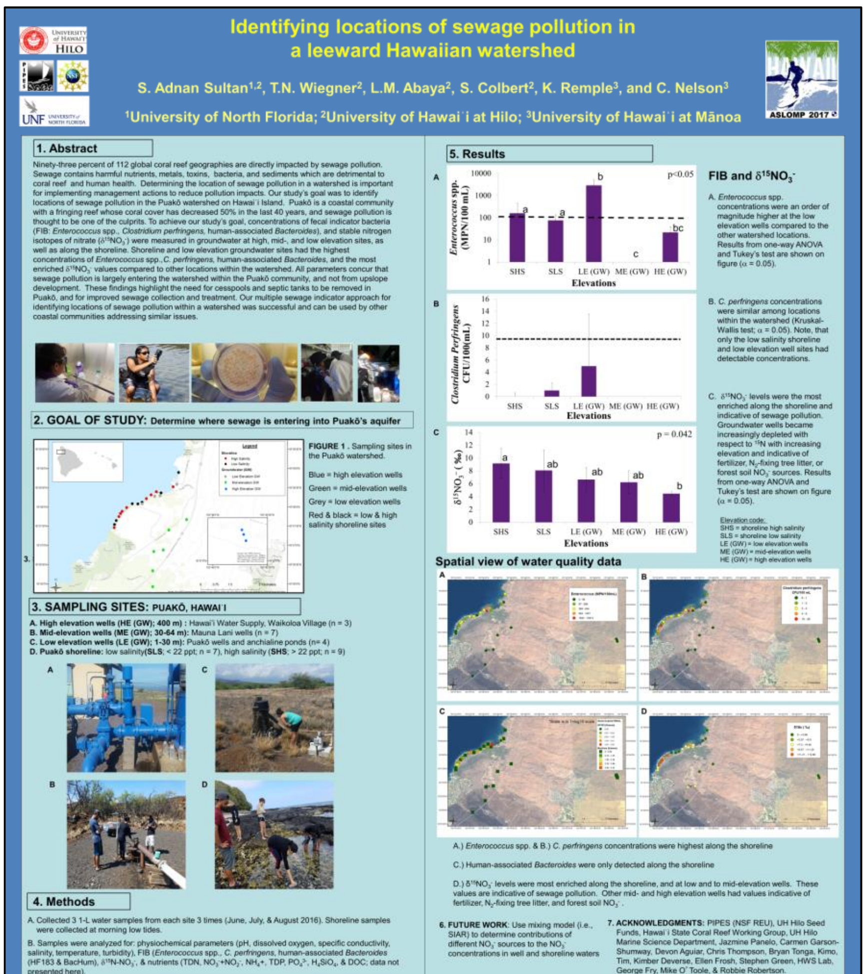

Figure 11. Saria Adnan Sultan's (summer 2016 intern) poster presented at the Association for the Sciences of Limnology and Oceanography (ASLO) conference in Honolulu, HI (February 28 –March 3, 2017). Her travel was supported by ASLO Minorities Program (ASLOMP) and UH Hilo PIPES program .

removal of sewage from the water table with a treatment plant, the resulting shoreline water would only be a mixture of groundwater and seawater.

4. Deliverables and Outcomes *(How did this project address critical management needs?)*

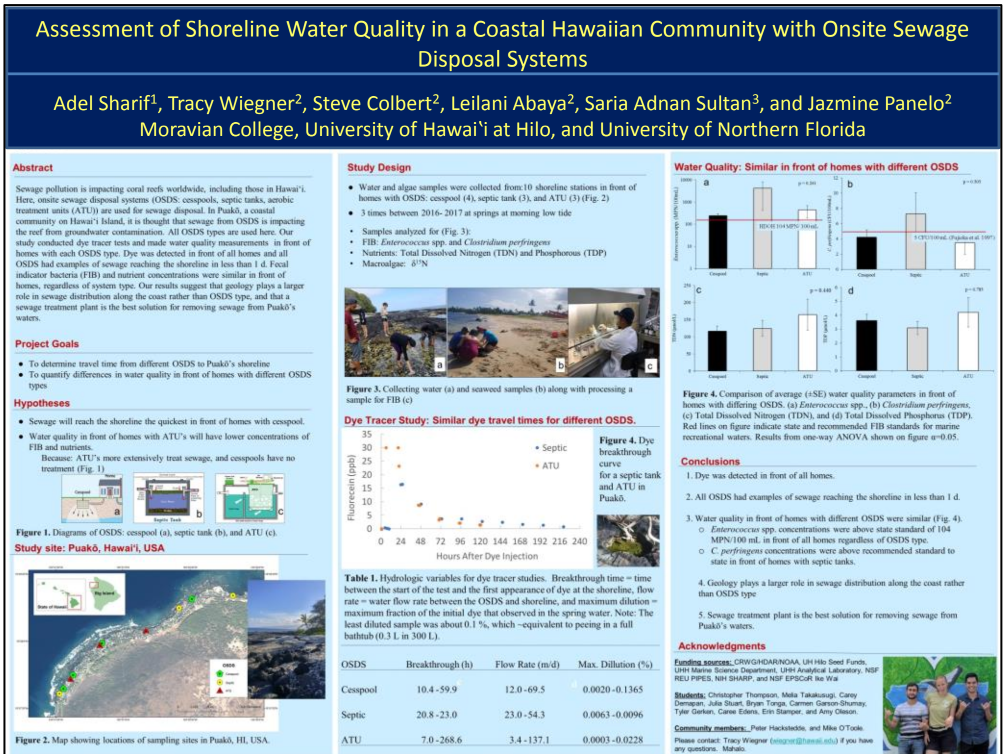

Figure 12. Adel Sharif's (summer 2017 intern) poster presented at the Association for the Sciences of Limnology and Oceanography (ASLO) conference in Victoria, Canada (June 2018). His travel was supported by UH Hilo PIPES program.

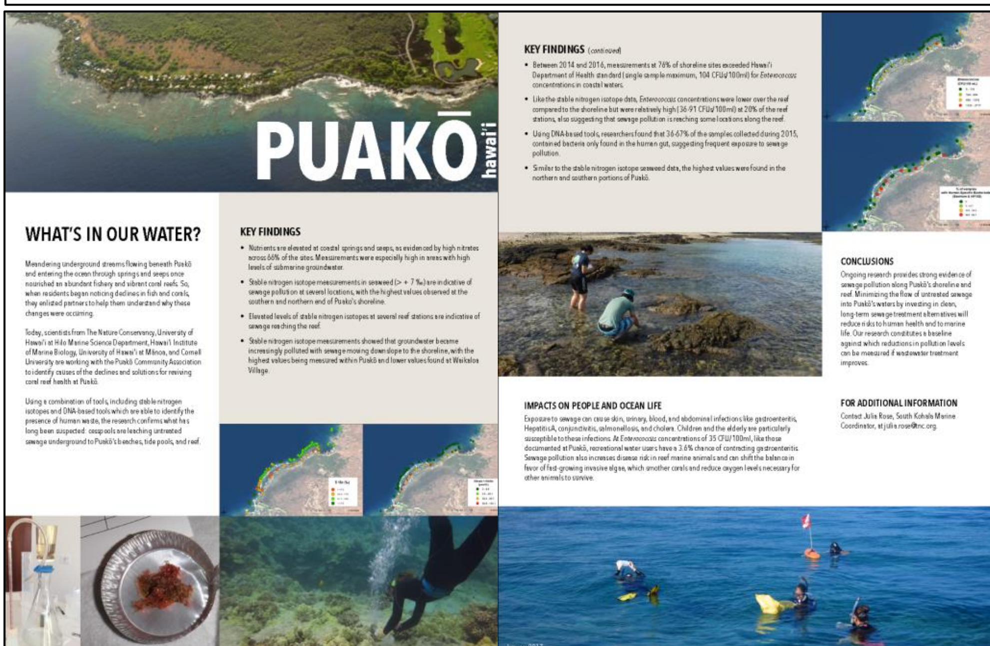

Figure 13. Community handout prepared in collaboration with The Nature Conservancy for the Puakō Wastewater Forum held January 2017. Meeting was organized by the Coral Reef Alliance.

Deliverables: Ten undergraduate students were trained on this project to date. They include six summer interns (Saria Adnan Sultan, Christopher Thompson, Adel Sharif, Carmen Garson-Shumay, Tyler Gerken, and Amy Olson; Fig. 10) supported by the National Science Foundation (NSF) Research Experiences for Undergraduates (REU) program at University of Hawaiʻi at Hilo [UH Hilo; Pacific Internship Programs for Exploring Science (PIPES) – https://hilo.hawaii.edu/uhintern/). They presented results from their internships at the end-of-the-summer symposium and wrote final reports. Early in 2017 (February – March 2017), one of the summer interns, Saria Adnan Sultan, presented findings from her internship in a poster at the ASLO conference in Honolulu, HI

(Fig. 11). Her travel was supported by ASLO Minorities Program (ASLOMP) and UH Hilo PIPES program. This past June (2018), Adel Sharif, our most recent summer intern, presented findings from his internship on this project at the ASLO Aquatic Sciences conference in Victoria, Canada (Fig. 12). His travel expenses were paid with funds from the UH Hilo PIPES program (NSF REU). We also had several other undergraduates working on this project, including: Carey Demapan (NSF Experimental Program to Stimulate Competitive Research (EPSCoR) Ike Wai scholars program 2016-2017, 2018 grant-employed student assistant), Melia Takakusagi (NIH SHARP intern), and Julia Stuart (grant-employed student assistant). These latter students assisted with field preparations and laboratory sample processing. Both Carey and Melia presented posters on their experiences working on the project in April 2017 as part of their respective internship programs.

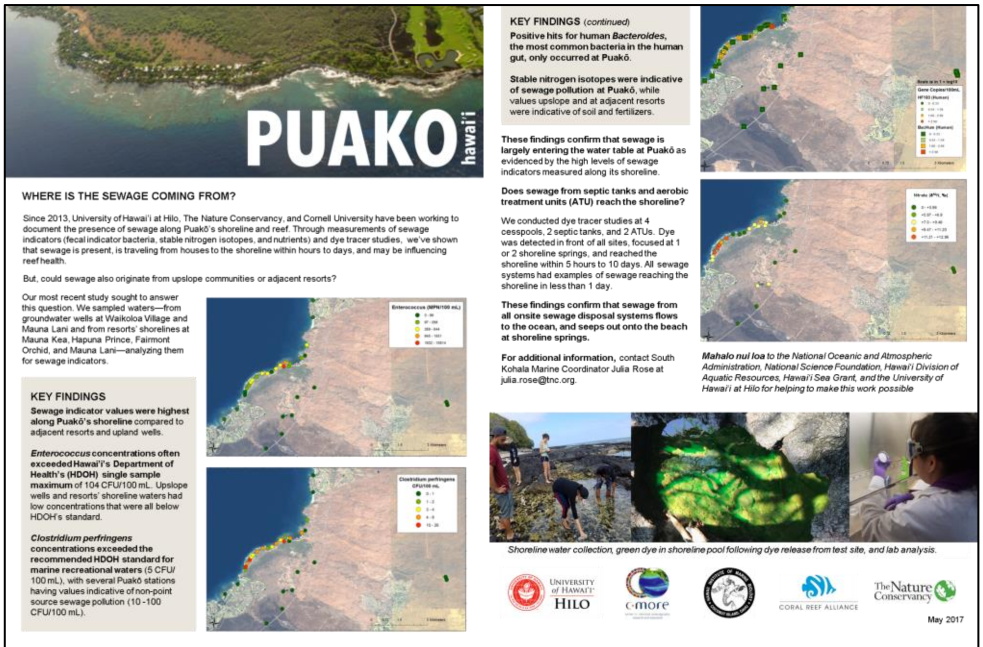

Figure 14. Community handout prepared in collaboration with The Nature Conservancy for the Puakō Community Association meeting in May 2017. Meeting was organized by the Coral Reef Alliance.

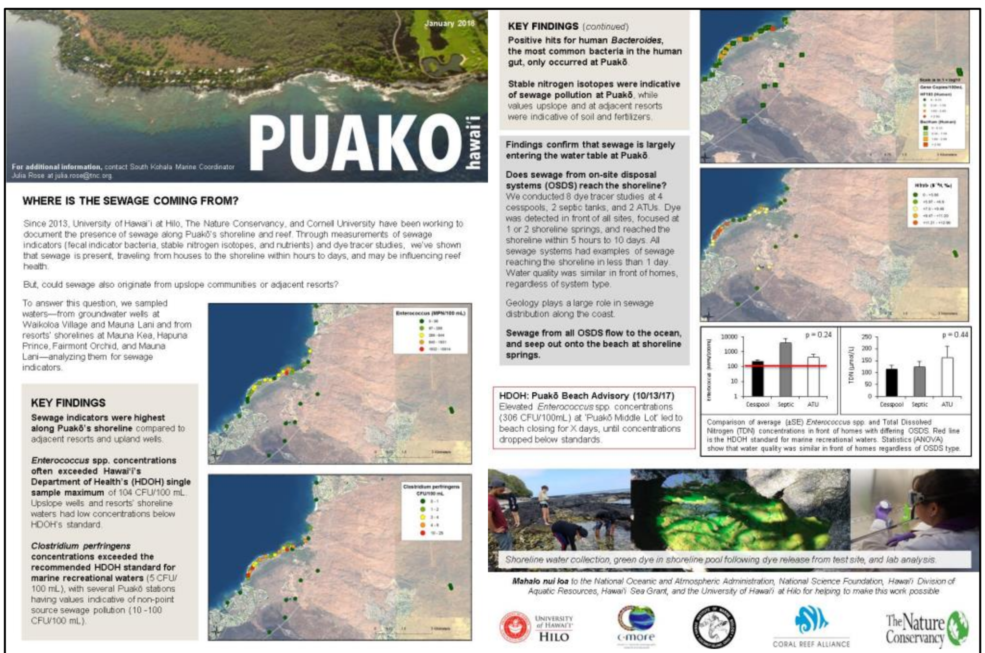

Figure 15. Community handout prepared in collaboration with The Nature Conservancy for the Puakō Wastewater Forum held January 2018. Meeting was organized by The Coral Reef Alliance.

We also produced three community handouts for the Puakō community in collaboration with The Nature Conservancy (TNC; Figs. 13 - 15). Two were for Puakō's Wastewater Symposium in January 2017 and 2018 (Figs. 13 and 15) and the other was for a community association meeting in May 2017 (Fig. 14). At this latter meeting, we gave a joint presentation on results from this project with TNC. In December 2017, Dr. Wiegner presented results from this project at the NOAA West Hawai'i

Symposium in Kona, HI. In January 2018, Dr. Colbert provided an update on this project to the Puakō community at a Coral Reef Alliance sponsored meeting, along with an updated handout (Fig. 15). In 2018, Drs. Wiegner and Colbert gave four presentations on this project at professional meetings: 1) HDOH Cesspool Update (June 2018), 2) Hawaiʻi Conservation Conference, Honolulu, HI (July 2018), 3) HDOH Joint Government Water Conference, Hilo, HI (August 2018), and 4) HDOH Joint Government Water Conference, Kailua Kona, HI (August 2018).

Outcomes: Results from ASLO poster were shared with the Puakō Community Association at a meeting in May (2017) to provide them with critical information regarding where sewage pollution is entering their aquifer (Fig. 14). An updated handout was shared with the community in January 2018 (Fig. 15). All parameters concur that sewage pollution is largely entering the watershed within the Puakō community, and not from upslope development or adjacent resorts. These findings highlight the need for OSDS (cesspools, septic tanks, and ATU) to be removed in Puakō, and for improved sewage collection and treatment at a centralized facility. This month (September 2018), a request to HDOH to change Puako's priority status for cesspool removal was submitted by Coral Reef Alliance, which included a letter from UH Hilo with data from this and early projects.

5. Obstacles or Delays: Dye tracer tests were delayed due to challenges in finding volunteer homeowners. A point of contact for arranging dye tracer tests with homeowners was established and all six tests have now been conducted.

Results from Northern Arizona University (NAU) for isotope analyses were delayed due to staff shortages and instrumentation downtime.

Bacteroides sample analysis is still pending due to staff shortages in the Nelson lab at UH Mānoa.

6. Future needs: There are no future needs as the project is finished.

7. Photo and caption (optional). Photos and captions are contained within figures.

8. Additional Sources of Funding: UH Hilo Seed Funds (from UH Hilo Research Council, Awarded to T. Wiegner), UHH Marine Science Department, UHH Analytical Laboratory (https://hilo.hawaii.edu/~analab/), and several UHH internship programs: [a]PIPES (https://hilo.hawaii.edu/uhintern/), [b]SHARP (http://www.uhhilo-sharp.org/), and [c]NSF EPSCoR Ike Wai (https://www.hawaii.edu/epscor/).

9. Field and Laboratory Assistance Provided By:

UH Hilo Technicians: Leilani Abaya, Jazmine Panelo, Caree Edens, and Erin Stamper.

Graduate students: Kristina Remple (UH Mānoa, SOEST).

UH Hilo Undergraduate Interns and Assistants: Saria Adan-Sultan[a], Christopher Thompson[a], Adel Sharif[a], Melia Takakusugi[b], Carey Demapan[c,d], Julia Stuart[d], Bryan Tonga[e], Carmen Garson-Shumay[a], Tyler Gerken[a], and Amy Oleson[a].

[a] UH Hilo NSF REU PIPES summer interns
[b] UH Hilo NIH SHARP interns
[c] UH Hilo NSF EPSCoR Ike Wai scholars
[d]Student assistant on DAR CRWG project
[e] UH Hilo Analytical Laboratory student assistant

Progress Report Prepared by: Tracy Wiegner, Leilani Abaya, and Steve Colbert

Signature of Point of Contact:

Tracy Wiegner

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