Title: A multi-indicator approach for identifying shoreline sewage pollution hotspots adjacent to coral reefs

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Abstract: Sewage pollution is contributing to the global decline of coral reefs. Identifying locations where it is entering waters near reefs is therefore a management priority. Our study documented shoreline sewage pollution hotspots in a coastal community with a fringing coral reef (Puakō, Hawai‘i) using dye tracer studies, sewage indicator measurements, and a pollution scoring tool. Sewage reached shoreline waters within 9 h to 3 d. Fecal indicator bacteria concentrations were high and variable, and $\delta^{15}N$ macroalgal values were indicative of sewage at many stations. Shoreline nutrient concentrations were two times higher than those in upland groundwater. Pollution hotspots were identified with a scoring tool using three sewage indicators. It confirmed known locations of sewage pollution from dye tracer studies. Our study highlights the need for a multi-indicator approach and scoring tool to identify sewage pollution hotspots. This approach will be useful for other coastal communities grappling with sewage pollution.

Key words: fecal indicator bacteria, Hawai‘i, macroalgae, nutrients, pollution score, stable nitrogen isotopes

Introduction

With more than 50% of the world's population living within coastal areas, sewage pollution has become a growing global problem that is largely unrecognized. Untreated sewage from cesspools and septic tanks is a concern for human and environmental health in rural areas (Lapointe et al. 1990, Paul et al. 2000, Whittier & El-Kadi 2014). Sewage pollution is a complex environmental problem because it is a cocktail containing elevated and potentially hazardous levels of pathogens, hydrocarbons, nutrients, toxins, organic and inorganic compounds, and endocrine disruptors (Wear & Vega Thurber 2015). For example, human exposure to sewage can result in skin and urinary tract infections, hepatitis, and gastroenteritis (Pinto 1999). Annually, there are over 120 million gastroenteritis cases worldwide associated with sewage contaminated waters (Shuval 2003). In addition, sewage pollution can have detrimental effects on coastal ecosystems (Wear & Vega Thurber 2015). Coral reefs, which are one of the most economically valuable and biologically diverse ecosystems in the world, are steadily declining from multiple stressors including sewage pollution (Wear & Vega Thurber 2015). Sewage pollution has been linked with increased coral disease prevalence and severity (Sutherland et al. 2010, Redding et al. 2013, Yoshioka et al. 2016). White pox disease in Caribbean corals is one well documented example where a human pathogen is found in sewage, *Serratia marcescens*, was shown to cause the disease (Sutherland et al. 2010), although this relationship is disputed (Lesser & Jarett 2014). Nutrient enrichments associated with sewage can stimulate benthic algal growth, resulting in a benthic phase shift from coral-to macroalgal-dominated reefs (Hunter & Evans 1995, Lapointe et al. 2005). These nutrients also alter coral growth rates, species distribution and abundance, and coral community diversity (Pastorok & Bilyard 1985, Parsons et al. 2008).

As the human population and associated coastal development continues to grow, monitoring water quality for sewage pollution is essential. Dye tracer studies provide irrefutable evidence that sewage from on-site sewage disposal systems (OSDS; i.e., cesspool, septic tanks) and treatment plants is entering and contaminating water bodies (Yates 1985, HDOH 1984, Glenn et al. 2013). These studies reveal hydrogeologic features connecting these treatment systems to nearshore waters, and are used to calculate

pollution transit times, flow rates, and dilution within aquifers. However, dye tracer studies are laborious with hourly, daily, and in some instances, monthly sample collections. They also occur at one location at a time, and thus, provide limited geographical information on where sewage is entering into the ocean. Hence, they are only generally conducted when it is suspected that sewage is entering the ocean from a specific site.

In contrast, measurements of fecal indicator bacteria (FIB) are a more widely used to detect sewage than dye tracer studies, and they serve as a proxy for assessing human health risks (Cabelli 1983, Prüss 1998). *Enterococcus spp*. is monitored in marine recreational waters by the United States Environmental Protection Agency (USEPA) and state health agencies. In tropical locations like Hawai‘i, a secondary indicator, *Clostridium perfringens,* is also assessed (Fujioka et al. 1997, Fujioka et al. 2015). Unlike *Enterococcus spp., C. perfringens* is an anaerobic, spore-forming bacterium that does not multiply in coastal waters, nor grows in tropical soils (Hardina & Fujioka 1991, Fung et al. 2007). Hence, it is thought to more accurately detect sewage pollution than *Enterococcus* spp. (Fujioka & Shizumura 1985, Hardina & Fujioka 1991, Fujioka et al. 1997).

Measurements of stable nitrogen (N) isotopic composition ($\delta^{15}N$) in macroalgal tissues is another method employed to detect sewage pollution in coastal waters (Umezawa et al. 2002, Savage 2005, Hsing-Jun Lin et al. 2007, Dailer et al. 2012, Wiegner et al. 2016). Macroalgae minimally discriminate between ^{14}N and ^{15}N. Therefore, they have similar isotopic compositions relative to their N sources (Savage 2005). Sewage, in particular, has a very distinct stable N isotopic composition compared to other N sources, i.e, fertilizers, soils, groundwater, and ocean water (*reviewed in* Wiegner et al 2016), and thus, has been successfully used to identify locations of shoreline sewage pollution (Umezawa et al. 2002, Savage 2005, Hsing-Jun Lin et al. 2007, Dailer et al. 2010, Dailer et al. 2012).

Nutrient concentrations are also commonly used to assess water quality. It has been shown that nutrient concentrations are significantly higher nearshore with known sewage pollution (Lapointe et al. 1990, Nelson et al. 2015). However, measuring

nutrients at the shoreline alone as a sewage indicator is not informative enough for management actions because of their numerous non-sewage watershed sources. Mixing plots of nutrient concentrations and salinity, a tool commonly used for examining mixing behavior and determining nutrient sources (freshwater vs. ocean) (Officer 1979), may be useful for identifying locations where sewage is entering coastal waters and increasing nutrient concentrations.

Due to spatial and temporal variability associated with different sewage indicators, assessing pollution from a single one can be misleading. For example, authorities are more likely to post beach advisories when using *Enterococcus* spp. rather than *C. perfringens* (Shibata et al. 2004). In addition to FIB, $\delta^{15}N$ in macroalgal tissues can be highly variable due to N inputs from different sources with differing N isotopic compositions (Ochua-Izaquirre & Soto-Jimenez 2015). Hence, it is imperative to measure multiple sewage indicators to determine spatial and temporal pollution patterns particularly when concerned with both human and ecosystem health. However, few studies have done this to date, with most only measuring two indicators simultaneously because methods are difficult, expensive, and time consuming (Knee et al. 2008a, Baker et al. 2010, Moynihan et al. 2012, Yoshioka et al. 2016).

Hawai‘i is an ideal location to develop a multiple sewage indicator approach as its coastal waters and coral reefs have been impacted by sewage pollution for decades (Pastorok & Bilyard 1985, Whittier & El-Kadi 2014). Presently, cesspools are the primary source of sewage pollution in rural areas, which comprise most of the state and are the location where the healthiest coral reefs are found. Hawai‘i uses cesspools more widely than any other state (USEPA 2013), and has only recently banned the installment of new ones (HDOH Administrative Rules-Title 11, HAR, 2016). Cesspools are particularly concerning in Hawai‘i where many of the homes are in close proximity to the water on highly porous substrate. As of 2014, there were over 110,000 OSDS in Hawai‘i State. On Hawai‘i Island alone, there are nearly 59,000 OSDS, with 49,000 being classified as cesspools (Whittier & El-Kadi 2014). A high-risk area where OSDS are likely impacting nearshore waters on Hawai‘i Island is Puakō (Whittier & El-Kadi 2014). Puakō is a coastal community that is home to some of the richest, most diverse reefs in

the state (Hayes et al. 1982). However, coral coverage has decreased from 80% in 1975 to 33% in 2010 (Minton et al. 2012), with concurrent decreases in fish abundance (49% - 69%), and increases in turf and macroalgal cover (38%) (HDAR 2013). Declining coral health and elevated disease prevalence and severity have also been documented (Couch et al. 2014, Yoshioka et al. 2016). While sewage pollution is thought to be one of the culprits contributing to these ecosystem changes, the link between these conditions and the presence of sewage has not been made.

The goal of this study was to develop a multiple sewage indicator approach to more accurately detect the presence of sewage in Puakō's nearshore waters. More specifically, we aimed to: 1) determine whether OSDS were hydrologically connected with coastal waters, 2) measure three sewage indicators including: FIB, $\delta^{15}N$ in macroalgal tissue, and nutrients along the shoreline, 3) identify locations of shoreline sewage pollution using mixing plots, and 4) pinpoint sewage pollution hotspots by developing a sewage pollution score.

Materials and Methods

Site Description

This study was conducted along the Puakō coastline in the South Kohala region of Hawai'i Island (Fig. 1), which is primarily comprised of basalt from the Mauna Loa Volcano. Annual rainfall ranges from 250 – 750 mm and infiltration of rainwater into the aquifer is high due to the permeable substratum. Average submarine groundwater discharge (SGD) at the shoreline ranges from 2083 - 2730 L m^{-1} h^{-1} (Paytan et al. 2006).

Puakō is a residential community along a 3.5 km stretch of coastline with 207 lots, of which 163 have homes. The population is growing at a rate of 6.9% per year (Minton et al. 2012). At Puakō, 47 homes have cesspools and 139 have conventional septic tanks with leach fields (Schott 2010). The entire coastline is accessible to the public and is frequently used for recreational activities such as fishing, surfing, SCUBA diving, and snorkeling. Presently, there is one development up-slope of Puakō, Waikoloa Village, which has 2,000 homes, with 1587 having OSDS; the remainder are connected to the sewer line (per. comm. Hawai'i Water Supply).

Dye Tracer Studies

Dye tracer studies were conducted to determine the hydraulic connectivity between OSDS at four oceanfront homes. Studies were conducted along the southern portion of Puakō's coastline where nearshore waters are relatively fresh. Three homes had cesspools, and one had a fractured aerobic treatment unit (ATU) tank (a type of OSDS that utilizes an aeration process). Two of the four homes were occupied during the studies. At each home, the closest point where dye could be delivered to the OSDS was identified. Fluorescein, a non-toxic organic dye was used for the studies. It has a strong fluorescence and detection levels as low as 1 ppb (Gaspar 1987, Reich et al 2001). For our studies, 500 - 1000 g of high purity fluorescein dye (Amresco Fluorescein Sodium Salt) was injected over ~ 10 h. Each hour, 50 or 100 g of dye were mixed with 20 L of tap water and slowly added to the OSDS. Additional tap water was added throughout the day and its volume recorded to calculate an initial dye concentration.

To sample for the presence of dye at the shoreline, five to six stations were identified in front of each home and adjacent properties (60-70 m of shoreline), representing three to four groundwater springs of varying salinity, and two stations with higher salinity and no apparent freshwater input. Water samples were collected at each station before and during dye tracer studies in opaque brown high density polyethylene bottles to prevent photodegradation, pre-rinsed with sample water, and stored at 4°C until analysis. During the first 12 h of the dye study, samples were collected every two h to identify any fast-flow pathways. Afterwards, two samples were collected at each station within an hour of the lowest-low tide each day for up to 14 d.

To quantify the concentration of fluorescein, samples were brought to room temperature, filtered (Whatman™ GF/F), and analyzed using a Turner AU10 fluorometer in the dark. The detection limit for our analysis was 0.95 ppb (USEPA 40 CFR 2011). When salinity was not measured in the field, conductivity of samples was measured in the laboratory (Orion Star) and converted to PSS-78 salinity (UNESCO 1981).

Shoreline Station Selection

To select shoreline stations for sewage pollution sampling, a salinity survey was conducted using a YSI 6600 V2 multi-parameter sonde interfaced with a Garmin etrex Global Positioning System. These shoreline stations were chosen prior to homes being

identified for dye tracer studies. This was completed in the summer of 2014 during low tides to capture maximum SGD input. From this survey, 16 shoreline stations were chosen with varying salinity; four stations were coincidentally in front of properties where dye tracer tests were conducted (Fig. 1).

FIB and Nutrient Analyses

Triplicate water samples were collected in sterile, acid washed, polypropylene plastic bottles on four dates (November 2014, March, June, and July 2015) at each station and analyzed for FIB, nutrients, and salinity. Sample processing was conducted within 6 h of collection. Samples were taken during low tide when SGD is highest, and near sunrise as sunlight reduces FIB survival (Fujioka et al. 1981). *Enterococcus* spp. was analyzed using the Enterolert MPN method (IDEXX Laboratories Inc.) following manufacture's recommendations of 10 mL sample and 90 mL sterile water. The analytical range for this method is from 1 to 2419 MPN/100 mL. All *Enterococcus* spp. concentrations were corrected for sample dilution during analysis. When no wells fluoresced blue in the QuantiTray, *Enterococcus* spp. concentrations were reported as 5 MPN/ 100 mL, one-half the detection limit of the method after correcting for sample dilution. No diluted *Enterococcus* spp. concentrations exceeded the upper detection limit of the Enterolert MPN method. *C. perfringens* was enumerated by filtering 100 mL of sample water with 0.45-µm pore size cellulose nitrate filters (Whatman™) and mCP medium (Acumedia, Baltimore, MD, USA) (Bisson & Cabelli 1979).

Water from one of the three samples was also filtered through a pre-combusted (500°C for 6 h) filter (GF/F Whatman™), and stored frozen until analysis for nutrient concentrations at the University of Hawai'i at Hilo's (UH Hilo) Analytical Laboratory. Nutrients were analyzed on a Pulse Technicon™ II autoanalyzer using standard methods (NO_3^- + NO_2^- [Detection Limit (DL) 0.07 µmol/L, USEPA 353.4], NH_4^+ [DL 0.36 µmol/L, USGS I-2525], PO_4^{3-} [DL 0.03 µmol/L, Technicon™ Industrial Method 155-71 W], total dissolved phosphorous (TDP) [DL 0.5 µmol/L, USGS I-4650-03], H_4SiO_4 [DL 1 µmol/L, USEPA 366]), and reference materials (NIST; HACH 307-49, 153-49, 14242-32, 194-49). Total dissolved nitrogen (TDN) was analyzed by high-temperature combustion, followed by chemiluminescent detection of nitric oxide (DL 5 µmol/L,

ASTM D5176, Shimadzu TOC-V, TNM-1) (Sharp et al. 2002). Salinity was measured at the time of water collection using an YSI Pro 2030 multi-parameter probe.

$\delta^{15}N$ Analyses

At the time of water sample collection, macroalgae with sufficient biomass to harvest were collected (~5 g) at all stations and analyzed for $\delta^{15}N$ (Fig. 1). Multiple species were collected at each station because a common macroalgal species did not exist among them. The number of species collected varied with station. Macroalgal tissues were placed on ice during transport to the laboratory, where tissues were rinsed with deionized water. Subsamples of macroalgae were preserved as voucher specimens and identified to the lowest taxonomic resolution using an Olympus™ CH30 microscope (Abbott 1999, Abbott & Huisman 2004). The remaining algal tissues from each station were combined for a composite sample. These tissues were dried at 60° C until a constant weight was achieved, ground and homogenized using a Wig-L-Bug grinding mill, and ~2 mg of the macroalgal tissue were folded in 4x6 mm tin capsules for stable isotope analysis. Macroalgal tissues were analyzed for $\delta^{15}N$ using a Thermo-Finnigan™ Delta V Advantage isotope ratio mass spectrometer (IRMS) with a Conflo III interface and a Costech™ ECS 4010 Elemental Analyzer located at the UH Hilo's Analytical Laboratory. Data were normalized to United States Geological Service (USGS) standard NIST 1547. Isotopic signatures are expressed as standard (δ) values, in units of parts per mil (‰), and calculated as $[(R_{sample} - R_{standard}) / R_{standard}] \times 1000$, where $R = {}^{15}N/{}^{14}N$.

To determine the sources of N being used by the macroalgae, $\delta^{15}N$ - NO_3^- of potential sources were measured. Nutrient concentrations ($NO_3^- + NO_2^-$, NH_4^+, and PO_4^{3-}) in these sources were also quantified. Sources sampled included: cesspools (n = 3), high elevation drinking water wells (n = 3), low elevation irrigation wells (n = 7), ambient seawater (n = 2), and soil (n = 3) from under Kiawe trees (*Prosopis pallida)*. Kiawe is an introduced N_2-fixing tree found widely on the leeward coast of Hawai'i Island, and contributes N to soil and groundwater (Dudley et al. 2014). Soil was collected directly under the Kiawe trees, dried, and then shaken overnight with reagent-grade water. N source water samples were collected at several locations to encompass spatial variability within Puakō's watershed. All N source samples were filtered through a 0.22-µm

cellulose acetate filter (Whatman™) and frozen until analysis. $\delta^{15}N$ - NO_3^- samples were analyzed on a Thermo-Finnigan™ Delta Plus IRMS with data normalized to USGS standards (USGS32, USGS34, USGS53) at Northern Arizona University's Colorado Plateau Stable Isotope Laboratory. IAEA-NO3 was used as a check standard. Fertilizer values used in this study were from a previous study on Hawai'i Island (Wiegner et al 2016). To determine their N sources, the $\delta^{15}N$ macroalgal tissue values were plotted relative to $\delta^{15}N$ source values (Derse et al. 2007, Wiegner et al. 2016).

Data Analyses

To determine if FIB, nutrients, and $\delta^{15}N$ values in macroalgal tissue differed among stations, a one-way analysis of variance (ANOVA) was used for each variable. Correlations were used to evaluate associations between FIB, nutrients, $\delta^{15}N$ values, and other water quality parameters. Data were tested for normality and equal variances. If assumptions were not met for parametric analyses, log transformations were used. All statistical analyses were conducted using Minitab17 (2010) with $\alpha = 0.05$.

Nutrient concentration data on mixing plots were compared to a theoretical mixing line connecting the freshwater and ocean end members. When nutrient concentration data overlaid the mixing line, the nutrient was characterized as having conservative behavior, where only dilution with seawater is affecting the nutrient concentration in the nearshore waters. When data fell above or below the mixing line, the nutrient was described as behaving non-conservatively, with some source adding the nutrient to the water or some process removing it during mixing.

Results

Dye Tracer Studies

Dye was visually observed at the shoreline in front of all four homes. Of the three to four springs sampled during each test, dye only appeared at one of the springs at the shoreline in front of the property with the OSDS being tested. The SGD at these springs dispersed over an area between 0.25 and 4 m^2. Initial dye breakthrough at the shoreline, calculated from the difference in time from when the dye was added to the OSDS until its first appearance at the shoreline, ranged from 9 h to 3 d. Three of the homes had comparable flow rates between 4 and 14 m/d; the OSDS at one home was faster, where

dye in SGD traveled 76 m/d. Based on dye dilution, 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 discharge at the shoreline.

Sewage Indicators

Contrasting patterns were seen among sewage indicators along shoreline stations. *Enterococcus* spp. concentrations ranged from 18 to 2777 MPN/100 mL, and significantly differed among stations ($p = 0.04$), with station 13 having the highest values, which were two times, to one or two orders of magnitude larger than other stations (Fig. 2, Table 1). *C. perfringens* concentrations ranged from 2 to 12 CFU/100 mL, and were similar among stations, averaging (mean ± SE) 5 ± 3 CFU/100 mL across all stations ($p = 0.06$) (Fig. 2, Table 1). The most prevalent macroalgal species along the shoreline were *Ulva fasciata*, *Cladophora* spp., and *Gelidiella acerosa*. The $\delta^{15}N$ in macroalgal tissue ranged from 4.23 to 11.88‰ across all stations, and significantly differed among stations ($p < 0.0001$) (Fig. 2, Table 1), with stations 3 and 4 having the highest values (Fig. 2). Six out of 16 stations fell within the sewage $\delta^{15}N$ - NO_3^- range (9.62 to 11.57‰) (Table 2), including stations 3, 4, 5, 6, 7, and 13 (Fig. 3). The remaining stations fell within the high and low elevation groundwater ranges (Fig. 3). $NO_3^- + NO_2^-$ concentrations were ~40 µmol/L lower in high elevation wells compared to the lower elevation ones (Table 2). In addition, PO_4^{3-} and NH_4^+ concentrations were similar between high and low elevation wells (Table 2). $NO_3^- + NO_2^-$, TDN, PO_4^{3-}, TDP, and H_4SiO_4 concentrations significantly differed among shoreline stations ($p < 0.001$) (Table 3). Station 4 had the highest concentrations, and station 15 had the lowest concentrations for all nutrients, except H_4SiO_4. H_4SiO_4 concentrations were highest at station 14, which was ~500 µmol/L higher than the lowest measured concentration (Table 3). NH_4^+ concentrations were similar across all stations ($p > 0.06$). Salinity also varied across stations ($p < 0.01$), with stations 2 and 14 being the freshest (Table 3). Nutrient concentrations ($NO_3^- + NO_2^-$, TDN, PO_4^{3-}, TDP, and H_4SiO_4,) were also inversely correlated with salinity ($p < 0.01$). Mixing plot analysis revealed conservative mixing of groundwater-derived nutrients ($NO_3^- + NO_2^-$, TDN, PO_4^{3-}, TDP, and H_4SiO_4) with seawater, except for a few stations that consistently fell well above the theoretical mixing

line (Fig. 4). These included stations 3, 4, and 7. NH_4^+ displayed non-conservative mixing (Fig. 4).

Associations between Sewage Indicators

Most sewage indicators were not correlated with each other. However, *C. perfringens* was positively correlated with NH_4^+ ($p = 0.02$) (Fig. 5), and $\delta^{15}N$ in macroalgal tissues was positively correlated with $NO_3^- + NO_2^-$ ($p < 0.001$), TDN ($p < 0.001$), and PO_4^{3-} concentrations ($p < 0.001$) (Fig. 6).

Discussion

Sewage Indicators

FIB are used by federal and state regulatory agencies to identify impaired recreational waters. At Puakō, *Enterococcus* spp. concentrations were highly variable across both space and time, with station 13 having the highest values. Additionally, when comparing our average values of *Enterococcus* spp. concentrations to Hawai‘i Department of Health's (HDOH) single sample maximum of 104 CFU/100 mL, 13 out of 16 stations exceeded this threshold, with the majority of these stations exceeding it two to three of the four sampling times, and two stations exceeding it on all four occasions (Fig 2). However, *Enterococcus* spp. concentrations have been shown to vary spatially, temporally, seasonally, and to be tidally influenced (Shibata et al. 2004, Maiga et al. 2009, Shibata et al. 2010, Nnane et al. 2011, Converse et al. 2012). In addition, *Enterococcus* spp. can persist in tropical soils and come from different animal sources (Hardina & Fujioka 1991, Byappanahalli & Fujioka 1998, Byappanahalli & Fujioka 2004, Ragosta et al. 2010), and thus, may not be indicative of sewage. However, soils are an unlikely source of *Enterococcus* spp. at Puakō as the area generally lacks soil, the substratum is primarily basalt, and shoreline concentrations were extremely high compared to state standards. While *C. perfringens* concentrations did not vary among stations, 11 of the 16 stations fell above the recommended standard to HDOH for marine recreational waters (5 CFU/100 mL) (Fujioka et al. 1997). Additionally, using the Fung/Fujioka *C. perfringens* scale for sewage pollution based on single sample maximums (Fung et al. 2007), only four of our stations (stations 7, 11, 14, and 15) were indicative of non-point sewage contamination (> 10 CFU/100 mL). The remaining five

stations fell below this range, and were classified as not being polluted by sewage. While only certain stations had *C. perfringens* concentrations within the range for non-point sewage pollution, the correlation between *C. perfringens* and NH_4^+ suggest that sewage pollution may be more pervasive, as these two parameters are associated with anaerobic conditions, which are often found in OSDS.

While FIB are used to detect sewage, their application is primarily for assessing human health hazards for recreational water users. In comparison, $\delta^{15}N$ values in macroalgal tissues are used to determine N sources to coastal waters including sewage (Costanzo et al. 2005, Lapointe et al. 2005, Savage 2005, Derse et al. 2007, Dailer et al. 2012). Typical sewage values range from +5 to +20‰ (*reviewed in* Wiegner et al. 2016), and values from cesspools in our study fell within this range (Table 3). $\delta^{15}N$ in macroalgal tissues along the Puakō shoreline ranged from 4.23 to 11.88‰, with six out of 16 shoreline stations falling within the range for sewage (Table 4). Stations 3 and 4 had the most enriched $\delta^{15}N$ macroalgal tissues, highlighting two potential sewage pollution hotspots. However, past studies have found that macroalgae assimilate N more rapidly under low NO_3^- concentrations (Fujita 1985), and that $\delta^{15}N$ in macroalgal tissue can be underestimated by up to 6‰ in waters with high NO_3^- concentrations (> 10 µmol/L) (Swart et al. 2014). All of the stations had $NO_3^- + NO_2^-$ concentrations exceeding 10 µmol/L, suggesting that the $\delta^{15}N$ macroalgal values may be underestimated. If this was the case, then all 16 stations fall within the sewage range. In contrast, other studies have found that tissue from opportunistic macroalgae reflects the nutrient concentrations in the water column (Fong et al. 1994). These macroalgae are often used as bioindicators as their tissues respond rapidly to N inputs (Duarte 1995, Cohen & Fong 2006). Two out of the three taxa (*U. fasciata*, *Cladophora* spp.) collected along the Puakō shoreline were opportunistic macroalgae. Opportunistic macroalgae taxa were not present at every station, possibly due to heavy grazing in the area. Hence, composite algal samples were analyzed, which included tissue from *G. acerosa*, a non-opportunistic species. Additionally, $NO_3^- + NO_2^-$, TDP, PO_4^{3-}, and TDN concentrations were nearly eight times greater at station 4 compared to all stations; this pattern was also seen with $\delta^{15}N$ in macroalgal tissues. These results further suggest that station 4 is a hotspot of non-point

sewage pollution at Puakō. The significant positive correlation between $\delta^{15}N$ in macroalgae and nutrient concentrations further suggest that some portion of the nutrients' concentrations are derived from sewage (Fig. 6).

Hydrology

At Puakō, a large portion of the nutrient concentration data for $NO_3^-+NO_2^-$, TDN, PO_4^{3-}, and TDP fell on the theoretical mixing line, with highest concentrations at the lowest salinities. This result suggests that high elevational groundwater is a source of nutrients to Puakō's coastal waters and that they are behaving conservatively as groundwater and ocean water mix at the shoreline. This pattern, in part, explains the lack of associations between sewage indicators and salinity, as a large portion of groundwater nutrients discharging at the shoreline is from a nutrient source other than sewage. The conservative mixing nutrient patterns observed at Puakō have been documented elsewhere on Hawai'i Island in coastal areas with SGD (Knee et al. 2008b, Knee et al. 2010).

In contrast to the majority of our shoreline stations, data for stations 3, 4, and 7 consistently fell above the theoretical mixing line, suggesting there is a localized source of nutrients in those areas. The likely source is the OSDS as our dye tracer studies demonstrated that OSDS at these stations were leaking, and that the travel time from the homes to the shoreline was 9 h to 3 d. Additionally, dye was only observed seeping out during low tide and was localized within 10 m (longshore direction) of the shoreline. $\delta^{15}N\text{-}NO_3^-$ at these shoreline stations clearly fell within our measured $\delta^{15}N\text{-}NO_3^-$ sewage range at Puakō (Table 2), as did the $\delta^{15}N$ in the macroalgal tissue (Fig. 3). These results provide insight to the hydrology and geology at Puakō, where the fracture system within the basalt determines the flow path of the sewage from the OSDS to the shoreline, and affects the time of travel. Two other factors affecting sewage inputs are weather and house occupancy. On the only rainy sampling day during this study (March 4, 2015), all three stations (3, 4, and 7) fell above the mixing line. This result illustrates that precipitation inputs may enhance the connection between the OSDS with the shoreline seeps through increased SGD. In contrast, there were some sampling dates on which nutrient concentrations for stations 3 and 7 fell on the mixing line. We suspect that on

these dates, homes at these stations were not occupied, and therefore, their OSDS were not being used.

The $\delta^{15}N\text{-}NO_3^-$ and NO_3^- concentration data from the groundwater wells and shoreline stations also provided another insight into the hydrology of the Puakō watershed and coastal nutrient sources. The $\delta^{15}N\text{-}NO_3^-$ became increasingly enriched in ^{15}N moving downslope to the Puakō shoreline. The change in the $\delta^{15}N\text{-}NO_3^-$ from the high to low elevational groundwater wells suggests a change in NO_3^- source from forest soil to sewage. It is possible that sewage is contaminating the lower elevational groundwater as an upslope development (Waikoloa Village) has over 2,000 homes with 1587 OSDS (per. comm. Hawai'i Water Supply). Additionally, NO_3^- concentrations increased ~40 mmol/L from the high to low elevational groundwater wells (Table 3). Lastly, the $\delta^{15}N$ enrichment in the $\delta^{15}N\text{-}NO_3^-$ from the lower elevational groundwater wells to the shoreline seeps suggests that additional sewage from Puakō homes is contaminating the groundwater before it is discharged along the shoreline. To understand the relative percent contributions of these two different communities to sewage pollution along Puakō's shoreline, more $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ data, as well as a mixing model capable of determining source contributions are needed (Wiegner et al. 2016). With this additional information, informed decisions about management actions can be made.

Development of a Sewage Pollution Score

As this study and others have shown, sewage indicators can provide conflicting information on the intensity and location of sewage pollution along the shoreline. Previous studies have confronted similar issues with their sewage indicator data (Shibata et al. 2004, Yoshioka et al. 2016). Hence, creating a sewage pollution score using several sewage indicators may be a more holistic way to assess sewage pollution in coastal waters. Water quality scores and indices have been used successfully in the past to assess healthy water quality conditions for both humans and ecosystems (Zambrano et al. 2009, Wang et al 2015). Interpolative mapping of water quality score or index values provides a simple and clear tool for managers and policymakers that allow them to relate human activities to water quality and identify areas in need of better management (Zambrano et al. 2009).

To better assess sewage pollution conditions along the Puakō shoreline, a scoring tool was developed using three sewage indicators (FIB, $\delta^{15}N$ macroalgae, and nutrients). The scoring tool had three levels for each indicator: level 1 = low, level 2 = medium, and level 3 = high. Levels for each indicator were based on established standards, literature information, or data from this study (Table 4). Specifically, the scoring tool used HDOH's geometric mean and the single sample maximum for *Enterococcus* spp. concentrations in marine waters, the Fung/Fujioka *C. perfringens* scale for sewage pollution, $\delta^{15}N$ values in macroalgal tissues for different NO_3^- sources (Table 2), and HDOH's water quality standards for nutrient concentrations in open coastal waters (NO_3^- + NO_2^-, NH_4^+, and TDP) (Table 4). Nutrient concentration standards for the wet criteria were used because the freshwater discharge along the Puakō shoreline ranged from 2083-2730 L m^{-1} h^{-1} (Paytan et al. 2006), which are an order of magnitude larger than the baseline for the wet criteria (> 294 L m^{-1} h^{-1}). Two dissolved inorganic forms of N were chosen for the score tool rather than TDN because it contains DON, and sewage-derived DON's contribution to eutrophication is only beginning to be investigated (Pehlivanoglu & Sedlak 2004, Urgun-Demirtas et al. 2008, Bronk et al. 2010, Filippino et al. 2011). TDP was used as the phosphorous water quality indicator since HDOH has no PO_4^{3-} water quality standard for open coastal waters (HDOH 2014). It should also be noted that a medium level in nutrient concentrations exceeds HDOH standards for open coastal waters' wet criteria.

Once each indicator was assigned a level (1-3) based on its measured value and our scoring tool (Table 4), 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* spp. and nutrient concentrations (Fung et al 2007, Dailer et al. 2010, Viau et al. 2011, Yoshioka et al. 2016). *Enterococcus* spp. concentrations received a medium weight (weight = 2) as HDOH uses this FIB to assess marine recreational water safety specifically for sewage pollution, but not the highest weight because concentrations fluctuate over short time scales (min to h)

and have other sources, like soils, in tropical areas (Hardina & Fujioka 1991, Byappanahalli & Fujioka 1998, Byappanahalli & Fujioka 2004). Nutrient concentrations received the lowest weight (weight = 1) since sewage pollution is known to increase them, but nutrients can also come from other sources within the watershed and concentrations can vary over short time scales (Lapointe et al. 1990, David et al. 2013, Nelson et al. 2015). The equation for calculating the overall sewage pollution score for each station was: (*C. perfringens* level x 3) + (δ^{15}N macroalgae level x 3) + (*Enterococcus* spp. level x 2) + (NO_3^-+NO_2^- level x 1) + (NH_4^+ level x 1) + (TDP level x 1). Sewage pollution score categories were: low = 11-17, medium = 18-25, and high = 26-33. The high end of the range for the low and medium sewage pollution score categories were calculated as the sum of low or medium scores for all indicators, respectively, except for nutrient concentrations, which were allowed to fluctuate up to the high level as inputs from non-sewage sources can result in high concentrations.

The stations with highest pollution sewage scores were stations 4 (score = 30), 6 (score = 26), and 7 (score = 27) (Fig. 7). Note, stations 4 and 7 are known locations of OSDS leakage from the dye tracer studies. These results confirm the effectiveness of our sewage pollution score in identifying hotspots of sewage pollution. Overall, three stations fell in the high category with the remainder in the medium category. This integrated approach identified sewage hotspots along the Puakō coastline, and locations where it is critical for homes to remove their cesspools and employ better sewage treatment technology. This map also provides information to the community on areas where residents and visitors may want to limit water exposure during recreational activities until sewage treatment is improved.

Conclusion

Globally, coral reefs are declining from multiple stressors, with sewage pollution being one of the most devastating (Wear & Vega Thurber 2015). Dye tracer studies confirmed locations of sewage pollution and provided information on the time of travel from homes to the shoreline. FIB and nutrient concentrations were high and variable along the Puakō shoreline, and δ^{15}N in macroalgal tissue were within the sewage range. However, data from different indicators were not always in agreement with one another

on the intensity and locations of sewage pollution. Hence, a novel pollution score was developed using these indicators to identify sewage hotspots. With sewage becoming a growing global threat in nearshore waters, being able to effectively assess its pollution is crucial for both human and marine ecosystem health. A multi-indicator approach for detecting sewage and this pollution scoring tool will allow other coastal communities to assess their water quality and take appropriate management actions, improving safety of recreational waters users and coastal ecosystem health.

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Table 1. Mean ± SE and [range] of *Enterococcus* spp.*, Clostridium perfringens*, and $\delta^{15}N$ in macroalgal tissues for shoreline stations at Puakō, Hawai'i. Superscript letters indicate significant groupings from One-way ANOVA and post-hoc Tukey's tests. $\alpha = 0.05$; n = 4 samplings.

Station	*Enterococcus* spp. (MPN/100 mL)	*C. perfringens* (CFU/100 mL)	$\delta^{15}N$ (‰)
1	18 ± 8^{b} [9-43]	2 ± 1 [0-4]	$6.38 \pm 0.15^{a-c}$ [6.03-6.65]
2	74 ± 25^{ab} [37-143]	2 ± 1 [0-4]	$7.54 \pm 0.18^{a-c}$ [7.04-7.90]
3	349 ± 162^{ab} [37-739]	5 ± 2 [1-10]	10.55 ± 0.15^{ab} [10.37-11.00]
4	237 ± 178^{ab} [47-770]	6 ± 2 [3-13]	11.88 ± 0.32^{a} [11.27-12.78]
5	1107 ± 861^{ab} [94-3674]	4 ± 1 [0-6]	$7.21 \pm 2.01^{a-c}$ [1.29-10.26]
6	1051 ± 570^{ab} [72-2546]	6 ± 2 [2-10]	$7.77 \pm 1.30^{a-c}$ [4.15-10.18]
7	170 ± 32^{ab} [104-257]	12 ± 5 [3-27]	$8.35 \pm 0.74^{a-c}$ [6.48-9.80]
8	738 ± 603^{ab} [62-2544]	7 ± 1 [3-10]	$5.48 \pm 0.37^{a-c}$ [5.06-6.58]
9	216 ± 120^{ab} [27-563]	3 ± 0 [2-3]	4.79 ± 0.53^{bc} [3.85-6.18]
10	122 ± 30^{ab} [66-202]	5 ± 1 [2-7]	4.54 ± 0.70^{c} [3.57-6.56]
11	315 ± 107^{ab} [15-495]	8 ± 3 [2-14]	$6.02 \pm 0.30^{a-c}$ [5.59-6.91]
12	676 ± 251^{ab} [121-1323]	5 ± 2 [2-8]	$6.43 \pm 0.65^{a-c}$ [4.90-8.04]
13	2777 ± 1806^{a} [17-7985]	2 ± 1 [0-3]	$7.74 \pm 1.92^{a-c}$ [4.80-13.12]
14	80 ± 36^{ab} [24-185]	10 ± 5 [1-20]	$5.94 \pm 0.47^{a-c}$ [5.08-7.28]
15	454 ± 132^{ab} [180-816]	9 ± 3 [2-13]	4.24 ± 0.51^{c} [3.62-5.77]
16	699 ± 554^{ab} [17-2338]	3 ± 1 [0-6]	4.23 ± 0.44^{c} [3.53-5.50]

Table 2. Mean ± SE of $\delta^{15}N$ - NO_3^- (‰) and NO_3^- + NO_2^-, PO_4^{3-}, and NH_4^+ concentrations (µmol/L) of N sources collected in the Puakō watershed, Hawai'i. (n = sample size)

N Source	n	$\delta^{15}N$-NO_3^-	NO_3^- + NO_2^-	NH_4^+	PO_4^{3-}
Cesspools	3	10.45 ± 0.58	20.76 ± 10.50	6370.00 ± 806.16	378.58 ± 16.59
Soil	3	2.13 ± 2.37	6366.67 ± 3682.46	594.54 ± 93.23	193.56 ± 141.56
Ocean	2	3.02 ± 0.79	1.43 ± 0.08	2.52 ± 0.55	0.11 ± 0.05
High elevation groundwater wells	3	4.76 ± 0.43	93.87 ± 4.35	4.84 ± 1.43	2.48 ± 0.19
Low elevation groundwater wells	7	7.03 ± 0.50	130.09 ± 6.70	4.82 ± 1.19	2.48 ± 0.19
Shoreline	3	11.95 ± 1.13	133.93 ± 64.68	1.47 ± 0.44	5.27 ± 1.57

Table 3. Mean ± SE and [range] of $NO_3^- + NO_2^-$, NH_4^+, TDN, PO_4^{3-}, TDP, H_4SiO_4 concentrations (µmol/L), and salinity for shoreline stations at Puakō, Hawai'i. Superscript letters indicate significant groupings from One-way ANOVA and post-hoc Tukey's tests. $\alpha = 0.05$; n = 4 samplings.

Station	$NO_3^- + NO_2^-$	NH_4^+	TDN	PO_4^{3-}	TDP	H_4SiO_4	Salinity
1	$27.87 \pm 4.09^{b-e}$ [18.10-36.79]	0.83 ± 0.15 [0.78-1.23]	$41 \pm 7^{c-f}$ [25-58]	0.44 ± 0.04^{fg} [0.33-0.51]	0.7 ± 0.1^{fg} [0.5-1.0]	$133 \pm 23^{a-c}$ [87-195]	$27.58 \pm 1.44^{a-c}$ [23.63-30.37]
2	149.94 ± 12.79^{ab} [129.62-187.09]	0.49 ± 0.11 [0.18-0.72]	159 ± 13^{ab} [139-195]	$2.24 \pm 0.24^{a-d}$ [1.62-2.73]	$2.9 \pm 0.3^{a-e}$ [2.2-3.5]	581 ± 155^{ab} [187-876]	7.12 ± 0.61^{e} [5.77-8.70]
3	$137.12 \pm 35.39^{a-c}$ [36.22-190.37]	1.95 ± 0.30 [1.04-2.29]	$154 \pm 39^{a-c}$ [41-217]	3.81 ± 0.92^{ab} [1.34-5.37]	4.3 ± 0.7^{ab} [2.4-5.1]	$377 \pm 124^{a-c}$ [112-646]	$16.26 \pm 3.96^{b-e}$ [9.50-25.73]
4	196.05 ± 28.14^{a} [125.66-263.07]	1.30 ± 0.05 [1.24-1.47]	221 ± 26^{a} [153-267]	7.42 ± 1.11^{a} [4.12-9.00]	8.3 ± 1.4^{a} [4.5-10.8]	501 ± 113^{ab} [172-683]	$15.25 \pm 2.30^{c-e}$ [9.10-20.20]
5	$46.92 \pm 8.73^{a-e}$ [23.44-65.52]	1.32 ± 0.16 [0.86-1.57]	$70 \pm 12^{a-f}$ [42-87]	$1.34 \pm 0.17^{b-f}$ [0.90-1.71]	$1.7 \pm 0.3^{b-f}$ [0.9-2.1]	$179 \pm 41^{a-c}$ [85-278]	$24.98 \pm 2.35^{a-d}$ [19.70-31.07]
6	26.78 ± 11.48^{de} [2.50-54.16]	1.22 ± 0.10 [1.03-1.46]	$44 \pm 16^{d-f}$ [23-86]	$0.66 \pm 0.21^{e-g}$ [0.25-1.17]	0.9 ± 0.2^{fg} [0.3-1.3]	95 ± 43^{c} [22-219]	30.77 ± 2.31^{a} [24.53-35.53]
7	$134.56 \pm 54.94^{a-d}$ [42.27-285.74]	1.69 ± 0.65 [0.46-2.90]	$131 \pm 43^{a-d}$ [53-241]	$3.08 \pm 0.44^{a-c}$ [2.12-3.83]	$3.4 \pm 0.5^{a-c}$ [2.2-4.5]	447 ± 132^{ab} [164-804]	$21.98 \pm 0.97^{a-d}$ [19.87-24.03]
8	$39.15 \pm 14.53^{c-e}$ [0.99-67.10]	2.40 ± 0.97 [1.00-0.33]	$59 \pm 19^{b-f}$ [12-99]	$0.70 \pm 0.23^{e-g}$ [0.52-1.07]	$1.0 \pm 0.2^{e-g}$ [0.6-1.6]	$253 \pm 83^{a-c}$ [31-416]	$20.60 \pm 4.90^{a-d}$ [14.10-35.17]
9	$69.74 \pm 9.06^{a-e}$ [47.81-91.92]	1.00 ± 0.33 [0.89-1.77]	$85 \pm 7^{a-e}$ [74-105]	$1.37 \pm 0.13^{b-f}$ [1.15-1.73]	$1.8 \pm 0.2^{b-f}$ [1.5-2.3]	$342 \pm 90^{a-c}$ [219-609]	15.28 ± 2.31^{cd} [8.53-18.53]
10	$56.72 \pm 17.48^{a-e}$ [11.59-94.94]	0.95 ± 0.27 [0.47-1.51]	$73 \pm 19^{b-f}$ [20-106]	$1.14 \pm 0.31^{c-g}$ [0.34-1.84]	$1.5 \pm 0.2^{b-f}$ [1.2-1.8]	$354 \pm 76^{a-c}$ [129-445]	15.03 ± 3.60^{de} [4.90-21.90]
11	16.52 ± 1.21^{de} [14.08-18.73]	0.96 ± 0.30 [0.18-1.45]	29 ± 4^{ef} [23-41]	$0.49 \pm 0.04^{e-g}$ [0.40-0.58]	0.8 ± 0.2^{fg} [0.3-1.3]	108 ± 27^{bc} [53-173]	28.30 ± 0.93^{ab} [26.07-30.60]
12	$35.80 \pm 4.37^{a-e}$ [25.62-46.59]	1.34 ± 025 [0.78-1.88]	$46 \pm 5^{b-f}$ [34-56]	$0.99 \pm 0.11^{c-g}$ [0.40-1.31]	$1.3 \pm 0.3^{c-g}$ [0.9-2.1]	$260 \pm 105^{a-c}$ [112-568]	$24.50 \pm 0.96^{a-d}$ [22.57-27.13]
13	$34.89 \pm 4.73^{a-e}$ [22.54-44.18]	1.21 ± 0.19 [0.73-1.56]	$49 \pm 7^{b-f}$ [35-67]	$1.64 \pm 0.28^{b-e}$ [0.91-2.29]	$1.9 \pm 0.2^{b-f}$ [1.7-2.4]	$207 \pm 23^{a-c}$ [167-267]	$23.96 \pm 2.00^{a-d}$ [19.90-28.27]
14	$89.08 \pm 5.48^{a-d}$ [75.93-101.22]	1.15 ± 0.29 [0.64-1.54]	$101 \pm 7^{a-d}$ [84-117]	$2.61 \pm 0.17^{a-c}$ [2.22-2.98]	$2.9 \pm 0.3^{a-d}$ [2.4-3.6]	652 ± 174^{a} [359-1018]	6.43 ± 0.63^{e} [5.33-8.07]
15	13.37 ± 2.80^{e} [5.73-19.24]	1.07 ± 0.17 [0.75-1.44]	22 ± 3^{f} [15-27]	0.39 ± 0.09^{g} [0.16-0.55]	0.6 ± 0.2^{g} [0.3-1.1]	$120 \pm 24^{a-c}$ [52-158]	29.94 ± 0.70^{a} [28.67-31.27]
16	$38.50 \pm 7.20^{a-e}$ [17.35-47.44]	0.63 ± 0.31 [0.18-1.51]	$46 \pm 4^{c-f}$ [34-52]	$0.81 \pm 0.13^{d-g}$ [0.45-1.09]	$1.1 \pm 0.3^{d-g}$ [0.6-2.0]	$323 \pm 86^{a-c}$ [142-552]	$17.13 \pm 3.44^{b-e}$ [7.94-24.53]

Table 4. Sewage indicators (fecal indicator bacteria = CFU/100 mL, $\delta^{15}N$ = ‰, and nutrients = µmol/L) used to evaluate water quality along the Puakō coastline, Hawai‘i. These indicators were ranked (low = 1, medium = 2, and high = 3), multiplied by a weight factor, and summed for a final sewage pollution score. * “Medium” nutrient concentration scores exceed HDOH standards (*see* discussion for details).

Sewage Indicator	Weight Factor	Low (1)	Medium (2)*	High (3)	Reference
Enterococcus spp.	2	0 - 35	36 - 104	105+	HDOH 2014
Clostridium perfringens	3	0 - 10	11 - 100	101 – 505+	Fung et al. 2007
$\delta^{15}N$ in macroalgae	3	0.00 – 5.99	6.00 – 10.99	11.00+	Current Study
$NO_3^- + NO_2^-$	1	0.0 – 0.4	0.5 – 1.0	1.1 – 1.8+	HDOH 2014
NH_4^+	1	0.00 – 0.25	0.26 – 0.61	0.62 – 1.07+	HDOH 2014
TDP	1	0.0 – 0.7	0.8 – 1.3	1.4 – 1.9+	HDOH 2014

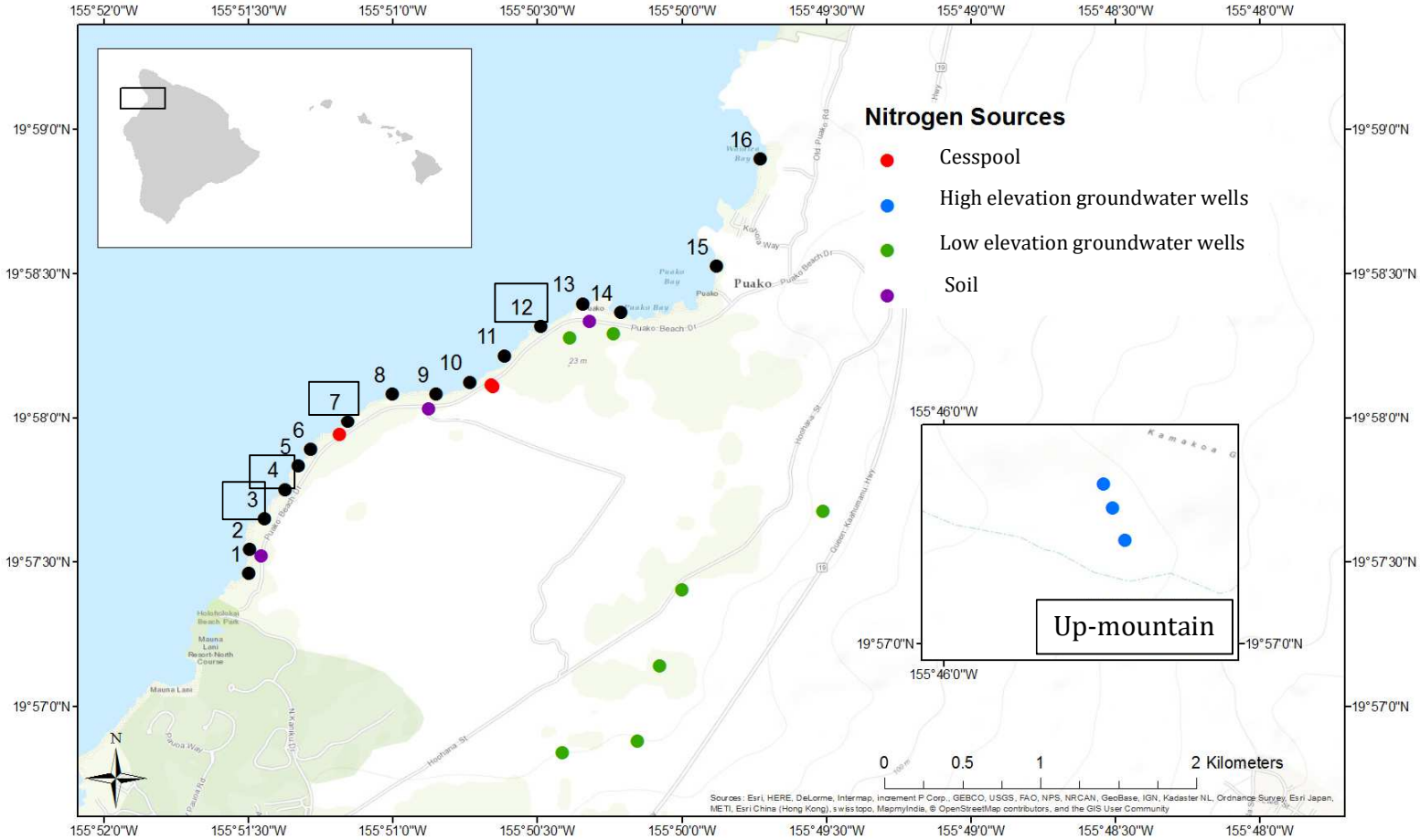

Figure 1. Samples for fecal indicator bacteria (FIB), nutrients, δ^{15} N in macroalgae, and physiochemical parameters were taken from 16 stations along the Puakō coastline, Hawai‘i (black circles). Dye tracer studies were conducted in close proximity to the stations outlined with squares. Nitrogen sources sampled include cesspools, high and low elevation groundwater wells, and soil (colored circles).

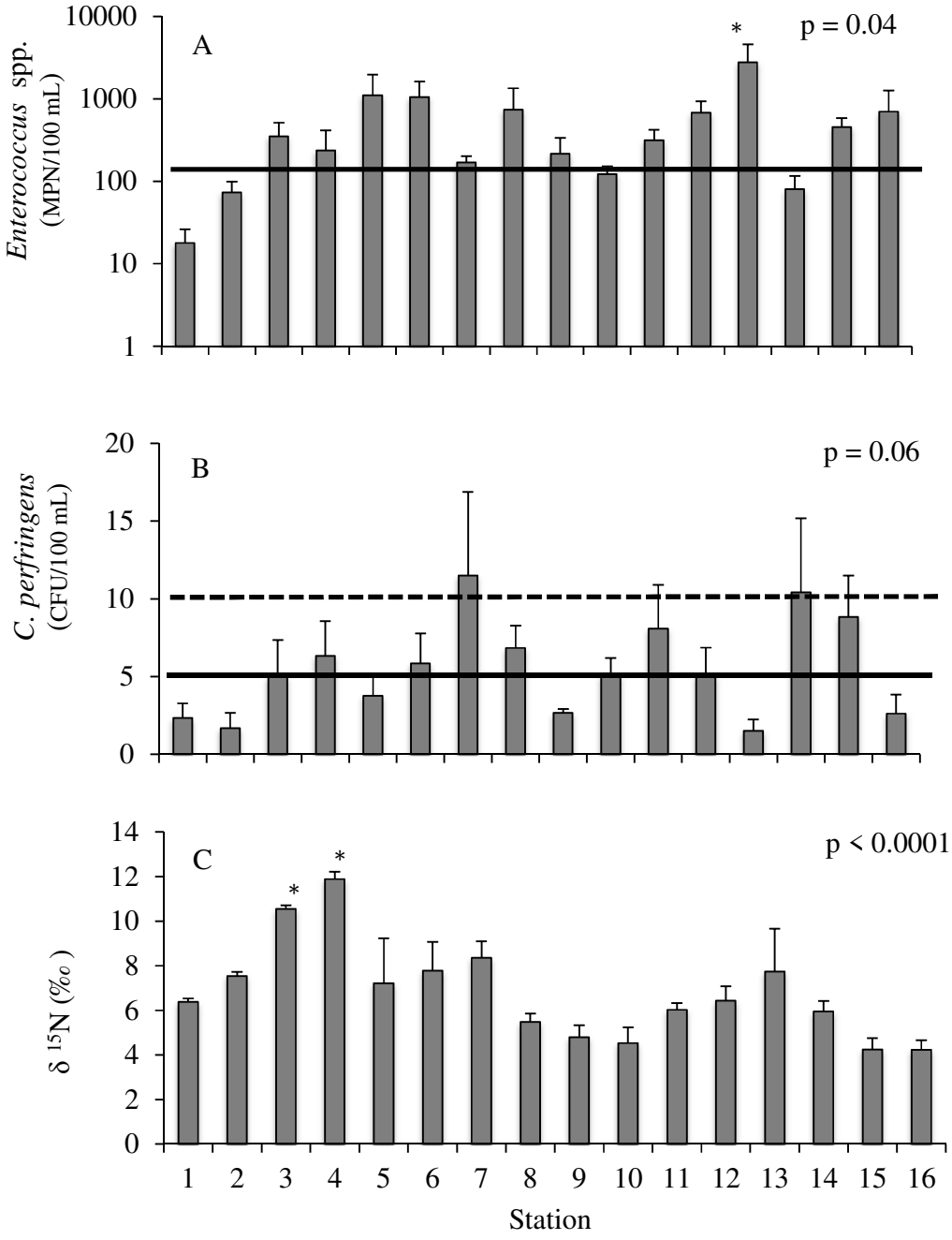

Figure 2. Mean ± SE of sewage indicators: (A) *Enterococcus* spp., (B) *Clostridium perfringens,* and (C) δ^{15} N (‰) of wild macroalgae at 16 shoreline stations at Puakō, Hawai'i. Hawai'i's Department of Health single sample maximum for *Enterococcus* spp. (104 CFU/100 mL) and a recommended marine recreational standard for *C. perfringens* (5 CFU/100 mL, Fujioka et al. 1997) are indicated by black lines. The dashed black line respresents the lowest benchmark level for non-point sewage pollution for *C. perfringens* (10 CFU/100 mL, Fung et al. 2007). Results from One-way ANOVA and Tukey's tests are shown on the figure, with * indicating significant differences ($\alpha = 0.05$).

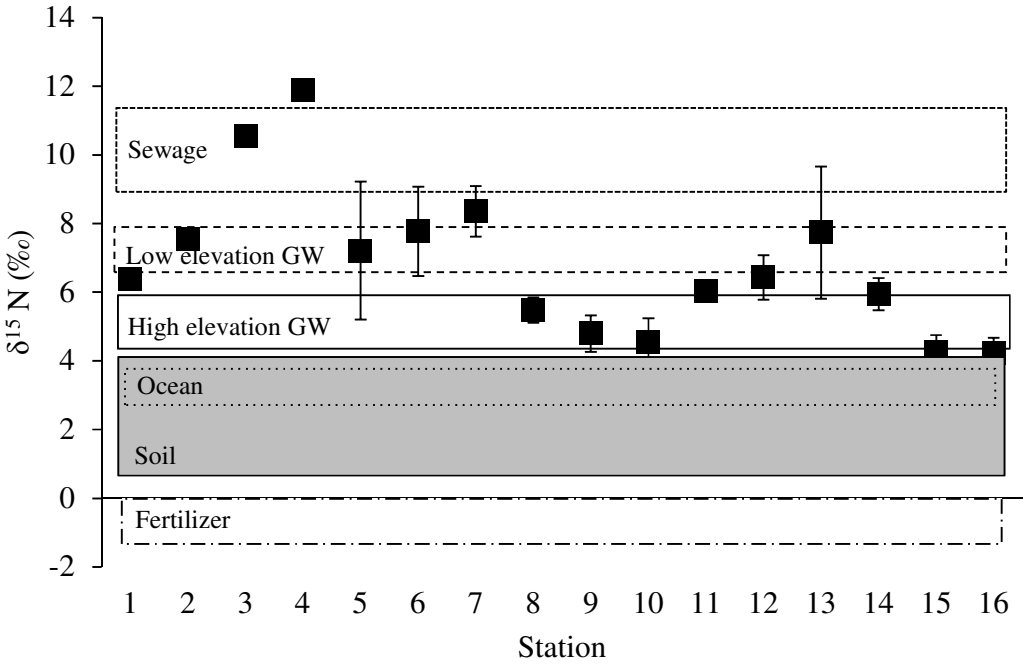

Figure 3. Mean ± SE δ^{15} N (‰) of wild macroalgae found at 16 stations in Puakō, Hawai‘i. Background areas represent (mean ± SE) δ^{15} NO_3^- of the N sources (fertilizer, soil, ocean, high elevation groundwater (GW) wells, low elevation GW wells, and sewage) measured as part of this study. Fertilizer values are from a previous study on Hawai‘i Island (Wiegner et al. 2016).

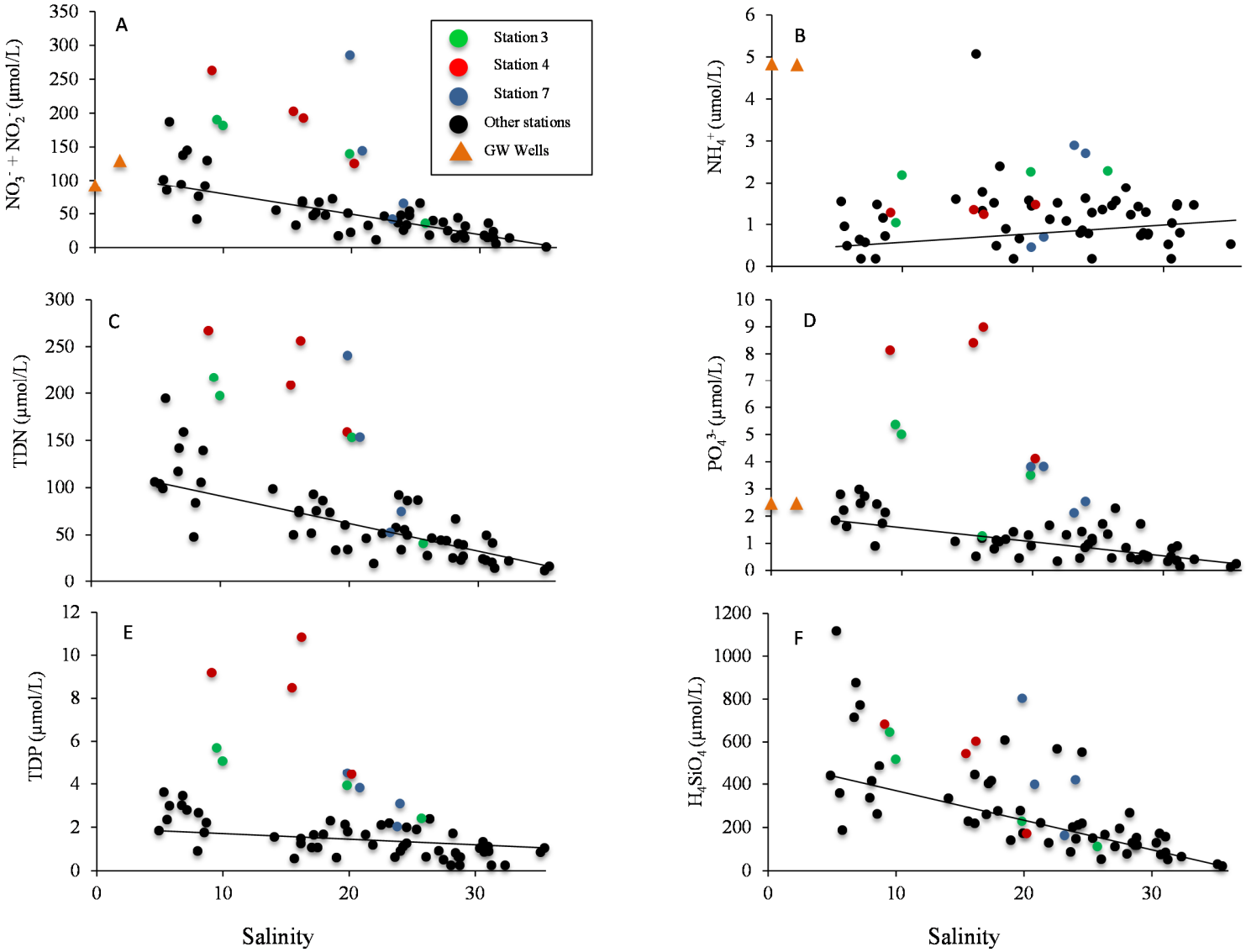

Figure 4. Mixing plots of nutrient concentrations and salinity along the Puakō shoreline, Hawai‘i: (A) NO_3^- + NO_2^-, (B) NH_4^+, (C) TDN, (D) PO_4^{3-}, (E) TDP, and (F) H_4SiO_4. Line represents theoretical mixing line, connecting freshest and saltiest shoreline samples. Groundwater samples from wells were only analyzed for NO_3^- + NO_2^-, NH_4^+, and PO_4^{3-}.

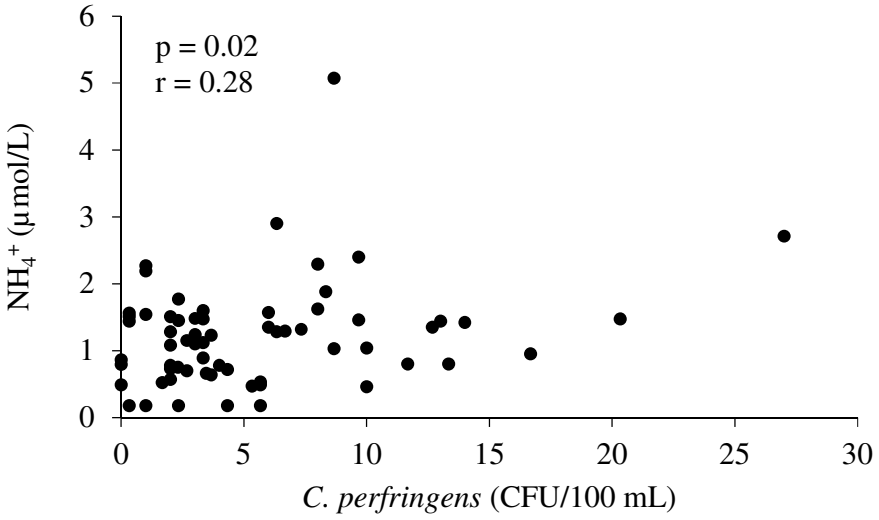

Figure 5. Correlation between *Clostridium perfringens* and NH_4^+along the Puakō shoreline, Hawai‘i.

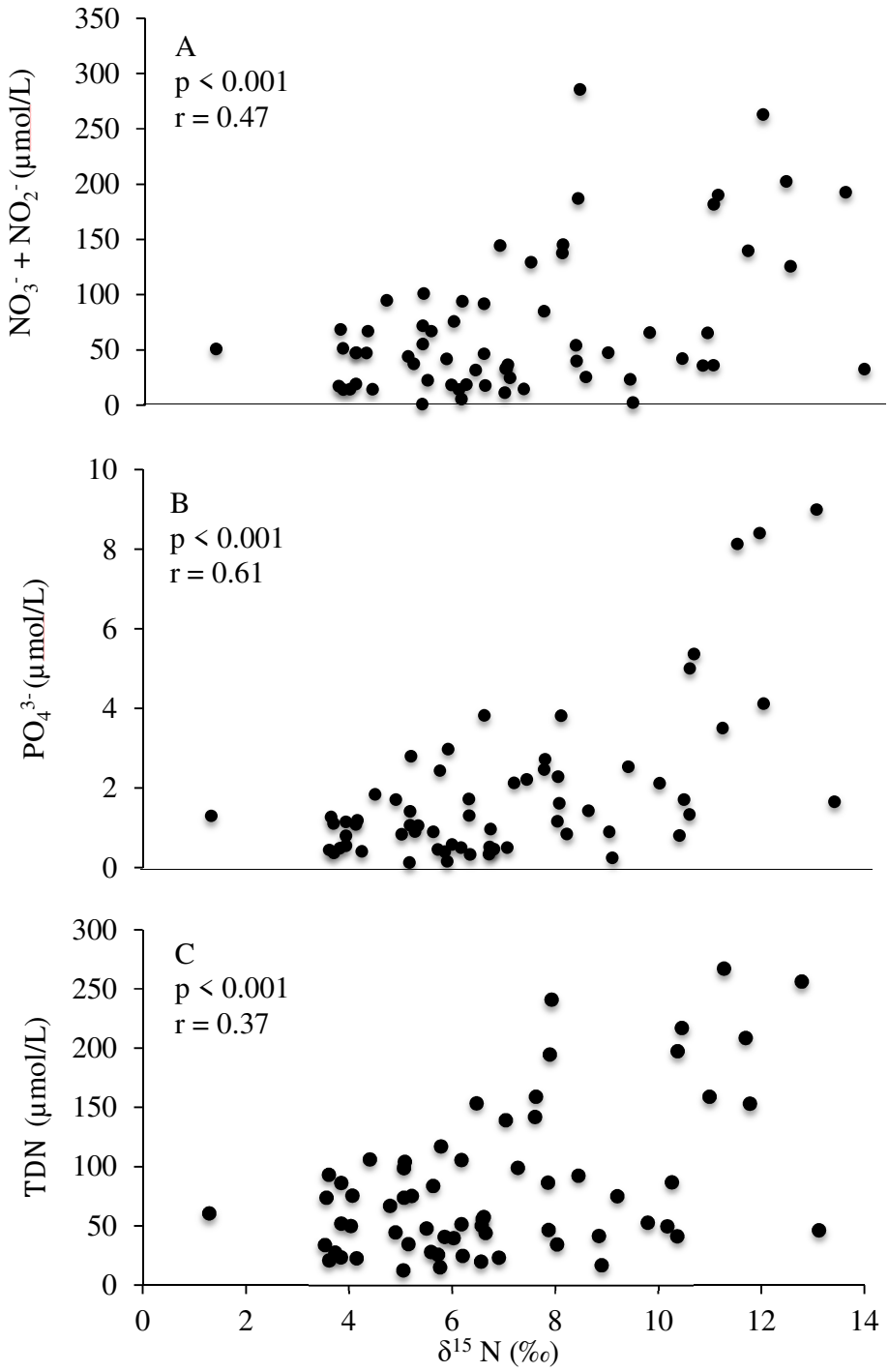

Figure 6. Correlations between δ^{15} N in macroalgal tissues and nutrient concentrations: (A) $NO_3^- + NO_2^-$, (B) PO_4^{3-}, and (C) TDN along the Puakō shoreline, Hawai‘i.

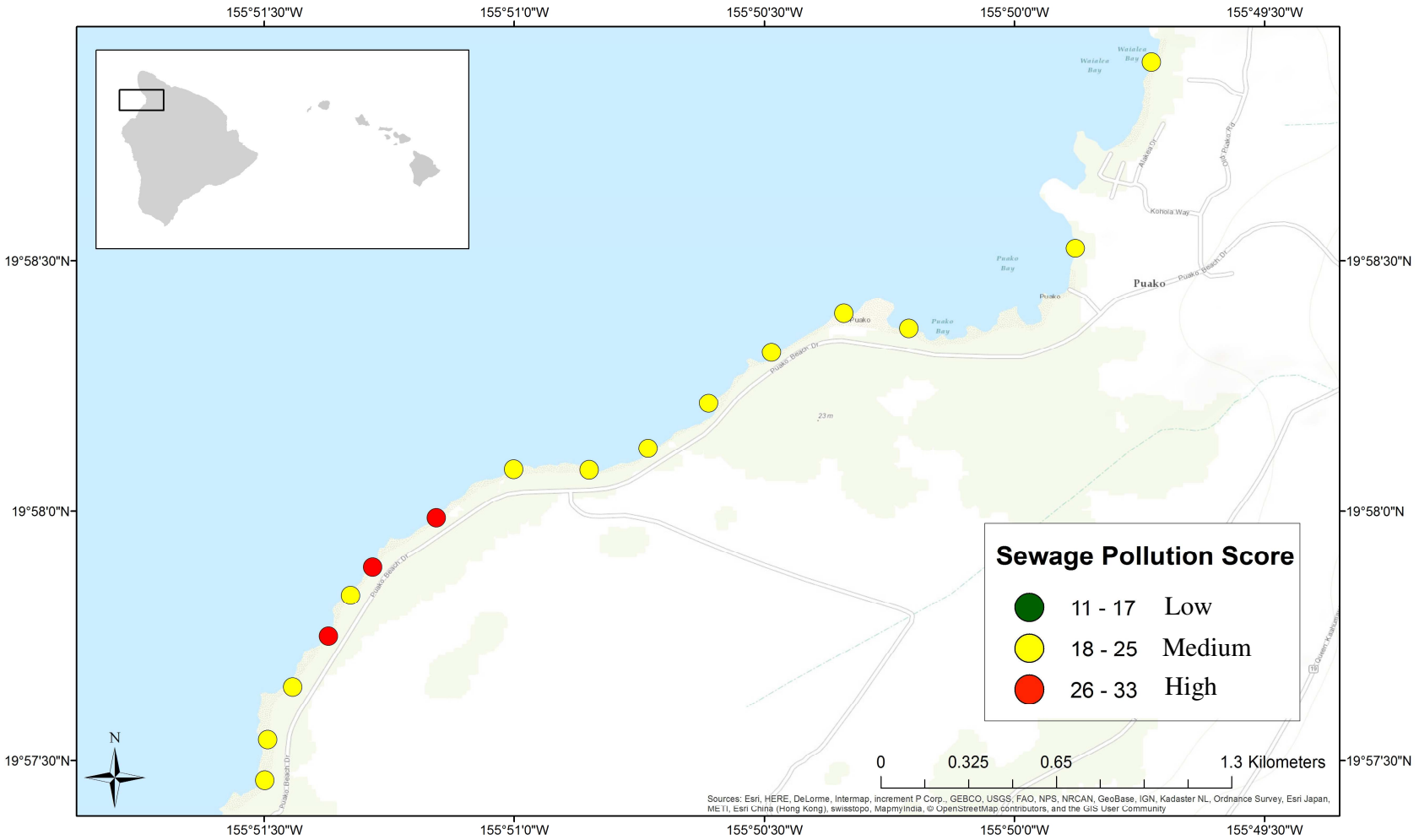

Figure 7. Sewage pollution scores for 16 stations along the Puakō shoreline, Hawai‘i, were based on established and recommended water quality standards, literature values for sewage indicators, and measured values from this study (fecal indicator bacteria, δ^{15} N in macroalgae, and nutrients). Sewage pollution score catergories are: Low = 11 - 17; Medium = 18 - 25; High = 26 - 33. Details on the calculation of the sewage pollution score are provided in the discussion section.

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