Title: Spatial distribution of sewage pollution on a Hawaiian coral reef

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Abstract: While sewage pollution is contributing to the global decline of coral reefs, its offshore extent and direct reef impacts from water column mixing and benthic seeps are poorly documented. We addressed this knowledge gap on a Hawaiian coral reef using sewage indicator and benthic cover measurements, macroalgal bioassays, and a pollution scoring tool. Fecal indicator bacteria (FIB) and nutrient concentrations were spatially variable in surface and benthic waters, with shoreline values being highest. Shoreline macroalgae $\delta^{15}N$ and %N indicated high nitrogen loads containing sewage, while offshore surface and benthic values suggested lower nitrogen loads from environmental sources. Coral cover was negatively correlated with FIB, macroalgal $\delta^{15}N$, and nutrient concentrations. Benthic salinity and temperature measurements detected daily tidal groundwater pulses which may explain these associations. While pollution scores revealed that sewage was largely concentrated along the shoreline, results showed some reached the reef and may be contributing to its declining condition.

Key words: coral reefs, fecal indicator bacteria, macroalgae, pollution score, sewage, stable nitrogen isotopes

Introduction

Sewage pollution is impacting coastal waters worldwide. It enters these water bodies from accidental spills or purposeful releases of sewage from treatment plants, injection wells, and effluent from onsite sewage disposal systems (OSDS, i.e., cesspools, septic tanks). Sewage pollution is a complex environmental problem impacting human and ecosystem health through release of pathogens (bacteria and viruses), nutrients, hydrocarbons, toxins, and endocrine disruptors (Wear & Vega Thurber 2015). Human exposure to sewage can result in skin and urinary tract infections, hepatitis, and gastroenteritis (Pinto et al. 1999). This pollution is also impacting coastal ecosystems, like coral reefs, which are one of the most economically valuable and biologically diverse ecosystems on Earth, but are steadily declining (Wear & Vega Thurber 2015). Increased coral disease prevalence and severity have been linked to sewage pollution (Sutherland et al. 2010, Redding et al. 2013, Vega Thurber et al. 2014, Yoshioka et al. 2016). For example, a human pathogen found in sewage, *Serratia marcescens*, was shown to cause White Pox disease that devastated coral in the Caribbean (Sutherland et al. 2010), although the relationship is disputed (Lesser & Jarett 2014). Elevated nutrients from sewage pollution alter coral growth and calcification rates, species distribution and abundance, and coral community diversity (Pastorok & Bilyard 1985, Reopanichkul et al. 2009). These nutrients are also associated with benthic phase shifts from coral- to maroalgal dominated reefs (Hunter & Evans 1995, Lapointe et al. 2005).

As coastal development increases with a growing human population, monitoring coastal waters for sewage pollution is critical to understanding its potential impacts. Fecal indicator bacteria (FIB) are the most widely used measurements for assessing human health risks related to sewage pollution (Cabelli 1983, Prüss 1998). The United States Environmental Protection Agency (USEPA) and state agencies currently monitor marine recreational waters for *Enterococcus* spp. In tropical areas like Hawai‘i, *Clostridium perfringens*, an anaerobic, spore-forming bacterium, is monitored as a secondary FIB as it, unlike *Enterococcus* spp., does not multiply in aerobic coastal waters or soils (Hardina & Fujioka 1991, Fujioka et al. 1997, Fung et al. 2007, Fujioka et al. 2015). Hence, *C. perfringens* is thought to more accurately detect sewage pollution than *Enterococcus* spp.

(Fujioka & Shizumura 1985, Hardina & Fujioka 1991, Fujioka et al. 1997), but it is measured less frequently outside of Hawai‘i because it is only a state approved FIB, and not a federal one (Fujioka et al. 2015). However, there are challenges when assessing recreational water quality using these two FIB because of the above stated differences. Thus, to better evaluate environmental conditions, FIB should be used in conjunction with other sewage indicators.

Measurements of stable nitrogen (N) isotopes ($\delta^{15}N$) in macroalgal tissue are also used 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 during nutrient uptake, and therefore, have stable isotopic compositions similar to their N sources (Savage 2005, Dudley et al. 2010). Sewage is highly enriched in ^{15}N, and thus, has a distinct stable isotopic composition compared to other N sources (i.e., fertilizers, soils, ocean water; *reviewed in* Wiegner et al. 2016). In sewage pollution studies, opportunistic macroalgal species, like *Ulva fasciata*, are often used as bioindicators because they have rapid nutrient uptake rates leading to increased growth under enriched conditions (Littler & Littler 1980, Abbott & Huisman 2004, Dailer et al. 2010, Dudley et al. 2010, Amato et al. 2016). More recently, in addition to collecting wild algal tissue for $\delta^{15}N$ analysis, researchers have conducted *in situ* macroalgal bioassays (Costanzo et al. 2001) to evaluate sewage and aquaculture pollution along shorelines, as well as within coastal water bodies and benthic environments (Costanzo et al. 2005, García-Sanz et al. 2011, Kaldy 2011, Dailer et al. 2012, Yoshioka et al. 2016). In some locations, $\delta^{15}N$ in macroalgal tissue can be highly variable due to differing isotopic compositions of N sources (Ochoa-Izaguirre & Soto-Jiménez 2015). Therefore, in some circumstances, evaluating sewage pollution based solely on $\delta^{15}N$ measurements in macroalgal tissue can be ambiguous.

Nutrient concentrations are also used to assess water quality relative to sewage pollution. Elevated nutrients are typically detected in sewage polluted areas (Wei & Huang 2010, Nelson et al. 2015, Amato et al. 2016). However, numerous non-sewage watershed sources affect nutrient concentrations. Thus, measuring them alone as sewage pollution indicators is not adequate for management applications.

Because of the limitations associated with each sewage indicator, researchers have recently begun measuring multiple ones (Knee et al. 2008, Baker et al. 2010, Moynihan et al. 2012, Yoshioka et al. 2016, Abaya et al. 2018) and using them to create pollution scores and indices for evaluating water quality (Zambrano et al. 2009, Wang et al. 2015, Abaya et al. 2018). These scores and indices have been successful in assessing water quality conditions for human and ecosystem health. Interpolative mapping of score and index values provides a simple and clear tool for managers and policy makers that allow them to relate human activities to water quality, and identify areas in need of better management (Zambrano et al. 2009).

The goal of our study was to assess the offshore spatial extent of sewage pollution in surface and benthic waters of a Hawaiian coral reef with measurements of several sewage indicators (FIB, nutrients), macroalgal bioassays ($\delta^{15}N$, %N), and a pollution scoring tool. Only a handful of macroalgal bioassay studies have examined sewage pollution offshore in surface and benthic waters, and even fewer that have used both FIB in combination with macroalgal bioassays (Dailer et al. 2010, Dailer et al. 2012, Amato et al. 2016, Yoshioka et al. 2016). Extending measurements of sewage indicators offshore and into benthic habitats is critical because sewage pollution can be transported offshore and enter coastal waters through benthic seeps. A recent study in a Hawaiian estuary found elevated concentrations of *Enterococcus* spp. ~ 2 km offshore (Wiegner et al. 2017). Another study conducted in Hawai‘i detected sewage in both offshore and benthic waters from $\delta^{15}N$ measurements using macroalgal bioassays (Dailer et al. 2010, Dailer et al. 2012). These studies highlight the need for determining the spatial distribution of sewage pollution offshore and in benthic habitats in order to improve water quality for human and coral reef health.

Materials and Methods

Site Description

This study was conducted in Puakō, a coastal community with a fringing coral reef ecosystem located in the South Kohala region of Hawai‘i Island, Hawai‘i, USA. This community includes more than 200 homes relying solely on OSDS, including: cesspools

155 (49), septic tanks (66), and aerobic/anaerobic treatment units (ATU, 23); there are 21 lots
156 where the type of OSDS is unknown and 43 vacant lots (Aqua Engineering 2015).
157 Because of the high number of OSDS and the proximity of the homes to the ocean, Puakō
158 is considered a high-risk area where sewage can affect nearshore waters (Whittier & El
159 Kadi 2014). Puakō's coral reef has also been designated by the state of Hawai'i as a
160 priority site for site-based action due to its rich diversity of corals (Hayes et al. 1982).
161 Decreases in coral coverage and fish abundances over the last 40 years (Minton et al.
162 2012, HDAR 2013, Kramer et al. 2016), as well as increases in coral disease prevalence
163 and severity have been documented (Couch et al. 2014a, Yoshioka et al. 2016). The
164 prevalence of coral growth anomalies is the highest observed in Hawai'i and greater than
165 reported for the Indo-Pacific region (Yoshioka et al. 2016). These ecosystem changes
166 highlight Puakō as an area of concern, and they are likely attributed, in part, to sewage
167 pollution. While recent studies have documented sewage pollution along Puakō's
168 shoreline (Yoshioka et al. 2016, Abaya et al. 2018), its spatial extent offshore in surface
169 and benthic waters has not yet been determined.
170 The hydrological connectivity between OSDS and the nearshore environment in
171 Puakō is quick, ranging from 5 h to 10 d (Abaya et al. 2018, Colbert et al. unpubl. data).
172 This is largely because the fractured basalt substrate has a high permeability. In addition,
173 while the area is arid (mean annual rainfall: 250 – 750 mm), submarine groundwater
174 discharge (SGD) is high, with rates ranging from 2083 to 2730 L m^{-1} h^{-1} (Paytan et al.
175 2006). SGD is responsible for transporting sewage effluent from the OSDS to the
176 shoreline and benthic habitats at Puakō.
177 **Study Design**
178 To determine the spatial extent of sewage pollution offshore of Puakō, as well as
179 inputs from benthic seeps that could directly impact the coral reef habitat, surface and
180 benthic waters were sampled for FIB and nutrient concentrations. Additionally, the green
181 macroalga, *Ulva fasciata,* was deployed as a bioassay for $\delta^{15}N$ and %N analyses at five
182 stations (Fig. 1). These stations included three zones (shoreline, bench, and reef slope)
183 and two water depths at each zone (surface and benthic) (Fig. 1). Benthic zones were
184 chosen based on physiography features. The bench zone was ~7 m deep, and on average,

196 m (range: 145 – 214 m) from the shoreline. The slope zone was ~15 m in depth, and on average, 267 m (201 – 304 m) from the shoreline. The bench and slope zones were ~65 m apart. Visibility in the water column was greater than 15 m. Collection of water samples and deployments of algal cages occurred once monthly in June and July 2015. Benthic water samples were collected ~0.5 m above the substrate.

FIB and Nutrient Analyses

Water samples were collected once during each macroalgal bioassay deployment at all zones and water depths, and analyzed for FIB, nutrient concentrations, and salinity in sterile, acid-washed, polypropylene plastic bottles. Samples were collected at 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 manufacturer's recommendations and procedures detailed in Wiegner et al. 2017. *Clostridium perfringens* was enumerated by filtering sample water through 0.45-µm pore size cellulose nitrate filters (Whatman™) and mCP medium (Acumedia, Baltimore, MD, USA) (Bisson & Cabelli 1979). Water samples for nutrient analyses were filtered through pre-combusted (500°C for 6 h) GF/F filters (Whatman™) and stored frozen until analysis at the University of Hawai'i at Hilo (UH Hilo) Analytical Laboratory. Nutrients in water samples were analyzed on a Pulse Technicon™ II autoanalyzer using standard methods and reference materials (NIST; HACH 307-49, 153-49, 14242-32, 194-49). These samples were analyzed for $NO_3^- + NO_2^-$ [Detection Limit (DL) 0.07 µmol/L, USEPA 353.4], NH_4^+ [DL 0.36 µmol/L, USGS I-2525], PO_4^{3-} [DL 0.03 µmol/L, Technicon Industrial Method 155-71 W], total dissolved phosphorous (TDP) [DL 0.5 µmol/L, USGS I-4650-03], and H_4SiO_4 [DL 1 µmol/L, USEPA 366]. Total dissolved nitrogen (TDN) was analyzed by high-temperature combustion, followed by chemiluminescent detection of nitric oxide (DL 5 µmol/L, Shimadzu TOC-V, TNM-1). Salinity was assessed using an YSI Pro 2030.

***Ulva fasciata* Bioassays & Analysis**

Ulva fasciata (Chlorophyta) was collected locally and acclimated to low nutrient water to ensure N stores within the thalli were depleted prior to bioassays. A sample of the *U. fasciata* was collected and preserved as a voucher for identification. Preliminary

studies determined that Instant-Ocean (salinity: 33-35) was the most suitable low-nutrient medium for the acclimation period. The lowest thalli tissue %N and $\delta^{15}N$ levels were observed within 3 d, with water changed every 24 h (Fig. 2). Thalli of *U. fasciata* were deployed in netted plastic cages (mesh size ~ 5 mm x 5 mm) creating a protective barrier from herbivores, while allowing sunlight and water movement within the cages. These cages were incubated in a grid-like pattern at the three zones for 7 d offshore of the Puakō coastline (Fig. 1). Within each zone, excluding the shoreline, replicate cages containing *U. fasciata* were placed at two depths: three at ~1 m below the surface with subsurface buoys (surface) and three above the reef substrate (~1 m) secured by weights (benthic) (Fig. 1). Approximately 4 g of acclimated *U. fasciata* were rinsed with reagent-grade water, spun-dried, and weighed before being placed in cages. In addition, to determine if there was a difference between deployed *U. fasciata* and nearby wild macroalgae, tissue samples of the latter were collected at all benthic zones during the June 2015 bioassay deployment. Macroalgae collected at the shoreline were primarily comprised of *U. fasciata*, *Cladophora* spp., *Gelidiella acerosa*, as well as 10 other unidentified species. Offshore, benthic macroalgae collected were comprised of *Pterocladiella* spp., *G. acerosa*, *Cladophora* spp., *U. fasciata, Laurencia* spp., *Hypnea musciformis*, and 11 other unidentified species, largely consisting of cyanobacteria and turf algae. Permits were obtained for macroalgal cage deployments from the Hawai‘i Division of Aquatic Resources (HDAR, Special Activities Permit 2016-11).

Following the bioassays, *U. fasciata* samples and adjacent wild algae collected were rinsed with reagent-grade water, dried at 60° C until a constant weight was achieved, ground, and homogenized using a Wig-L-Bug grinding mill. For stable isotope analysis, 2 mg of the macroalgal tissues were folded in 4x6-mm tin capsules. These tissues were analyzed for $\delta^{15}N$ and %N using a Thermo-Finnigan™ Delta V Advantage isotope ratio mass spectrometer with a Conflo III interface and a Costech™ ECS 4010 Elemental Analyzer located at the UH Hilo Analytical Laboratory. Data were normalized to United States Geological Survey (USGS) standard NIST 1547. Isotopic signatures are expressed as standard (δ) values, in units of parts per mil (‰), and calculated as: $[(R_{sample} - R_{standard}) / R_{standard}] \times 1000$, where $R = {}^{15}N/{}^{14}N$. To determine N sources utilized by

macroalgae, $\delta^{15}N$ in their tissues were plotted relative to $\delta^{15}N\text{-}NO_3^-$ source values (Derse et al. 2007) which were determined in a concurrent and earlier study (Wiegner et al. 2016, Abaya et al. 2018).

Benthic Surveys

Benthic surveys were conducted at each benthic algal cage deployment station, where a 1.0-m x 0.70-m quadrat was haphazardly placed at four locations immediately surrounding each cage and then photographed ~ 0.5 m above the substrate with a Canon G12-series Powershot camera. Four images were taken at each shoreline, bench, and slope zone cage location. Benthic image analysis was adapted from the National Park Service Pacific Island Network Inventory & Monitoring program benthic monitoring protocol (Brown et al. 2011). Benthic cover was analyzed using an image point-count method with the open-source program PhotoGrid (Bird 2001). For each image, 50 points were overlaid, and the benthic substrate below each point was identified to the lowest possible taxon, including coral, algae (turf and macroalgae), crustose coralline algae (CCA), and other substrate types. Point identifications were pooled into major benthic categories for comparison among stations. Note, benthic surveys were conducted prior to the 2015 bleaching event at Puakō (Kramer et al. 2016).

Benthic Water Properties

To characterize benthic water properties during macroalgal bioassay cage deployments, a CTD (Seabird 37 SMP), measuring pressure, temperature, and salinity was deployed 6/13/2015-6/19/2015 and 7/11/2015-7/19/2015. The instrument was placed on the seafloor at the shallow end of the bench zone (10.7 m water depth) within a stainless steel frame that allowed for open water flow. Manufacture's protocols for instrument calibration, deployment, and recovery were followed.

Statistical Analyses

To examine differences among sewage indicators (FIB, algal tissue $\delta^{15}N$ and %N, nutrients) among zones, general linear models (GLM) were used. One model examined surface water patterns extending from the shoreline to offshore. The second model examined benthic patterns from the shoreline to offshore. To determine differences between depths, a nested GLM was used, where depth was nested within zones to

account for variability at each station. In addition, to determine differences between initial and final $\delta^{15}N$ and %N in *U. fasciata,* a GLM and a one-way analysis of variance (ANOVA) were used with initial values compared with shoreline, as well as surface and benthic offshore zones. $\delta^{15}N$ in *U. fasciata* was also compared to $\delta^{15}N$ of NO_3^- sources within the Puakō watershed (Abaya et al. 2018). To determine if differences in $\delta^{15}N$ and %N existed between adjacent wild macroalgae and deployed *U. fasciata,* two-sample t-tests were used. Correlations examined associations between sewage indicators ($\delta^{15}N$ and %N in *U. fasciata*, *Enterococcus* spp., and *C. perfringens*) in surface and benthic waters with other water quality parameters. Shoreline values were included in the correlation analyses for both surface and benthic waters. Correlation analysis was also used to examine associations with % benthic coral and turf algal cover with each other, as well as sewage indicators and other water quality parameters. Data were tested for normality and equal variances; if assumptions for parametric analyses were not met, log, log + 1, and rank transformations were applied, and data were reassessed (Potvin & Roff 1993). Statistical analyses were conducted using Minitab 16™ ($\alpha = 0.05$).

Results

Surface and Benthic Waters Spatial Patterns

Enterococcus spp. concentrations were similar among zones in surface waters (Fig. 3A); however, they did significantly differ among benthic zones ($p = 0.04$; Fig. 3D). The greatest differences in the benthos were detected between shoreline (average ±SE, 302 MPN/100 mL ± 306) and slope (35 MPN/100 mL ± 22) zones, which were approximately an order of magnitude different. In offshore waters, *Enterococcus* spp. concentrations were similar between surface and benthic waters. In contrast, *C. perfringens* concentrations differed significantly among zones in both surface ($p = 0.01$) and benthic waters ($p < 0.01$). In surface waters, the largest differences were detected between shoreline (8 CFU/100 mL ± 4) and slope (2 CFU/100 mL ± 1) zones (Fig. 3B). Shoreline *C. perfringens* concentrations were also significantly higher (8 CFU/100 mL ± 4) compared to benthic bench (1 CFU/100 mL ± 1) and benthic slope (1 CFU/100 mL ±

304 0) waters (Fig. 3E). In offshore waters, *C. perfringens* concentrations were similar in
305 surface and benthic waters.
306 Nutrient concentrations (NO_3^- + NO_2^-, NH_4^+, TDN, PO_4^{3-}, TDP, and H_4SiO_4)
307 were highest at the shoreline ($p \leq 0.02$) (Table 1) and lower offshore, with surface and
308 benthic waters at the bench and slope zones having similar concentrations. Salinity also
309 varied among zones in both surface ($p < 0.01$, range = 29.95 – 34.62) and benthic
310 waters ($p < 0.01$, range = 31.03 – 35.00), with the shoreline being the freshest (lowest)
311 (18.52 ± 3.08). Surface water *C. perfringens* concentrations were positively correlated
312 with most nutrient and *Enterococcus* spp. concentrations, while the latter was only
313 positively correlated with NO_3^-+NO_2^- (Table 2). All surface water nutrient concentrations
314 were positively correlated with each other, and negatively correlated with salinity (Table
315 2). Benthic water *C. perfringens* concentrations were positively correlated with
316 *Enterococcus* spp. and H_4SiO_4 concentrations (Table 3). Most benthic nutrient
317 concentrations were positively correlated with each other, except for NH_4^+ and H_4SiO_4,
318 and negatively correlated with salinity (Table 3).
319 Initial and post-bioassay $\delta^{15}N$ and %N in *U. fasciata* values differed among
320 zones. Both $\delta^{15}N$ and %N differed at the shoreline ($p \leq 0.01$), where post-bioassay
321 values were ~2‰ and 0.5% higher, respectively (Fig. 4). Post-bioassay $\delta^{15}N$ and %N in
322 *U. fasciata* varied significantly by zone in both surface ($p < 0.01$) and benthic waters ($p <$
323 0.01) (Fig. 3C, F; Fig. 4). Shoreline values were the highest (5.61‰ ± 0.77, 2.76% ±
324 0.47) compared to slope (surface = 4.32‰ ± 0.34, 1.45% ± 0.13; benthic = 4.18‰ ± 0.34,
325 1.60% ± 0.15) and bench (surface = 3.92‰ ± 0.46, 1.62% ± 0.07; benthic = 3.71‰ ±
326 0.29, 1.90% ± 0.11). In offshore waters, $\delta^{15}N$ and %N were similar in surface and benthic
327 waters. Values for both surface and benthic $\delta^{15}N$ for *U. fasciata* samples fell within the
328 $\delta^{15}N$ - NO_3^- range for soil, seawater, and low elevation groundwater at Puakō (Abaya et
329 al. 2018) (Fig. 5). Surface *U. fasciata* $\delta^{15}N$ and %N were both positively correlated with
330 each other, as well as NO_3^-+NO_2^- and H_4SiO_4, and negatively correlated with salinity
331 (Table 2). In addition, surface *U. fasciata* %N was positively correlated with the
332 remaining nutrients (NH_4^+, TDN, PO_4^{3-}, and TDP; Table 2). Benthic *U. fasciata* $\delta^{15}N$ and
333 %N were both positively correlated with NO_3^-+NO_2^-, TDN, PO_4^{3-}, and H_4SiO_4

concentrations (Table 3). Benthic *U. fasciata* $\delta^{15}N$ was also positively correlated with TDP and *C. perfringens* concentrations, while %N was positively correlated with NH_4^+ concentrations (Table 3). Both $\delta^{15}N$ and %N in the benthic *U. fascita* were negatively correlated with salinity (Table 3).

Collected Wild Macroalgae vs. Deployed *U. fasciata*

Wild macroalgae collected adjacent to bioassay locations had similar $\delta^{15}N$ and %N values to those in the *U. fasciata* deployed in cages at the benthic zones, except for those along the slope ($p = 0.026$, $p < 0.0001$; Fig. 6A). At the slope, wild algae were more enriched in ^{15}N and had a higher %N content than the deployed *U. fasciata*, approximately 2‰ and 1% higher, respectively (Fig. 6A, B).

Benthic Cover

Shoreline substratum consisted primarily of turf algae and basalt (Table 4). Benthic cover at the bench and slope stations consisted of turf algae, coral, and CCA, with turf algae comprising the greatest percentage in both zones (Table 4). Coral cover increased with increasing distance from shore, with coral comprising ~40% of the slope zone's benthic cover (Table 4). Percent coral cover had significant negative correlations with both FIB, $\delta^{15}N$ and %N in the deployed *U. fasciata* tissue, and most benthic nutrient concentrations (NO_3^-+NO_2^-, TDN, PO_4^{3-}, and H_4SiO_4) (Table 4). Percent coral cover was also positively correlated with salinity ($r = 0.76$, $p = 0.001$). Percent turf algal cover had a significant negative correlation with $\delta^{15}N$ ($r = -0.73$, $p = 0.002$) in the deployed *U. fasciata* tissue and benthic *C. perfringens* concentrations ($r = -0.57$, $p = 0.03$); it was not correlated with %N in the *U. fasciata* tissue nor benthic *Enterococcus* spp. and nutrient concentrations. Turf algae and coral cover were not correlated ($p = 0.120$).

Benthic Water Properties

At 10.7 m water depth, salinity varied by 0.16 (SD ± 0.05) each day, with a daily minimum salinity that typically occurred 1.8 (SD ± 0.7) h after the lowest-low tide (Fig. 7). Temperature displayed a diurnal signal, with warming during the day and cooling at night. Between June and July 2015, the water temperature and salinity increased by 0.44°C and 0.29, respectively (Table 5).

Discussion

Onshore – Offshore Sewage Indicator Patterns

FIB concentrations are generally spatially variable within a water body, with the highest values observed closer to shore, and the different FIB are often correlated with one another (Paul et al. 1995, Griffen et al. 1999, Shibata et al. 2004, Bonkosky et al. 2009, Lisle et al. 2014). Similarly, FIB concentrations documented in our study were spatially variable in both surface and benthic waters. Spatially and temporally variable *Enterococcus* spp. concentrations have been previously reported for Puakō (Couch et al. 2014b, Yoshioka et al. 2016). In our study, surface water *Enterococcus* spp. concentrations were similar across zones (Fig. 3A). However, benthic water *Enterococcus* spp. concentrations were significantly higher along the shoreline compared to bench and slope zones (Fig. 3D). *C. perfringens* concentrations in both surface and benthic waters were greatest at the shoreline, with the greatest concentration difference between shoreline and slope zones (Fig. 3B, E). *Enterococcus* spp. and *C. perfringens* concentrations were correlated in both the surface and benthic waters at Puakō, suggesting they have similar sources. The spatial variability of FIB concentrations at Puakō is similar to those reported for other coastal water bodies (Shibata et al. 2004). In the Florida Keys, *Enterococcus* spp. and *C. perfringens* concentrations were highly variable, with the highest concentrations nearshore (Paul et al. 1995). A recent study in Hawai‘i found a similar spatial pattern for these two FIB, and that high concentrations could be detected ~2 km from shore, illustrating substantial offshore pollution transport (Wiegner et al. 2017).

State and federal governments have developed FIB concentration standards to evaluate the risk of water users in contracting gastroenteritis (Fujioka et al. 2015). At Puakō, the average *Enterococcus* spp. concentrations across both sampling periods exceeded the Hawai‘i Department of Health (HDOH) single sample maximum standard (104 CFU/100 mL) in surface waters in all three zones (inclusive of SE), as well as benthic bench zone waters (Fig. 3A, D). At the shoreline, *Enterococcus* spp. concentrations were three times greater than this standard. A concurrent study at Puakō also found 13 of their 16 shoreline stations had *Enterococcus* spp. concentrations that exceeded this standard (Abaya et al. 2018), while an earlier study observed that they

394 rarely exceeded it (Yoshioka et al. 2016). Difference in findings between these studies
395 may be related to the time of day at which the samples were collected as sunlight can
396 inactivate FIB cells (Fujioka et al. 1981). Additionally, averages across all zones in
397 surface and benthic waters were higher than HDOH's geometric mean standard for
398 marine recreational waters of 35 CFU/ 100 mL, where a water user's chance of
399 contracting gastroenteritis is 3.6% (Fujioka et al. 2015). Shoreline *C. perfringens*
400 concentrations also exceeded the recommended standard to HDOH for marine
401 recreational waters of 5 CFU/100 mL (Fig. 3B, Fujioka et al. 1997), and fell within the
402 range reported for non-point source sewage pollution (10 – 100 CFU/100 mL, Fung et al.
403 2007). These results suggest that Puakō's shoreline and surface waters, as well as some
404 benthic waters may be contaminated with sewage.
405 Similar to FIB, nutrient concentrations are often spatially variable within water
406 bodies with sewage pollution, with highest values generally observed along shorelines
407 where homes have OSDS, including those in Hawai'i (Wei & Huang 2010, Nelson et al.
408 2015, Amato et al. 2016, Wiegner et al. 2016). Our study and a concurrent one found
409 that nutrient concentrations at Puakō followed this pattern, with highest concentrations
410 documented along the shoreline, and then decreasing with increasing distance offshore
411 (Couch et al. 2014b). Surface water nutrient concentrations were correlated with both
412 *Enterococcus* spp. and *C. perfringens* concentrations suggesting they may be from
413 sewage (Table 2), whereas benthic nutrient concentrations were not correlated with FIB
414 (Table 3). Compared to most other studies conducted in Hawai'i, shoreline NO_3^- + NO_2^-
415 concentrations were five to ten times greater than those reported on other islands or
416 locations on Hawai'i Island with known sewage inputs (Wiegner et al. 2013, Nelson et al.
417 2015, Wiegner et al. 2016, Wiegner et al. 2017). Additionally, shoreline nutrient
418 concentrations (NO_3^- + NO_2^-, NH_4^+, and PO_4^{3+}) measured at Puakō as part of this study
419 and Abaya et al. 2018 are the highest reported to date, and in some cases, they are 40
420 times greater than previously reported values (Knee et al. 2010). Salinity measurements
421 indicated that SGD was greatest along the shoreline where nutrient concentrations were
422 highest, but that SGD was also transported offshore in surface waters and discharging at
423 benthic seeps (Table 1). These results concur with many studies on the importance of

SGD as a nutrient to coastal waters, especially in coral reef environments (Johannes 1980, Johannes & Hearn 1985, Paytan et al. 2006, Street et al. 2008, Knee et al. 2010).

Like FIB and nutrients, $\delta^{15}N$ macroalgal values have also been reported to decrease offshore from known areas of sewage, and to be lower in benthic waters than surface ones (Lapointe et al. 2005, Derse et al. 2007, Baker et al. 2010, Dailer et al. 2010, Yoshioka et al. 2016). %N in macroalgal tissues also show a similar pattern, but are not reported as often as $\delta^{15}N$, and benthic measurements are rare (García-Sanz et al. 2010, García-Sanz et al. 2011, Barr et al. 2013, Amato et al. 2016, Yoshioka et al. 2016). Additionally, several studies have found $\delta^{15}N$ in benthic organismal tissues (macroalgae, coral, sea fans, sea grass, and sponges) to be correlated with *Enterococcus* spp. concentrations (Baker et al. 2010, Moynihan et al. 2012, Yoshioka et al. 2016). In this study and Abaya et al. (2018), macroalgal $\delta^{15}N$ was not correlated with *Enterococcus* spp. concentrations, which were paired by zone and water depth (Tables 2 and 3, Abaya et al. 2018). This contrasts with the Yoshioka et al.'s (2016) study at Puakō, which found macroalgal $\delta^{15}N$ in deployed in benthic cages correlated with shoreline *Enterococcus* spp. concentrations. We did, however, observe a pattern of decreasing $\delta^{15}N$ and %N macroalgal values with distance offshore in both surface and benthic waters (Fig. 3C, F). This pattern reinforces an earlier finding at Puakō where macroalgal $\delta^{15}N$ was highest along the shoreline compared to the reef (Yoshioka et al. 2016). Our higher shoreline $\delta^{15}N$ and %N macroalgal values in comparison to offshore surface water values suggests that there is little transport of sewage offshore, and that it is substantially diluted with ocean water before reaching offshore locations.

CTD measurements aided in interpreting our water quality and macroalgal tissue measurements, with respect to water column mixing and the presence of benthic seeps. The average decrease in benthic salinity of 0.16 (SD ± 0.05) from high to low tide was likely from dilution of seawater with fresh groundwater, which comprised 0.45% of the water at that time. While small, these tidal pulses exposed the reef substrate to water with 0.5 to 1.6 µmol/L more NO_3^-+NO_2^- than ambient conditions, as the fresh groundwater at the shoreline has concentrations between 110 - 355 µmol/L (Abaya et al. 2018). These land-based nutrients likely support primary production on the reef and may

454 explain some of the more enriched $\delta^{15}N$ macroalgal values in the benthos. Similarly, any
455 of the other pollutants from OSDS can also impact the reef at this tidal frequency. The
456 tidal drop in water column height is unlikely to be responsible for transporting the fresher
457 surface water to the benthos as the salinity gradient at 10 m water depth is about 0.01/m,
458 similar to other West Hawai'i sites with high SGD (Grossman et al. 2010, Wiegner et al.
459 unpubl. data). Instead, turbulent processes, including wave action, strong tidal currents,
460 and winds that can enhance vertical mixing and reduce stratification, were more likely
461 responsible for the observed patterns (Simpson et al. 1990, Jones et al. 2008).
462 Alternatively, downward secondary flows may develop as a result of wave action and
463 periodic reef topography (Rogers et al. 2015).

464 **Sewage Indicator Patterns in Surface and Benthic waters**

465 Sewage floats at the surface because it is largely comprised of freshwater (Wear
466 & Vega Thurber 2015). Therefore, we hypothesized that our sewage indicator values
467 would be higher in surface waters compared to benthic ones due to density stratification.
468 This pattern was previously observed on Maui Island at the location of an offshore
469 injection well that discharges sewage through benthic seeps (Dailer et al. 2012). Here,
470 sewage rose to the surface resulting in more enriched $\delta^{15}N$ macroalgal values. However,
471 during large wave mixing events, $\delta^{15}N$ macroalgal values indicated that sewage became
472 more concentrated in the benthos than in the surface waters (Dailer et al. 2010). In
473 contrast, our study found that sewage indicator (FIB, nutrient concentrations, $\delta^{15}N$
474 macroalgae) values were similar in surface and benthic waters. This results further
475 supports that sewage is entering the ocean at shoreline seeps and is substantially diluted
476 with ocean water before reaching offshore locations.

477 **Sewage Indicators Associations with Benthic Cover**

478 One way to investigate if sewage pollution may be impacting coral reefs is to
479 examine associations of benthic cover or coral health with sewage indicators.
480 Surprisingly, there are only a few studies that have conducted this type of analysis (Baker
481 et al. 2007, Parsons et al. 2008, Redding et al. 2013, Amato et al. 2016, Yoshioka et al.
482 2016). At Puakō, we found that percent coral cover was significantly and negatively
483 correlated with both FIB, macroalgal $\delta^{15}N$ and %N, and several nutrients (Table 3). This

484 result concurs with an earlier Puakō study that found a strong negative relationship
485 between coral cover and benthic-deployed macroalgal $\delta^{15}N$ (Yoshioka et al. 2016), and
486 another study in West Hawai‘i which found a positive relationship between macroalgal
487 $\delta^{15}N$ and percent dead coral cover (Parsons et al. 2008). Together, these findings suggest
488 sewage pollution may be contributing to the declining coral cover at Puakō. Increased
489 coral disease due to sewage pollution could be one possible contributor as macroalgae
490 and soft coral $\delta^{15}N$ were positively related to coral disease severity in Guam (Redding et
491 al. 2013). This relationship, however, was not observed at Puakō (Yoshioka et al. 2016),
492 although coral growth anomaly pressure (prevalence x severity) was shown to
493 significantly increase with NO_3^-+NO_2^- concentrations (Couch et al. 2014b). Other studies
494 have also found positive relationships between growth anomaly pressure and N
495 concentrations (Kuta & Richardson 2002, Kaczmarsky & Richardson 2011). Algal
496 overgrowth from sewage nutrients is another possible contributor to coral cover decline,
497 as it is commonly observed in locations where herbivore abundance has declined (Hughes
498 1994, Roger & Miller 2006, Rodgers et al. 2015). However, macroalgal cover at Puakō
499 was negligible, and turf and coral cover were not inversely correlated (Table 4).
500 Additionally, benthic turf cover was negatively associated with macroalgal $\delta^{15}N$ and *C.*
501 *perfringens* concentrations, and not correlated with other sewage indicators including
502 *Enterococcus* spp. and nutrient concentrations (Table 3). Likewise, macroalgal $\delta^{15}N$ was
503 not correlated with either percent turf algae or macroalgae cover in another West Hawai‘i
504 study (Parsons et al. 2008). Our results suggest that sewage pollution is not stimulating
505 algal overgrowth of the coral, although an earlier Puakō study suggests that algal
506 overgrowth may contribute to or exacerbate declining reef health (Couch et al. 2014a).
507 **N Sources and Loading**
508 Macroalgal tissue $\delta^{15}N$ values are commonly used to determine N sources to
509 coastal areas (Umezawa et al. 2002, Savage 2005, Lin et al. 2007, Dailer et al. 2012,
510 Wiegner et al. 2016). They can also be used in conjunction with %N to evaluate coastal N
511 loading, on a relative scale (low, medium, high) (Barr et al. 2013, Amato et al. 2016).
512 Generally, highly enriched $\delta^{15}N$ macroalgal values (+7 to +20‰ and higher) are
513 indicative of sewage pollution (*reviewed in* Wiegner et al. 2016), and these are most

514 commonly observed along the shoreline (Derse et al. 2007, Dailer et al. 2012, García-
515 Sanz et al. 2011). Macroalgal tissue %N is indicative of the recent nutritional history of a
516 plant, reflecting N availability to the algae over short time scales (days to weeks)
517 (Atkinson & Smith 1983). This higher %N in the macroalgal tissue reflects a plant's
518 exposure to higher N loadings (Barr et al. 2013, Amato et al. 2016). Like $\delta^{15}N$, %N
519 values are generally higher along the shoreline or near offshore pollution sources (García-
520 Sanz et al. 2010, García-Sanz et al. 2011, Barr et al. 2013, Amato et al. 2016).
521 In our study, $\delta^{15}N$ macroalgal values in shoreline and offshore surface and benthic
522 waters fell within the range for soil, seawater, and low elevation groundwater NO_3^-. This
523 assessment agrees with the $\delta^{15}N$ range reported for macroalgae exposed to N from
524 fertilizer/natural/mixed N sources (Amato et al. 2016). In a concurrent study (Abaya et
525 al. 2018), six out of 16 shoreline stations in Puakō had macroalgal $\delta^{15}N$ within the
526 sewage range; note, our five stations in this study are included in this number, and two of
527 them were previously reported to be in the sewage range. Additionally, our shoreline *U.*
528 *fasciata* $\delta^{15}N$ values may be underestimated by up to ~6‰ due to the high NO_3^-
529 concentrations (> 10 µmol/L). Under these conditions, macroalgae discriminate more
530 between ^{15}N and ^{14}N during nutrient uptake (Swart et al. 2014). If this occurred in our
531 study, then all of our shoreline $\delta^{15}N$ macroalgal values fall within sewage range
532 determined for Puakō (Abaya et al. 2018). Dye tracer studies at Puakō have confirmed
533 the presence of sewage at several locations along the shoreline (Abaya et al. 2018,
534 Colbert et al. unpubl. data), including one station in this current study. Thus, our
535 shoreline $\delta^{15}N$ macroalgal tissue values likely reflect sewage contamination.
536 Unfortunately, the relative percent contribution of sewage to the shoreline N pollution
537 load at Puakō cannot be determined from macroalgal $\delta^{15}N$ measurements alone, and this
538 information is crucial for assessing current and future N inputs from sewage, especially
539 following a sewage collection and treatment upgrade project. To obtain this information,
540 a study measuring $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- in coastal waters and N sources using a mixing
541 model to partition out N sources' contributions is needed (Xue et al. 2009, Wiegner et al.
542 2016).

While we could not partition out the N load to Puakō's shoreline and offshore surface and benthic waters with our macroalgal $\delta^{15}N$ measurements, %N in the macroalgal tissues was used to assess the relative N loading. %N decreased offshore in both surface and benthic waters (Fig. 4B), and was correlated with $\delta^{15}N$ of the deployed *U. fasciata* tissue, TDN, NH_4^+, and NO_3^-+NO_2^- (Tables 2, 3). These results illustrate that macroalgal tissue %N reflected N concentrations in surface and benthic waters at Puakō, and that it is a good indicator of water quality conditions. Accordingly, macroalgal %N tissue values along Puakō's shoreline are representative of medium to high N loading, while offshore values are indicative of low to medium loading (Amato et al. 2016). Note, Amato et al.'s (2016) conceptual model does not have actual N amounts assigned to the relative N loading level categories (low, medium, and high). Our macroalgal %N tissue measurements further support our supposition that sewage is largely entering the shoreline at groundwater seeps.

Sewage pollution score mapping

Our high sewage indicator values show that the shoreline was most contaminated with sewage compared to other zones and water depths. However, these individual sewage indicator measurements do not agree on which shoreline station was the most contaminated with sewage, or how they compare to offshore surface and benthic water sampling locations. To identify potential sewage hotspots, Abaya et al. (2018)'s sewage pollution score was employed. This scoring system uses sewage indicators measured in our study, including: *Enterococcus* spp., *C. perfringens*, $\delta^{15}N$ in macroalgae tissue, and nutrient concentrations (NO_3^- + NO_2^-, NH_4^+, TDP), and applies established water quality standards and/or literature values indicative of sewage contamination to establish relative pollution levels (Abaya et al. 2018). Sewage indicator levels for each sampling location were then multiplied by a weight factor to distinguish its reliability as a sewage indicator. Sewage pollution scores were calculated using the following equation: Sewage pollution score = (*C. perfringens* level x 3) + ($\delta^{15}N$ macroalgae level x 3) + (*Enterococcus* spp. level x 2) + (NO_3^-+NO_2^- level x 1) + (NH_4^+ level x 1) + (TDP level x 1). Sewage pollution score categories were: 'low' = 11-17, 'medium' = 18-25, and 'high' = 26-33.

572 The shoreline stations had medium and low sewage pollution scores, with stations
573 1 and 3 being potential hotspots with the highest scores (Fig. 8). These stations were
574 previously identified as hotspots in Abaya et al. 2018, with station 3 as a known location
575 of a leaking OSDS. Offshore, the majority of the stations had low pollution scores, with
576 only station 1 possibly being contaminated with sewage (Fig. 8). Future studies need to
577 combine sewage pollution scores with coral health indices in order to establish stronger
578 links between sewage pollution and coral health.
579
580 **Conclusion**
581 Our study used a multi-technique approach and pollution scoring system to
582 document the offshore spatial extent of sewage pollution in surface and benthic waters of
583 a Hawaiian coral reef ecosystem. We found that sewage was largely concentrated along
584 the shoreline. However, daily tidal groundwater pulses to the benthos were detected,
585 which may be delivering sewage and other land-based pollutants to the reef. The negative
586 correlations between coral cover with FIB, macroalgal $\delta^{15}N$, and nutrient concentrations
587 support this supposition, and suggest that sewage may be contributing to the reef's
588 declining condition. Overall, our study demonstrates that measurements of multiple
589 sewage indicators, as well as physical characterization of benthic water properties are
590 necessary for assessing sewage impacts to coral reefs. Our successful approach may help
591 researchers and natural resource managers in other locations better assess the spatial
592 impacts of sewage to their reef habitats.

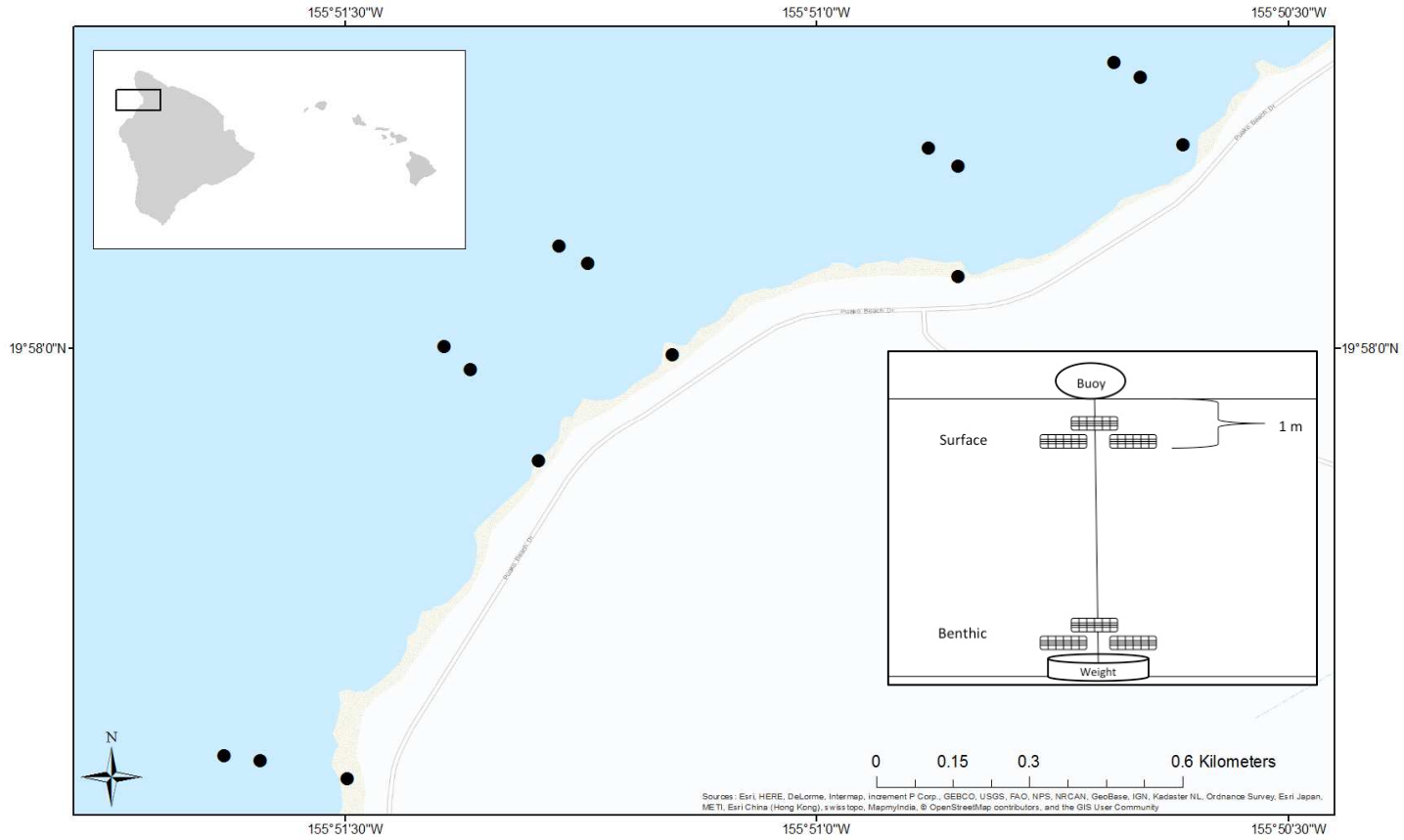

Figure 1. Location of water sample collection (for FIB and nutrients) and macroalgal cage bioassay deployments (for $\delta^{15}N$ and %N in *Ulva fasciata*) in Puakō, Hawai‘i (black circles). Water and macroalgal samples were taken at three zones (shoreline, bench, and slope) to determine the spatial extent of sewage pollution in surface and benthic waters offshore. Diagram of macroalgal cage deployment design is shown in lower right corner of figure.

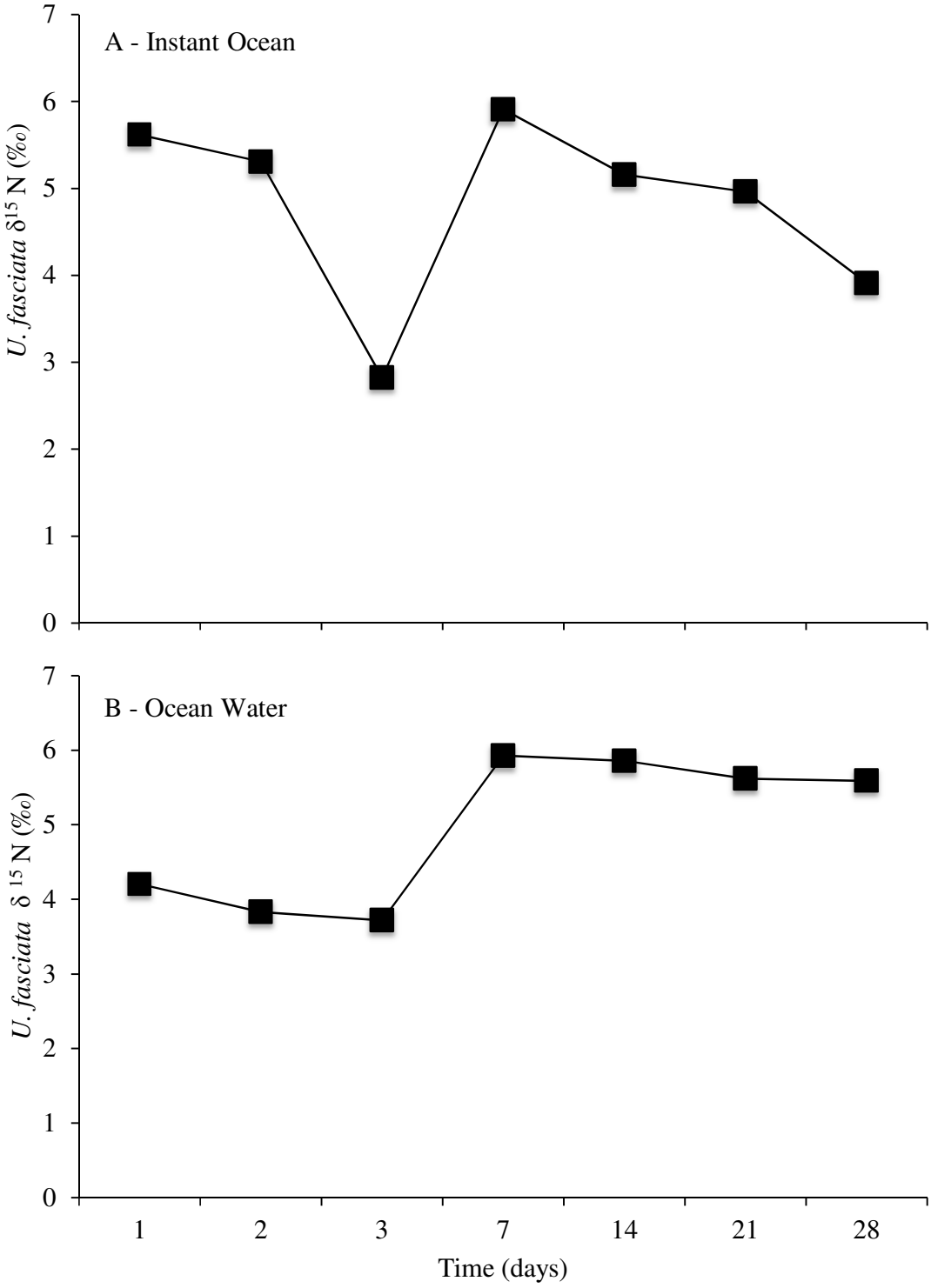

Figure 2. Changes in $\delta^{15}N$ of *Ulva fasciata* tissue during purging experiments to decrease internal stores of nitrogen using (A) Instant Ocean™ and (B) local ocean water.

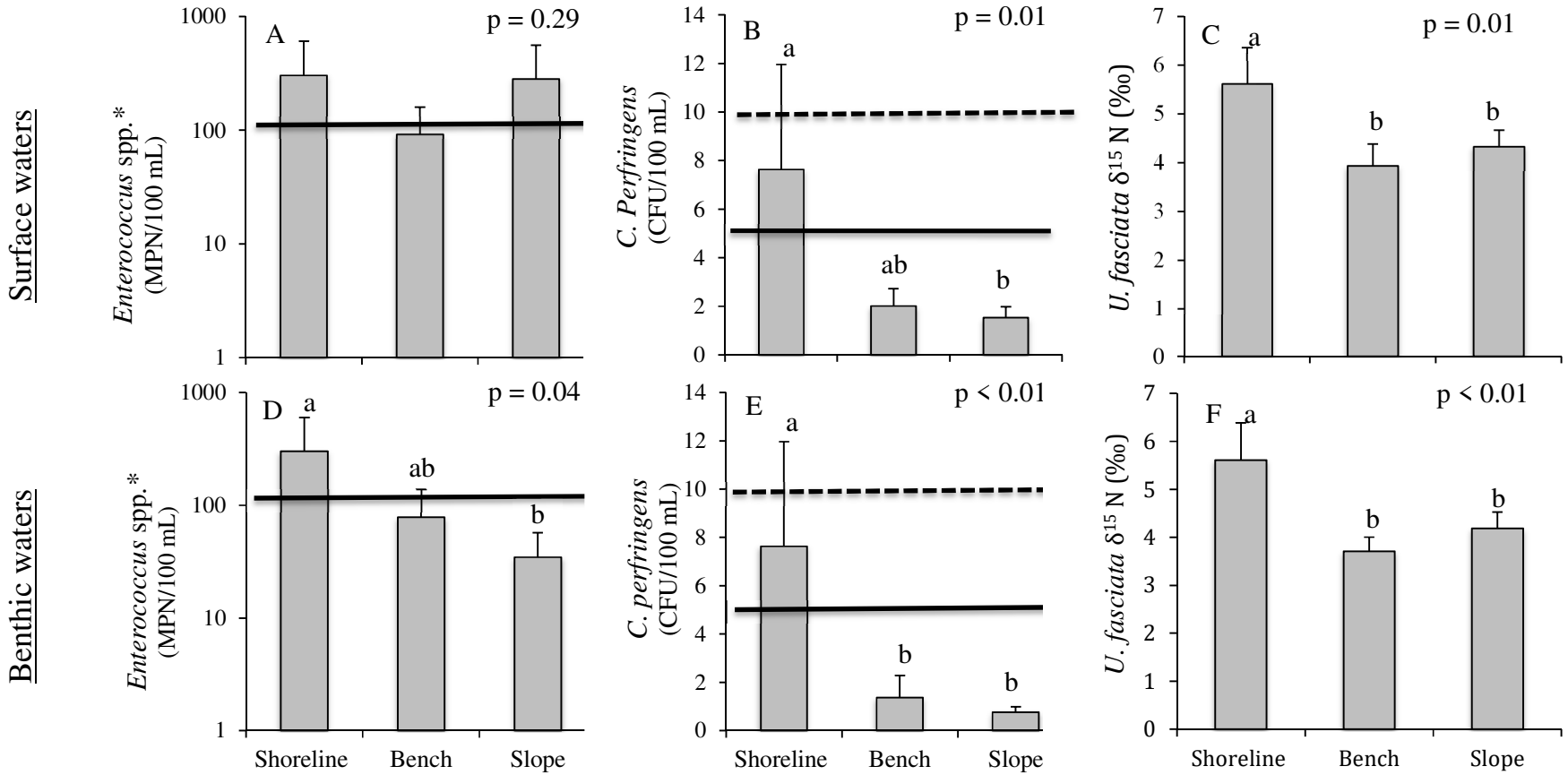

Figure 3. Average ± SE of sewage indicators (A, D) *Enterococcus* spp. (*logged scale), (B, E) *Clostridium perfringens, and* (C, F) δ^{15}N in *Ulva fasciata* collected within three zones (shoreline, bench, and slope) in both surface and benthic waters in Puakō, Hawai‘i. Black lines represent the Hawai‘i’s Department of Health’s single sample maximum for *Enterococcus* spp. (104 CFU/100 mL) and Fujioka et al.’s recommendation (1997) for *C. perfringens* in marine recreational waters (5 CFU/100 mL). Dashed lines represent non-point source sewage contamination level of 10 CFU/100 mL for *C. perfringens* (Fung et al. 2007). Results from GLM and Tukey’s test are shown, with different letters indicating significant differences ($\alpha = 0.05$). FIB n =10. Sample size varied for δ^{15}N in *U. fasciata* in both surface waters (shoreline, n =9; bench, n =6; slope, n =10) and benthic waters (shoreline, n = 9; bench, n = 8; slope, n = 10).

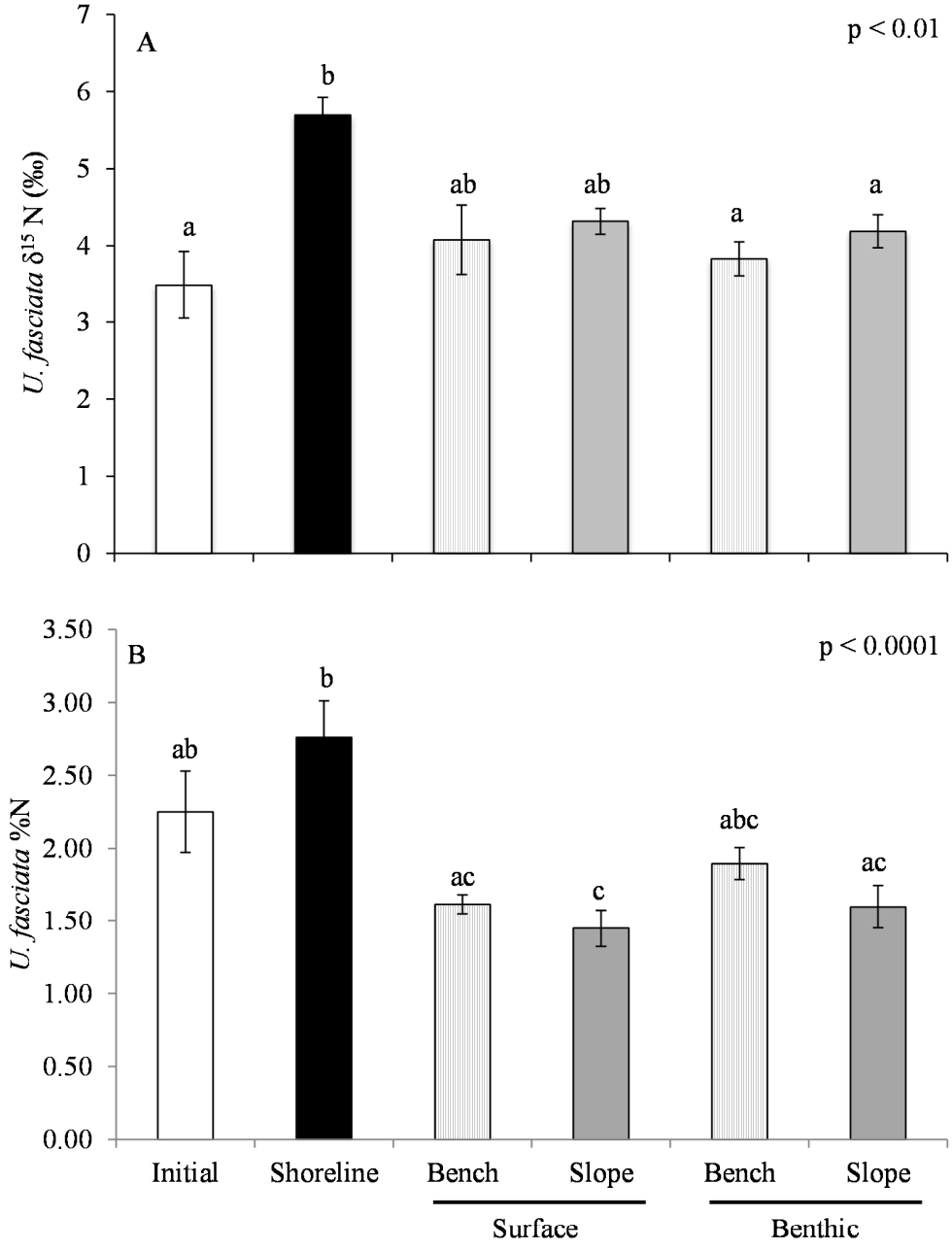

Figure 4. Average ± SE(A) δ^{15} N and (B) %N of *U. fasciata* tissue pre-(initial) and post-macroalgal bioassay deployments within three benthic zones (shoreline, bench, and slope) and two depths (surface and benthic) in Puakō, Hawai'i. Results from GLM and a one way-ANOVA are shown on figure. Shared lettering indicates no significant differences in Tukey's post hoc test. Sample size varied (initial, n = 11; shoreline, n = 5; surface bench, n = 4; surface slope, n = 5; benthic bench, n = 5; benthic slope, n = 5). α = 0.05.

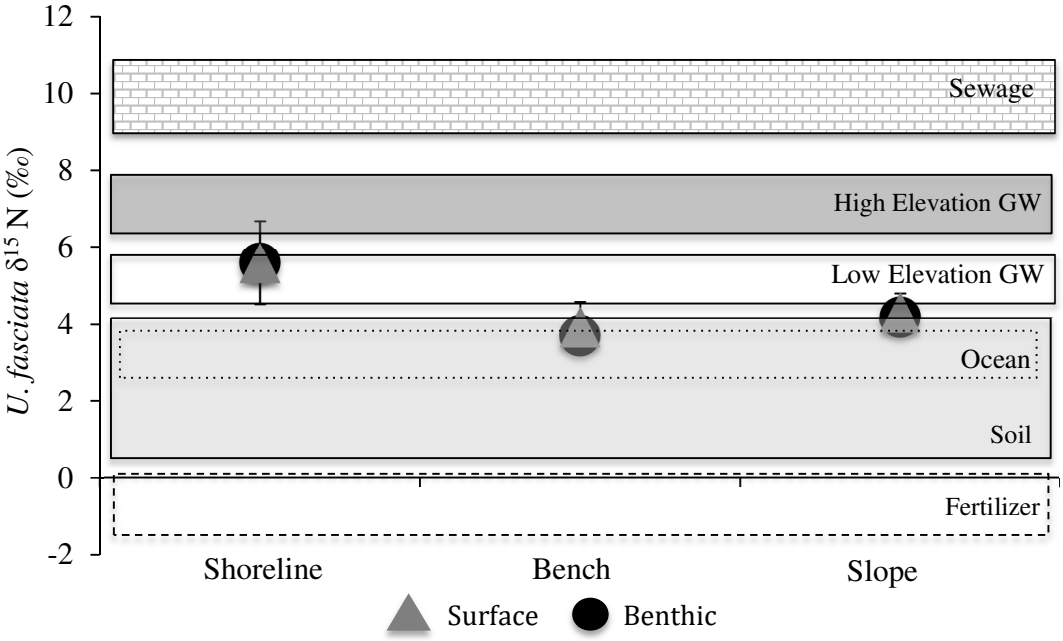

Figure 5. Average ± SE δ^{15} N (‰) of *Ulva fasciata* deployed during macroalgal cage bioassay deployments within three benthic zones (shoreline, bench, and slope) in Puakō, Hawai‘i. Background areas represent average ± SE of δ^{15} N – NO_3^- of the N sources taken in a companion study at Puakō (Abaya et al. submitted) and fertilizer from another study on Hawai‘i Island (Wiegner et al. 2016). Surface samples are represented by grey triangles and benthic samples by black circles.

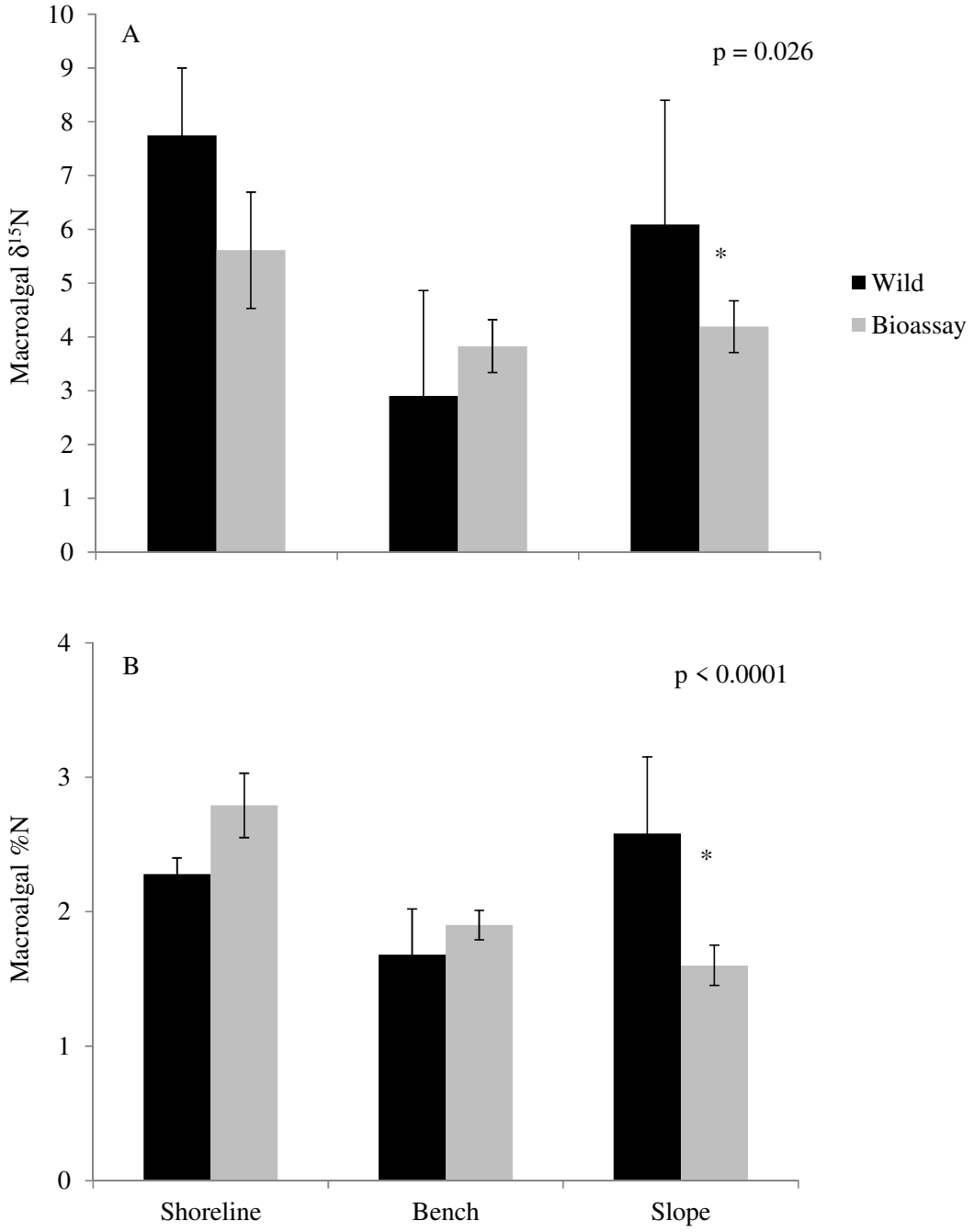

Figure 6. Average ± SE (A) δ^{15} N and (B) %N of benthic wild macroalgae (composite species samples) and post macroalgal bioassay deployment *Ulva fasciata* tissue within three benthic zones (shoreline, bench, and slope) in Puakō, Hawai‘i. Two-sample t-tests were used to compare differences between wild and deployed algae within a zone. *indicates a significant differences between wild and deployed algae (α =0.05).

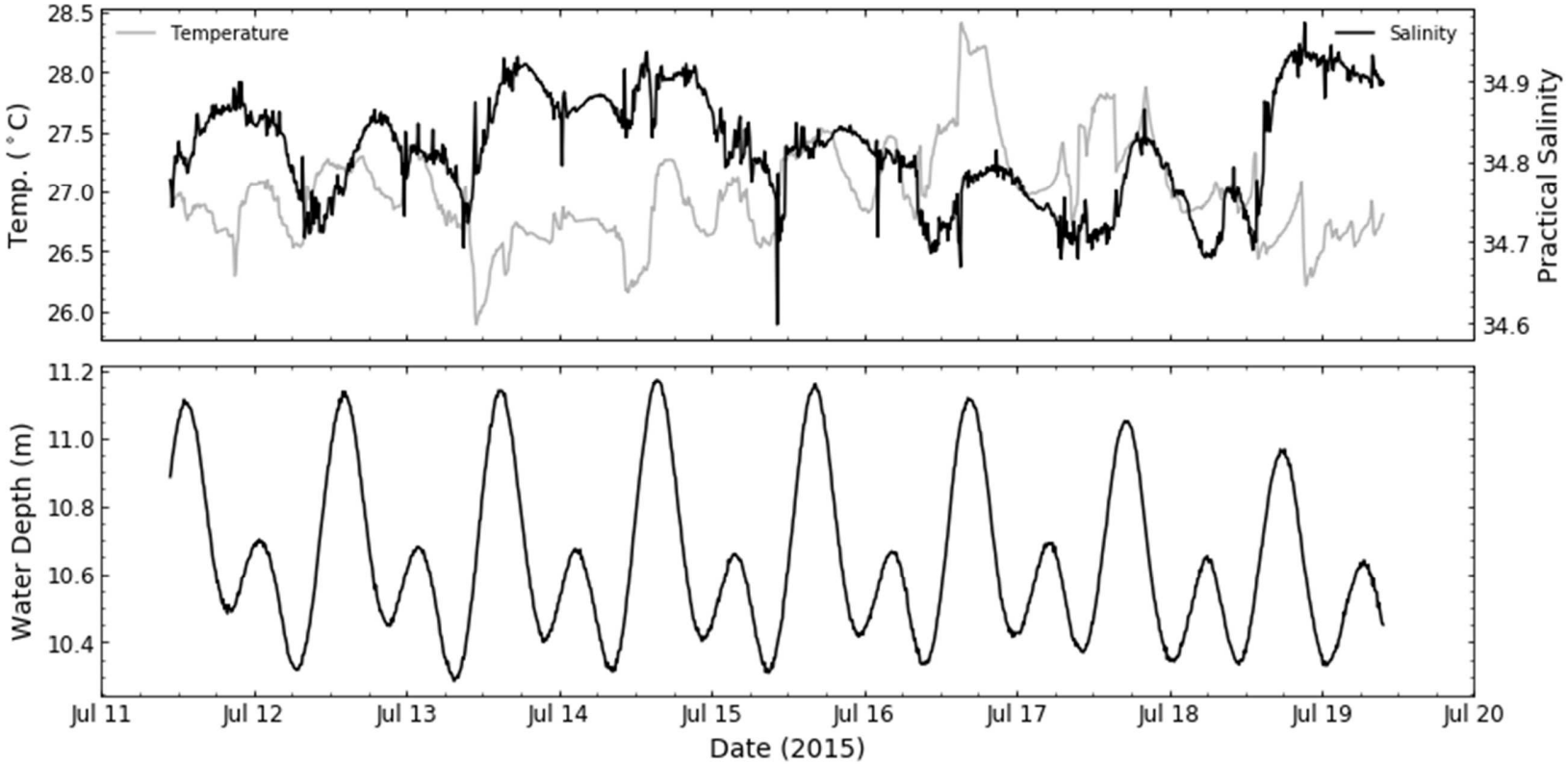

Figure 7. Benthic water properties recorded at 10 min intervals during July 2015 at Puakō, Hawai‘i, during macroalgal bioassay cage deployments. CTD was deployed at the transition zone between the bench and slope zones, at ~ 10.7 m water depth.

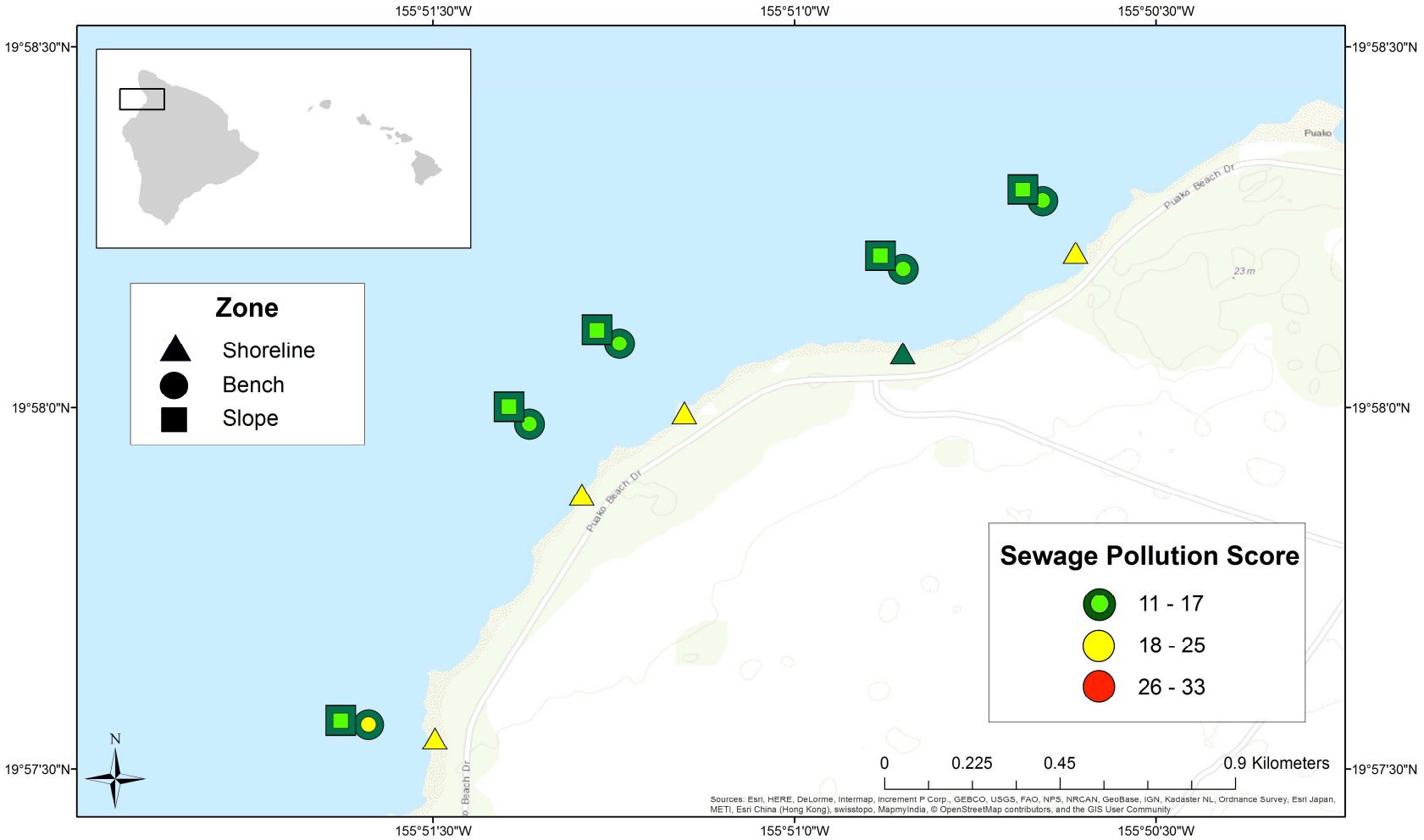

Figure 8. Map of sampling stations at Puakō, Hawai‘i, with their sewage pollution scores. Smaller shapes represent surface stations and larger ones benthic stations. Different shapes represent different zones Scores were calculated using established and recommended water quality standards and literature values for sewage indicators. Sewage pollution scores represent the following categories: Low (all shades of green) = 11 – 17; Medium (yellow) = 18 – 25; High (red) = 26 – 33.

Table 1. Average ± SE and [range] of nutrient concentrations (μmol/L) and salinity for surface and benthic water samples among zones (shoreline, bench, and slope) in Puakō, Hawai'i. A GLM was used to determine differences among zones and between depths, and superscript letters indicate grouping from post hoc Tukey's test. $\alpha = 0.05$; $n = 10$.

Zone	$NO_3^- + NO_2^-$	NH_4^+	TDN	PO_4^{3-}	TDP	H_4SiO_4	Salinity
Shoreline	66.87 ± 11.47^a	1.52 ± 0.16^a	73 ± 11^a	1.67 ± 0.22^a	1.98 ± 0.22^a	439 ± 74^a	18.52 ± 3.08^a
	[11.59 – 139.72]	[0.18 – 3.05]	[21 – 121]	[0.47 – 2.56]	[0.70 – 3.25]	[154 – 617]	[3.78 – 29.63]
Surface							
Bench	1.43 ± 0.26^b	0.57 ± 0.14^b	10 ± 1^b	0.14 ± 0.03^b	0.64 ± 0.13^b	7 ± 3^b	33.26 ± 1.11^b
	[0.83 – 1.84]	[0.18 – 1.56]	[8 – 12]	[0.02 – 0.27]	[0.25 – 1.23]	[1 – 21]	[29.95 – 34.47]
Slope	1.23 ± 0.18^b	0.38 ± 0.11^b	9 ± 1^b	0.12 ± 0.02^b	0.59 ± 0.11^b	5 ± 1^b	34.24 ± 0.41^b
	[0.40 – 2.14]	[0.18 – 1.06]	[7 – 13]	[0.02 – 0.24]	[0.25 – 0.96]	[1 – 11]	[33.75 – 34.62]
Benthic							
Bench	1.10 ± 0.13^b	0.50 ± 0.12^b	10 ± 1^b	0.18 ± 0.05^b	0.58 ± 0.11^b	2 ± 1^b	33.55 ± 0.95^b
	[0.53 – 2.06]	[0.18 – 1.23]	[7 – 13]	[0.02 – 0.49]	[0.25 – 0.94]	[1 – 5]	[31.03 – 35.00]
Slope	1.57 ± 0.51^b	1.10 ± 0.53^{ab}	9 ± 1^b	0.24 ± 0.11^b	0.94 ± 0.29^b	1 ± 0^b	34.46 ± 0.30^b
	[1.01 – 6.09]	[0.18 – 5.58]	[7 – 13]	[0.02 – 1.13]	[0.25 – 3.25]	[1 – 1]	[34.22 – 34.85]

Table 2. Correlation test results for surface water quality parameters and *Ulva faciata* tissue measurements from surface macroalgal bioassay cage deployments in Puakō, Hawai`i. $\alpha = 0.05$. *Enterococcus* = *Enterococcus* spp., *C. perfringens* = *Clostridium perfringens*.

	Statistic	Salinity	*Enterococcus*	*C. perfringens*	$\delta^{15}N$	%N	$NO_3^- + NO_2^-$	NH_4^+	TDN	PO_4^{3-}	TDP
Enterococcus	r	-0.2012									
	p	0.4721									
C. perfringens	r	-0.4360	0.5138								
	p	0.1048	0.0501								
$\delta^{15}N$	r	-0.6275	0.4720	0.3816							
	p	0.0163	0.0884	0.1782							
%N	r	-0.9311	0.3281	0.4938	0.7982						
	p	<0.0001	0.2521	0.0727	0.0006						
$NO_3^- + NO_2^-$	r	-0.6041	0.5563	0.5223	0.6051	0.7194					
	p	0.0171	0.0313	0.0458	0.0218	0.0037					
NH_4^+	r	-0.6343	0.2233	0.5376	0.4773	0.6516	0.7580				
	p	0.0111	0.4237	0.0388	0.0844	0.0116	0.0011				
TDN	r	-0.8275	0.2253	0.3654	0.5127	0.7633	0.7607	0.7875			
	p	0.0001	0.4195	0.1805	0.0608	0.0015	0.0010	0.0005			
PO_4^{3-}	r	-0.6517	0.2106	0.5159	0.4546	0.6926	0.8215	0.8541	0.8933		
	p	0.0085	0.4513	0.0490	0.1025	0.0060	0.0002	<0.0001	<0.0001		
TDP	r	-0.7558	0.1862	0.5519	0.4737	0.7325	0.6899	0.6967	0.7882	0.7576	
	p	0.0011	0.5063	0.0329	0.0871	0.0029	0.0044	0.0039	0.0005	0.0011	
H_4SiO_4	r	-0.6381	0.4679	0.5637	0.5977	0.7738	0.9393	0.7011	0.8143	0.8592	0.7703
	p	0.0105	0.0786	0.0286	0.0240	0.0012	<0.0001	0.0036	0.0002	<0.0001	0.0008

Table 3. Correlation test results for benthic water quality parameters, *Ulva faciata* tissue measurements from benthic macroalgal bioassay cage deployments, and benthic cover surveys in Puakō, Hawai`i. $\alpha = 0.05$. *Enterococcus* = *Enterococcus* spp., *C. perfringens* = *Clostridium perfringens*.

		Salinity	*Enterococcus*	*C. perfringens*	$\delta^{15}N$	%N	$NO_3^- + NO_2^-$	NH_4^+	TDN	PO_4^{3-}	TDP	H_4SiO_4	% Turf Cover
Enterococcus	r	-0.6167											
	p	0.0143											
C. perfringens	r	-0.5622	0.5747										
	p	0.0291	0.0250										
$\delta^{15}N$	r	-0.6667	0.4338	0.6424									
	p	0.0066	0.1062	0.0098									
%N	r	-0.7536	0.3037	0.3630	0.7131								
	p	0.0012	0.2712	0.1836	0.0028								
$NO_3^- + NO_2^-$	r	-0.7811	0.3730	0.4754	0.7019	0.7194							
	p	0.0006	0.1708	0.0733	0.0035	0.0037							
NH_4^+	r	-0.4508	0.1915	0.1858	0.3485	0.6516	0.7850						
	p	0.0917	0.4941	0.5074	0.2030	0.0116	0.0005						
TDN	r	-0.7321	0.2497	0.3959	0.6282	0.7633	0.7936	0.6443					
	p	0.0019	0.3694	0.1441	0.0121	0.0015	0.0004	0.0096					
PO_4^{3-}	r	-0.8043	0.4124	0.4576	0.5527	0.6926	0.8819	0.7886	0.7668				
	p	0.0003	0.1266	0.0863	0.0326	0.0060	<0.0001	0.0005	0.0009				
TDP	r	-0.4790	0.0826	0.3892	0.6672	0.7325	0.6807	0.6948	0.5201	0.6404			
	p	0.0708	0.7699	0.1516	0.0066	0.0029	0.0052	0.0040	0.0469	0.0101			
H_4SiO_4	r	-0.7491	0.3269	0.5339	0.6478	0.7738	0.6924	0.3979	0.9104	0.6996	0.3979		
	p	0.0013	0.2344	0.0404	0.0090	0.0012	0.0042	0.1419	<0.0001	0.0037	0.1419		
% Turf cover	r	0.2050	-0.2478	-0.5704	-0.7267	-0.4582	-0.2796	-0.1291	-0.3976	-0.2641	-0.4289	-0.4641	
	p	0.4635	0.3733	0.0264	0.0021	0.0859	0.3128	0.6467	0.1422	0.3414	0.1106	0.0814	
% Coral cover	r	0.7613	-0.5194	-0.6519	-0.5792	-0.7697	-0.6279	-0.3298	-0.7747	-0.6261	-0.3695	-0.8797	0.4190
	p	0.0001	0.0472	0.0084	0.0236	0.0008	0.0122	0.2313	0.0007	0.0125	0.1753	<0.0001	0.1200

Table 4. Average ±SE [range] percent benthic cover at macroalgal bioassay cage deployment locations in Puakō, Hawai'i. Macroalgal bioassay cages were deployed in June and July 2015. CCA stands for crustose coraline algae.

	Zone		
Substrate	Shoreline	Bench	Slope
Basalt	32.80 ± 0.07 [14.50 - 54.50]	0.00 ± 0.00 [0.00 - 0.00]	0.00 ± 0.00 [0.00 - 0.00]
Coral	0.00 ± 0.00 [0.00 - 0.00]	18.10 ± 5.91 [1.00 - 35.50]	38.30 ± 0.04 [22.50 - 45.00]
CCA	1.30 ± 0.01 [0.00 - 6.50]	13.90 ± 3.71 [0.00 - 20.00]	15.60 ± 0.03 [8.50- 29.50]
Turf	59.38 ± 0.06 [44.50- 77.50]	62.30 ± 6.81 [37.50 - 79.00]	42.50 ± 0.05 [32.00 - 61.50]
Macroalgae	0.00 ± 0.00 [0.00 - 0.00]	0.00 ± 0.00 [0.00 - 0.00]	0.00 ± 0.00 [0.00 - 0.00]
Limestone	2.20 ± 0.01 [0.00 - 6.50]	1.50 ± 1.26 [0.00 - 6.50]	0.00 ± 0.00 [0.00 - 0.00]
Sand	1.20 ± 0.01 [0.00 - 5.00]	3.80 ± 3.80 [0.00 - 19.00]	3.50 ± 0.03 [0.00 - 14.50]
Invertebrates	0.00 ± 0.00 [0.00-0.00]	0.40 ± 0.29 [0.00 - 1.50]	0.10 ± 0.00 [0.00 - 0.50]

Table 5. Average ±SD [variability] benthic water properties during June and July 2015 macroalgal bioassay cage deployments at Puakō, Hawai‘i. Measurements were recorded every 10 min for a total of 739 observations during June, and 1147 during July. SD is reported here instead of SE because of the large number of observations.

	Month (2015)	
Parameter	June	July
Water temp. (°C)	26.53 ± 0.20 [0.54 ± 0.30]	26.97 ± 0.42 [1.20 ± 0.33]
Salinity	34.53 ± 0.03 [0.12 ± 0.05]	34.82 ± 0.07 [0.19 ± 0.05]
Depth (m)	10.78 ± 0.25 [0.89 ± 0.02]	10.64 ± 0.24 [0.81 ± 0.06]
Water density (kg/m^3)	1022.50 ± 0.01 [0.22 ± 0.14]	1022.61 ± 0.03 [0.40 ± 0.13]

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