



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 $\delta^{15}\text{N}$ 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.

1. Introduction

With > 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 and 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 and 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 and 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 and 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 and 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 and Evans, 1995; Lapointe et al., 2005). These nutrients also alter coral growth rates, species distribution and abundance, and coral community diversity (Pastorok and 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

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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 and Fujioka, 1991; Fung et al., 2007). Hence, it is thought to more accurately detect sewage pollution than *Enterococcus* spp. (Fujioka and Shizumura, 1985; Hardina and Fujioka, 1991; Fujioka et al., 1997).

Measurements of stable nitrogen (N) isotopic composition ($\delta^{15}\text{N}$) in macroalgal tissues is another method employed to detect sewage pollution in coastal waters (Umezawa et al., 2002; Savage, 2005; 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; 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, $\delta^{15}\text{N}$ in macroalgal tissues can be highly variable due to N inputs from different sources with differing N isotopic compositions (Ochoa-Izaguirre and Soto-Jiménez, 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 and Bilyard, 1985; Whittier and 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 and El-Kadi, 2014). A high-risk area where OSDS are likely impacting nearshore waters on Hawai'i Island is Puakō (Whittier and 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, $\delta^{15}\text{N}$ 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.

2. Materials and methods

2.1. 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 to 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 to 2730 $\text{L m}^{-1} \text{h}^{-1}$ (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 2000 homes, with 1587 having OSDS; the remainder are connected to the sewer line (per. comm. Hawai'i Water Supply).

2.2. 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

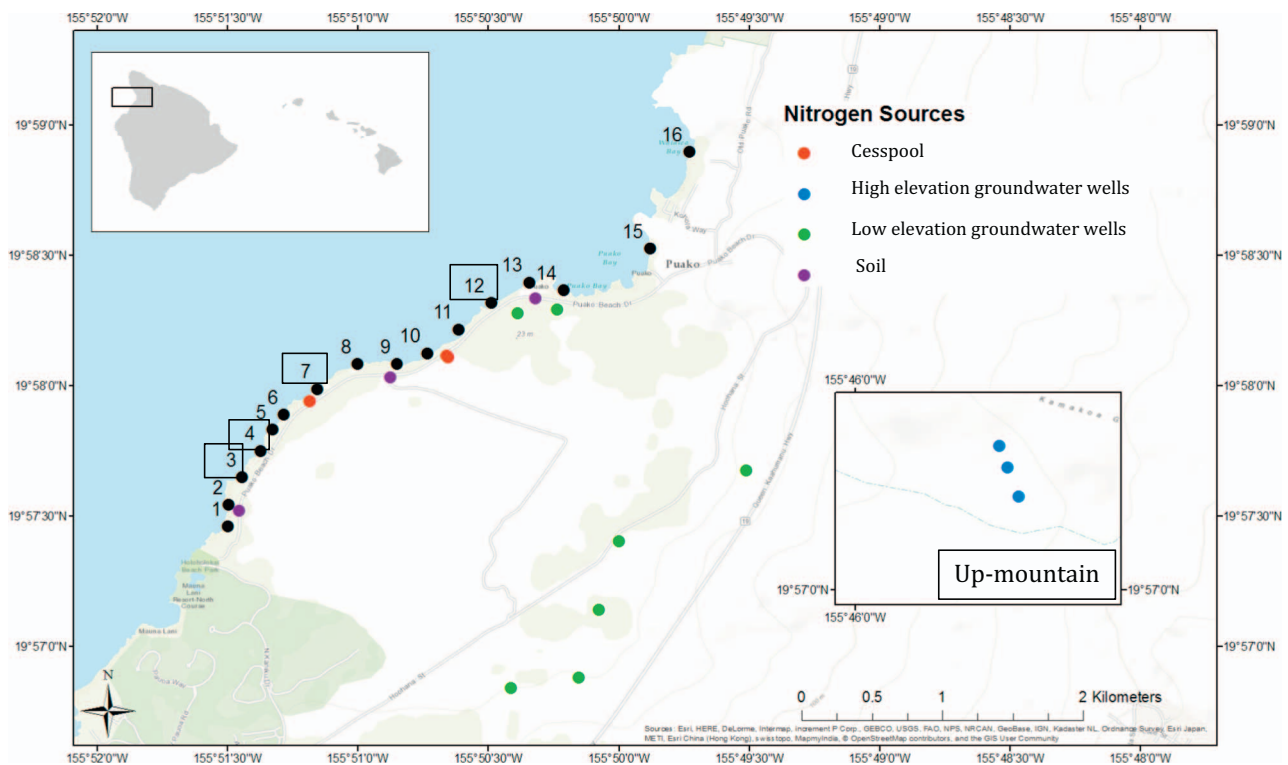


Fig. 1. Samples for fecal indicator bacteria (FIB), nutrients, $\delta^{15}\text{N}$ in macroalgae, and physiochemical parameters were taken from 16 stations along the Puako coastline, Hawaii (black circles). Dye tracer studies were conducted in close proximity to the stations outlined with squares. Nitrogen sources sampled include cesspools, high and low elevation groundwater wells, and soil (colored circles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(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 2 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).

2.3. 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).

2.4. 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 and 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 ($\text{NO}_3^- + \text{NO}_2^-$ [Detection Limit (DL) 0.07 $\mu\text{mol/L}$, USEPA 353.4], NH_4^+ [DL 0.36 $\mu\text{mol/L}$, USGS I-2525], PO_4^{3-} [DL 0.03 $\mu\text{mol/L}$, Technicon™ Industrial Method 155-71 W], total dissolved phosphorous (TDP) [DL 0.5 $\mu\text{mol/L}$, USGS I-4650-03], H_4SiO_4 [DL 1 $\mu\text{mol/L}$, 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 $\mu\text{mol/L}$, ASTM D5176, Shimadzu TOC-V, TNM-1) (Sharp et al., 2002). Salinity was measured at the time of water collection using a YSI Pro 2030 multi-parameter probe.

2.5. $\delta^{15}\text{N}$ analyses

At the time of water sample collection, macroalgae with sufficient biomass to harvest were collected (~5 g) at all stations and analyzed for

$\delta^{15}\text{N}$ (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 and 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 4 × 6 mm tin capsules for stable isotope analysis. Macroalgal tissues were analyzed for $\delta^{15}\text{N}$ 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 $[(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$, where $R = {}^{15}\text{N}/{}^{14}\text{N}$.

To determine the sources of N being used by the macroalgae, $\delta^{15}\text{N}$ - NO_3^- of potential sources were measured. Nutrient concentrations ($\text{NO}_3^- + \text{NO}_2^-$, 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. $\delta^{15}\text{N}$ - 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 $\delta^{15}\text{N}$ macroalgal tissue values were plotted relative to $\delta^{15}\text{N}$ source values (Derse et al., 2007; Wiegner et al., 2016).

2.6. Data analyses

To determine if FIB, nutrients, and $\delta^{15}\text{N}$ 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, $\delta^{15}\text{N}$ 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.

3. Results

3.1. 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 m². 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 fresh-water at the shoreline varied from < 0.02 to 0.14%, depending on how much mixing occurred before discharge at the shoreline.

3.2. 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 \pm 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 $\delta^{15}\text{N}$ 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

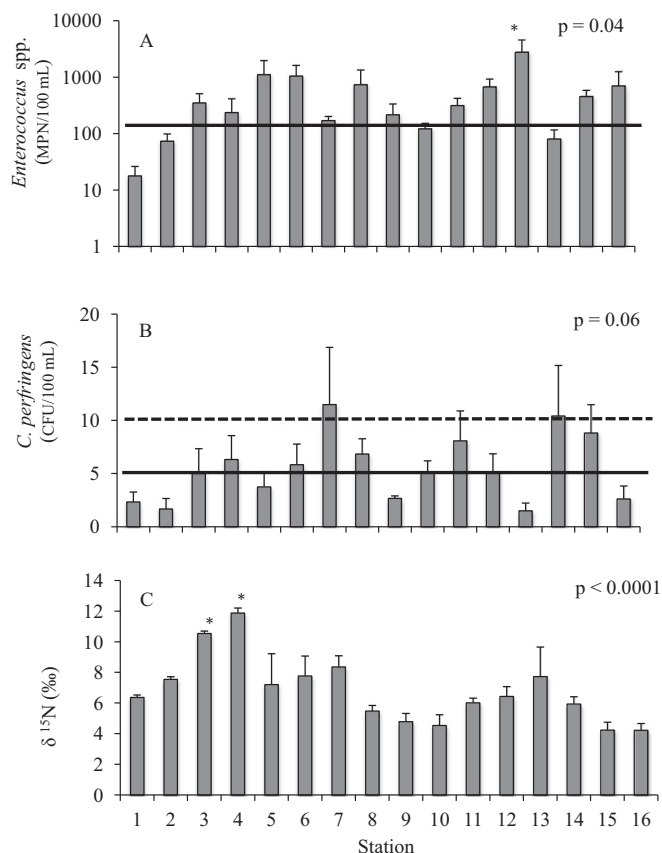


Fig. 2. Mean \pm SE of sewage indicators: (A) *Enterococcus* spp., (B) *Clostridium perfringens*, and (C) $\delta^{15}\text{N}$ (‰) of wild macroalgae at 16 shoreline stations at Puakō, Hawai'i. Hawai'i's Department of Health single sample maximum for *Enterococcus* spp. (104 CFU/100 mL, Fujioka et al., 1997) and a recommended marine recreational standard for *C. perfringens* (5 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 ($\alpha = 0.05$).

Table 1

Mean \pm SE and [range] of *Enterococcus* spp., *Clostridium perfringens*, and $\delta^{15}\text{N}$ 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	<i>Enterococcus</i> spp. (MPN/100 mL)	<i>C. perfringens</i> (CFU/100 mL)	$\delta^{15}\text{N}$ (‰)
1	18 \pm 8 ^b [9–43]	2 \pm 1 [0–4]	6.38 \pm 0.15 ^{a-c} [6.03–6.65]
2	74 \pm 25 ^{ab} [37–143]	2 \pm 1 [0–4]	7.54 \pm 0.18 ^{a-c} [7.04–7.90]
3	349 \pm 162 ^{ab} [37–739]	5 \pm 2 [1–10]	10.55 \pm 0.15 ^{ab} [10.37–11.00]
4	237 \pm 178 ^{ab} [47–770]	6 \pm 2 [3–13]	11.88 \pm 0.32 ^a [11.27–12.78]
5	1107 \pm 861 ^{ab} [94–3674]	4 \pm 1 [0–6]	7.21 \pm 0.20 ^{a-c} [1.29–10.26]
6	1051 \pm 570 ^{ab} [72–2546]	6 \pm 2 [2–10]	7.77 \pm 1.30 ^{a-c} [4.15–10.18]
7	170 \pm 32 ^{ab} [104–257]	12 \pm 5 [3–27]	8.35 \pm 0.74 ^{a-c} [6.48–9.80]
8	738 \pm 603 ^{ab} [62–2544]	7 \pm 1 [3–10]	5.48 \pm 0.37 ^{a-c} [5.06–6.58]
9	216 \pm 120 ^{ab} [27–563]	3 \pm 0 [2–3]	4.79 \pm 0.53 ^{bc} [3.85–6.18]
10	122 \pm 30 ^{ab} [66–202]	5 \pm 1 [2–7]	4.54 \pm 0.70 ^c [3.57–6.56]
11	315 \pm 107 ^{ab} [15–495]	8 \pm 3 [2–14]	6.02 \pm 0.30 ^{a-c} [5.59–6.91]
12	676 \pm 251 ^{ab} [121–1323]	5 \pm 2 [2–8]	6.43 \pm 0.65 ^{a-c} [4.90–8.04]
13	2777 \pm 1806 ^a [17–7985]	2 \pm 1 [0–3]	7.74 \pm 1.92 ^{a-c} [4.80–13.12]
14	80 \pm 36 ^{ab} [24–185]	10 \pm 5 [1–20]	5.94 \pm 0.47 ^{a-c} [5.08–7.28]
15	454 \pm 132 ^{ab} [180–816]	9 \pm 3 [2–13]	4.24 \pm 0.51 ^c [3.62–5.77]
16	699 \pm 554 ^{ab} [17–2338]	3 \pm 1 [0–6]	4.23 \pm 0.44 ^c [3.53–5.50]

within the sewage $\delta^{15}\text{N}$ - 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). $\text{NO}_3^- + \text{NO}_2^-$ concentrations were $\sim 40 \mu\text{mol/L}$ 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). $\text{NO}_3^- + \text{NO}_2^-$, 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 $\sim 500 \mu\text{mol/L}$ 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 ($\text{NO}_3^- + \text{NO}_2^-$, 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 ($\text{NO}_3^- + \text{NO}_2^-$, TDN, PO_4^{3-} , TDP, and H_4SiO_4) with seawater, except for a few stations that consistently

Table 2

Mean \pm SE of $\delta^{15}\text{N}$ - NO_3^- (‰) and $\text{NO}_3^- + \text{NO}_2^-$, PO_4^{3-} , and NH_4^+ concentrations ($\mu\text{mol/L}$) of N sources collected in the Puakō watershed, Hawai'i. (n = sample size).

N Source	n	$\delta^{15}\text{N}$ - NO_3^-	$\text{NO}_3^- + \text{NO}_2^-$	NH_4^+	PO_4^{3-}
Cesspools	3	10.45 \pm 0.58	20.76 \pm 10.50	6370.00 \pm 806.16	378.58 \pm 16.59
Soil	3	2.13 \pm 2.37	6366.67 \pm 3682.46	594.54 \pm 93.23	193.56 \pm 141.56
Ocean	2	3.02 \pm 0.79	1.43 \pm 0.08	2.52 \pm 0.55	0.11 \pm 0.05
High elevation groundwater wells	3	4.76 \pm 0.43	93.87 \pm 4.35	4.84 \pm 1.43	2.48 \pm 0.19
Low elevation groundwater wells	7	7.03 \pm 0.50	130.09 \pm 6.70	4.82 \pm 1.19	2.48 \pm 0.19
Shoreline	3	11.95 \pm 1.13	133.93 \pm 64.68	1.47 \pm 0.44	5.27 \pm 1.57

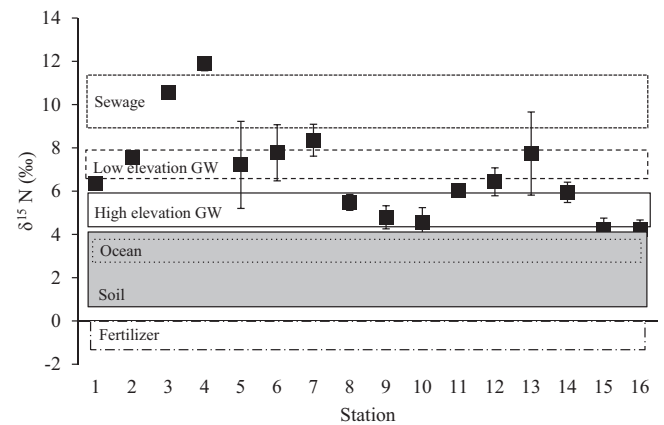


Fig. 3. Mean \pm SE $\delta^{15}\text{N}$ (‰) of wild macroalgae found at 16 stations in Puakō, Hawai'i. Background areas represent (mean \pm SE) $\delta^{15}\text{N}$ of the N sources (fertilizer, soil, ocean, high elevation groundwater (GW) wells, low elevation GW wells, and sewage) measured as part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

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

3.3. 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 $\delta^{15}\text{N}$ in macroalgal tissues was positively correlated with $\text{NO}_3^- + \text{NO}_2^-$ ($p < 0.001$), TDN ($p < 0.001$), and PO_4^{3-} concentrations ($p < 0.001$) (Fig. 6).

4. Discussion

4.1. 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 and Fujioka, 1991; Byappanahalli and Fujioka, 1998; Byappanahalli and 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

Table 3

Mean \pm SE and [range] of $\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ , TDN, PO_4^{3-} , TDP, H_4SiO_4 concentrations ($\mu\text{mol/L}$), 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	$\text{NO}_3^- + \text{NO}_2^-$	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 \pm 0.15 [0.78–1.23]	41 \pm 7 ^{c-f} [25–58]	0.44 \pm 0.04 ^{fg} [0.33–0.51]	0.7 \pm 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 \pm 12.79 ^{ab} [129.62–187.09]	0.49 \pm 0.11 [0.18–0.72]	159 \pm 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 \pm 155 ^{ab} [187–876]	7.12 \pm 0.61 ^e [5.77–8.70]
3	137.12 \pm 35.39 ^{a-c} [36.22–190.37]	1.95 \pm 0.30 [1.04–2.29]	154 \pm 39 ^{a-c} [41–217]	3.81 \pm 0.92 ^{ab} [1.34–5.37]	4.3 \pm 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 \pm 28.14 ^a [125.66–263.07]	1.30 \pm 0.05 [1.24–1.47]	221 \pm 26 ^a [153–267]	7.42 \pm 1.11 ^a [4.12–9.00]	8.3 \pm 1.4 ^a [4.5–10.8]	501 \pm 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 \pm 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 \pm 11.48 ^{de} [2.50–54.16]	1.22 \pm 0.10 [1.03–1.46]	44 \pm 16 ^{d-f} [23–86]	0.66 \pm 0.21 ^{c-g} [0.25–1.17]	0.9 \pm 0.2 ^{fg} [0.3–1.3]	95 \pm 43 ^c [22–219]	30.77 \pm 2.31 ^a [24.53–35.53]
7	134.56 \pm 54.94 ^{a-d} [42.27–285.74]	1.69 \pm 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 \pm 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 \pm 0.97 [1.00–0.33]	59 \pm 19 ^{b-f} [12–99]	0.70 \pm 0.23 ^{c-g} [0.52–1.07]	1.0 \pm 0.2 ^{c-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-c} [47.81–91.92]	1.00 \pm 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 \pm 2.31 ^{cd} [8.53–18.53]
10	56.72 \pm 17.48 ^{a-e} [11.59–94.94]	0.95 \pm 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 \pm 3.60 ^{de} [4.90–21.90]
11	16.52 \pm 1.21 ^{de} [14.08–18.73]	0.96 \pm 0.30 [0.18–1.45]	29 \pm 4 ^{ef} [23–41]	0.49 \pm 0.04 ^{c-g} [0.40–0.58]	0.8 \pm 0.2 ^{fg} [0.3–1.3]	108 \pm 27 ^{bc} [53–173]	28.30 \pm 0.93 ^{ab} [26.07–30.60]
12	35.80 \pm 4.37 ^{a-e} [25.62–46.59]	1.34 \pm 0.25 [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 \pm 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 \pm 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 \pm 174 ^a [359–1018]	6.43 \pm 0.63 ^e [5.33–8.07]
15	13.37 \pm 2.80 ^e [5.73–19.24]	1.07 \pm 0.17 [0.75–1.44]	22 \pm 3 ^f [15–27]	0.39 \pm 0.09 ^g [0.16–0.55]	0.6 \pm 0.2 ^g [0.3–1.1]	120 \pm 24 ^{a-c} [52–158]	29.94 \pm 0.70 ^a [28.67–31.27]
16	38.50 \pm 7.20 ^{a-e} [17.35–47.44]	0.63 \pm 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]

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, $\delta^{15}\text{N}$ 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). $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ in macroalgal tissue can be underestimated by up to 6‰ in waters with high NO_3^- concentrations ($> 10 \mu\text{mol/L}$) (Swart et al., 2014). All of the stations had $\text{NO}_3^- + \text{NO}_2^-$ concentrations exceeding $10 \mu\text{mol/L}$, suggesting that the $\delta^{15}\text{N}$ 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 and 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, $\text{NO}_3^- + \text{NO}_2^-$, 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ in macroalgae and nutrient concentrations further suggest that some portion of the nutrients' concentrations are derived from sewage (Fig. 6).

4.2. Hydrology

At Puakō, a large portion of the nutrient concentration data for $\text{NO}_3^- + \text{NO}_2^-$, 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,

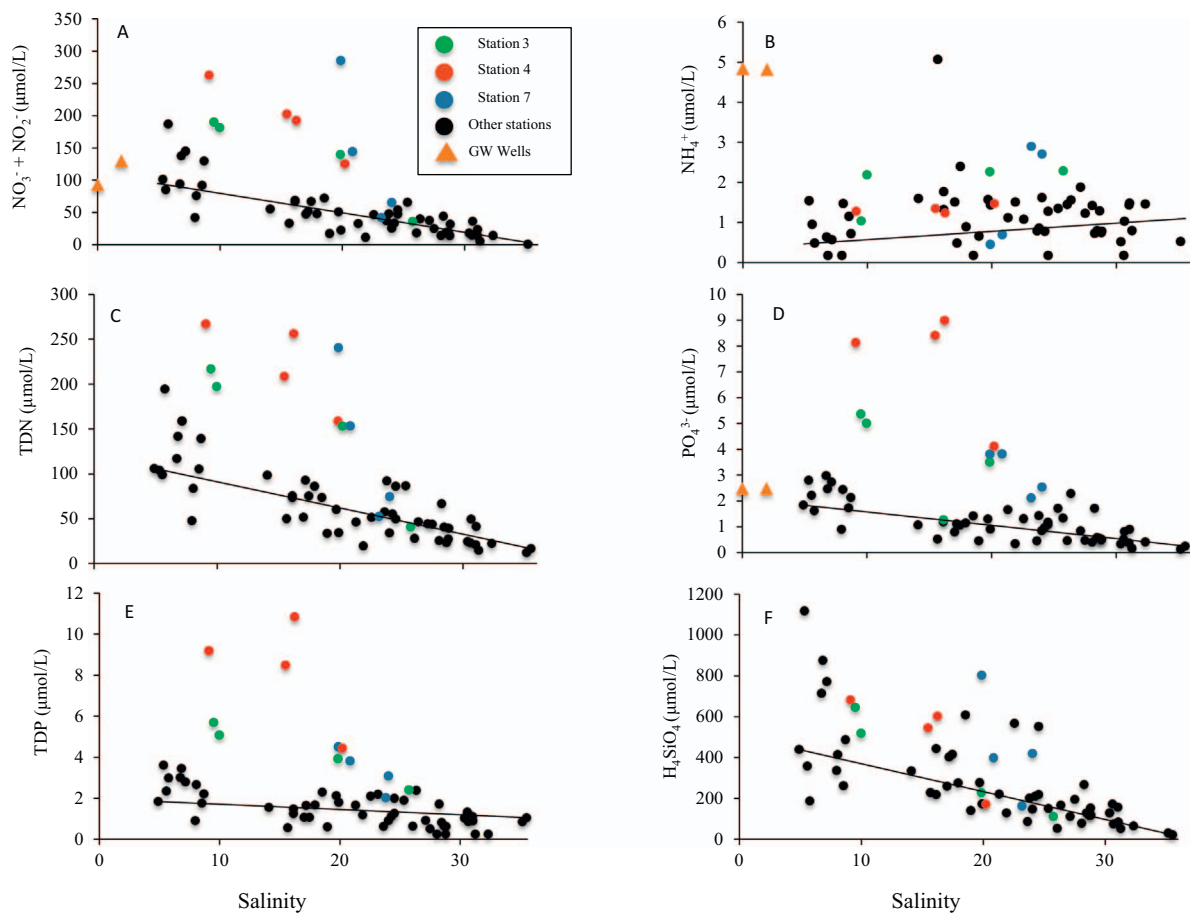


Fig. 4. Mixing plots of nutrient concentrations and salinity along the Puakō shoreline, Hawaii: (A) $\text{NO}_3^- + \text{NO}_2^-$, (B) NH_4^+ , (C) TDN, (D) PO_4^{3-} , (E) TDP, and (F) H_4SiO_4 . Line represents theoretical mixing line, connecting freshest and saltiest shoreline samples. Groundwater samples from wells were only analyzed for $\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ , and PO_4^{3-} .

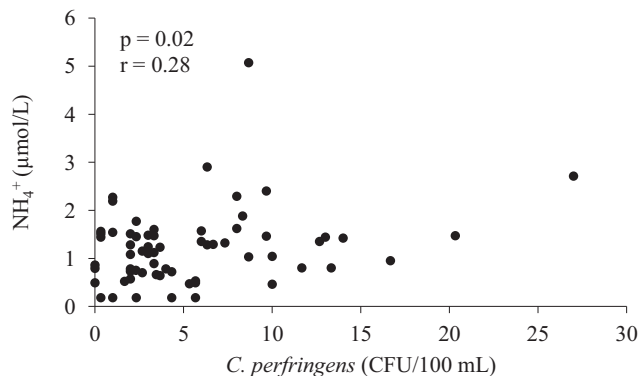


Fig. 5. Correlation between *Clostridium perfringens* and NH_4^+ along the Puakō shoreline, Hawaii.

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. $\delta^{15}\text{N}\text{-NO}_3^-$ at these shoreline stations clearly fell within our measured $\delta^{15}\text{N}\text{-NO}_3^-$ sewage range at Puakō (Table 2), as did the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}\text{-NO}_3^-$ 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 $\delta^{15}\text{N}\text{-NO}_3^-$ became increasingly enriched in ^{15}N moving downslope to the Puakō shoreline. The change in the $\delta^{15}\text{N}\text{-NO}_3^-$ 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 2000 homes with 1587 OSDS (per. Comm. Hawaii Water Supply). Additionally, NO_3^- concentrations increased ~ 40 mmol/L from the high to low elevational groundwater wells (Table 3). Lastly, the $\delta^{15}\text{N}$ enrichment in the $\delta^{15}\text{N}\text{-NO}_3^-$ 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 $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ 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.

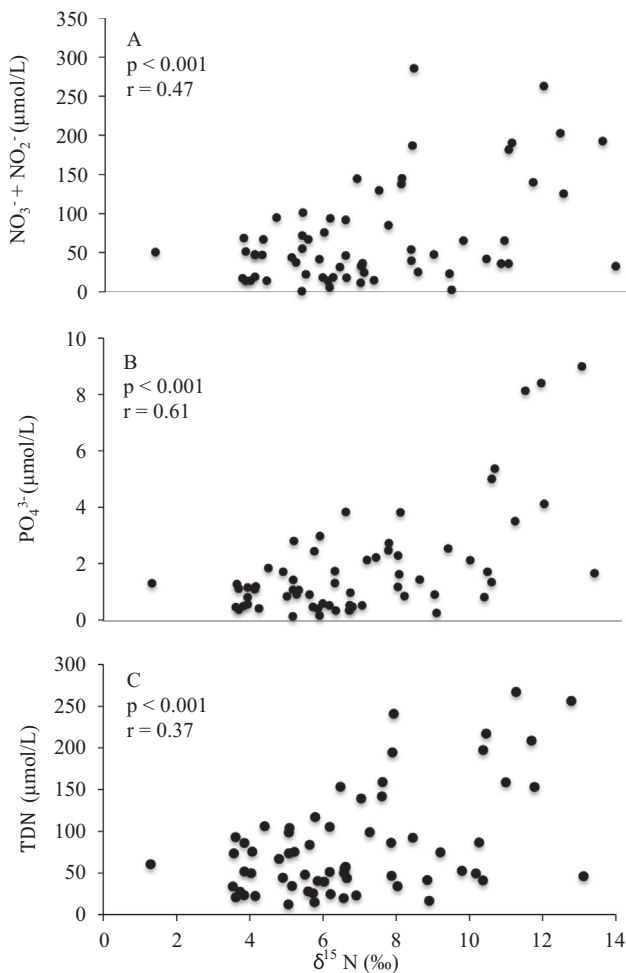


Fig. 6. Correlations between $\delta^{15}\text{N}$ in macroalgal tissues and nutrient concentrations: (A) $\text{NO}_3^- + \text{NO}_2^-$, (B) PO_4^{3-} , and (C) TDN along the Puakō shoreline, Hawai'i.

4.3. 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).

Table 4

Sewage indicators (fecal indicator bacteria = CFU/100 mL, $\delta^{15}\text{N}$ = ‰, and nutrients = $\mu\text{mol/L}$) 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
<i>Enterococcus</i> spp.	2	0–35	36–104	105 +	HDOH, 2014
<i>Clostridium perfringens</i>	3	0–10	11–100	101–505 +	Fung et al., 2007
$\delta^{15}\text{N}$ in macroalgae	3	0.00–5.99	6.00–10.99	11.00 +	Current study
$\text{NO}_3^- + \text{NO}_2^-$	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

To better assess sewage pollution conditions along the Puakō shoreline, a scoring tool was developed using three sewage indicators (FIB, $\delta^{15}\text{N}$ 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, $\delta^{15}\text{N}$ values in macroalgal tissues for different NO_3^- sources (Table 2), and HDOH's water quality standards for nutrient concentrations in open coastal waters ($\text{NO}_3^- + \text{NO}_2^-$, 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 to 2730 $\text{L m}^{-1} \text{h}^{-1}$ (Paytan et al., 2006), which are an order of magnitude larger than the baseline for the wet criteria ($> 294 \text{ L m}^{-1} \text{h}^{-1}$). 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 and 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 $\delta^{15}\text{N}$ 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 and Fujioka, 1991; Byappanahalli and Fujioka, 1998; Byappanahalli and 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 $\times 3$) + ($\delta^{15}\text{N}$ macroalgae level $\times 3$) + (*Enterococcus* spp. level $\times 2$) + ($\text{NO}_3^- + \text{NO}_2^-$ level $\times 1$) + (NH_4^+ level $\times 1$) + (TDP level $\times 1$). 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.

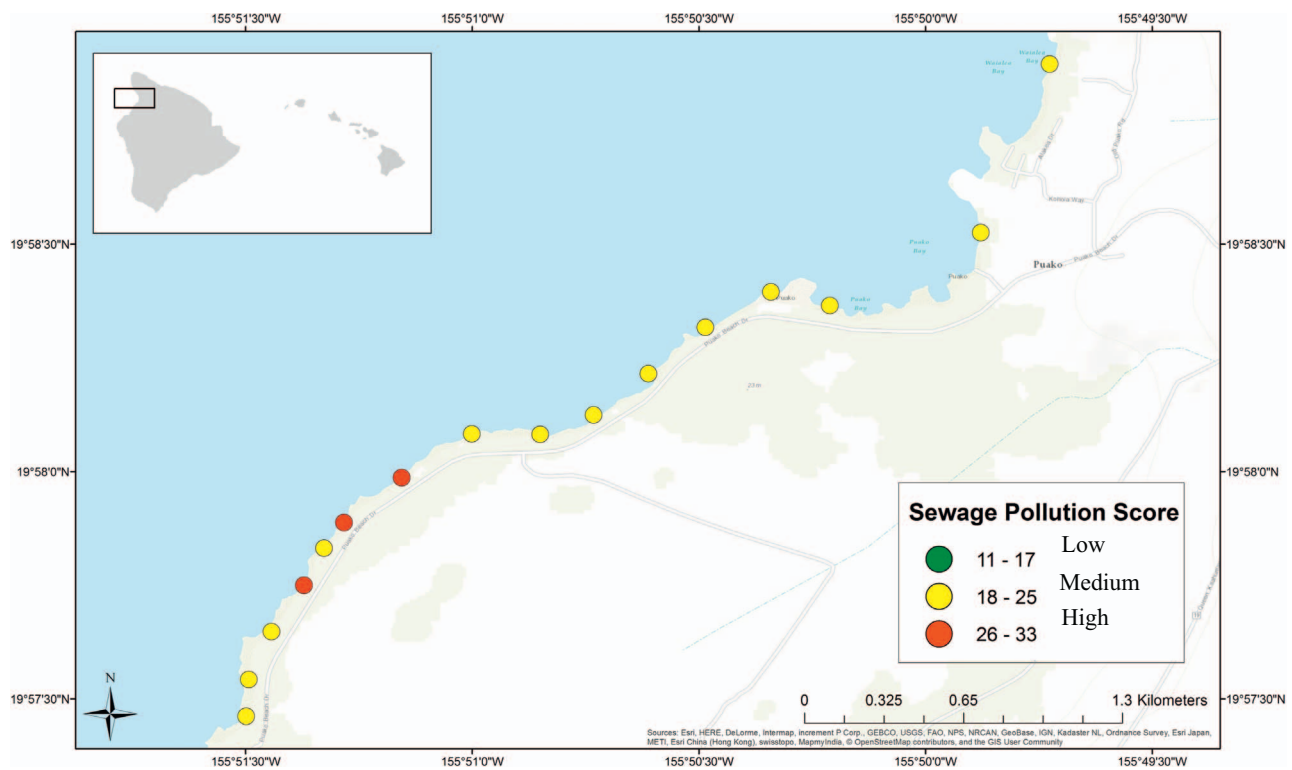


Fig. 7. Sewage pollution scores for 16 stations along the Puakō shoreline, Hawai'i, were based on established and recommended water quality standards, literature values for sewage indicators, and measured values from this study (fecal indicator bacteria, $\delta^{15}\text{N}$ in macroalgae, and nutrients). Sewage pollution score categories 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.

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.

5. Conclusion

Globally, coral reefs are declining from multiple stressors, with sewage pollution being one of the most devastating (Wear and 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 $\delta^{15}\text{N}$ 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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References

- Abbott, I.A., 1999. *Marine Red Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu, Hawai'i.
- Abbott, I.A., Huisman, J.M., 2004. *Marine Green and Brown Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu, Hawai'i (Honolulu, Hawai'i).
- Baker, D.M., Jordán-Dahlgren, E., Maldonado, M.A., Harvell, C.D., 2010. Sea fan corals provide a stable isotope baseline for assessing sewage pollution in the Mexican Caribbean. *Limnol. Oceanogr.* 55 (5), 2139–2149.
- Bisson, J.W., Cabelli, V.J., 1979. Membrane filter enumeration method for *Clostridium perfringens*. *Appl. Environ. Microbiol.* 37, 55–66.
- Bronk, D.A., Roberts, Q.N., Sanderson, M.P., Canuel, E.A., Hatcher, P.G., Mesfioui, R., Filippino, K.C., Mulholland, M.R., Love, N.G., 2010. Effluent organic nitrogen (EON): bioavailability and photochemical and salinity-mediated release. *Environ. Sci. Technol.* 44 (15), 5830e5835.
- Byappanahalli, M., Fujioka, R., 1998. Evidence that tropical soil environment can support

- the growth of *Escherichia coli*. Water Sci. Technol. 38, 171–174.
- Byappanahalli, M., Fujioka, R., 2004. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. Water Sci. Technol. 50, 27–32.
- Cabelli, V.J., 1983. Health Effects Criteria for Marine Recreational Waters. U.S. Environmental Protection Agency EPA-600/1-80-031, Cincinnati, OH. <http://www.epa.gov/microbes/online.htm>.
- Cohen, R.A., Fong, P., 2006. Using opportunistic green macroalgae as indicators of nitrogen supply and sources to estuaries. Ecol. Appl. 16, 1405–1420.
- Converse, R.R., Griffith, J.F., Noble, R.T., Haugland, R.A., Schiff, K.C., Weisberg, S.B., 2012. Correlation between quantitative PCR and culture-based methods for measuring *Enterococcus* spp. over various temporal scales at three California marine beaches. Appl. Environ. Microbiol. 78, 1237–1242.
- Costanzo, S.D., Udy, J., Longstaff, B., Jones, A., 2005. Using nitrogen stable isotope ratios ($\delta^{15}\text{N}$) of macroalgae to determine the effectiveness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. Mar. Pollut. Bull. 51, 212–217.
- Couch, C., Garriques, J., Barnett, C., Preskitt, L., Cotton, S., Giddens, J., Walsh, W., 2014. Spatial and temporal patterns of coral health and disease along leeward Hawai'i Island. Coral Reefs 33, 693–704.
- Dailer, M.L., Knox, R.S., Smith, J.E., Napier, M., Smith, C.M., 2010. Using $\delta^{15}\text{N}$ values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Mar. Pollut. Bull. 60, 655–671.
- Dailer, M.L., Ramey, H.L., Saephan, S., Smith, C.M., 2012. Algal $\delta^{15}\text{N}$ values detect a wastewater effluent plume in nearshore and offshore surface waters and three-dimensionally model the plume across a coral reef on Maui, Hawai'i, USA. Mar. Pollut. Bull. 64, 207–213.
- David, A., Tournoud, M., Perrin, J., Rosain, D., Rodier, C., Salles, C., Bancon-Montigny, C., Picot, B., 2013. Spatial and temporal trends in water quality in a Mediterranean temporary river impacted by sewage effluents. Environ. Monit. Assess. 185, 2517–2534.
- Derse, E., Kne, K.L., Wankel, S.D., Kendall, C., Berg, C.J., Paytan, A., 2007. Identifying sources of nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae. Environ. Sci. Technol. 41, 5217–5223.
- Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41, 87–112.
- Dudley, B., Hughes, F., Ostertag, B., 2014. Groundwater availability mediates the ecosystem effects of an invasion of *Prosopis pallida*. Ecol. Appl. 24, 1954–1971.
- Filippino, K., Mulholland, M., Bernhardt, P., Boneillo, G., Morse, R., Semcheski, M., Marshall, H., Love, N., Roberts, Q., Bronk, D., 2011. The bioavailability of effluent-derived organic nitrogen along an estuarine salinity gradient. Estuar. Coasts 34 (2), 269e280.
- Fong, P., Donohoe, R., Zedler, J., 1994. Nutrient concentration in tissue of the macroalga *Enteromorpha* as a function of nutrient history: an experimental evaluation using field microcosms. Mar. Ecol. Prog. Ser. 106, 273–281.
- Fujioka, R.S., Shizumura, L.K., 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J (Water Pollut Control Fed) 57, 986–992.
- Fujioka, R., Hashimoto, H., Siwak, E., Young, R., 1981. Effect of sunlight on survival of indicator bacteria in seawater. Appl. Environ. Microbiol. 41, 690–696.
- Fujioka, R., Roll, B., Byappanahalli, N., 1997. Appropriate recreational water quality standards for Hawai'i and other tropical regions based on concentrations of *Clostridium perfringens*. Proc. Water Environ. Fed. 4, 405–411.
- Fujioka, R.S., Solo-Gabriele, H.M., Byappanahalli, M.N., Kirs, M., 2015. US recreational water quality criteria: a vision for the future. Int. J. Environ. Res. Public Health 12 (7), 7752–7776.
- Fujita, R.M., 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J. Exp. Mar. Biol. Ecol. 92, 283–301.
- Fung, D.Y.C., Fujioka, R., Vijayavel, K., Sato, D., Bishop, D., 2007. Evaluation of Fung double tube test for *Clostridium perfringens* and easyphage test for f-specific RNA coliphages as rapid screening tests for fecal contamination in recreational waters of Hawai'i. J Rapid Meth Aut Mic 15, 217–229.
- Gaspar, E., 1987. Modern Trends in Tracer Hydrology. I and II CRC Press, Inc., Boca Raton, FL.
- Glenn, C.R., Whittier, R.B., Dailer, M.L., Dulaiova, H., El-Kadi, A.I., Fackrell, J., Kelly, J.L., Waters, C.A., Sevadjan, J., 2013. Lahaina Groundwater Tracer Study – Lahaina, Maui, Hawai'i, Final Report. (prepared for the State of Hawai'i Department of Health, the U.S. Environmental Protection Agency, and the U.S. Army Engineer Research and Development Center).
- Hardina, C., Fujioka, R., 1991. Soil: the environmental source of *Escherichia coli* and *Enterococci* in Hawai'i's streams. Environ. Toxicol. Water Qual. 6, 185–195.
- Hayes, T., Hourigan, T., Jazwinski, S., Johnson, S., Parrish, J., Walsh, D., 1982. The Coastal Resources, Fisheries and Fishery, Ecology of Puakō, West Hawai'i. Hawai'i Cooperative Fishery Research Unit 81-1, pp. 1–245.
- HDAR, 2013. South Kohala Reefs in Dire Straits. (Public informational sheet).
- HDOH, 1984. Interim Report of a Baseline Study on Water Quality at Kapoho Bay, Hawai'i.
- HDOH, 2014. State of Hawai'i Water Quality Monitoring and Assessment Report. HDOH CWA §305(b).
- HDOH Administrative Rules-Title 11, HAR, 2016. Hawaii Administrative Rules Title 11, Department of Health, Chapter 62, Wastewater System. HDOH, pp. 11–62.
- Hunter, C., Evans, C., 1995. Coral reefs in Kane'ohe Bay, Hawai'i: two centuries of western influence and two decades of data. Bull. Mar. Sci. 57, 501–515.
- Knee, K., Layton, B., Street, J., 2008a. Sources of nutrients and fecal indicator bacteria to nearshore water on the north shore of Kauai (Hawai'i USA). Estuar. Coasts 31, 607–622.
- Knee, K., Street, J., Grossman, E., Paytan, A., 2008b. Submarine ground-water discharge and fate along the coast of Kaloko-Honokōhau National Historical Park, Island of Hawai'i – part 2, spatial and temporal variations in salinity, radium-isotope activity, and nutrient concentrations in coastal waters, December 2003–April 2006. In: USGS Scientific Investigations Report, pp. 2008–5128.
- Knee, K., Street, J., Boehm, A., Paytan, A., 2010. Nutrient inputs to the coastal ocean from submarine groundwater discharge in a groundwater dominated system: relation to land use (Kona coast, Hawaii, USA). Limnol. Oceanogr. 55 (3), 1105–1122.
- Lapointe, B., O'Connell, J., Garrett, G., 1990. Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry 10, 289–307.
- Lapointe, B., Barile, P., Wynne, M., Yentsch, C., 2005. Reciprocal *Caulerpa* invasion: Mediterranean native *Caulerpa olivieri* in the Bahamas supported by human nitrogen enrichment. Aquat. Invaders 16, 1–5.
- Lesser, M.P., Jarrett, J.K., 2014. Culture-dependent and culture-independent analyses reveal no prokaryotic community shifts or recovery of *Serratia marcescens* in *Acropora palmata* with white pox disease. FEMS Microbiol. Ecol. 88 (3), 457–467.
- Lin, H.-J., Wu, C.-Y., Kao, S.-J., Kao, W.-Y., Meng, P.-J., 2007. Mapping anthropogenic nitrogen through point sources in coral reefs using $\delta^{15}\text{N}$ in macroalgae. Mar. Ecol. Prog. Ser. 335, 95–109.
- Maiga, Y., Wethe, J., Denyigba, K., Ouattara, A.S., 2009. The impact of pond depth and environmental conditions on sunlight inactivation of *Escherichia coli* and *Enterococci* in wastewater in a warm climate. Can. J. Microbiol. 55, 1364–1374.
- Minton, D., Conklin, E., Weiant, P., Wiggins, C., 2012. Forty Years of Decline on Puakō's Coral Reefs - A Review of Historical and Current Data (1970–2010) the Nature Conservancy, Honolulu, HI. pp. 1–140.
- Moynihn, M.A., Baker, D.M., Mmochi, A.J., 2012. Isotopic and microbial indicators of sewage pollution from Stone Town, Zanzibar, Tanzania. Mar. Pollut. Bull. 64 (7), 1348–1355.
- Nelson, C., Donahue, M., Dulaiova, H., Goldberg, S., La Valle, F., Lubarsky, K., Miyano, J., Richardson, C., Silbiger, N., Thomas, F., 2015. Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. Mar. Chem. 177, 232–243.
- Nnane, D.E., Ebdon, J.E., Taylor, H.D., 2011. Integrated analysis of water quality parameters for cost-effective faecal pollution management in river catchments. Water Res. 45, 2235–2246.
- Ochoa-Izaguirre, M.J., Soto-Jiménez, M.F., 2015. Variability in nitrogen stable isotope ratios of macroalgae: consequences for the identification of nitrogen sources. J. Phycol. 51, 46–65.
- Officer, C.B., 1979. Discussion on the behavior of non-conservative dissolved constituents in estuaries. Estuar. Coast. Shelf Sci. 9, 91–94.
- Parsons, M.L., Walsh, W.J., Settlemyer, C.J., White, D.J., Ballauer, J.M., Ayotte, P.M., Osada, K.M., Carman, B., 2008. A multivariate assessment of the coral ecosystem health of two embayments on the lee of the island of Hawai'i. Mar. Pollut. Bull. 56, 1138–1149.
- Pastorok, R., Bilyard, G., 1985. Effects of sewage pollution on coral-reef communities. Mar. Ecol. Prog. Ser. 21, 175–189.
- Paul, J.H., McLaughlin, M.R., Griffin, D.W., Lipp, E.K., Stokes, R., Rose, J.B., 2000. Rapid movement of wastewater from on-site disposal systems into surface waters in the Lower Florida Keys. Estuar. Coasts 23 (5), 662–668.
- Paytan, A., Shellenbarger, G.G., Street, J.H., Gonnea, M.E., Davis, K., Young, M.B., Moore, W.S., 2006. Submarine groundwater discharge: an important source of new inorganic nitrogen to coral reef ecosystems. Limnol. Oceanogr. 51, 343–348.
- Pehlivanoglu, E., Sedlak, D.L., 2004. Bioavailability of wastewater derived organic nitrogen to the alga *Selenastrum capricornutum*. Water Res. 38 (14e15), 3189e3196.
- Pinto, B., 1999. Characterization of faecal streptococci as indicators of faecal pollution and distribution in the environment. Lett. Appl. Microbiol. 29, 258–263.
- Prüss, A., 1998. Review of epidemiological studies on health effects from exposure to recreational water. Int. J. Epidemiol. 27, 1–9.
- Ragosta, G., Evensen, C., Atwill, E.R., Walker, M., Ticktin, T., Asquith, A., Tate, K., 2010. Casual connections between water quality and land use in a rural tropical island watershed. EcoHealth 7, 105–113.
- Redding, J.E., Myers-Miller, R.L., Baker, D.M., Fogel, M., Raymundo, L.J., Kim, K., 2013. Link between sewage-derived nitrogen pollution and coral disease severity in Guam. Mar. Pollut. Bull. 73 (1), 57–63.
- Reich, C., Shinn, E.A., Hickey, C., Tihansky, A.B., 2001. Tidal and meteorological influences on shallow marine groundwater flow in the upper Florida Keys. In: Porter, J., Porter, K.C. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys, an Ecosystem Handbook. CRC Press.
- Savage, C., 2005. Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. Ambio 34, 145–150.
- Schott, E., 2010. Mauna Lani, DW 'Aina Le'a and Puakō/Wailea Bay Wastewater Assessment. Professional Report to the Puakō Community Association Phase Onepp. 1–47.
- Sharp, J.H., Rinker, K.R., Savidge, K.B., Abell, J., Benaim, J.Y., Bronk, D., Burdige, D.J., Cauwet, G., Chen, W., Doval, M.D., Hansell, D., 2002. A preliminary methods comparison for measurement of dissolved organic nitrogen in seawater. Mar. Chem. 78 (4), 171–184.
- Shibata, T., Solo-Gabriele, H., Fleming, L.E., Elmira, S., 2004. Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. Water Res. 38, 3119–3131.
- Shibata, T., Solo-Gabriele, H., Sinigalliano, C.D., Gidley, M.L., Plano, L.R.W., Fleisher, J.M., Wang, J.D., Elmira, S.M., He, G., Wright, M.E., Abdelzacher, A.M., Ortega, C., Wanless, D., Garza, A.C., Kish, J., Scott, T., Hollenback, J., Backer, L.C., Fleming, L.E., 2010. Evaluation of conventional and alternative monitoring methods for a recreational marine beach with nonpoint source of fecal contamination. Environ. Sci. Technol. 44, 8175–8181.

- Shuval, H., 2003. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. *J. Water Health* 1, 53–64.
- Sutherland, K.P., Porter, J.W., Turner, J.W., Thomas, B.J., Looney, E.E., Luna, T.P., Meyers, M.K., Futch, J.C., Lipp, E.K., 2010. Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, *Acropora palmata*. *Environ. Microbiol.* 12 (5), 1122–1131.
- Swart, P.K., Evans, S., Capo, T., Altabet, M.A., 2014. The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate. *Biogeosciences* 11, 6147–6157.
- Umezawa, Y., Miyajima, T., Yamamuro, M., Kayanne, H., Koike, I., 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by $\delta^{15}\text{N}$ in macroalgae. *Limnol. Oceanogr.* 47, 1405–1416.
- UNESCO, 1981. Background papers and supporting data on the practical salinity scale 1978. In: UNESCO Technical Papers in Marine Science. 37.
- Urgun-Demirtas, M., Sattayatewa, C., Pagilla, K.R., 2008. Bioavailability of dissolved organic nitrogen in treated effluents. *Water Environ. Res.* 80 (5), 397–406.
- USEPA, 2013. Cesspools in Hawai'i. <http://www.epa.gov/region9/water/groundwater/uic-hicesspools.html>, Accessed date: 8 February 2014.
- Viau, E., Goodwin, K., Yamahara, K., Layton, B., Sassoubre, L., Burns, S., Tong, H., Wong, S., Lu, Y., Boehm, A., 2011. Bacterial pathogens in Hawaiian coastal streams associations with fecal indicators, land cover, and water quality. *Water Res.* 45, 3279–3290.
- Wang, Y., Wilson, J.M., Vanbriesen, J.M., 2015. The effect of sampling strategies on assessment of water quality criteria attainment. *J. Environ. Manag.* 154, 33–39.
- Wear, S., Vega Thurber, R., 2015. Sewage pollution: mitigation is key for coral reef stewardship. *Ann. N. Y. Acad. Sci.* 1–16.
- Whittier, R., El-Kadi, A., 2014. Human Health and Environmental Risk Ranking of On-site Sewage Disposal Systems for the Hawaiian Islands of Kaua'i, Moloka'i, Maui, and Hawai'i. http://health.hawaii.gov/wastewater/files/2015/09/OSDS_NI.pdf.
- Wiegner, T.N., Mokiao-Lee, A., Johnson, E.E., 2016. Identifying nitrogen sources to thermal tide pools in Kapoho, Hawai'i, U.S.A, using a multi-stable isotope approach. *Mar. Pollut. Bull.* 103, 63–71.
- Yates, M.V., 1985. Septic tank density and groundwater contamination. *Groundwater* 23 (5), 586–591.
- Yoshioka, R.M., Kim, C.J.S., Tracy, A.M., Most, R., Harvell, C.D., 2016. Linking sewage pollution and water quality to spatial patterns of *Porites lobata* growth anomalies in Puakō, Hawai'i. *Mar. Pollut. Bull.* 104, 313–321.
- Zambrano, L., Contreras, V., Mazari-Hiriart, M., Zarco-Arista, A., 2009. Spatial heterogeneity of water quality in a highly degraded tropical freshwater ecosystem. *Environ. Manag.* 43, 249–263.