

**Dilution of sewage pollution in the coastal waters of Hilo, Hawai'i, USA; an area  
with high river and groundwater inputs.**

Presented to the Faculty of the  
Tropical Conservation Biology and Environmental Science Graduate Program

University of Hawai'i at Hilo  
In partial fulfillment for the requirements of the degree of

Master of Science  
in  
Tropical Conservation Biology and Environmental Science

August 2022

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## **Acknowledgements**

I am extremely grateful for everyone that has helped me to complete my master's thesis work, from planning to field work to the final analyses of the data. Dr. Tracy Wiegner and Dr. Steven Colbert both assisted in every step of the research process and taught me everything that I know of the many hydrological processes that take place in the watershed where I was raised. Dr. Karla McDermid also played a major role in helping me to identify the different macroalgal species that were frequently found at the shoreline stations that were monitored throughout this study. Together we were able to use many indicators of sewage to efficiently assess water quality and sewage pollution vectors along the shoreline of Hilo, Hawai'i.

Field work would not have been possible without the help of the many hands that assisted in collecting and processing water and macroalgal samples. Shayla Waiki assisted in collecting and processing every sample that was collected in this study. Nicolas Storie, a graduate student at the University of Hawai'i at Mānoa, helped with field work and processed samples for human-associated *Bacteroidetes* using qPCR techniques. Devon Aguiar assisted with planning and field work during the early stages of this project. Three student interns also contributed over the course of the project: Darriene Kealoha, Kaitlin Villafuerte, and Brooke Enright. Field and lab assistance was also provided by volunteers, Finn Reil and Walter Boger.

I would also like to thank Erik Johnson and the University of Hawai'i at Hilo Analytical Lab for their services in processing samples. Deborah Ombac from the Hilo Wastewater Treatment Plant collected monthly influent, effluent, and manhole samples for analysis. Blayne Castillo from the Hawai'i County Department of Water Supply provided well water samples for analysis. The Keaukaha Community Association, Leleiwi Community Association, and the Hilo community allowed us to sample throughout the shoreline of the place they call home.

Finally, I would like to thank the University of Hawai'i Sea Grant College Program and the Hau'oli Mau Loa Foundation for funding my master's thesis work and graduate studies, respectively. I truly feel that none of this work would have been possible without the support of the abovementioned people, programs, foundations, and communities.

Mahalo.

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## **List of Abbreviations**

**Chl a:** Chlorophyll a.

**DL:** Detection limit.

**DOC:** Dissolved organic carbon.

**FIB:** Fecal indicator bacteria.

**GW:** Groundwater, in reference as an N-source.

**HDOH:** Hawai'i Department of Health.

**IRMS:** Isotope ratio mass spectrometry.

**HGW:** High groundwater-influence, stations that had an average salinity <10 and had a visible spring.

**LGW:** Low groundwater-influence, stations that had an average salinity >10 and had a visible spring.

**NOAA:** National Oceanic and Atmospheric Agency

**OSDS:** On-site sewage disposal systems, in reference to systems and as an N-source.

**RI:** River influenced, stations that were at river mouths or in the immediate sediment plume of river mouths.

**STP:** Sewage treatment plant, in reference as an N-source.

**qPCR:** Quantitative polymerase chain reaction.

**TDN:** Total dissolved nitrogen.

**TDP:** Total dissolved phosphorus.

**USGS:** United States Geological Survey.

$\delta^{15}\text{N}$ : Delta notation for the nitrogen-15 isotope signifying the enrichment of  $^{15}\text{N}$  to  $^{14}\text{N}$ .

$\delta^{18}\text{O}$ : Delta notation for oxygen-18 isotope signifying the enrichment of  $^{18}\text{O}$  to  $^{16}\text{O}$ .

## **Abstract**

Sewage pollution threatens human and ecosystem health, and its presence can be masked by dilution from freshwater inputs or ocean mixing. This project assessed sewage pollution in the coastal waters of Hilo, Hawai'i, an area with high freshwater inputs impacted by on-site sewage disposal systems (OSDS) and an offshore sewage treatment plant (STP) outfall. Twenty shoreline stations were sampled from July 2020 to October 2021 for multiple sewage indicators: fecal indicator bacteria (FIB), nutrients, and stable isotopes in macroalgae and nitrate in water, many of which were incorporated into a sewage pollution scoring tool. Rivers and groundwater were assessed as vectors of sewage pollution and sewage indicator values were compared to water quality standards. Nutrient standards were exceeded in all waters, while FIB standards were exceeded in river-influenced areas. River areas had the highest FIB,  $\text{NH}_4^+$ , Chl a, turbidity values, and the highest probability of having stations with a medium-level sewage impact. Groundwater areas had the highest nutrient concentrations and groundwater was the dominant source of  $\text{NO}_3^-$  to all freshwater-influence groups as indicated by the stable isotope mixing model. Low sewage indicator values and pollution scores at stations with recently confirmed connectivity to OSDS suggest that freshwater is diluting sewage along the shoreline. This study emphasizes the importance of including a variety of sewage indicators to account for variability influenced by area-specific environmental factors, such as high freshwater input or differing hydrogeology, when assessing the influence of sewage pollution on water quality.



## **Introduction**

The growing human population has exacerbated sewage pollution in coastal waters, impacting both human and environmental health (Pruss, 1998; Wade et al., 2003). Untreated sewage harbors high concentrations of nutrients, pathogens, endocrine disruptors, heavy metals, and other toxins (Wear & Vega Thurber, 2015). Annually, an estimated 50 million cases of respiratory illness and 120 million cases of gastrointestinal illness are linked to swimming in sewage-contaminated waters, and the antibiotic-resistant bacteria associated with sewage often increases the difficulty of treating infection (Shuval, 2003; Fleisher et al., 2010; Boopathy, 2017; Gerken et al., 2021). Algal blooms are well-documented in response to nutrient inputs from sewage outfalls and phase shifts have occurred from coral to algae-dominated communities after long-term sewage inputs (Pastorok & Bilyard, 1985; Hunter & Evans, 1995; Lapointe et al., 2005; Lapointe & Bedford, 2010). Additionally, algal communities have shown changes in dominant species in areas of known sewage pollution – increases in opportunistic green algae or cyanobacteria (Pastorok & Bilyard, 1985; Soltan et al., 2001; Guinda et al., 2008). Nutrients from sewage pollution increase the prevalence of coral disease and add stress to coral reef ecosystems (Hunter & Evans, 1995). Sewage-derived pathogens have been linked to coral disease, hindering their ability to recover from other environmental stressors (Sutherland et al., 2010). Short-term recovery of coral reefs has been observed after reducing point-source sewage pollution, but full recovery can take years to decades and some changes are irreversible (Pastorok & Bilyard, 1985; Hunter & Evans, 1995). If sewage is not effectively managed in coral reef areas, further water quality degradation is inevitable.

Sewage collection and treatment are handled differently in urban and rural areas. Sewer systems collect sewage in urban areas, where it is transported to sewage treatment plants (STP) for treatment, and then disposed of through outfalls and injection wells. In rural areas, onsite-sewage disposal systems (OSDS: cesspools, septic tanks, aerobic treatment units) are a common method of sewage disposal. Cesspools, an antiquated type of OSDS, are unlined, underground holes used to dispose of domestic sewage. Hawai'i is the last state in the United States to ban new cesspool construction and has set the goal of eliminating all cesspools by 2050 (Act 125; Ige, 2017).

Feasibility of removing cesspools and switching to the use of a STP has been impeded by the remote location of many homes currently utilizing cesspools. In many of these remote locations, the highly variable, and often very permeable substrata with different hydrologies has also hindered conversion to more modern types of OSDS. Hence, area-specific strategies are needed for the cesspool conversion effort (HDOH, 2020).

To document sewage pollution in coastal waters, researchers use a variety of sewage indicators and have found them to have varying effectiveness. Mezzacapo et al. (2021) suggested that sewage pollution may be difficult to detect where there are significant effects of dilution; either by freshwater inputs or ocean mixing, or nutrient uptake. Fecal indicator bacteria (FIB) are commonly used by monitoring agencies to assess recreational waters for the presence of sewage. *Enterococcus* spp. is used by the United States Environmental Protection Agency (USEPA) and states' departments of health to assess marine recreational waters. In tropical settings, *Enterococcus* spp. can survive and multiply in tropical soils and waters, confounding interpretations of concentrations relative to sewage pollution (Hardina & Fujioka, 1991; Pinto et al., 2002; Enns et al., 2012; Fujioka et al., 2015). Because of this, an additional FIB has been adopted by the Hawai'i Department of Health (HDOH), *Clostridium perfringens*. This bacterium is considered to be more indicative of sewage pollution, because it is found in the human gut and cannot multiply in oxic environments; however, *C. perfringens* is also found in the guts of other warm-blooded animals and can survive in soils and rivers (Niilo, 1980; Madema et al., 1997; Boehm et al., 2009; Fujioka et al., 2015; Miller-Pierce & Rhoades, 2019; Gerken et al., 2022). More recently, genetic markers have been used to identify human-specific gut bacteria, most commonly from the genus *Bacteroides* using the HF183 marker, although geographic variability of the human gut microbiome influences the effectiveness of this approach (Zhang et al., 2012; Ahmed et al., 2020).

Nutrient concentrations are high in sewage and often elevated in areas polluted by sewage, but nutrients can have many non-sewage sources within a watershed, making nutrient sources difficult to distinguish (Pastorok & Bilyard, 1985). Stable isotopes:  $\delta^{15}\text{N}$  of macroalgae and  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  from water samples, have been used as indicators of sewage in other areas of Hawai'i Island, on other Hawaiian Islands, and worldwide, because isotope ratios often vary among nitrogen sources and

therefore can be distinguished from one another (Costanzo et al., 2005; Dailer et al., 2010; Lapointe et al., 2015; Wiegner et al., 2016; Abaya et al., 2018a; Wiegner et al., 2021). However, in some instances, nitrogen sources can have overlapping stable isotope values not allowing them to be distinguished from one another. Because of the limitations of each individual indicator of sewage pollution, a recent study has incorporated multiple indicators into a scoring tool to better evaluate sewage pollution in coastal waters and found the tool to be successful in predicting sewage pollution hotspots such as at springs with known connections to OSDS (Abaya et al., 2018a).

One location in Hawai'i experiencing sewage pollution is Hilo, the population center of Hawai'i Island. Hilo has a STP with an offshore outfall, a high number of OSDS, rivers flowing through urban areas into an enclosed portion of its bay, and large rates of groundwater discharge. Hilo is currently recognized as a priority three category in the cesspool conversion plan by the HDOH cesspool conversion working group, "having sensitive waters that are potentially impacted by cesspools" (HDOH, 2017). These risk factors threaten the Hilo community, who use these waters to fish and gather for subsistence, and for surfing, canoe paddling, swimming, and other cultural practices (Puniwai et al., 2016). Previous studies of Hilo have examined sewage pollution under various river flow conditions, but the study of groundwater-influenced areas has been limited (Wiegner et al., 2013; Carlson & Wiegner, 2016; Wiegner et al., 2016; Economy et al., 2019). Two studies have also documented the presence of FIB in soils of various land use areas, which may contribute to surface runoff during heavy rain events (Economy et al., 2019; Gerken et al., 2022).

The presence of many sewage pollution risk factors in Hilo, the potential negative impacts of sewage pollution to human and coral reef health, and the limited research of this topic here, has inspired this study, which aimed to gain a comprehensive understanding of sewage pollution along Hilo's shoreline. A multi-indicator approach to assess sewage presence was developed by Abaya et al. (2018a), but this method has never been applied to East Hawai'i or other areas influenced by both groundwater and river inputs. The objectives: 1) to evaluate the relative importance of two sewage pollution vectors, groundwater and rivers, and 2) to examine the efficacy of sewage indicators in a tropical setting with many freshwater inputs. This work should inform the

HDOH cesspool conversion working group on the current status of sewage pollution in Hilo and provide aid in prioritizing areas for cesspool removal. A better understanding of the accuracy of sewage indicators and the importance of incorporating multiple indicators should aid future studies of sewage pollution in tropical settings.

## **Methods**

### ***Site Description***

The town of Hilo is within the *moku* (district) of Hilo, located on the east side of Hawai'i Island, HI, USA, and is home to approximately 43,263 residents (U.S. Census Bureau, 2021). The study area for this project includes five *ahupua'a* (traditional land division) in the Hilo Bay watershed - starting from the east: Waiākea, Pi'ihonua, Pu'u'eo, 'Alae, and Kaiwiki, encompassing the slopes of Mauna Loa and Mauna Kea volcanoes, two major rivers: Wailuku and Wailoa, and multiple streams (Figure 1). Groundwater recharge in the Hilo watershed is one of the largest in the Hawaiian chain, estimated to be 409 m<sup>3</sup>/d/m, but sewage input through groundwater has not been well investigated here (Engott, 2011). A 3.07-km breakwater divides the southern part of the Hilo Bay shoreline, providing shelter to the Port of Hilo, but also trapping sediment and pollutants from two major rivers within the sheltered area by reducing ocean recharge (M&E Pacific, 1980; U.S. Army Corps of Engineers, 2015). Hilo has several sources of sewage pollution to its coastal waters. The single municipal STP is permitted to discharge effluent, treated by fixed-film secondary treatment with disinfection by chlorination (Figure 1). Effluent from the Hilo STP is discharged approximately 1.3 km offshore through an outfall pipe (U.S. EPA Region 9, 2010; Higuchi, 2014). This STP services ~30% of homes and the remainder use OSDS, primarily cesspools. Hilo has 12,272 OSDS, 10,635 of which are classified as cesspools (Whittier & El Kadi, 2014). Elevated FIB and nutrient concentrations have been documented in Hilo Bay and its rivers (Wiegner et al., 2013; Wiegner et al., 2017; Economy et al., 2019). There are currently five sites monitored by the HDOH Beach monitoring program (HDOH, 2019) and an additional seven sites were previously monitored by the Surfrider Foundation Blue Water Task Force, although this latter program is no longer active (Surfrider Foundation, 2022).

Hilo has a tropical climate, with wet winter (October-April) and dry summer (May-October) seasons, experiencing an average annual rainfall of nearly 7000 mm at high elevations and about 3000 mm near sea level (Giambelluca et al., 2013). The Honoli'i Stream watershed has an area of 39.2 km<sup>2</sup> and a maximum elevation of 1867 m, consisting predominantly of conservation land, followed by agricultural, and urban at 78.4%, 21%, and 0.6%, respectively (Parham et al., 2008). The Wailuku River watershed is the largest in the State of Hawai'i, with an area of 653.2 km<sup>2</sup> and a maximum elevation of 4200 m (Parham et al., 2008). The dominant land use of this watershed is conservation, followed by agricultural, and urban at 76.9%, 22.4%, and 0.7%, respectively (Parham et al., 2008). The Wailoa River watershed encompasses 255.4 km<sup>2</sup> and has a maximum elevation of 2964 m, with land use consisting of 70.9% conservation, followed by 20.4% agricultural, and 8.7% urban near the lower reaches (Parham et al., 2008). The low-lying Keaukaha watershed has a maximum elevation of 39 m, covering 6.6 km<sup>2</sup>, of which 61.9% is urban and 38.1% is agricultural land use type (Parham et al., 2008).

### ***Shoreline Sampling***

Sampling took place at 20 stations spanning approximately 15 km of the Hilo shoreline. Stations were selected based on observations of groundwater discharge from field salinity surveys, county beach park status, and community significance. Shoreline sampling took place over two consecutive days each month from July 2020 to October 2021. Stations were sampled on the lowest possible tides of each month to minimize dilution of groundwater with seawater and within 2 h of sunrise to reduce sunlight inactivation of FIB (Sinton et al., 2002). Triplicate water samples were collected from each station in sterilized, acid-washed 1-L polypropylene bottles. Salinity and temperature were measured in the field using a calibrated YSI ProSolo. Macroalgal species present at each station were haphazardly collected based on availability and proximity to the water sample collection point. Water and macroalgae samples were stored on ice until processed in the laboratory.

## Laboratory Analyses

*Enterococcus* spp. were quantified using the Enterolert MPN method with a 1:9 dilution (10 mL sample: 90mL sterile water) (IDEXX Laboratories Inc.). Samples below the detection limit of this method were assumed to have a value of 5 MPN/100 mL, half of the detection limit at this dilution. *Clostridium perfringens* was quantified by filtering 100 mL of sample through a 0.45- $\mu$ m pore size NO<sub>3</sub><sup>-</sup> filter (Whatman™) and plating the filter onto membrane-*Clostridium perfringens* (m-CP) agar base (Oxoid Ltd., Basingstoke, Hampshire, England) (Bisson & Cabelli, 1979). All FIB culture samples were processed and incubated within 6 h of sample collection. Samples from each station from July 2020 to December 2020 were also analyzed for the HF183 marker for human-associated *Bacteroides*. For *Bacteroides* analyses, 400 mL of water samples were filtered onto sterile 0.20- $\mu$ m pore size MCE filters (Advantec MFS, Inc.), dried, and stored at -80 °C until DNA extraction. Bacterial DNA was extracted using the FastDNA spin kit for soil (MP Biomedicals) with a slight modification to manufacturer protocol: cells were lysed using a MP Biomedicals FastPrep bead beater for ~2 min. Extracted DNA was stored at -40 °C until sequencing using an established HF183 quantitative polymerase chain reaction (qPCR) assay (Haugland et al., 2010; Green et al., 2014).

Samples for nutrient analyses were taken from one of the triplicate bottles from each station. Water was filtered through a muffled (600 °C, 5 h) GF/F filter (Whatman, 0.7- $\mu$ m pore size) and stored at 0 °C until analysis. Nutrient samples were analyzed with a Lachat Quikchem 8500, FIA autoanalyzer for NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> [Detection Limit (DL) 0.07  $\mu$ mol/L, USEPA 353.2], NH<sub>4</sub><sup>+</sup> [DL 0.36  $\mu$ mol/L, USEPA 349], TDP [DL 0.25  $\mu$ mol/L, USGS I-4650-03, USEPA 365.5], PO<sub>4</sub><sup>3-</sup> [DL 0.03  $\mu$ mol/L, USEPA 365.5], and H<sub>4</sub>SiO<sub>4</sub> [DL 1  $\mu$ mol L<sup>-1</sup>, USEPA 366]. Samples that were below the detection limit were recorded as concentrations half the limit of detection. Total dissolved nitrogen (TDN) was analyzed by high-temperature combustion, followed by chemiluminescent detection of nitric oxide using a Shimadzu TNM-1 (DL 5  $\mu$ mol/L, ASTM D5176). Filters from nutrient sample processing were stored in amber-colored plastic vials at -80 °C until analysis for Chlorophyll *a* (Chl *a*). Filters were analyzed using a Turner 10-AU fluorometer with an analytical range from 0-20  $\mu$ g Chl *a*/L with dilutions up to 1:4 (1 extract:4 90% acetone) (USEPA 445.0).

Additionally, water from each station was filtered through a 0.45- $\mu\text{m}$  nylon filter (Corning) for  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}\text{-NO}_3^-$  analysis, using a Thermo-GasBench II (Thermo Electron) interfaced to an isotope ratio mass spectrometer (IRMS). Isotope data were normalized to United States Geological Survey (USGS) standards (USGS32, USGS34, USGS53) using the method developed by McIlvin & Altabet (2005) and expressed as per mil (‰) (Eq. 1).

$$\text{Eq. 1 } \delta^{15}\text{N}, \delta^{18}\text{O} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000, R = {}^{15}\text{N}/{}^{14}\text{N}, {}^{18}\text{O}/{}^{16}\text{O}$$

Nutrient and  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  analyses were conducted by the University of Hawai'i at Hilo (UH Hilo) Analytical Lab. Turbidity and pH were measured using a HACH 2100Q Sw (Version 1.03/9 #14100C035910) and an ORION STAR A325 Sw (Revision 3.04 meter #G12945), respectively.

Composite macroalgal samples from each station were rinsed with deionized water to remove debris. A voucher of each species present in the sample was preserved in 4% formalin for identification using morphological and anatomical characteristics. The remaining samples were dried at 60 °C until a constant weight was achieved. Dried, composite algal samples were ground and homogenized using a Wig-L-Bug grinding mill, because a common species was not present at all stations. Ground algal samples were folded into 3.5x5 mm foil tins for stable isotope analysis at the University of Hawai'i Hilo Analytical Lab. Macroalgae samples were analyzed for  $\delta^{15}\text{N}$  using a Thermo Delta V IRMS. Nitrogen isotope data were normalized to USGS 40 ( $\delta^{15}\text{N}$  vs Air = -4.5‰) and USGS 41 ( $\delta^{15}\text{N}$  vs Air = 47.6‰), accurate to 0.2‰, and expressed as ‰N and  $\delta^{15}\text{N}$  vs  $\text{N}_2$  in air.

The documented variability of nutrient uptake and  $\delta^{15}\text{N}$  values among algal species, justified the use of composite samples as a sewage indicator (Martinez et al., 2012; Bailes & Gröcke, 2020). Individual species were separated before processing when three or more species were available from the same station. Preserved samples were identified using a Leica ZOOM 2000 dissecting microscope, an Olympus BX41 compound microscope, and identification books (Abbott, 1999; Abbott & Huisman, 2004). A species list for all specimens was created (Appx. I-1).

### **Station Groupings**

Stations were grouped according to the freshwater water source that most influenced their water quality. The most influential freshwater source was determined using salinity data collected at each sampling and physical attributes of the station. River-influenced (RI) stations were located at river mouths or in the visible sediment plume of river mouths. Stations with an average salinity >10 throughout the study period, despite having a visible spring, were considered to have a low groundwater-influence (LGW). Stations with an average salinity <10 that had a visible spring were considered to have a high groundwater-influence (HGW). Five stations were RI, seven LGW, and eight HGW. Sewage indicator measurements were also examined for temporal trends between wet and dry weather periods. Weather periods were based on peaks in USGS stream discharge data from Honoli'i Stream and Wailuku River (Honoli'i: USGS 16717000 <https://waterdata.usgs.gov/usa/nwis/uv?16717000>, Wailuku: USGS 16704000 <https://waterdata.usgs.gov/usa/nwis/uv?16704000>) and National Oceanic and Atmospheric Agency (NOAA) National Weather Service data from the Hilo International Airport rain gauge (Hilo International Airport 87 <https://www.weather.gov/wrh/Climate?wfo=hfo>) gathered for each monthly pair of sampling days during the study period (Figure 1). Peaks in these datasets were generally between November and March, with 24 h rainfall values ranging from 24.80-108.20 mm and streamflow values ranging from 8.04-64.56 m<sup>3</sup>/s.

### **Geospatial analysis**

A 0.8-km radius buffer was created around shoreline station points in ArcGIS Pro (2.8.0) to include ~1-km<sup>2</sup> of inland area. The total number of OSDS within the station buffers were isolated using the intersect tool on the "osds\_haw" layer (2010) available from HDOH (2017). OSDS density was calculated as the sum of OSDS within the ~1-km<sup>2</sup> land area buffer for each station (Figure 2; Table 2).

### **Statistical analyses:**

*Enterococcus* spp., *C. perfringens*, nutrient concentrations and OSDS density of each freshwater-influence group were directly compared to their applicable HDOH



(2014; 2019) state water quality standards for recreational marine waters, estuaries, and embayments, and those recommended to HDOH (Yates, 1985; Fujioka et al., 2015). Each freshwater-influence group was also assigned a sewage pollution score using the method developed by Abaya et al. (2018a) that weights each indicator based on its reliability related to sewage pollution (Eq. 2).

$$\text{Eq 2. } (C. \textit{perfringens} \text{ level} \times 3) + (\delta^{15}\text{N macroalgae level} \times 3) + (Enterococcus \text{ spp. level} \times 2) + (\text{NO}_3^- + \text{NO}_2^- \text{ level} \times 1) + (\text{NH}_4^+ \text{ level} \times 1) + (\text{TDP level} \times 1)$$

Standards for nutrient concentrations used in the sewage pollution scoring tool were modified for estuaries and embayments under wet conditions based on the environmental conditions in this study (HDOH, 2014); the original score was designed using standards for open coastal waters as that research was conducted in Puakō, Hawai'i. Sewage indicators used in the sewage pollution scoring tool were *C. perfringens*,  $\delta^{15}\text{N}$  macroalgae, *Enterococcus* spp.,  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ , and TDP (Table 1). Final sewage pollution scores were placed into low (11-17), medium (18-25), and high (26-33) categories.

The assumptions of parametric statistical tests, normality and equal variance, were examined using Shapiro-Wilk and Levene tests, respectively. When assumptions for parametric tests were not met, data were transformed, or nonparametric tests were used and data were presented using medians and range. A Kruskal-Wallis test was used to determine if the *Enterococcus* spp., *C. perfringens*, nutrient concentrations, %N-macroalgae,  $\delta^{15}\text{N}$ -macroalgae, and sewage pollution scores differed among freshwater-influence groups. Wilcoxon rank sum tests with a Bonferroni correction were used to determine differences among groups if a significant difference was found. One-sample t-tests and one-sample Wilcoxon rank sum tests were used to assess differences between the  $\delta^{15}\text{N}$  of individual macroalgal species from a similar station and the  $\delta^{15}\text{N}$  of composite samples at the same stations. Two-sample Wilcoxon rank-sum tests were used to determine differences between wet and dry weather periods. Spearman's rank correlation tests were used to determine if there were any associations between measured indicators, salinity, temperature, turbidity, or OSDS

density. All statistical tests were completed using the program R (version 4.0.3) with  $\alpha = 0.05$  or 0.017 when a Bonferroni correction was applied.

### ***Stable Isotope Mixing Model***

The percent contribution of specific N sources to the  $\text{NO}_3^-$  pool at each station were determined using the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in water in a stable isotope mixing model in the program R, the “simmr” package (version 0.4.5). This model uses Bayesian methods and  $\text{NO}_3^-$  concentration dependence to estimate coefficients that are proportions of each source to the total sample (Parnell et al., 2013). Normality of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values was tested using the Shapiro-Wilk normality test. Potential  $\text{NO}_3^-$  sources sampled were effluent from the Hilo STP pre-outfall manhole (STP,  $n=13$ ) and outfall discharge plume ( $n=4$ ), inland groundwater from drinking water wells (GW,  $n=7$ ), and ocean water ( $n=3$ ) (Appx. II-1). Stable isotope data for OSDS on Hawai'i Island were obtained from Wiegner et al. (2016) and Abaya et al. (2018a) consisting of cesspools, septic systems, and anaerobic treatment units (OSDS,  $n=17$ ).

$\text{NO}_3^-$  concentrations for the outfall discharge plume and ocean water were below the minimum requirement for stable isotope analysis and could not be used as  $\text{NO}_3^-$  sources. Bi-plots were created using average and corrected average values of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  for each potential source to remove any sources with substantial overlap from the mixing model. Two models were created to separate stations within the breakwater and beyond the breakwater, because although permeable, it is unlikely that the STP outfall would be a  $\text{NO}_3^-$  source to areas within the breakwater. The first model analyzed contributions of GW, OSDS, and STP sources to stations in the HGW and LGW groups. The second model analyzed sources of GW and OSDS to stations in the HGW, LGW, and RI groups. Both models were run a second time, considering contributions of sources to each station within the model to assess trends that may have been obscured by grouping. Mixing model results are presented as Bayesian 50% credibility intervals to account for a larger range of variability.

## Results

### **Comparison of Sewage Indicators Among Freshwater-Influence Groups**

#### *Fecal Indicator Bacteria*

The two measured FIB varied among freshwater-influence groups. *Enterococcus* spp. concentrations significantly differed among groups ( $\chi^2_{(2)} = 15.13$ ,  $p < 0.001$ ), and values were 253% and 215% higher in the RI group compared to the LGW ( $p < 0.001$ ) and HGW groups ( $p = 0.002$ ) (Table 2). *Clostridium perfringens* concentrations were low among all freshwater-influence groups, but differed significantly among groups ( $\chi^2_{(2)} = 14.46$ ,  $p < 0.001$ ), with the highest median concentration being 0.58 CFU/100 mL in the RI group compared to 0 CFU/100 mL in both the HGW ( $p < 0.001$ ) and LGW groups (Table 2). No human-associated *Bacteroides* were detected in shoreline samples using the HF183 marker.

#### *Nutrients*

Nutrient concentrations varied among freshwater-influence groups.  $\text{NO}_2^- + \text{NO}_3^-$  and TDN concentrations significantly differed among groups ( $\chi^2_{(2)} = 97.68$ ,  $p < 0.001$ ;  $\chi^2_{(2)} = 73.80$ ,  $p < 0.001$ ), with concentrations up to 203% and 78% higher in the HGW group than the LGW and RI groups ( $p < 0.001$ ,  $p < 0.001$ ) (Table 2).  $\text{NH}_4^+$  concentrations also differed among groups ( $\chi^2_{(2)} = 40.85$ ,  $p < 0.001$ ), with concentrations up to 82% higher in the RI group than the LGW and HGW groups ( $p < 0.001$ ) (Table 2).  $\text{PO}_4^{3-}$  and TDP concentrations were significantly higher, up to 252% and 90%, respectively, in the HGW group compared to the LGW ( $p < 0.001$ ,  $p < 0.001$ ) and RI ( $p < 0.001$ ,  $p < 0.001$ ) groups ( $\chi^2_{(2)} = 140.86$ ,  $p < 0.001$ ;  $\chi^2_{(2)} = 97.50$ ,  $p < 0.001$ ) (Table 2).  $\text{H}_4\text{SiO}_4$  concentrations were also significantly higher, up to 113%, in the HGW group compared to the other freshwater-influence groups ( $p < 0.001$ ) ( $\chi^2_{(2)} = 82.72$ ,  $p < 0.001$ ).

#### *Chlorophyll a & Turbidity*

Chlorophyll *a* concentrations differed among freshwater-influence groups ( $\chi^2_{(2)} = 33.11$ ,  $p < 0.001$ ), with concentrations up to 272% higher in the RI group than the HGW ( $p < 0.001$ ) and LGW groups (Table 2). Turbidity also differed among these groups ( $\chi^2_{(2)}$

= 91.34,  $p < 0.001$ ), with values in the RI group up to 316% higher than the LGW ( $p < 0.01$ ) and HGW groups ( $p < 0.001$ ).

### *Macroalgae*

Thirty-two identifiable macroalgal species were collected for  $\delta^{15}\text{N}$  and %N analyses (Appx. I-1). Macroalga from Phylum Rhodophyta were most prevalent, with *Ahnfeltiopsis concinna*, *Chondrus* sp., and *Polyopes hakalauensis* making up nearly half of all samples collected. Percent N-content in macroalgae was similar at five of six stations where individual and composite samples were compared (Appx. I-2). The  $\delta^{15}\text{N}$ -macroalgae was also similar at five of the six stations where individual species and composite samples were compared (Appx. I-3). Macroalgal  $\delta^{15}\text{N}$  values significantly differed among all freshwater-influence groups ( $\chi^2_{(2)} = 18.65$ ,  $p < 0.001$ ), with the values in the RI group up to 143% higher than the LGW ( $p < 0.001$ ) and HGW ( $p < 0.001$ ) groups (Figure 3A). Percent N content in macroalgae also differed significantly among all groups ( $\chi^2_{(2)} = 14.74$ ,  $p = 0.001$ ), and was 4% higher in the HGW group compared to the LGW group ( $p = 0.001$ ) (Figure 3B). Macroalgal  $\delta^{15}\text{N}$  fell below the cutoff of +5.9‰, considered by Abaya et al. (2018a) to be the low range for  $\delta^{15}\text{N-NO}_3^-$  of sewage, at all stations. Four stations were above the cutoff of +4.0‰ that is considered by Smith et al. (2021) to have moderate sewage influence (Appx. III-2). Macroalgal %N at 12 stations was above the 2% considered by Amato et al. (2016) to indicate anthropogenic nutrient loading (Appx. III-2).

### *Sewage Pollution Scores*

All freshwater-influence groups had a similar median sewage pollution score of 17, falling into the low-impacted category ( $p = 0.17$ ) (Figure 4). Sewage pollution scores at individual stations ranged from a one-time minimum of 11 to as high as 21 at many stations and all stations fell within the medium-impacted category at least once. Median sewage pollution scores for two stations were in the medium category (Appx. III-1). The probability of a station falling in the medium category was highest in the RI group, at 35(±23)%, followed by the HGW, then LGW groups at 28(±14)% and 24(±12)%, respectively.

### **Weather-related Patterns of Sewage Indicators**

*Clostridium perfringens* concentrations were significantly higher during dry periods with a median concentration of 0.33 CFU/100 mL compared to 0.00 CFU/100 mL during wet periods ( $W = 13298$ ,  $p = 0.002$ ) (Figure 5A). *Enterococcus* spp. concentrations did not differ between weather periods and were 0.34 MPN/100 mL lower during dry periods. Concentrations of  $\text{NH}_4^+$  were also significantly higher during dry periods ( $W = 14730$ ,  $p = 0.03$ ) (Figure 5B). Aside from  $\text{NH}_4^+$ , nutrient concentrations were generally higher during wet periods, but only  $\text{H}_4\text{SiO}_4$ , was significantly higher ( $W = 8605$ ,  $p < 0.001$ ) (Figure 5C). Chlorophyll *a* was also significantly higher during wet periods ( $W = 16224$ ,  $p < 0.001$ ) (Figure 5D). Macroalgae  $\delta^{15}\text{N}$  and %N were similar between wet and dry periods ( $p > 0.05$ ).

### **Associations Between Sewage Indicators**

Salinity, temperature, and turbidity were negatively correlated with most nutrient concentrations:  $\text{NO}_2^- + \text{NO}_3^-$  ( $p < 0.001$ ),  $\text{PO}_4^{3-}$  ( $p < 0.001$ ),  $\text{H}_4\text{SiO}_4$  ( $p < 0.001$ ), TDP ( $p < 0.001$ ), and TDN ( $p < 0.001$ ), and were positively correlated with  $\text{NH}_4^+$  ( $p < 0.001$ ) (Figure 6). Salinity, temperature, and turbidity were also negatively correlated with OSDS density ( $p < 0.025$ ) and %N of macroalgae ( $p < 0.005$ ) and were positively correlated with  $\delta^{15}\text{N}$ -macroalgae ( $p < 0.022$ ). OSDS density was negatively correlated with *C. perfringens* ( $p = 0.032$ ),  $\text{NH}_4^+$  ( $p = 0.002$ ), and Chl *a* ( $p = 0.005$ ), and positively correlated with  $\text{NO}_2^- + \text{NO}_3^-$  ( $p = 0.028$ ),  $\text{PO}_4^{3-}$  ( $p = 0.039$ ), TDP ( $p = 0.001$ ), and TDN ( $p = 0.021$ ) (Figure 6). *Clostridium perfringens* was positively correlated with *Enterococcus* spp. ( $p = 0.012$ ),  $\text{NH}_4^+$  ( $p < 0.001$ ), and Chl *a* ( $p = 0.001$ ). Chlorophyll *a* concentrations were also significantly correlated with all nutrient measurements ( $p < 0.001$ ). All nutrient concentrations were significantly correlated to one another, except  $\text{NH}_4^+$  and TDN (Figure 6).

### **Nutrient Source Contributions**

The dominant  $\text{NO}_3^-$  source to stations beyond the breakwater, with three potential sources, was GW, contributing 99% and 98% to the HGW and LGW groups, respectively (Figure 7). Sewage from both OSDS and STP contributed 1% to these

groups (Figure 7). For groups within the breakwater, with two potential sources, GW was also the dominant source, contributing 96%, 94%, and 95% to the HGW, LGW, and RI groups, respectively with OSDS contributing a maximum of 6% to the LGW group. (Figure 8). Trends among individual stations showed a more even distribution of source contributions. The highest contributions were from GW, contributing 46-97% of  $\text{NO}_3^-$  to all stations with the highest contribution to a station in the RI group (Figure 9). Sewage from OSDS contributed 4-16%, with the highest contribution at a station in the RI group (Figure 10). Sewage from the STP contributed 14-40% of  $\text{NO}_3^-$ , with the highest contribution to a station in the LGW group (Figure 11).

## **Discussion**

### ***Sewage Pollution Vectors***

Rivers are known vectors of sewage pollution to the coastal environment. Sewage-associated bacteria and viruses are present in contaminated rivers at concentrations that increase the risk of contracting gastrointestinal and respiratory illness as well as skin infections (Shuval, 2003; Viau et al., 2011, Economy et al., 2019; Gerken et al., 2021). In addition to pathogens, rivers often have elevated concentrations of nutrients, mainly  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , and silica, in comparison to coastal waters (Knee et al., 2008). Sewage indicator measurements suggest that rivers make a large contribution to sewage pollution in Hilo. The RI group had the highest FIB,  $\text{NH}_4^+$ , and Chl *a* concentrations, as well as the highest  $\delta^{15}\text{N}$ -macroalgal and turbidity values (Figure 3A; Table 2). Additionally, stations in the RI group had the highest probability of having a sewage pollution score in the medium-impacted sewage pollution category. Measurements of these sewage indicators fell within the range of those previously found in Hilo studies near rivers (Wiegner et al., 2017; Economy et al., 2019). Groundwater comprises all baseflow discharge in rivers, likely explaining the high  $\text{NO}_3^-$  contribution of groundwater to the RI group determined by the stable isotope mixing model (Shopka & Derry, 2012). Consistent with the findings of this study, rivers have been identified as major sources of sewage to shoreline waters in other parts of the world; however, in some instances, groundwater-influenced groups had higher sewage indicator values than the RI group (Knee et al., 2008; Wiegner et al., 2013; Wiegner et

al., 2017; Economy et al., 2019). Although sewage pollution scores were similar among freshwater-influence groups, these findings suggest that localized sewage inputs can have significant effects on the level of sewage pollution in an area.

Sewage contamination of groundwater, especially from OSDS, is a growing concern in areas with large groundwater discharge rates and high densities of OSDS (Smith et al., 2021). OSDS-derived sewage pollution can be exacerbated in areas with a highly permeable substratum, such as the fractured basalt of volcanic islands (ArandaCirerol et al., 2006; Abaya et al., 2018a; Wiegner et al., 2021). Groundwater has also been seen to contribute low concentrations of FIB and high concentrations of nutrients to surf zones along sandy beaches in California and Mexico (Boehm et al., 2004; ArandaCirerol et al., 2006; de Saiyes et al., 2008). In Hawai'i, groundwater has been found to transport high concentrations of both FIB and nutrients to the shoreline, and elevated  $\delta^{15}\text{N}$  macroalgal values are frequently observed in close proximity to shoreline springs of sewage-impacted areas (Abaya et al., 2018a; Amato et al., 2020; Panelo et al., 2022). Studies have confirmed connections between OSDS and the shoreline at many locations through groundwater using dye-tracer tests (Abaya et al., 2018a; Wiegner et al., 2021, Waiki, 2022). Groundwater-influenced groups in this study were characterized by extremely high concentrations of nutrients, but lower FIB concentrations and low  $\delta^{15}\text{N}$  macroalgal values, suggesting that sewage may be diluted by groundwater or that non-sewage nutrients are present. Previous research has documented elevated *Enterococcus* spp. concentrations in surf zones of groundwater-influenced areas rather than at groundwater seeps and springs (Boehm et al., 2004). In this study, groundwater appears to provide a constant low-level input of *Enterococcus* spp. and *C. perfringens*. Positive correlations between two measured FIB (*Enterococcus* spp. and *C. perfringens*) and turbidity suggests that continued low-level groundwater input of FIB may accumulate in sediments and be resuspended during high surf events, a possibility that was not investigated in this study.

Dye-tracer tests provide irrefutable evidence of sewage from OSDS at the shoreline (Abaya et al., 2018a; Mezzacapo et al., 2021; Wiegner et al., 2021). Waiki (2022) found sewage from OSDS was discharging at springs along the Hilo shoreline from dye-tracer tests. All springs where dye emerged were in close proximity to

groundwater stations in the HGW group in this study, documenting that sewage from OSDS is present. Despite the known connectivity between OSDS and four stations in this study, FIB and  $\delta^{15}\text{N}$ -macroalgae concentrations were low, nutrient concentrations were not elevated in comparison to other stations, and mixing model results also suggested that OSDS made low  $\text{NO}_3^-$  contributions at these stations. The groundwater influenced station with the highest OSDS contribution was station 9 in the LGW group, a beach park protected by a small breakwater with homes adjacent to its parking lot (Appx. III-1; Figure 10). However, this station also had the highest contribution from the STP, agreeing with the thought that sewage pollution is more evident when ocean mixing is reduced (Mezzacapo et al., 2021).

Dilution of sewage indicators may obscure obvious evidence of sewage pollution in coastal waters (Mezzacapo et al., 2021). Many areas with well-documented sewage pollution on other Hawaiian islands and throughout the world have elevated sewage indicator levels in comparison to the sewage indicator measurements in this study (Hunter & Evans, 1995; Mallin et al., 2007; de Saiyes et al., 2008; Abaya et al., 2018a). Dilution of treated effluent is commonly relied upon for disposal into streams across the United States and in ocean outfalls to reduce the concentration of harmful pollutants (Hunt et al., 2010; Rice & Westerhoff, 2017). Total groundwater recharge from the Onomea and Hilo watersheds included in the study area is estimated to be nearly 609  $\text{m}^3/\text{d}/\text{m}$  compared to a total of 479  $\text{m}^3/\text{d}/\text{m}$  for all watersheds encompassing west Hawai'i Island and 8  $\text{m}^3/\text{d}/\text{m}$  for the entire island of O'ahu (Engott, 2011; Engott et al., 2015). The low FIB concentrations at shoreline stations may be caused by dilution of sewage by high-nutrient groundwater, driving the trends seen in freshwater-influence groups. Mixing model results attribute nearly all  $\text{NO}_3^-$  in groups to groundwater, with the lowest contribution to the RI group receiving 95% of  $\text{NO}_3^-$  from groundwater. Temporal trends support this as well, with nutrient concentrations generally higher during wet periods and FIB generally higher during dry periods, contrary to past studies of two rivers in Hilo Bay (Wiegner et al., 2013; Wiegner et al., 2017; Economy et al., 2019). Previous studies have observed higher FIB concentrations during storm events and wet weather periods because of higher transport of FIB through groundwater and rivers, especially during the first flush after heavy rain and the added effect of surface runoff of



watershed sources (Economy et al., 2019; Ahmed et al., 2020, Gerken et al., 2022). These temporal effects were not observed in this study because there may be a constant flow of freshwater diluting OSDS-derived sewage or little surface runoff at stations. Significant increases in  $\text{NH}_4^+$  concentrations were observed during dry periods and were also positively correlated with salinity, suggesting that ocean waters may have a higher influence on stations during dry periods. All other nutrients were negatively correlated to salinity, temperature, and turbidity, which are indicators of ocean influence contrasting the fresh, cold, and less turbid groundwater emerging from springs and seeps.

### ***Sewage Indicators***

Monitoring of FIB can provide insight into the risk associated with swimming in sewage-contaminated waters (Cabelli, 1989; Viau et al., 2011; Sunger et al., 2018). *Enterococcus* spp. are thought to be indicative of pathogens related to sewage and consistent with the “Fung-Fujioka scale” for *C. perfringens*, but this similarity was not seen in the results of this study (Vaiu et al., 2011). The Fung/Fujioka scale specifies the presence of non-point source sewage pollution when environmental *C. perfringens* concentrations are greater than 10 CFU/100 mL and considers areas below this level to be uncontaminated (Fung et al., 2007). In the current study, observed *Enterococcus* spp. concentrations were often extremely high and would have prompted beach closures for exceeding the statistical threshold value of 130 MPN/100 mL (HDOH, 2019). The *Enterococcus* spp. standard of 35 MPN/100 mL was exceeded in the RI group, but not in groundwater groups, and was within the lower end of the range for two of three rivers previously studied in Hilo (Economy et al., 2019; HDOH, 2019). The *C. perfringens* standard of 5 CFU/100 mL was not exceeded in any freshwater influence group and this FIB was generally not detected (Fujioka et al., 2015). Past studies of rivers in Hilo and Florida found much higher concentrations of *C. perfringens*, ranging from 0-45 CFU/100 mL (Futch et al., 2011; Wiegner et al., 2017; Economy et al., 2019). The incorporation of *C. perfringens* usually contradicted the high levels of *Enterococcus* spp. observed, although these FIB had a positive correlation. This is likely because *C. perfringens* is more indicative of sewage pollution and has a lower probability of having

an environmental source (Fujioka et al., 2015; Miller-Pierce & Rhoads, 2019). Many studies have agreed that *Enterococcus* spp., although found in high concentrations in human sewage, is not a reliable sewage indicator in tropical settings and that the incorporation of other FIB is beneficial (Boehm et al, 2009; Fujioka et al., 2015; Abaya et al., 2018a; Economy et al., 2019; Gerken et al., 2022). Genetic markers and qPCR techniques are being increasingly used to identify and quantify human-specific FIB (Amato et al., 2020). Regional dietary trends can lead to spatial variability in the human-gut microbiome and a better understanding is needed to develop area-specific markers to identify sewage pollution (Jia et al., 2019). The absence of human-associated *Bacteroides* in shoreline samples may be a result of spatial variability of the human-gut microbiome or the general low FIB concentrations due to dilution that were observed in this study, as hits for the HF183 marker have been observed in Hilo (Wiegner et al., 2017).

Eutrophication of coastal waters can result in algal blooms and anoxic zones, adding stress to coral reef ecosystems that play important economic and cultural roles (Mallin et al., 2007; Lapointe et al., 2015). Nitrogen and phosphorus concentrations are often elevated in water bodies impacted by sewage pollution, but also in areas impacted by agricultural and urban activities (Burford et al., 2012). Freshwater on young volcanic islands is known to contain high levels of silica, resulting from the weathering of the basalt substrata (Schopka & Derry, 2012; Adolf et al., 2019). HDOH nutrient standards for estuaries and embayments were exceeded by tens to thousands of percent in all freshwater-influence groups. Nitrogen concentrations observed at river stations were similar to previous studies in Hilo rivers and concentrations at groundwater stations were more than twice as high as river stations, but much lower than areas with known anthropogenic disturbances in Maui and California (de Saiyes et al., 2008; Wiegner et al., 2013; Amato et al., 2016; Wiegner et al., 2017). Nutrient concentrations measured in sewage impacted areas in Maui have exceeded HDOH standards by nearly 100,000%, with concentrations of TDN, TDP, and  $\text{PO}_4^{3-}$  up to 525.9, 5.25, and 4.90  $\mu\text{mol/L}$ , respectively (Amato et al., 2016). Observed phosphorus concentrations were also similar to previous studies at river influenced stations in the current study and much lower than has been documented at areas known to be impacted by human nutrient

input in Maui (Wiegner et al., 2013; Wiegner et al., 2017; Amato et al., 2016). Silica concentrations were within the range of those previously recorded for groundwater and rivers in Hawai'i and were consistent with the freshwater-influence groupings (Knee et al., 2008; Wiegner et al., 2017; Abaya et al., 2018a). Freshwater, especially groundwater, is a major source of nutrients to the Hilo shoreline, but levels are not indicative of sewage pollution.

Macroalgae are a useful tool in assessing the impacts of sewage pollution in coastal environments. The  $\delta^{15}\text{N}$  and %N-macroalgae are often elevated in areas impacted by human sewage pollution through comparison of both collected and deployed algal samples (Costanzo et al., 2005; Dailer et al., 2010; Dailer et al., 2012; Abaya et al., 2018a; Amato et al., 2020; Wiegner et al., 2021). Comparisons of both single species and composite samples have been employed in previous studies (Lapointe et al., 2005; Dailer et al., 2012; Wiegner et al., 2016; Abaya et al., 2018a; Wiegner et al., 2021). This study found low variability in the  $\delta^{15}\text{N}$  and %N-macroalgae between individual species and composite samples collected from the same stations with multiple species present. The %N- and  $\delta^{15}\text{N}$ -macroalgae both differed between individual species and composite samples at one of six stations in this study (Appx. I-2F; Appx. I-3A). Both stations that differed in one measurement were similar in the other measurement. Amato et al. (2016) suggest that areas with  $\delta^{15}\text{N}$ -macroalgae greater than 7‰ and N-content greater than 2% are indicative of anthropogenic nutrient loading. Macroalgae collected throughout this study were frequently in excess of 2% N-content, as high as 3%-N, suggesting an anthropogenic impact, but the highest  $\delta^{15}\text{N}$ -macroalgae value was 5.2‰. Swart et al. (2014) suggested that  $\delta^{15}\text{N}$ -macroalgae may be underestimated by as much as 6‰ when ambient  $\text{NO}_2^- + \text{NO}_3^-$  concentrations exceed 10  $\mu\text{mol/L}$  because of shifts in the N fractionation mechanism of macroalgae. Most stations in this study had concentrations of  $\text{NO}_2^- + \text{NO}_3^-$  greater than this threshold on many occasions, and if accounted for, all stations would have had values more similar to other areas impacted by sewage on Hawai'i Island, in Florida, and in Australia, but lower values than recorded in wastewater plumes of Maui (Costanzo et al., 2005; Lapointe et al., 2005; Dailer et al., 2012; Abaya et al., 2018a; Wiegner et al., 2021). Additionally, the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  of inland groundwater from drinking water wells was

much lower in the current study than other studies in west Hawai'i Island (Amato et al., 2016; Abaya et al., 2018a). If the difference in  $\delta^{15}\text{N}$ -macroalgae values and the  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  of inland groundwater were considered, rather than the  $\delta^{15}\text{N}$ -macroalgae values themselves,  $\delta^{15}\text{N}$ -macroalgae values recorded in the current study are elevated within the range of previous studies, in relation to the background  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  of groundwater.

The stable isotopes,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$ , have been used to estimate sewage pollution source contributions in estuarine and coastal environments using stable isotope mixing models (Wiegner et al., 2016; Wiegner et al., 2021; Panelo et al., 2022). Previous studies on Hawai'i Island have estimated that groundwater is the dominant source of  $\text{NO}_3^-$  with sewage from OSDS contributing up to 36% and STP effluent contributing up to 26% of  $\text{NO}_3^-$  at the shorelines studied (Wiegner et al., 2021; Panelo et al., 2022). The results of this study are consistent with these previous studies, suggesting that diluted sewage from OSDS and the STP are present in low concentrations throughout the study sites. The elevated range of  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  of STP sewage effluent from +7.0 to +20.0‰ seen in the literature was not observed in effluent from the Hilo STP, (-7.3 to +12.5‰) (reviewed in Wiegner et al., 2016). OSDS sewage samples used as a source for the mixing models were located in other areas of Hawai'i Island and the  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  of sewage from OSDS in Hilo may have been lower, given the difference seen in the STP values.

Different sewage indicators can provide contradictory conclusions of the extent of sewage pollution in a given area. In tropical settings, the fact that *Enterococcus* spp. can survive and multiply in the environment often warrants the use of secondary FIB (Fujioka et al., 2015). The sewage pollution scoring tool of Abaya et al. (2018a) placed a high emphasis on *C. perfringens* and  $\delta^{15}\text{N}$ -macroalgae for their specificity to sewage pollution, but also included other indicators to gain a more comprehensive understanding of sewage pollution. As was seen in this current study, sewage pollution can be present in low concentrations, even when dye tracer tests confirm connectivity of OSDS to shoreline springs (Waiki, 2022), and especially in areas of high freshwater flow where dilution is a factor. *Clostridium perfringens* was mostly absent or in low concentrations in shoreline samples and  $\delta^{15}\text{N}$ -macroalgae was often below the range for

sewage at all stations. All groups fell in the low category when grouped by freshwater influence, but two stations were in the medium category individually.  $\delta^{15}\text{N}$ -macroalgal values would have been within the range of sewage if the underestimation of  $\delta^{15}\text{N}$  in  $\text{NO}_2^- + \text{NO}_3^-$ -rich settings was considered, raising the sewage pollution scores of all stations into the medium category and increasing variability in the sewage pollution scores of all three freshwater-influence groups. Nutrient indicators used in the sewage pollution scoring tool also exceeded the high level on most occasions, often by many orders of magnitude, and varied among freshwater-influence groups, which was not accounted for in this tool.

## **Conclusions**

Freshwater dilution of sewage from both river and groundwater discharge make it challenging for sewage to be detected in the environment. Different sewage indicators measured in this study provided contrasting results, further emphasizing the importance of incorporating many sewage indicators to assess sewage presence in the environment. Sewage was present in the coastal waters of Hilo, and although difficult to detect, the use of a sewage pollution scoring tool considering multiple indicators demonstrated that Hilo is impacted by low-level sewage pollution. Commonly used sewage indicators need to be assessed with an area-based understanding of environmental factors to fully illustrate the extent of sewage pollution in an area of interest. Results of this study will provide insight for decision making regarding sewage pollution and upcoming discussions related to cesspool conversions, specifically for Hilo, as well as other areas relying on OSDS. The HDOH priority three ranking for cesspool conversion in Hilo should be reevaluated, given the documented presence of sewage pollution in Hilo's nearshore waters, and the impacts of sewage pollution in Hilo should be assessed. Future studies of sewage pollution should aim to include a variety of sewage indicators, with an area-based understanding of environmental factors, to gain a more comprehensive understanding of the influence of sewage pollution vectors on water quality.

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## Tables

**Table 1.** Sewage indicators used to calculate a sewage pollution score for each freshwater-influence group with the weight factor for each indicator determined by the relationship to sewage and level values shown and referenced within the table.

Sewage Indicator	Weight Factor	Low	Medium	High	Reference
<i>C. perfringens</i> (CFU/100 mL)	3	0.00-10.00	10.01-100.00	100.01+	Fujioka et al., 2015
Macroalgal $\delta^{15}\text{N}$ (‰)	3	0.00-5.99	6.00-10.99	11.00+	Abaya et al., 2018a
<i>Enterococcus</i> spp. (MPN/100 mL)	2	0.00-35.00	35.01-129.99	130.00+	HDOH, 2019
$\text{NO}_2^- + \text{NO}_3^-$ ( $\mu\text{mol/L}$ )	1	0.00-0.60	0.61-1.43	1.44+	HDOH, 2014
$\text{NH}_4^+$ ( $\mu\text{mol/L}$ )	1	0.00-0.43	0.44-0.93	0.94+	HDOH, 2014
TDP ( $\mu\text{mol/L}$ )	1	0.00-0.81	0.82-1.61	1.62+	HDOH, 2014

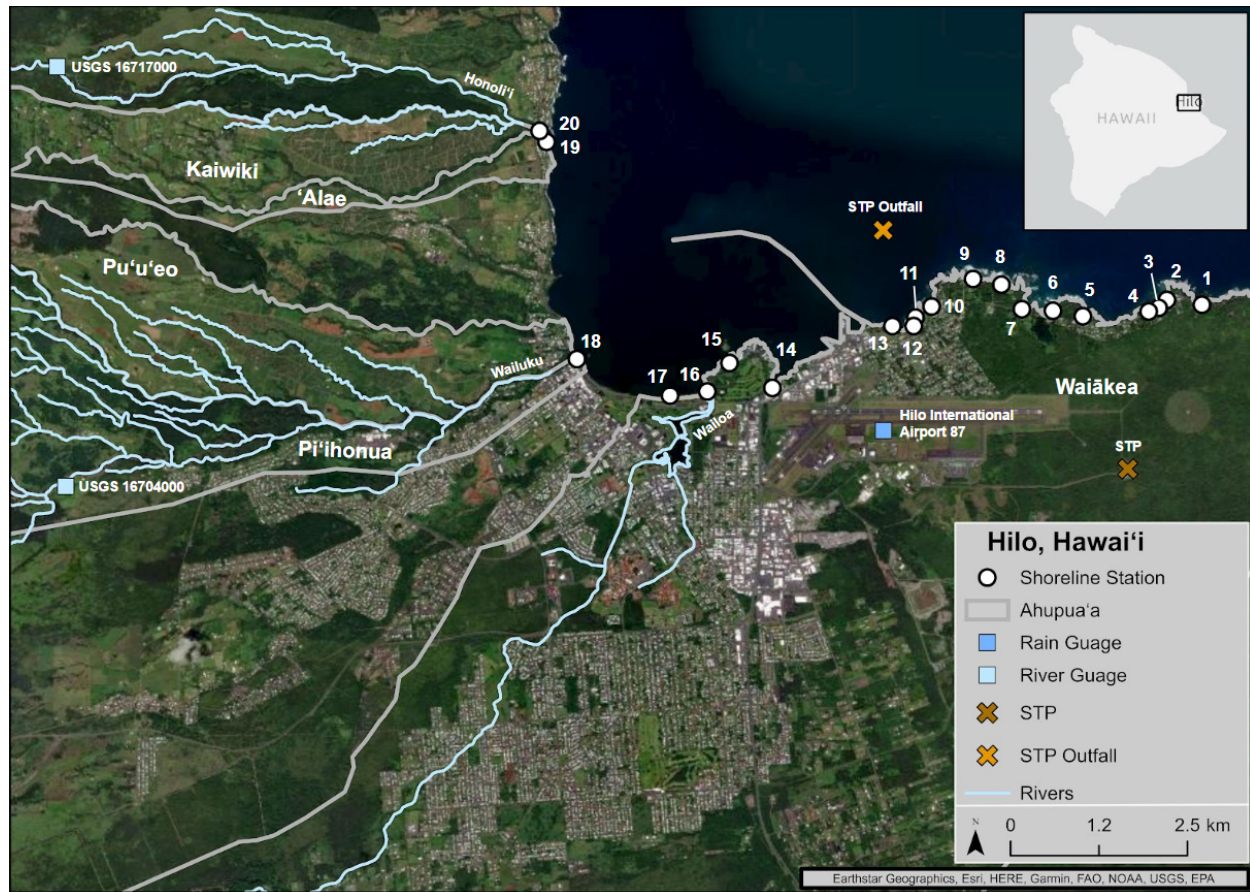
**Table 2.** Median [range] fecal indicator bacteria (FIB, \*/left), nutrients (center), water quality concentrations (right), and OSDS density (far right) along the Hilo, Hawai'i, shoreline compared to standards (bottom) implemented and recognized by the Hawai'i Department of Health. Bold values indicate a measurement that exceeded its respective standard. Note, that the FIB, nutrient, and water quality standards are geometric means and values from this study are presented as medians to more accurately portray the spread of the data. RI represents river influenced groups, HGW and LGW represent groups influenced by high and low volume groundwater discharge, respectively. Letters represent groupings determined by Wilcoxon Rank Sum tests after applying a Bonferroni correction for multiple comparisons ( $\alpha = 0.017$ ).

Freshwater Influence Group	<i>Enterococcus</i> spp. MPN/100 mL	<i>C. perfringens</i> CFU/100 mL	NO <sub>2</sub> <sup>-</sup> + NO <sub>3</sub> <sup>-</sup> (μmol/L)	NH <sub>4</sub> <sup>+</sup> (μmol/L)	TDN (μmol/L)	PO <sub>4</sub> <sup>3-</sup> (μmol/L)	TDP (μmol/L)	H <sub>4</sub> SiO <sub>4</sub> (μmol/L)	Chl <i>a</i> (μg/L)	Turbidity (NTU)	OSDS Density (OSDS/km <sup>2</sup> )
HGW	18.67 <sup>b</sup> [5.00-6546.00]	0.00 <sup>b</sup> [0.00-7.00]	<b>27.19<sup>a</sup></b> [7.00-66.10]	<b>0.83<sup>b</sup></b> [0.18-11.70]	<b>35.29<sup>a</sup></b> [18.00-118.84]	1.83 <sup>a</sup> [0.40-3.48]	<b>2.35<sup>a</sup></b> [0.98-4.54]	408.03 <sup>a</sup> [116.09-759.16]	0.67 <sup>b</sup> [0.00-108.47]	0.49 <sup>c</sup> [0.06-19.70]	<b>20</b> [12-112]
LGW	16.67 <sup>b</sup> [5.00-865.67]	0.00 <sup>ab</sup> [0.00-7.67]	<b>16.18<sup>b</sup></b> [0.44-48.76]	<b>1.49<sup>a</sup></b> [0.18-9.57]	<b>23.72<sup>b</sup></b> [8.27-84.74]	1.14 <sup>b</sup> [0.02-2.64]	<b>1.81<sup>b</sup></b> [0.13-6.05]	259.39 <sup>b</sup> [6.71-599.58]	1.28 <sup>a</sup> [0.05-44.37]	1.10 <sup>b</sup> [0.16-34.90]	<b>16.5</b> [1-63]
RI	<b>58.83<sup>a</sup></b> [5.00-724.33]	0.58 <sup>a</sup> [0.00-9.67]	<b>8.97<sup>c</sup></b> [0.04-49.65]	<b>1.51<sup>a</sup></b> [0.18-4.89]	<b>19.81<sup>b</sup></b> [2.50-98.54]	0.52 <sup>c</sup> [0.02-1.76]	<b>1.24<sup>c</sup></b> [0.13-15.22]	191.25 <sup>b</sup> [3.85-712.85]	<b>2.49<sup>a</sup></b> [0.00-38.10]	<b>2.04<sup>a</sup></b> [0.21-78.00]	<b>43</b> [0-81]
Standard/ Recommendation	35.00	5.00	0.17, 0.13	0.43	14.28**	-	0.81	-	*1.50, *2.00	1.50	15
Reference	HDOH, 2019	Fujioka et al., 2015	HDOH, 2014	HDOH, 2014	HDOH, 2014	-	HDOH, 2014	-	HDOH, 2014	HDOH, 2014	Yates, 1985

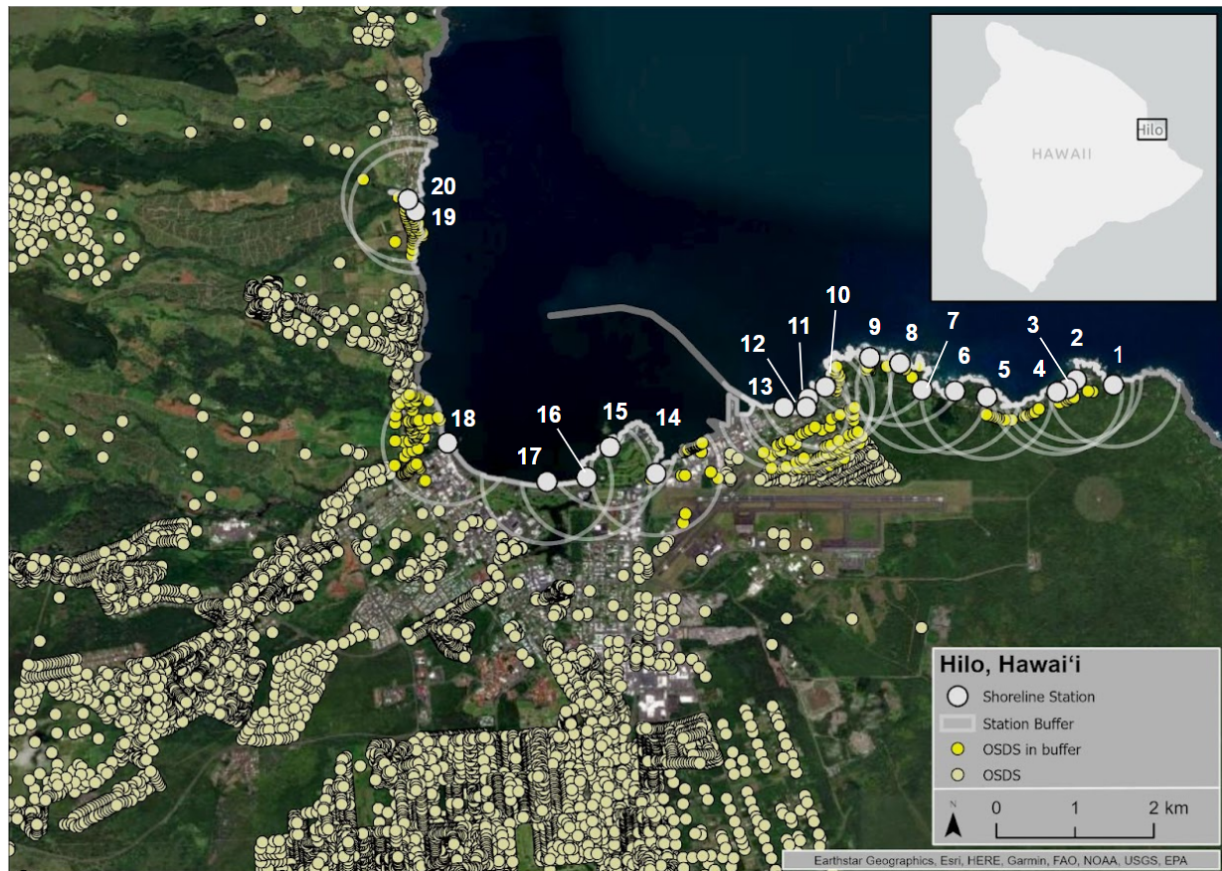
\*1.50 is the HDOH standard for embayments and 2.00 is the standard for estuaries. Standards for all other indicators are the same for embayments under wet conditions and estuaries.

\*\*14.28 is the HDOH standard for total nitrogen, while total dissolved nitrogen was measured in this study.

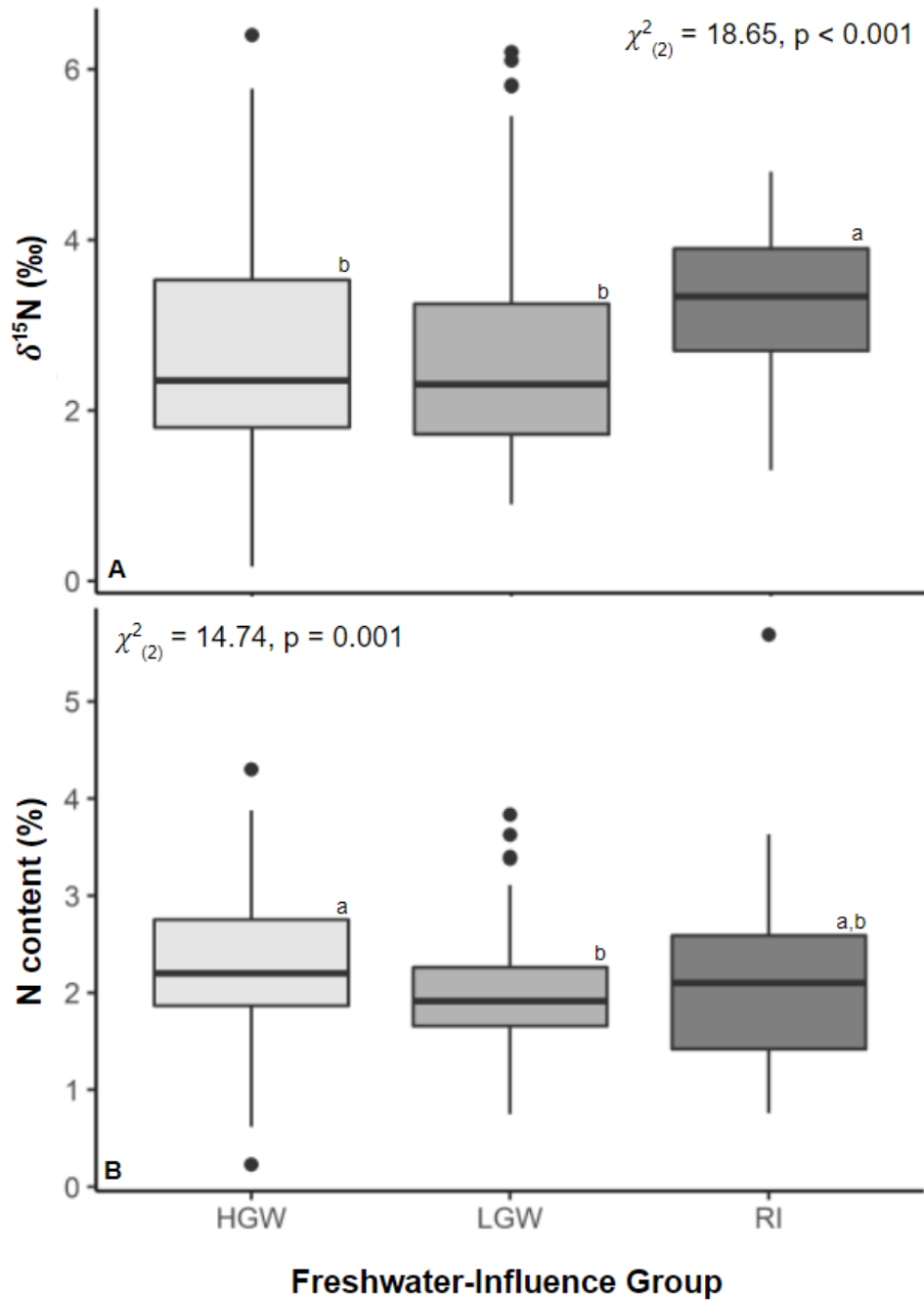
## Figures



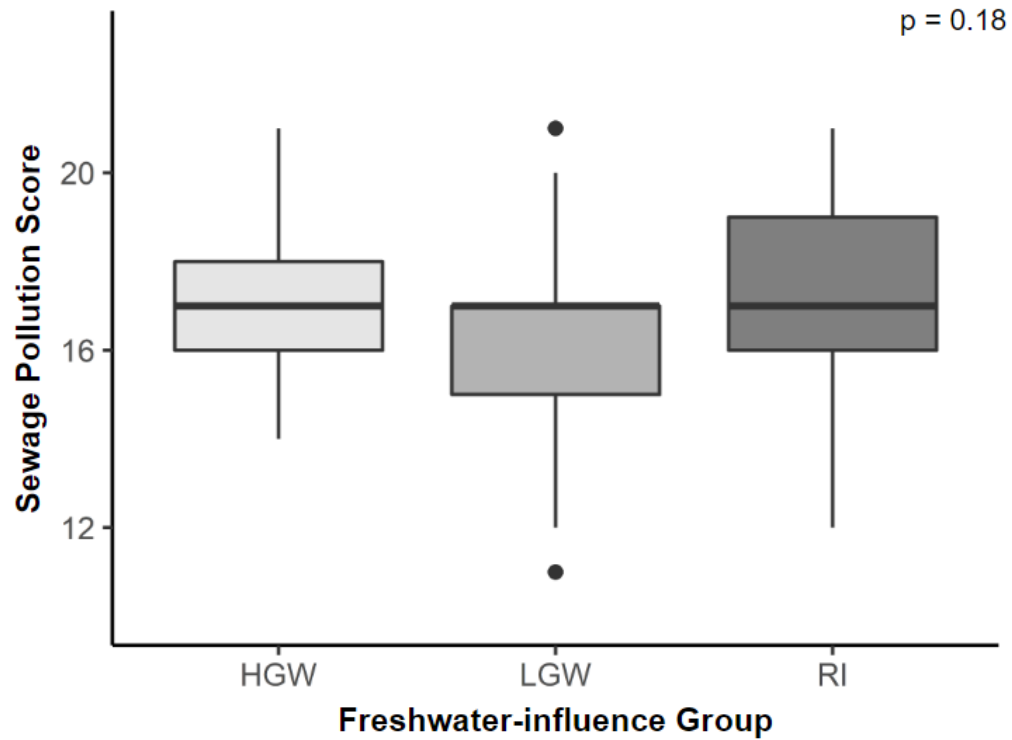
**Figure 1.** Map of 20 stations in Hilo, Hawai'i, sampled for sewage pollution indicators from July 2020 to October 2021 showing *ahupua'a* (Traditional land divisions), the Hilo STP, the STP outfall, rivers, river gauges, and rain gauges included in the study.



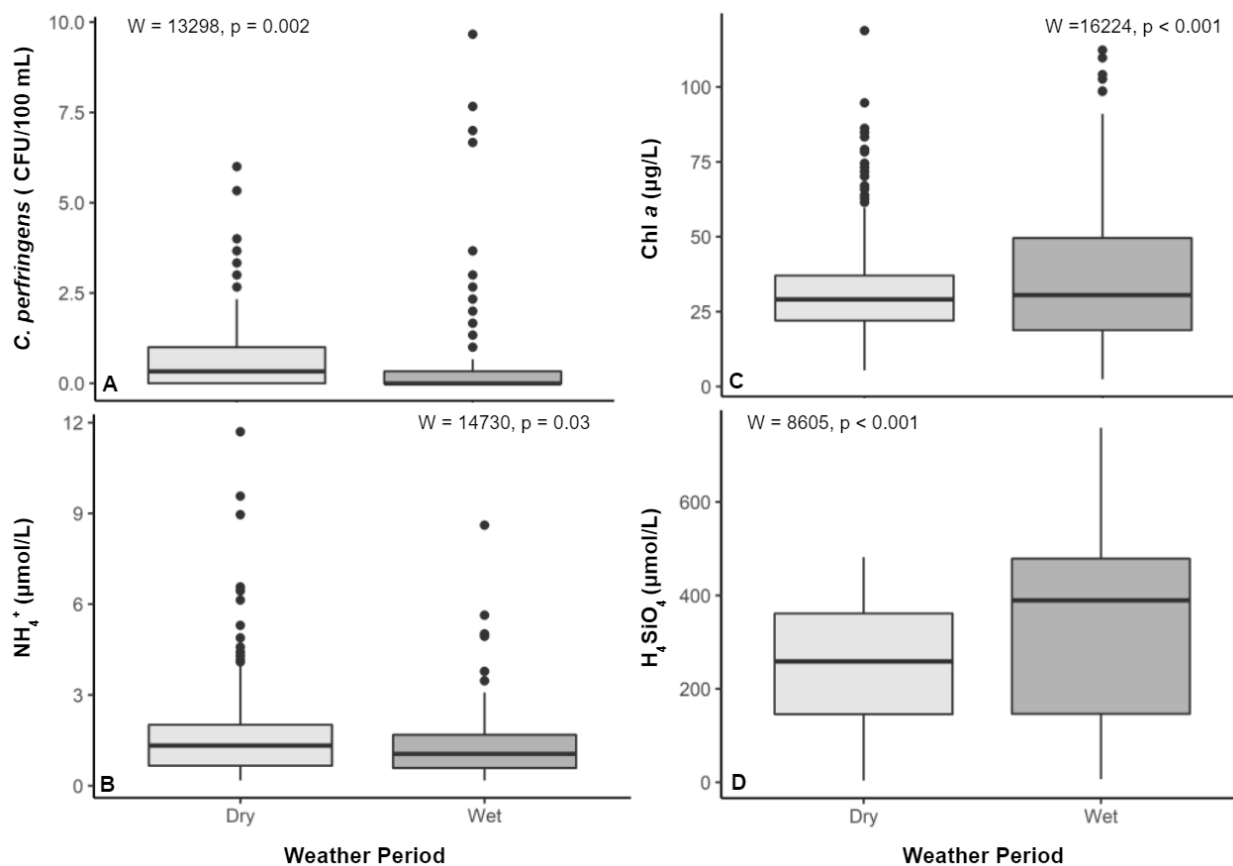
**Figure 2.** Map of  $\sim 1 \text{ km}^2$  buffer of stations in Hilo and OSDS (HDOH, 2017; available from State of Hawai'i Office of Planning and Sustainable Development) within a station buffer (bright yellow) and beyond station buffers (light yellow).



**Figure 3.** Median concentrations of (A)  $\delta^{15}\text{N}$  and (B) %N in composite macroalgal samples collected in Hilo, Hawai'i, from July 2020 to October 2021 along the Hilo, Hawai'i, shoreline from three freshwater-influence groups (High Groundwater Influenced (HGW), Low Groundwater Influenced (LGW), and River Influenced (RI)). Results of Kruskal Wallis tests are displayed on the figures ( $\chi^2_{(\text{degrees of freedom})}$ ,  $\alpha = 0.05$ ) with lower case letters indicating the significant groupings determined by Wilcoxon rank sum tests with a Bonferroni correction for multiple comparisons ( $\alpha = 0.017$ ).



**Figure 4.** Median sewage pollution scores among freshwater-influence groups (High Groundwater Influenced (HGW), Low Groundwater Influenced (LGW), and River Influenced (RI)) calculated from sewage indicator measurements made in Hilo, Hawai'i, from July 2020 to October 2021. Results of Kruskal Wallis tests are displayed on the figures ( $\alpha = 0.05$ ; Table 1).

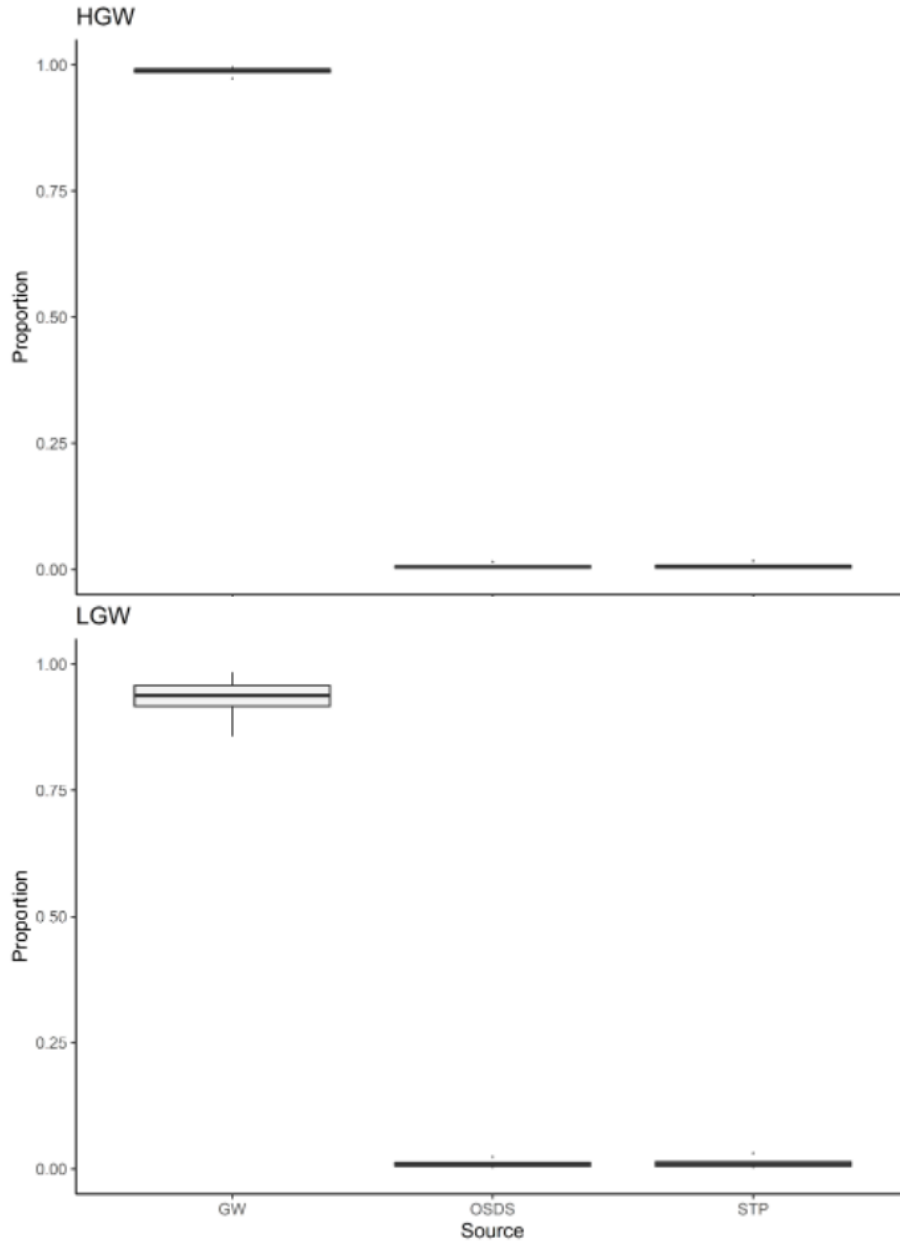


**Figure 5.** Median concentrations of: **(A)** *Clostridium perfringens*, **(B)**  $\text{NH}_4^+$ , **(C)** Chlorophyll *a*, and **(D)**  $\text{H}_4\text{SiO}_4$ , from measurements made between July 2020 and October 2021 along the Hilo, Hawai'i, shoreline during dry and wet weather periods determined by rainfall and local seasonal trends. The results of Wilcoxon rank sum tests are displayed on the figures ( $\alpha = 0.05$ ).

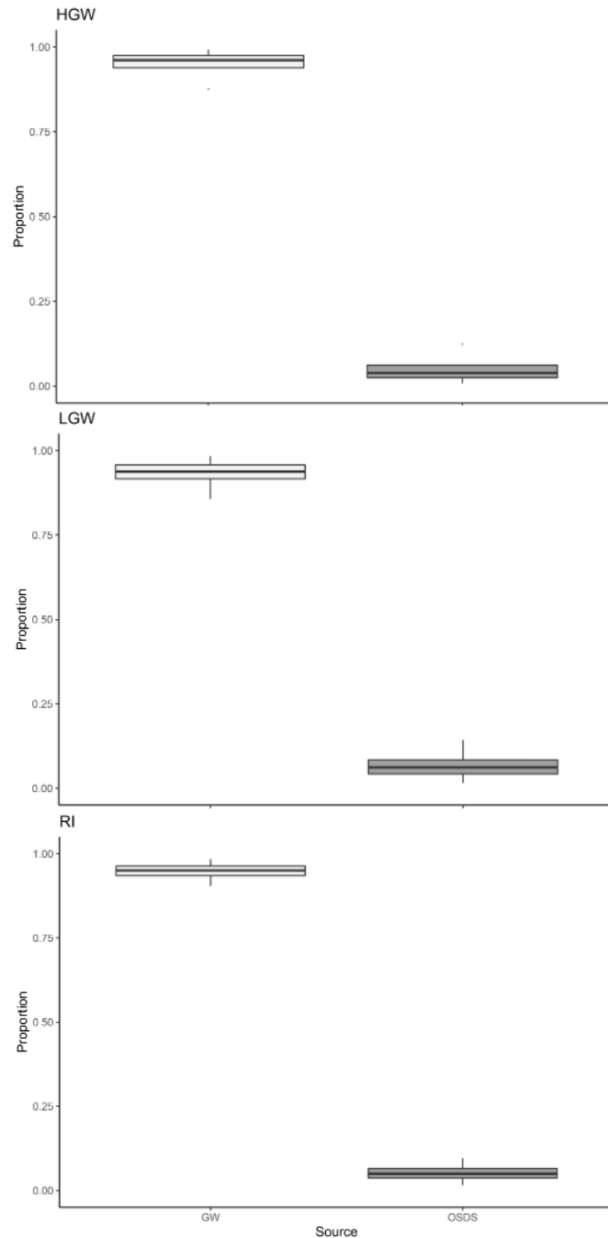


**Figure 6.** Correlations between sewage indicators, salinity, temperature, turbidity, and OSDS density from measurements made in Hilo, Hawai'i, from July 2020 to October 2021. Spearman's rho ( $\rho$ ) is shown on each tile with red and blue tiles representing positive and negative correlations, respectively, and asterisks indicating a significant result ( $\alpha = 0.05$ ).

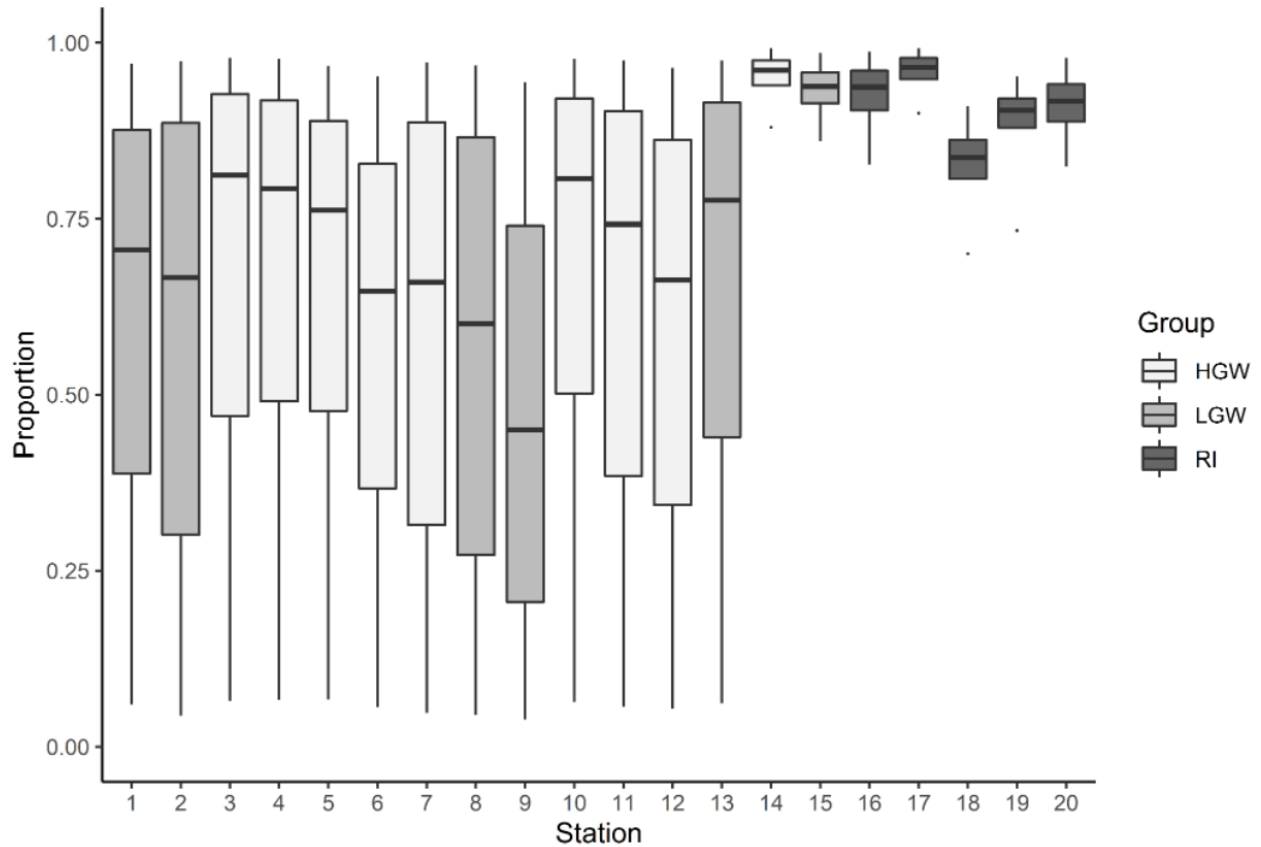




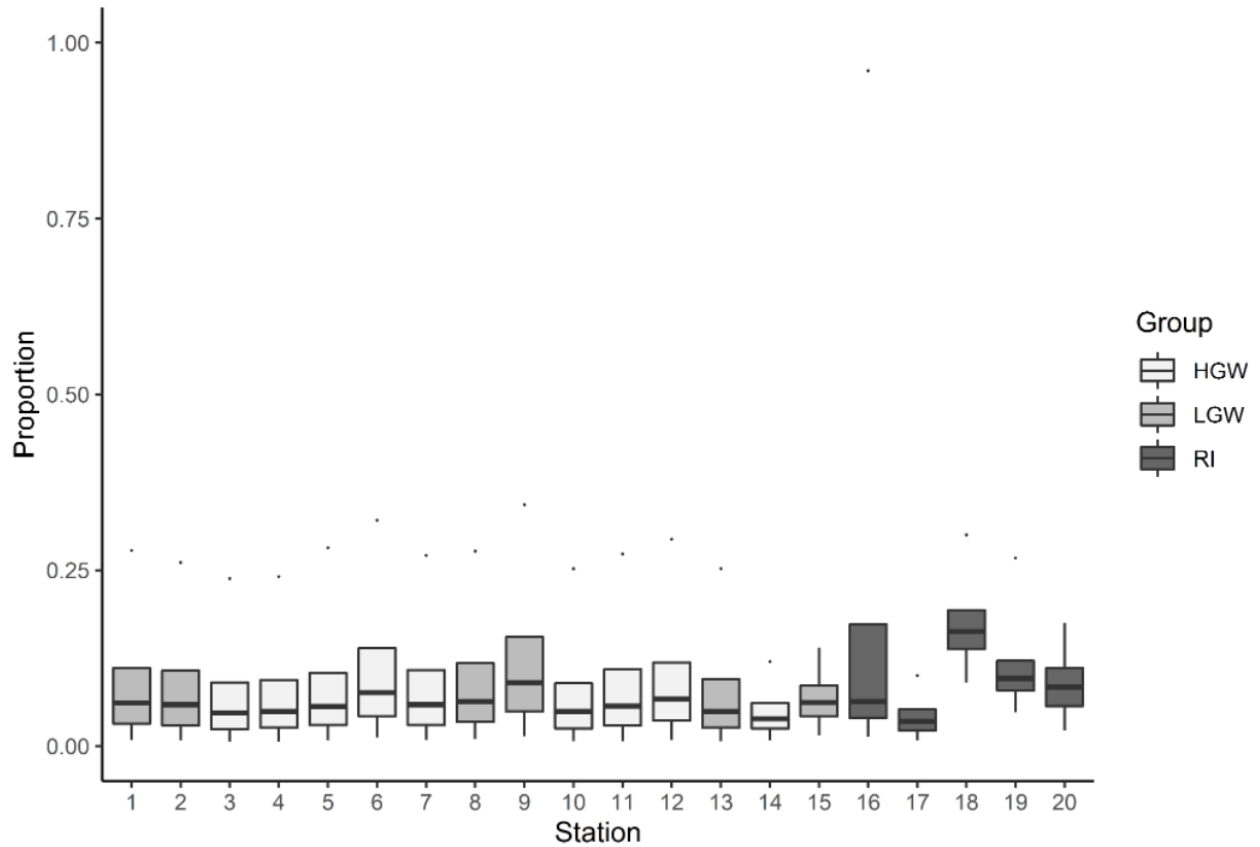
**Figure 7.** Simmr mixing model results displaying the proportion (%) that potential sources (groundwater sampled from drinking water wells (GW), sewage from on-site sewage disposal systems (OSDS), and sewage from the Hilo STP outfall manhole (STP)) contributed to the  $\text{NO}_3^-$  pool of stations, beyond the breakwater, in two freshwater-influence groups ((**HGW**) High Groundwater Influence, (**LGW**) Low Groundwater Influence).



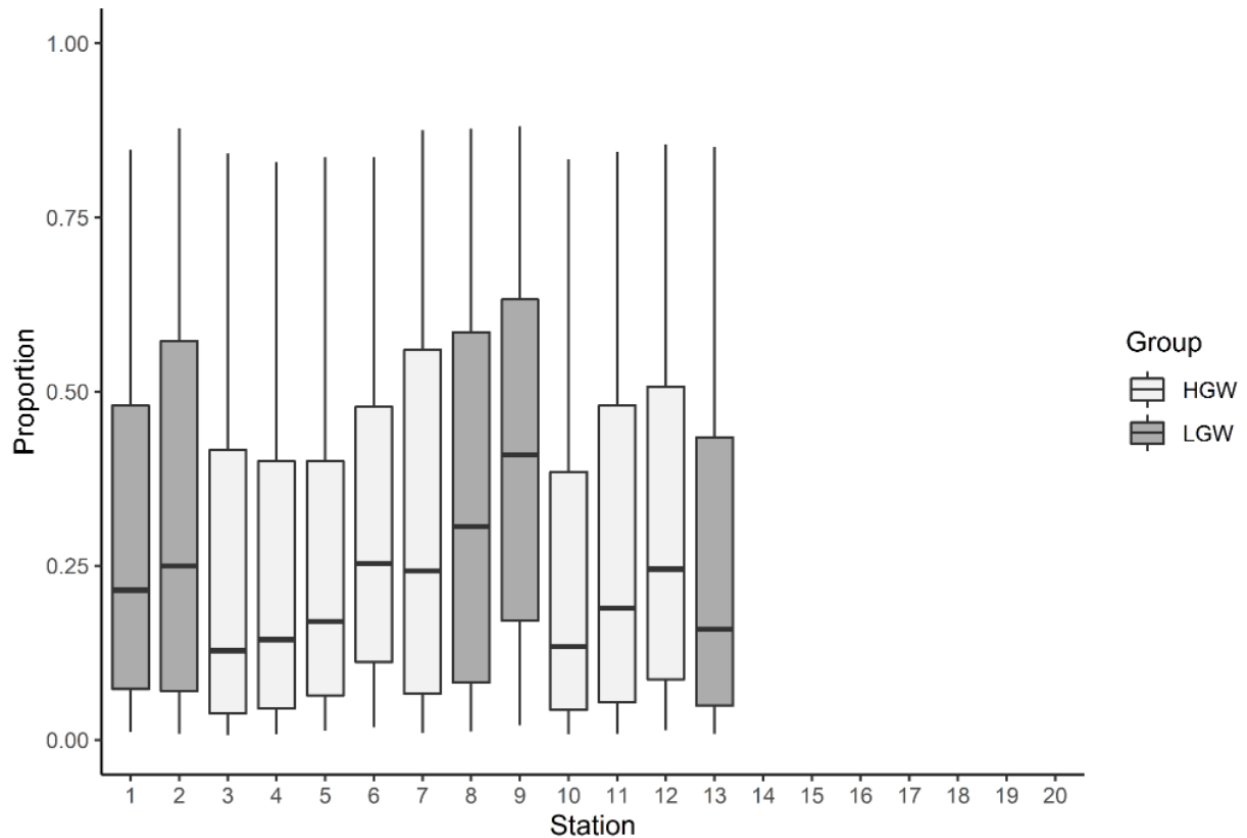
**Figure 8.** Simmr mixing model results displaying the proportion (%) that potential sources (groundwater sampled from drinking water wells (GW) and sewage from on-site sewage disposal systems (OSDS)) contributed to the  $\text{NO}_3^-$  pool of stations, within the breakwater, in three freshwater-influence groups ((**HGW**) High Groundwater Influence, (**LGW**) Low Groundwater Influence, and (**RI**) River Influenced).



**Figure 9.** Estimated proportion contributions of groundwater from drinking water wells (GW) to shoreline stations in Hilo, Hawai'i, derived from Bayesian stable isotope mixing models (simmr) using  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values. Boxed area represents 25% and 75% credible quantiles, horizontal lines indicate the 50% quantile, and vertical lines and outliers indicate minimum (2.5%) and maximum (97.5%) proportion values.



**Figure 10.** Estimated proportion contributions of sewage from on-site sewage disposal systems (OSDS) to shoreline stations in Hilo, Hawai'i, derived from Bayesian stable isotope mixing models (simmr) using  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  values. Boxed area represents 25% and 75% credible quantiles, horizontal lines indicate the 50% quantile, and vertical lines and outliers indicate minimum (2.5%) and maximum (97.5%) proportion values.



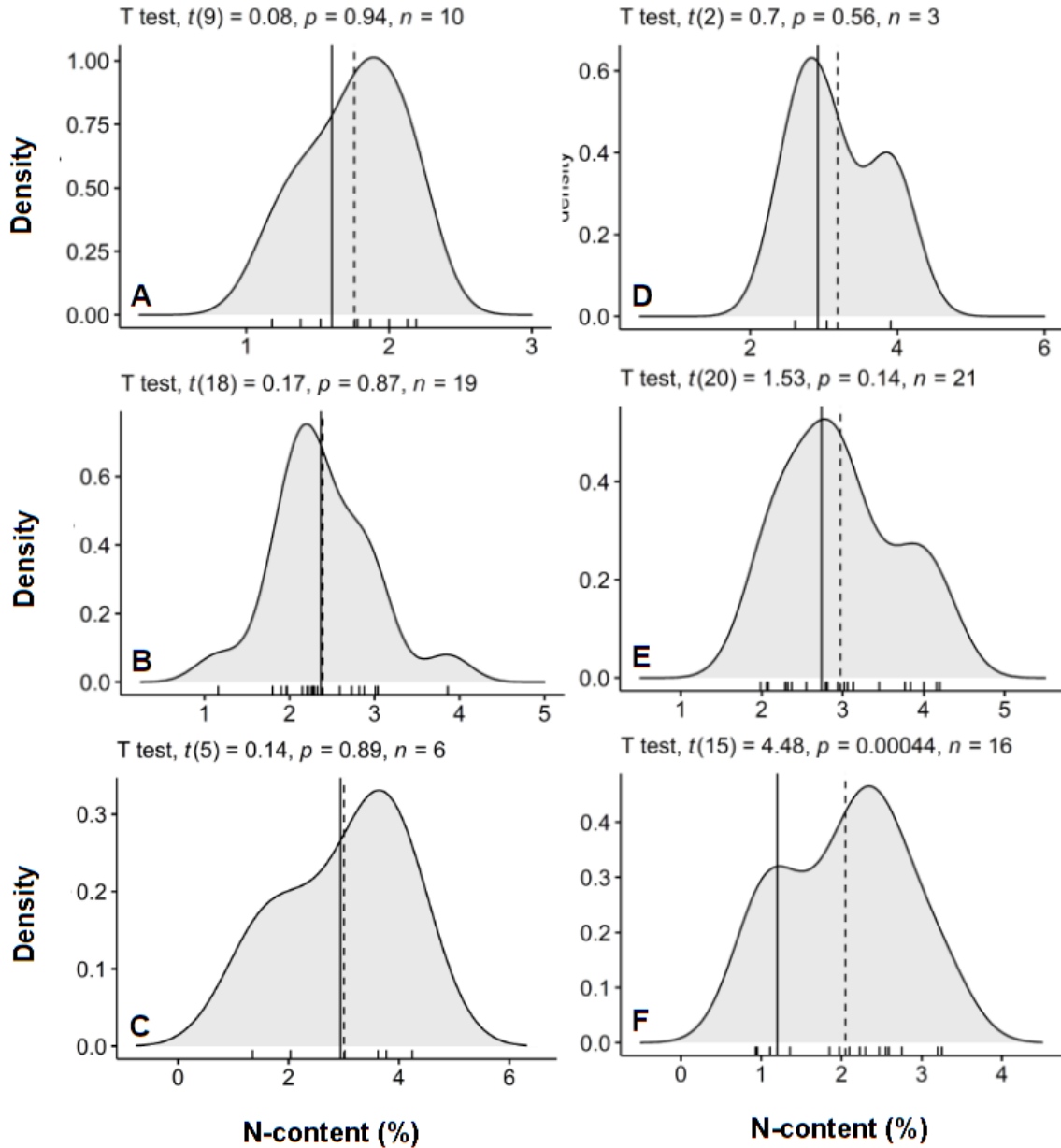
**Figure 11.** Estimated proportion contributions of sewage from the Hilo sewage treatment plant outfall (STP) to shoreline stations in Hilo, Hawai'i, derived from Bayesian stable isotope mixing models (simmr) using  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}\text{-NO}_3^-$  values. Boxed area represents 25% and 75% credible quantiles, horizontal lines indicate the 50% quantile, and vertical lines and outliers indicate minimum (2.5%) and maximum (97.5%) proportion values.

## Appendices

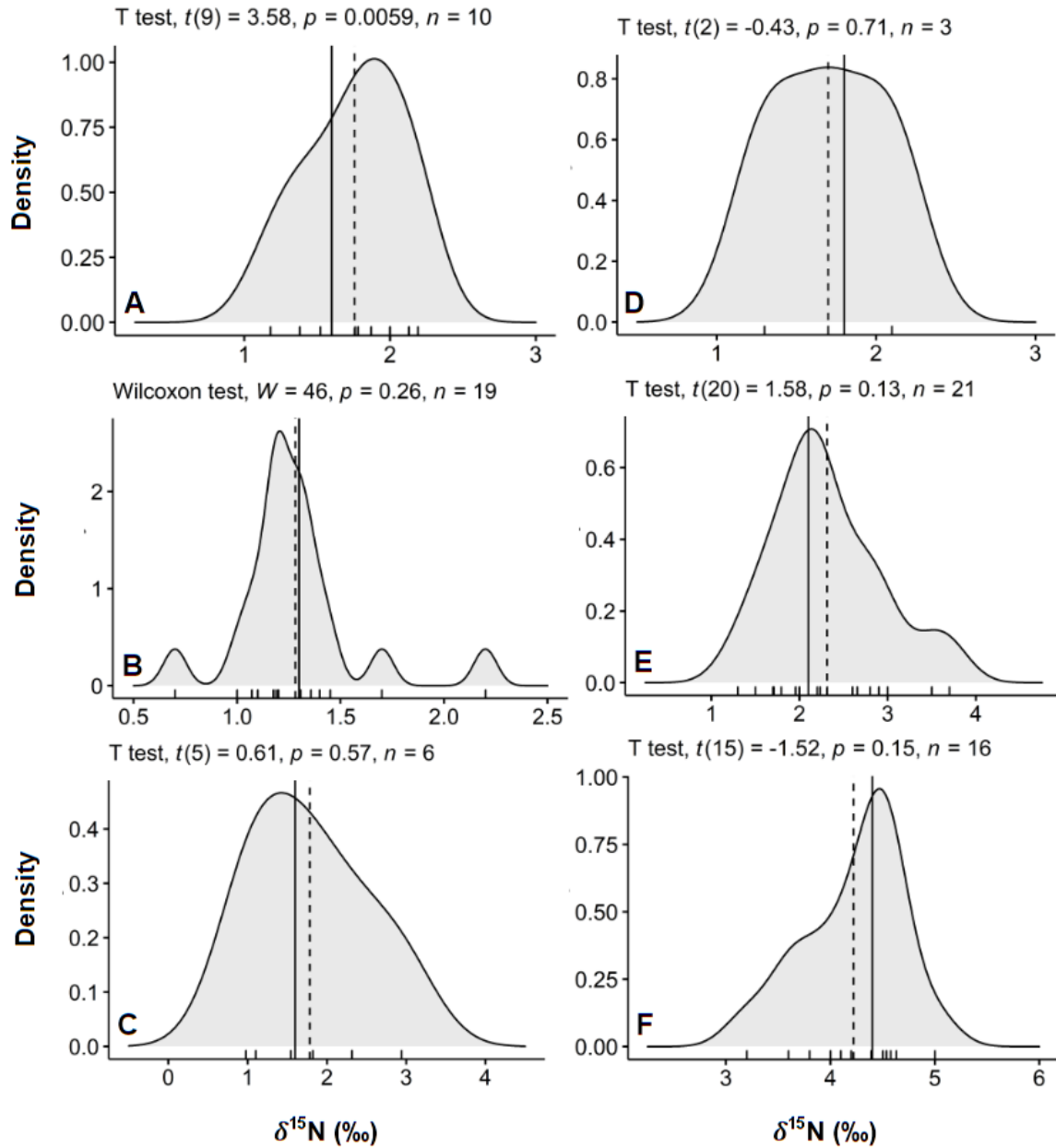
### Appendix I: Algae Species and Analyses

**Appx. I-1.** Macroalgae, diatoms, and cyanobacteria collected from shoreline stations from January 2021 to June 2021. Algae were identified to the lowest taxonomic level and are listed in alphabetical order.

Phylum	Species	Occurrences
Rhodophyta	-	<b>314</b>
Rhodophyta	<i>Ahnfeltiopsis concinna</i>	62
Rhodophyta	<i>Ahnfeltiopsis flabelliformis</i>	26
Rhodophyta	<i>Ahnfeltiopsis pygmaea</i>	27
Rhodophyta	<i>Amphiroa valonioides</i>	1
Rhodophyta	<i>Chondrus sp.</i>	61
Rhodophyta	<i>Gelidiella myrioclada</i>	15
Rhodophyta	<i>Gelidium pusillum</i>	8
Rhodophyta	<i>Gelidium reediae</i>	1
Rhodophyta	<i>Gelidium sp.</i>	1
Rhodophyta	<i>Gracilaria salicornia</i>	11
Rhodophyta	<i>Grateloupia filicina</i>	1
Rhodophyta	<i>Grateloupia hawaiiiana</i>	1
Rhodophyta	<i>Hypnea spinella</i>	17
Rhodophyta	<i>Polyopes hakalauensis</i>	50
Rhodophyta	<i>Polysiphonia howei</i>	7
Rhodophyta	<i>Pyropia vietnamensis</i>	7
Rhodophyta	<i>Portieria homemannii</i>	8
Rhodophyta	<i>Pterocladia caerulescens</i>	2
Rhodophyta	<i>Pterocladia capillacea</i>	8
Chlorophyta	-	<b>58</b>
Chlorophyta	<i>Chaetomorpha antennina</i>	7
Chlorophyta	<i>Cladophora socialis</i>	1
Chlorophyta	<i>Enteromorpha flexuosa</i>	2
Chlorophyta	<i>Rhizoclonium africanum</i>	1
Chlorophyta	<i>Rhizoclonium grande</i>	6
Chlorophyta	<i>Rhizoclonium implexum</i>	1
Chlorophyta	<i>Rhizoclonium riparium</i>	24
Chlorophyta	<i>Ulva clathrata</i>	1
Chlorophyta	<i>Ulva compressa</i> Linneaus	13
Chlorophyta	<i>Ulva expansa</i>	1
Chlorophyta	<i>Ulva intestinalis</i>	1
Phaeophyta	-	<b>15</b>
Phaeophyta	<i>Colpomenia sinuosa</i>	12
Phaeophyta	<i>Padina sanctae-crucis</i>	3
Bacillariophyta	-	<b>39</b>
Cyanobacteria	-	<b>17</b>



**Appx. I-2.** Density plots showing the results of one-sample t-tests comparing mean %N values of individual macroalgal species (dashed line) to the mean %N value of composite samples (solid line) at stations 2 (**A**), 3 (**B**), 8 (**C**), 10 (**D**), 11 (**E**), and 18 (**F**) with  $\alpha = 0.05$ .



**Appx. I-3.** Density plots showing the results of one-sample tests comparing mean  $\delta^{15}\text{N}$  values of individual macroalgal species (dashed line) to the mean  $\delta^{15}\text{N}$  value of composite samples (solid line) at stations 2 (A), 3 (B), 8 (C), 10 (D), 11 (E), and 18 (F) with  $\alpha = 0.05$ . Medians and the results of a one-sample Wilcoxon test are displayed for station 3 (B) with  $\alpha = 0.05$ .



## Appendix II: Potential Sources in Stable Isotope Mixing Model

**Appx. II-1.** Average ( $\pm$  standard deviation) values for stable isotopes of nitrate and nutrient concentrations of potential sources used in the stable isotope mixing model to determine nitrate contributions to freshwater-influence groups. OSDS data are obtained from Wiegner et al. (2016) and Abaya et al. (2018a).

Source	n	$\delta^{15}\text{N-NO}_3^-$ (‰)	$\delta^{18}\text{O-NO}_3^-$ (‰)	$\text{NO}_2^- + \text{NO}_3^-$ ( $\mu\text{mol/L}$ )	$\text{NH}_4^+$ ( $\mu\text{mol/L}$ )	TDN ( $\mu\text{mol/L}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{mol/L}$ )	TDP ( $\mu\text{mol/L}$ )
OSDS	17	13.1 ( $\pm 2.1$ )	16.6 ( $\pm 5.2$ )	16.10 ( $\pm 43.90$ )	-	-	-	-
Outfall Manhole	13	3.6 ( $\pm 7.4$ )	4.48 ( $\pm 2.7$ )	145.25 ( $\pm 73.10$ )	514.09 ( $\pm 132.09$ )	968.16 ( $\pm 334.46$ )	46.39 ( $\pm 12.21$ )	58.45 ( $\pm 12.29$ )
Inland Groundwater	8	0.7 ( $\pm 0.6$ )	2.5 ( $\pm 1.1$ )	25.17 ( $\pm 6.64$ )	1.02 ( $\pm 0.55$ )	28.76 ( $\pm 6.34$ )	2.49 ( $\pm 1.09$ )	3.45 ( $\pm 1.32$ )
Outfall Discharge Plume	3	-	-	1.63 ( $\pm 1.22$ )	4.19 ( $\pm 2.65$ )	16.21 ( $\pm 3.54$ )	0.35 ( $\pm 0.50$ )	1.69 ( $\pm 1.09$ )
Ocean	3	-	-	0.92 ( $\pm 0.46$ )	1.35 ( $\pm 0.35$ )	9.21 ( $\pm 3.15$ )	0.02 ( $\pm 0.00$ )	1.19 ( $\pm 0.62$ )

## Appendix III: Summary Station Data

**Appx. III-1.** Median [range] fecal indicator bacteria (left), nutrient (center-left), chlorophyll *a*, and turbidity (center) measurements, OSDS density (center-right) and sewage pollution scores (right) at twenty stations along the Hilo, Hawai'i, shoreline from July 2020 to October 2021. Stations are grouped by freshwater-influence (left) and bold values indicate an exceedance of its respective standard, if applicable (bottom).

Station Name (Number)	Group	<i>Enterococcus</i> spp. (MPN/100 mL)	<i>C. perfringens</i> (CFU/100 mL)	NO <sub>2</sub> + NO <sub>3</sub> <sup>-</sup> (μmol/L)	NH <sub>4</sub> <sup>+</sup> (μmol/L)	TDN (μmol/L)	PO <sub>4</sub> <sup>3-</sup> (μmol/L)	TDP (μmol/L)	Chl <i>a</i> (μg/L)	Turbidity (NTU)	OSDS Density (OSDS/km <sup>2</sup> )	Sewage Pollution Score
Lehia (1)	LGW	13.33 [5.00-67.00]	0.00 [0.00-2.33]	<b>11.44</b> [1.72-17.80]	<b>1.49</b> [0.18-6.45]	<b>19.88</b> [12.12-62.46]	1.23 [0.16-2.64]	<b>1.47</b> [0.43-3.71]	<b>2.07</b> [0.22-11.21]	0.80 [0.27-5.53]	7	16.5 [13-19]
Ka'iliwai (2)	LGW	23.67 [5.00-466.67]	0.50 [0.00-4.00]	<b>16.12</b> [8.16-22.98]	<b>1.40</b> [0.18-4.42]	<b>24.60</b> [14.89-46.44]	1.45 [0.45-2.07]	<b>2.19</b> [0.74-3.63]	1.34 [0.24-38.10]	1.04 [0.18-34.90]	14	17 [15-21]
Lelelwi (3)	HGW	<b>383.83</b> [5.00-6546]	0.00 [0.00-0.33]	<b>19.69</b> [13.30-28.06]	<b>0.85</b> [0.18-2.71]	<b>26.73</b> [19.00-70.46]	1.92 [0.75-3.43]	<b>2.31</b> [1.66-3.38]	0.11 [0.00-0.56]	0.49 [0.06-1.30]	15	17 [15-19]
Wai'olena (4)	HGW	32.33 [5.00-242.33]	0.00 [0.00-2.67]	<b>22.25</b> [14.90-25.49]	<b>1.10</b> [0.18-1.80]	<b>28.11</b> [20.47-88.28]	1.90 [0.99-2.69]	<b>2.17</b> [1.49-3.06]	<b>3.56</b> [0.67-31.82]	0.82 [0.10-19.70]	<b>20</b>	17 [15-20]
Lalakea (5)	HGW	11.67 [5.00-397.00]	0.00 [0.00-3.00]	<b>31.63</b> [11.51-41.74]	<b>0.70</b> [0.18-3.06]	<b>35.74</b> [25.74-77.12]	2.02 [0.60-2.31]	<b>2.44</b> [1.46-3.49]	0.16 [0.02-14.23]	0.36 [0.12-1.03]	<b>18</b>	16 [15-20]
Carlsmith (6)	HGW	27.33 [5.00-813.33]	0.00 [0.00-1.33]	<b>23.98</b> [11.45-29.05]	<b>0.60</b> [0.18-5.64]	<b>29.37</b> [18.00-63.90]	1.57 [0.40-2.87]	<b>1.88</b> [1.02-2.93]	0.66 [0.01-1.98]	0.44 [0.15-1.51]	<b>17</b>	17 [14-21]
Hale o Lono (7)	HGW	56.33 [6.67-355.67]	0.17 [0.00-2.00]	<b>20.73</b> [14.90-29.41]	<b>0.85</b> [0.18-2.70]	<b>25.01</b> [14.15-69.06]	1.84 [0.93-2.27]	<b>2.07</b> [1.19-3.23]	<b>2.62</b> [0.20-12.21]	0.81 [0.19-3.07]	12	17.5 [15-21]
Yacht Club (8)	LGW	20.00 [5.00-769.33]	0.00 [0.00-1.33]	<b>18.04</b> [1.06-30.44]	<b>1.11</b> [0.62-8.61]	<b>22.03</b> [12.38-127.74]	1.23 [0.18-2.27]	<b>1.38</b> [0.13-3.11]	1.23 [0.17-15.91]	1.21 [0.25-4.04]	<b>19</b>	16.5 [14-21]
Onakahakaha (9)	LGW	6.67 [5.00-700.0]	0.00 [0.00-7.67]	<b>12.92</b> [0.44-28.58]	<b>1.39</b> [0.18-3.53]	<b>18.45</b> [8.27-65.98]	0.47 [0.02-1.84]	<b>0.89</b> [0.13-6.05]	<b>3.31</b> [0.18-44.37]	<b>1.76</b> [0.37-12.80]	<b>37</b>	16 [11-21]
Chock's (10)	HGW	20.00 [6.67-3039.00]	0.00 [0.00-2.00]	<b>31.50</b> [11.29-48.33]	<b>0.83</b> [0.18-5.02]	<b>40.88</b> [5.00-102.66]	1.66 [0.59-2.65]	<b>2.32</b> [1.87-3.91]	0.20 [0.00-4.48]	0.41 [0.10-2.67]	<b>55</b>	17 [15-21]
Puhi Bay Ocean (11)	HGW	15.00 [5.00-123.00]	0.50 [0.00-2.00]	<b>31.57</b> [24.22-42.93]	<b>0.64</b> [0.18-1.65]	<b>42.79</b> [5.00-112.34]	1.84 [0.48-2.39]	<b>2.38</b> [0.98-3.49]	0.63 [0.06-26.22]	0.49 [0.09-1.19]	<b>73</b>	17 [14-19]
Puhi Bay Cove (12)	HGW	13.33 [5.00-364.33]	0.00 [0.00-7.00]	<b>32.86</b> [22.56-44.32]	<b>0.56</b> [0.18-1.93]	<b>40.12</b> [24.66-91.06]	1.89 [0.66-3.31]	<b>2.33</b> [2.01-4.41]	0.67 [0.01-108.47]	0.45 [0.07-4.79]	<b>112</b>	16 [15-21]
Puhi Bay Outfall (13)	LGW	13.33 [5.00-865.67]	0.00 [0.00-3.67]	<b>25.75</b> [18.42-46.13]	<b>0.45</b> [0.18-3.08]	<b>34.81</b> [19.06-84.74]	1.74 [0.39-2.58]	<b>2.32</b> [1.79-4.72]	0.21 [0.05-4.47]	0.53 [0.16-7.24]	<b>63</b>	16.5 [15-20]
Reed's Bay (14)	HGW	10.00 [5.00-382.00]	0.00 [0.00-1.00]	<b>31.65</b> [7.00-58.04]	<b>0.63</b> [0.18-2.73]	<b>37.15</b> [20.83-109.74]	1.94 [0.44-3.48]	<b>2.40</b> [1.67-3.99]	1.45 [0.00-39.67]	0.63 [0.14-3.69]	<b>25</b>	17 [15-21]
Lili'uokalani (15)	LGW	23.67 [5.00-135.00]	1.50 [0.00-6.00]	<b>6.40</b> [3.15-14.71]	<b>2.41</b> [0.50-4.99]	<b>17.06</b> [8.38-97.42]	0.47 [0.02-1.37]	0.59 [0.13-2.29]	1.30 [0.52-10.76]	<b>3.07</b> [0.60-8.64]	1	17 [15-20]
Wailoa (16)	RI	<b>90.00</b> [5.00-724.33]	0.83 [0.00-9.67]	<b>32.31</b> [23.96-49.65]	<b>1.35</b> [0.58-2.62]	<b>38.19</b> [13.34-98.54]	1.14 [0.66-1.76]	<b>1.31</b> [0.77-2.93]	1.36 [0.00-8.07]	0.88 [0.21-2.51]	1	18.5 [15-21]
Bayfront (17)	RI	34.33 [8.33-186.67]	0.50 [0.00-2.67]	<b>12.07</b> [4.69-24.70]	<b>1.61</b> [0.18-2.22]	<b>15.13</b> [2.50-113.74]	0.39 [0.15-0.87]	<b>0.87</b> [0.13-2.03]	<b>4.71</b> [0.11-26.33]	<b>5.05</b> [1.28-76.20]	0	-
Wailuku (18)	RI	<b>69.67</b> [10.00-557.67]	0.17 [0.00-5.33]	<b>1.42</b> [0.04-10.87]	<b>1.76</b> [0.18-4.09]	11.20 [5.45-146.25]	0.08 [0.02-0.25]	0.40 [0.13-15.22]	1.46 [0.13-4.71]	<b>2.14</b> [0.28-78.00]	<b>81</b>	16.5 [12-20]
Honoli'i Beach (19)	RI	22.00 [5.00-286.67]	0.67 [0.00-2.67]	<b>2.50</b> [0.79-6.13]	<b>1.80</b> [0.88-3.26]	<b>14.97</b> [7.30-140.58]	0.26 [0.04-0.71]	<b>1.10</b> [0.45-3.76]	1.50 [0.00-6.50]	<b>2.15</b> [0.75-47.20]	<b>43</b>	17 [15-21]
Honoli'i Stream (20)	RI	34.00 [5.00-429.00]	0.00 [0.00-3.67]	<b>9.85</b> [6.00-15.08]	<b>0.87</b> [0.18-2.21]	<b>16.97</b> [10.14-70.12]	1.04 [0.44-1.53]	<b>1.45</b> [0.57-2.21]	<b>4.59</b> [0.30-38.10]	<b>1.88</b> [0.73-13.40]	<b>43</b>	17 [14-21]
Standard		35.00	5.00	0.17, 0.13	0.43	14.28	-	0.81	1.50, 2.00*	1.50	15	-
Reference		HDOH, 2019	Fujioka et al., 2015	HDOH, 2014	HDOH, 2014	HDOH, 2014	-	HDOH, 2014	HDOH, 2014	HDOH, 2014	Yates, 1985	-

\*HDOH standards for Chl *a* differ between embayments (left) and estuaries (right).

**Appx. III-2.** Median [range] macroalgal %N and  $\delta^{15}\text{N}$  values and mean ( $\pm$ standard deviation)  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of nitrate in water from samples collected at 20 stations along the Hilo, Hawai'i, shoreline from July 2020 to October 2021.

Station Name (Number)	Group	N-content (%)	$\delta^{15}\text{N}$ (‰)	$\delta^{15}\text{N-NO}_3^-$ (‰)	$\delta^{18}\text{O-NO}_3^-$ (‰)
Lehia (1)	LGW	1.75 [1.06-3.38]	1.90 [1.00-2.50]	0.3 ( $\pm 5.5$ )	-1.0 ( $\pm 4.7$ )
Ka'iliwai (2)	LGW	1.91 [0.75-2.32]	1.72 [0.90-2.30]	-1.2 ( $\pm 6.8$ )	-1.9 ( $\pm 3.8$ )
Lelewi (3)	HGW	2.32 [1.96-2.94]	1.12 [0.70-2.10]	-1.4 ( $\pm 5.0$ )	-1.8 ( $\pm 4.8$ )
Wai'olena (4)	HGW	1.90 [0.89-2.99]	1.30 [0.30-2.40]	-0.9 ( $\pm 5.0$ )	-1.5 ( $\pm 5.0$ )
Lalakea (5)	HGW	2.56 [1.49-2.95]	4.16 [3.19-6.40]	0.9 ( $\pm 5.2$ )	-2.2 ( $\pm 3.5$ )
Carlsmith (6)	HGW	2.19 [1.58-3.68]	4.76 [4.00-5.35]	1.4 ( $\pm 5.5$ )	-1.2 ( $\pm 5.3$ )
Hale o Lono (7)	HGW	1.25 [0.23-2.05]	2.45 [1.80-5.23]	-1.5 ( $\pm 6.3$ )	0.0 ( $\pm 4.7$ )
Yacht Club (8)	LGW	2.31 [0.95-3.63]	2.09 [1.38-3.10]	-0.8 ( $\pm 6.5$ )	-0.6 ( $\pm 4.7$ )
Onakahakaha (9)	LGW	1.31 [0.93-2.56]	2.90 [1.20-4.50]	-1.8 ( $\pm 5.7$ )	2.9 ( $\pm 5.3$ )
Chock's (10)	HGW	3.02 [2.01-4.30]	2.10 [1.69-2.74]	-0.9 ( $\pm 4.8$ )	-0.3 ( $\pm 5.1$ )
Puhi Bay Ocean (11)	HGW	3.00 [1.40-3.76]	2.10 [1.60-2.50]	-1.3 ( $\pm 4.9$ )	-0.8 ( $\pm 6.1$ )
Puhi Bay Cove (12)	HGW	2.37 [1.35-3.65]	2.70 [1.95-4.20]	0.4 ( $\pm 5.7$ )	-1.1 ( $\pm 4.8$ )
Puhi Bay Outfall (13)	LGW	1.98 [1.56-2.38]	2.76 [2.10-3.70]	-0.3 ( $\pm 5.5$ )	-0.7 ( $\pm 4.1$ )
Reed's Bay (14)	HGW	1.73 [1.31-3.48]	3.20 [0.17-5.77]	0.5 ( $\pm 6.3$ )	-0.3 ( $\pm 4.7$ )
Lili'uokalani (15)	LGW	2.06 [1.65-3.83]	5.20 [3.50-6.20]	1.4 ( $\pm 4.6$ )	3.3 ( $\pm 3.7$ )
Wailoa (16)	RI	2.40 [1.78-5.69]	3.60 [1.70-4.60]	3.4 ( $\pm 4.9$ )	0.5 ( $\pm 4.3$ )
Bayfront (17)	RI	-	-	-0.1 ( $\pm 4.3$ )	1.0 ( $\pm 4.3$ )
Wailuku (18)	RI	1.24 [1.04-2.28]	4.10 [2.90-4.80]	3.2 ( $\pm 1.2$ )	7.9 ( $\pm 4.3$ )
Honoli'i Beach (19)	RI	2.70 [1.15-3.63]	3.02 [2.47-3.55]	2.2 ( $\pm 0.7$ )	7.5 ( $\pm 3.9$ )
Honoli'i Stream (20)	RI	2.09 [0.76-3.04]	2.45 [1.30-2.99]	1.3 ( $\pm 4.4$ )	4.2 ( $\pm 3.7$ )