Marine Pollution Bulletin 130 (2018) 335–347

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Spatial distribution of sewage pollution on a Hawaiian coral reef

Leilani M. Abaya[a,b], Tracy N. Wiegner[b,*], James P. Beets[b], Steven L. Colbert[b], Kaile'a M. Carlson[b,c], K. Lindsey Kramer[b,d]

[a] Tropical Conservation Biology and Environmental Science Graduate Program, University of Hawai'i at Hilo, 200 W. Kawili St., Hilo, HI 96720, USA
[b] Marine Science Department, University of Hawai'i at Hilo, 200 W. Kawili St, Hilo, HI 96720, USA
[c] National Park Service, Kaloko-Honokōhau NHP, 73-4786 Kanalani St., #14, Kailua Kona, HI 96743, USA
[d] Pacific Cooperative Studies Unit – Hawai'i Division of Aquatic Resources, 75-308B Kealakehe Pkwy, Kailua Kona, HI 96740, USA

ARTICLE INFO

Keywords:
Coral reefs
Fecal indicator bacteria
Macroalgae
Pollution score
Sewage
Stable nitrogen isotopes

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.

1. 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 and 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 and 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 and Jarett, 2014). Elevated nutrients from sewage pollution alter coral growth and calcification rates, species distribution and abundance, and coral community diversity (Pastorok and Bilyard, 1985; Reopanichkul et al., 2009). These nutrients are also associated with benthic phase shifts from coral- to maroalgal dominated reefs (Hunter and 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 (Harinda and 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 and Shizumura, 1985; Harinda and 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

* Corresponding author.
E-mail addresses: labaya@hawaii.edu (L.M. Abaya), wiegner@hawaii.edu (T.N. Wiegner), beets@hawaii.edu (J.P. Beets), colberts@hawaii.edu (S.L. Colbert), kailea_carlson@nps.gov (K.M. Carlson), k.lindsey.kramer@gmail.com (K.L. Kramer).

https://doi.org/10.1016/j.marpolbul.2018.03.028
Received 22 September 2017; Received in revised form 16 March 2018; Accepted 16 March 2018

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 and Littler, 1980; Abbott and 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 and 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 and 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.

2. Materials and methods

2.1. 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 (49), septic tanks (66), and aerobic/anaerobic treatment units (ATU, 23); there are 21 lots where the type of OSDS is unknown and 43 vacant lots (Aqua Engineering, 2015). Because of the high number of OSDS and the proximity of the homes to the ocean, Puakō is considered a high-risk area where sewage can affect nearshore waters (Whittier and El-Kadi, 2014). Puakō's coral reef has also been designated by the state of Hawai'i as a priority site for site-based action due to its rich diversity of corals (Hayes et al., 1982). Decreases in coral coverage and fish abundances over the last 40 years (Minton et al., 2012; HDAR, 2013; Kramer et al., 2016), as well as increases in coral disease prevalence and severity have been documented (Couch et al., 2014a; Yoshioka et al., 2016). The prevalence of coral growth anomalies is the highest observed in Hawai'i and greater than reported for the Indo-Pacific region (Yoshioka et al., 2016). These ecosystem changes highlight Puakō as an area of concern, and they are likely attributed, in part, to sewage pollution. While recent studies have documented sewage pollution along Puakō's shoreline (Yoshioka et al., 2016; Abaya et al., 2018), its spatial extent offshore in surface and benthic waters has not yet been determined.

The hydrological connectivity between OSDS and the nearshore environment in Puakō is quick, ranging from 5 h to 10 d (Abaya et al., 2018; Colbert et al., unpubl. data). This is largely because the fractured basalt substrate has a high permeability. In addition, while the area is arid (mean annual rainfall: 250–750 mm), submarine groundwater discharge (SGD) is high, with rates ranging from 2083 to 2730 $L\,m^{-1}\,h^{-1}$ (Paytan et al., 2006). SGD is responsible for transporting sewage effluent from the OSDS to the shoreline and benthic habitats at Puakō.

2.2. Study design

To determine the spatial extent of sewage pollution offshore of Puakō, as well as inputs from benthic seeps that could directly impact the coral reef habitat, surface and benthic waters were sampled for FIB and nutrient concentrations. Additionally, the green macroalga, *Ulva fasciata*, was deployed as a bioassay for $\delta^{15}N$ and %N analyses at five stations (Fig. 1). These stations included three zones (shoreline, bench, and reef slope) and two water depths at each zone (surface and benthic) (Fig. 1). Benthic zones were 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 > 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.

2.3. 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

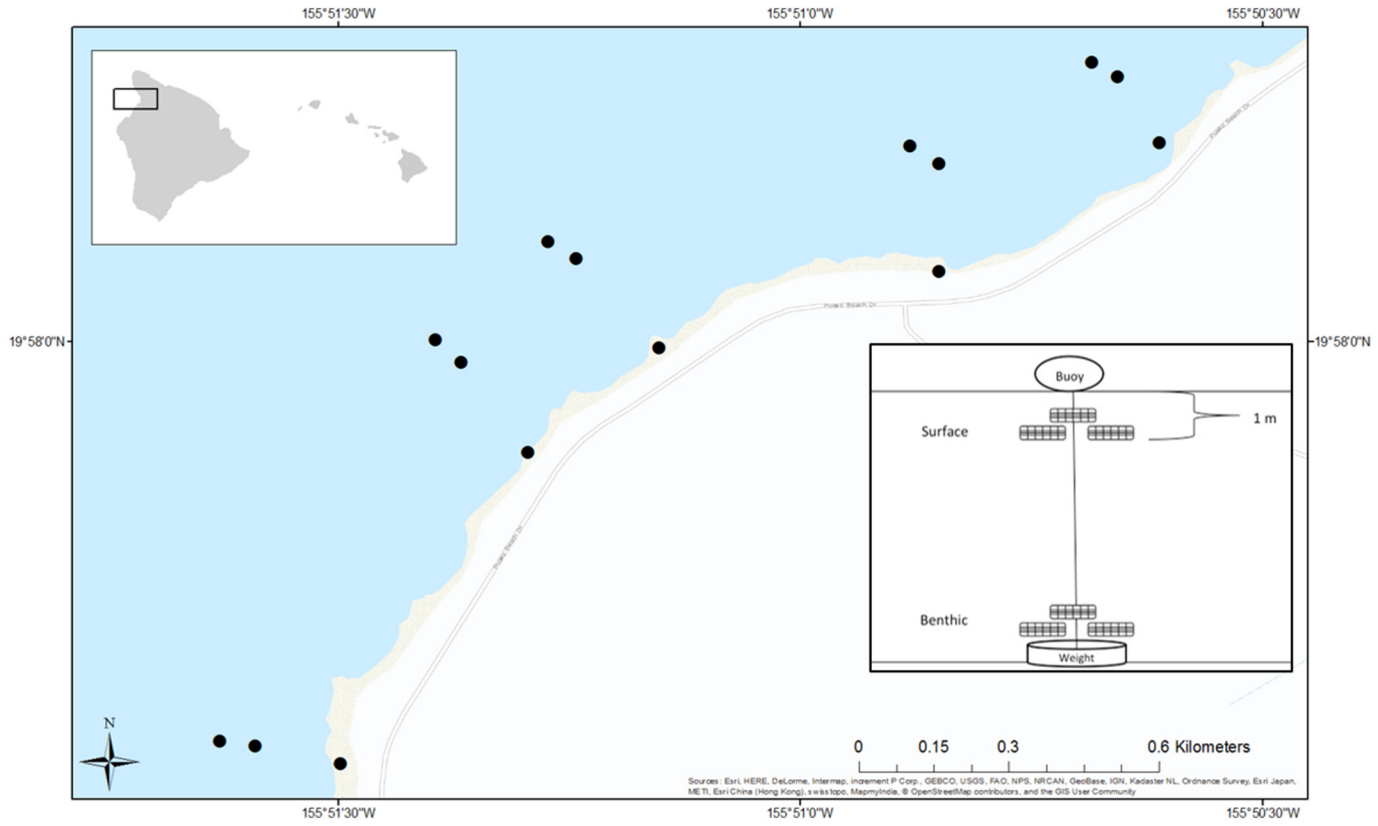

Fig. 1. Location of water sample collection (for FIB and nutrients) and macroalgal cage bioassay deployments (for $\delta^{15}N$ and %N in *Ulva fasciata*) in Puakō, Hawai'i (black circles). Water and macroalgal samples were taken at three zones (shoreline, bench, and slope) to determine the spatial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

0.45-μm pore size cellulose nitrate filters (Whatman™) and mCP medium (Acumedia, Baltimore, MD, USA) (Bisson and 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, 14,242–32, 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.

2.4. *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 × 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 4 × 6-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).

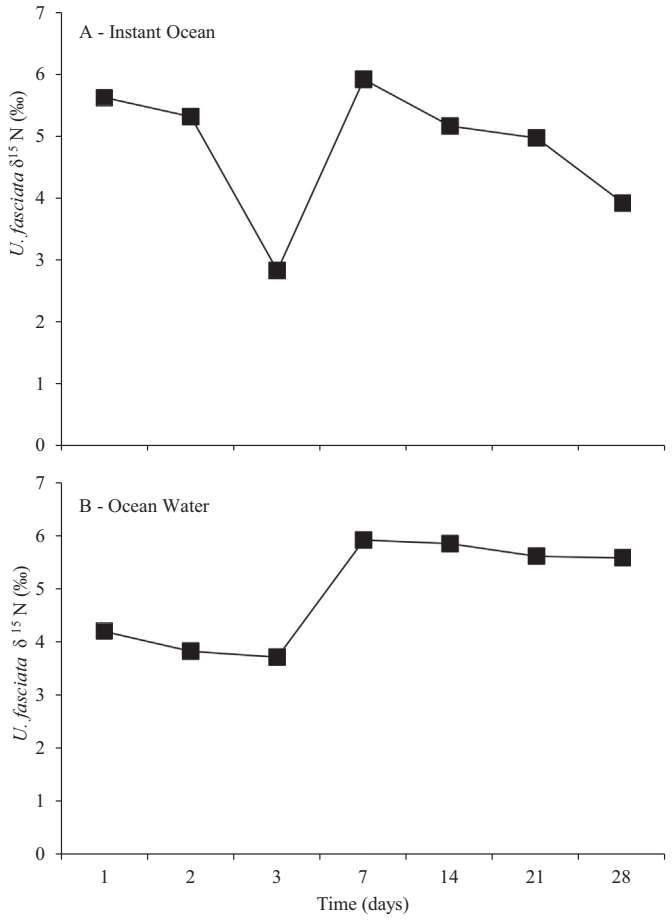

Fig. 2. Changes in $\delta^{15}N$ of *Ulva fasciata* tissue during purging experiments to decrease internal stores of nitrogen using (A) Instant Ocean™ and (B) local ocean water.

2.5. Benthic surveys

Benthic surveys were conducted at each benthic algal cage deployment station, where a 1.0-m × 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).

2.6. 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.

2.7. 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 and Roff, 1993). Statistical analyses were conducted using Minitab 16™ ($\alpha = 0.05$).

3. Results

3.1. 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 ± 0) waters (Fig. 3E). In offshore waters, *C. perfringens* concentrations were similar in surface and benthic waters.

Nutrient concentrations ($NO_3^- + NO_2^-$, NH_4^+, TDN, PO_4^{3-}, TDP, and H_4SiO_4) were highest at the shoreline ($p \leq 0.02$) (Table 1) and lower offshore, with surface and benthic waters at the bench and slope zones having similar concentrations. Salinity also varied among zones in both surface ($p < 0.01$, range = 29.95–34.62) and benthic waters ($p < 0.01$, range = 31.03–35.00), with the shoreline being the freshest (lowest) (18.52 ± 3.08). Surface water *C. perfringens* concentrations were positively correlated with most nutrient and *Enterococcus* spp. concentrations, while the latter was only positively correlated with $NO_3^- + NO_2^-$ (Table 2). All surface water nutrient concentrations were positively correlated with each other, and negatively correlated with salinity (Table 2). Benthic water *C. perfringens* concentrations were positively correlated with *Enterococcus* spp. and H_4SiO_4 concentrations (Table 3). Most benthic nutrient concentrations were positively correlated with each other, except for NH_4^+ and H_4SiO_4, and negatively correlated with salinity (Table 3).

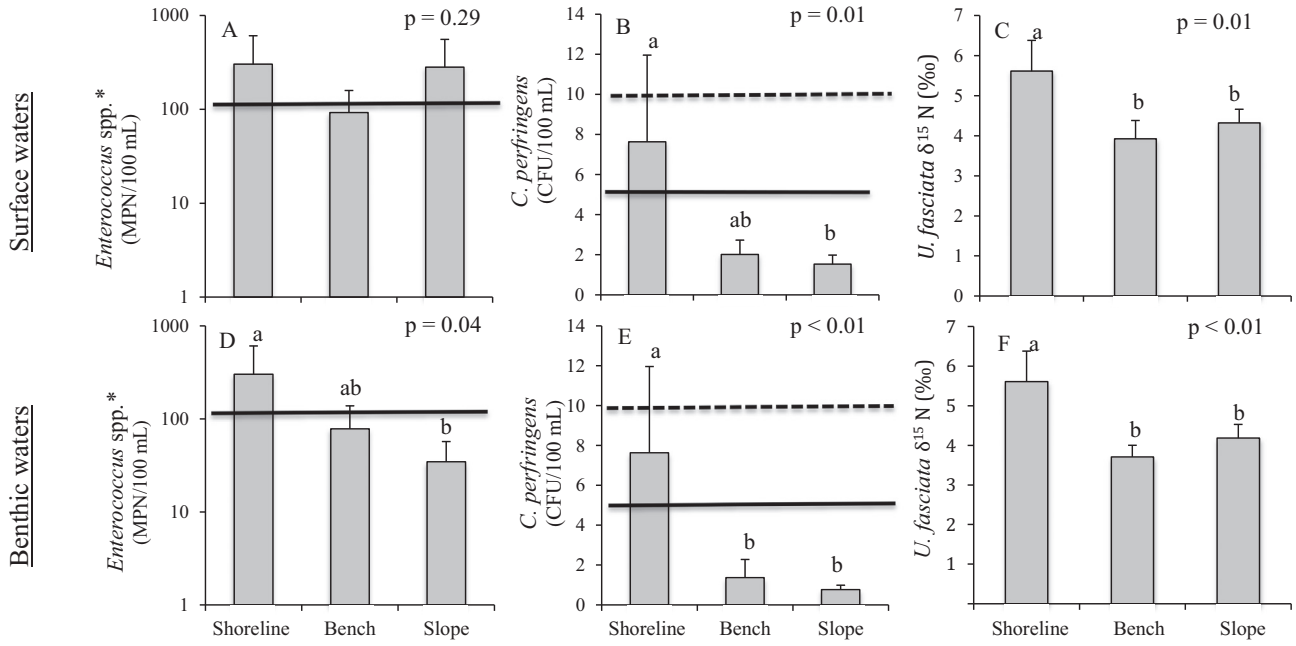

Fig. 3. Average ± SE of sewage indicators (A, D) *Enterococcus* spp. (*logged scale), (B, E) *Clostridium perfringens*, *and* (C, F) $\delta^{15}N$ in *Ulva fasciata* collected within three zones (shoreline, bench, and slope) in both surface and benthic waters in Puakō, Hawai'i. Black lines represent the Hawai'i's Department of Health's single sample maximum for *Enterococcus* spp. (104 CFU/100 mL) and Fujioka et al.'s (1997) recommendation 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 (α = 0.05). FIB $n = 10$. Sample size varied for $\delta^{15}N$ 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).

Initial and post-bioassay $\delta^{15}N$ and %N in *U. fasciata* values differed among zones. Both $\delta^{15}N$ and %N differed at the shoreline ($p \leq 0.01$), where post-bioassay values were ~2‰ and 0.5% higher, respectively (Fig. 4). Post-bioassay $\delta^{15}N$ and %N in *U. fasciata* varied significantly by zone in both surface ($p < 0.01$) and benthic waters ($p < 0.01$) (Fig. 3C, F; Fig. 4). Shoreline values were the highest (5.61‰ ± 0.77, 2.76% ± 0.47) compared to slope (surface = 4.32‰ ± 0.34, 1.45% ± 0.13; benthic = 4.18‰ ± 0.34, 1.60% ± 0.15) and bench (surface = 3.92‰ ± 0.46, 1.62% ± 0.07; benthic = 3.71‰ ± 0.29, 1.90% ± 0.11). In offshore waters, $\delta^{15}N$ and %N were similar in surface and benthic waters. Values for both surface and benthic $\delta^{15}N$ for *U. fasciata* samples fell within the $\delta^{15}N$ - NO_3^- range for soil, seawater, and low elevation groundwater at Puakō (Abaya et al., 2018) (Fig. 5). Surface *U. fasciata* $\delta^{15}N$ and %N were both positively correlated with each other, as well as $NO_3^- + NO_2^-$ and H_4SiO_4, and negatively correlated with salinity (Table 2). In addition, surface *U. fasciata* %N was positively correlated with the remaining nutrients (NH_4^+, TDN, PO_4^{3-}, and TDP; Table 2). Benthic *U. fasciata* $\delta^{15}N$ and %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).

3.2. 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).

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

3.3. 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 ($p = 0.120$).

3.4. 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).

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. α = 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

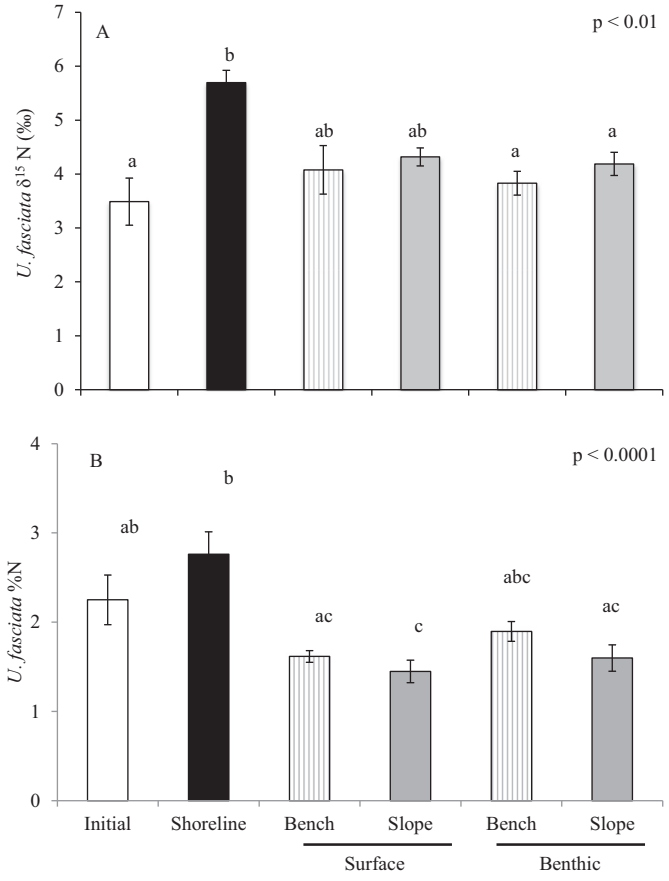

Fig. 4. Average ± SE(A) $\delta^{15}N$ and (B) %N of *U. fasciata* tissue pre-(initial) and post-macroalgal bioassay deployments within three benthic zones (shoreline, bench, and slope) and two depths (surface and benthic) in Puakō, Hawai'i. Results from GLM and a one way-ANOVA are shown on figure. Shared 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). $\alpha = 0.05$.

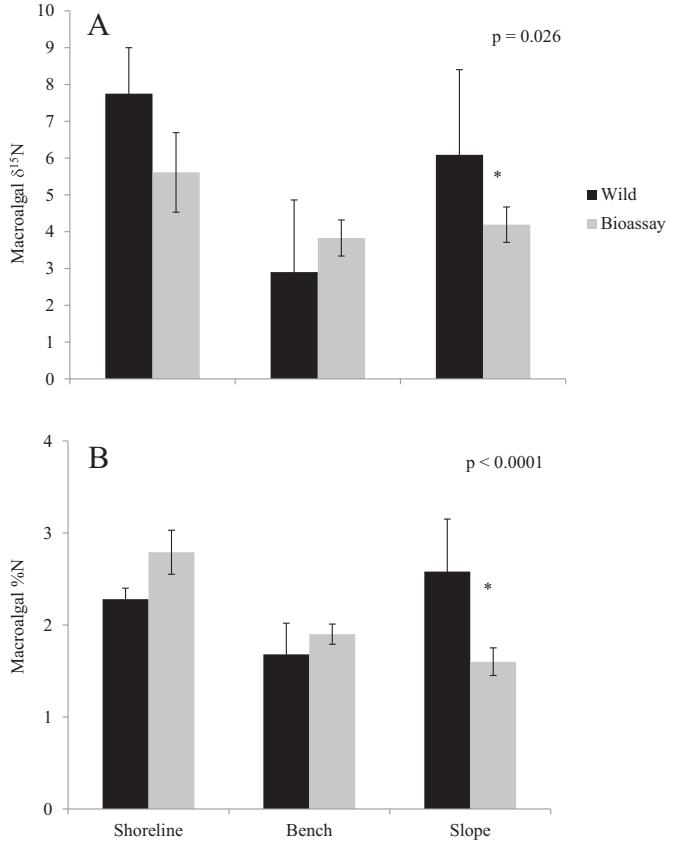

Fig. 6. Average ± SE (A) $\delta^{15}N$ and (B) %N of benthic wild macroalgae (composite species samples) and post macroalgal bioassay deployment *Ulva fasciata* tissue within three benthic zones (shoreline, bench, and slope) in Puakō, Hawai'i. Two-sample *t*-tests were used to compare differences between wild and deployed algae within a zone. * indicates a significant differences between wild and deployed algae ($\alpha = 0.05$).

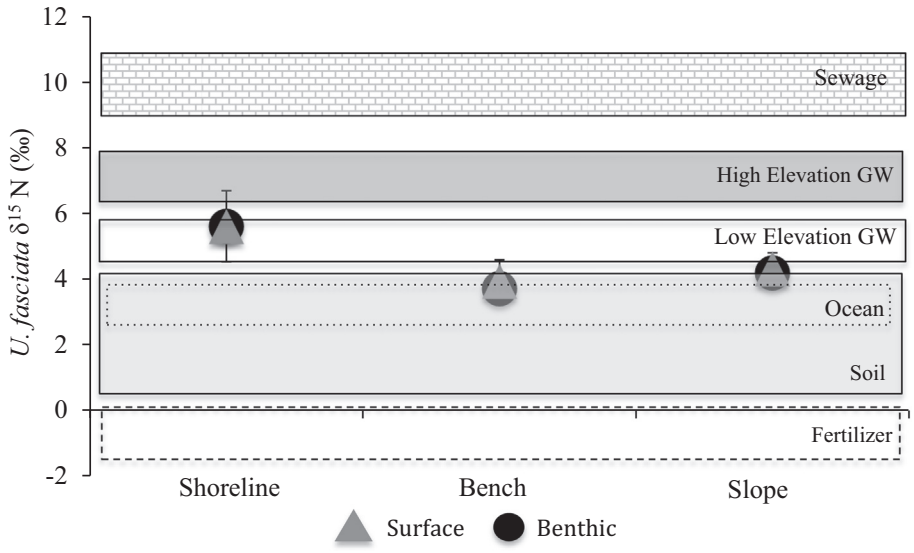

Fig. 5. Average ± SE $\delta^{15}N$ (‰) of *Ulva fasciata* deployed during macroalgal cage bioassay deployments within three benthic zones (shoreline, bench, and slope) in Puakō, Hawai'i. Background areas represent average ± SE of $\delta^{15}N{-}NO_3^-$ of the N sources taken in a companion study at Puakō (Abaya et al., 2018) and fertilizer from another study on Hawai'i Island (Wiegner et al., 2016). Surface samples are represented by gray triangles and benthic samples by black circles.

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.

Substrate	Zone		
	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.

Parameter	Month (2015)	
	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]

4. Discussion

4.1. 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, Griffin et al., 1999, Shibata et al., 2004, Lisle et al., 2004; Bonkosky et al., 2009). 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

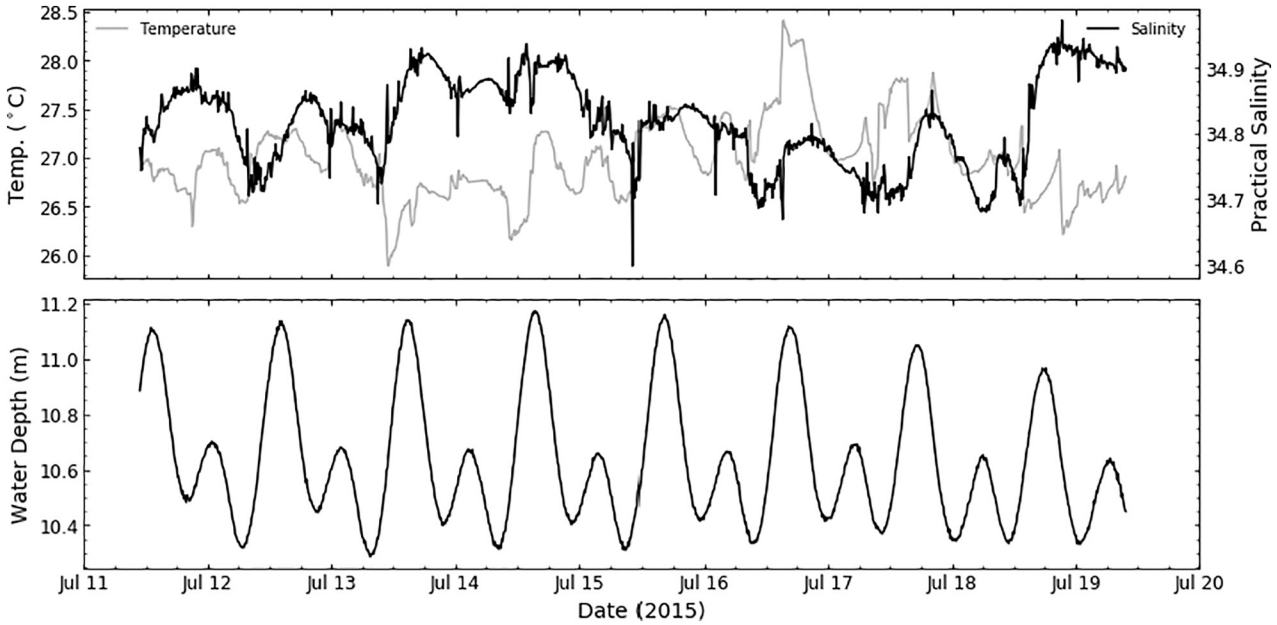

Fig. 7. Benthic water properties recorded at 10 min intervals during July 2015 at Puakō, Hawai'i, during macroalgal bioassay cage deployments. CTD was deployed at the transition zone between the bench and slope zones, at ~10.7 m water depth.

they rarely exceeded it (Yoshioka et al., 2016). Difference in findings between these studies may be related to the time of day at which the samples were collected as sunlight can inactivate FIB cells (Fujioka et al., 1981). Additionally, averages across all zones in surface and benthic waters were higher than HDOH's geometric mean standard for marine recreational waters of 35 CFU/ 100 mL, where a water user's chance of contracting gastroenteritis is 3.6% (Fujioka et al., 2015). Shoreline *C. perfringens* concentrations also exceeded the recommended standard to HDOH for marine recreational waters of 5 CFU/100 mL (Fig. 3B, Fujioka et al., 1997), and fell within the range reported for non-point source sewage pollution (10–100 CFU/100 mL, Fung et al., 2007). These results suggest that Puakō's shoreline and surface waters, as well as some benthic waters may be contaminated with sewage.

Similar to FIB, nutrient concentrations are often spatially variable within water bodies with sewage pollution, with highest values generally observed along shorelines where homes have OSDS, including those in Hawai'i (Wei and Huang, 2010; Nelson et al., 2015; Amato et al., 2016; Wiegner et al., 2016). Our study and a concurrent one found that nutrient concentrations at Puakō followed this pattern, with highest concentrations documented along the shoreline, and then decreasing with increasing distance offshore (Couch et al., 2014b). Surface water nutrient concentrations were correlated with both *Enterococcus* spp. and *C. perfringens* concentrations suggesting they may be from sewage (Table 2), whereas benthic nutrient concentrations were not correlated with FIB (Table 3). Compared to most other studies conducted in Hawai'i, shoreline $NO_3^- + NO_2^-$ concentrations were five to ten times greater than those reported on other islands or locations on Hawai'i Island with known sewage inputs (Wiegner et al., 2013; Nelson et al., 2015; Wiegner et al., 2016; Wiegner et al., 2017). Additionally, shoreline nutrient concentrations ($NO_3^- + NO_2^-$, NH_4^+, and PO_4^{3+}) measured at Puakō as part of this study and Abaya et al., 2018 are the highest reported to date, and in some cases, they are 40 times greater than previously reported values (Knee et al., 2010). Salinity measurements indicated that SGD was greatest along the shoreline where nutrient concentrations were highest, but that SGD was also transported offshore in surface waters and discharging at 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 and 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 and 355 μmol/L (Abaya et al., 2018). These land-based nutrients likely support primary production on the reef and may explain some of the more enriched $\delta^{15}N$ macroalgal values in the benthos. Similarly, any of the other pollutants from OSDS can also impact the reef at this tidal frequency. The tidal drop in water column height is unlikely to be responsible for transporting the fresher surface water to the benthos as the salinity gradient at 10 m water depth is about 0.01/m, similar to other West Hawai'i sites with high SGD (Grossman et al., 2010, Wiegner et al. unpubl. data). Instead, turbulent processes, including wave action, strong tidal currents, and winds that can enhance vertical mixing and reduce stratification, were more likely responsible for the observed patterns (Simpson et al., 1990; Jones et al., 2008). Alternatively, downward secondary flows may develop as a result of wave action and periodic reef topography (Rogers et al., 2015).

4.2. Sewage indicator patterns in surface and benthic waters

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

4.3. Sewage indicators associations with benthic cover

One way to investigate if sewage pollution may be impacting coral reefs is to examine associations of benthic cover or coral health with sewage indicators. Surprisingly, there are only a few studies that have conducted this type of analysis (Parsons et al., 2008; Baker et al., 2010; Redding et al., 2013; Amato et al., 2016; Yoshioka et al., 2016). At Puakō, we found that percent coral cover was significantly and negatively correlated with both FIB, macroalgal $\delta^{15}N$ and %N, and several nutrients (Table 3). This result concurs with an earlier Puakō study that found a strong negative relationship between coral cover and benthic-deployed macroalgal $\delta^{15}N$ (Yoshioka et al., 2016), and another study in West Hawai'i which found a positive relationship between macroalgal $\delta^{15}N$ and percent dead coral cover (Parsons et al., 2008). Together, these findings suggest sewage pollution may be contributing to the declining coral cover at Puakō. Increased coral disease due to sewage pollution could be one possible contributor as macroalgae and soft coral $\delta^{15}N$ were positively related to coral disease severity in Guam (Redding et al., 2013). This relationship, however, was not observed at Puakō (Yoshioka et al., 2016), although coral growth anomaly pressure (prevalence x severity) was shown to significantly increase with $NO_3^- + NO_2^-$ concentrations (Couch et al., 2014b). Other studies have also found positive relationships between growth anomaly pressure and N concentrations (Kuta and Richardson, 2002; Kaczmarsky and Richardson, 2011). Algal overgrowth from sewage nutrients is

another possible contributor to coral cover decline, as it is commonly observed in locations where herbivore abundance has declined (Hughes, 1994; Rogers and Miller, 2006; Rodgers et al., 2015). However, macroalgal cover at Puakō was negligible, and turf and coral cover were not inversely correlated (Table 4). Additionally, benthic turf cover was negatively associated with macroalgal $\delta^{15}N$ and *C. perfringens* concentrations, and not correlated with other sewage indicators including *Enterococcus* spp. and nutrient concentrations (Table 3). Likewise, macroalgal $\delta^{15}N$ was not correlated with either percent turf algae or macroalgae cover in another West Hawai'i study (Parsons et al., 2008). Our results suggest that sewage pollution is not stimulating algal overgrowth of the coral, although an earlier Puakō study suggests that algal overgrowth may contribute to or exacerbate declining reef health (Couch et al., 2014a).

4.4. N sources and loading

Macroalgal tissue $\delta^{15}N$ values are commonly used to determine N sources to coastal areas (Umezawa et al., 2002; Savage, 2005; Lin et al., 2007; Dailer et al., 2012; Wiegner et al., 2016). They can also be used in conjunction with %N to evaluate coastal N loading, on a relative scale (low, medium, and high) (Barr et al., 2013; Amato et al., 2016). Generally, highly enriched $\delta^{15}N$ macroalgal values (+7 to +20‰ and higher) are indicative of sewage pollution (*reviewed in* Wiegner et al., 2016), and these are most commonly observed along the shoreline (Derse et al., 2007; Dailer et al., 2012; García-Sanz et al., 2011). Macroalgal tissue %N is indicative of the recent nutritional history of a plant, reflecting N availability to the algae over short time scales (days to weeks) (Atkinson and Smith, 1983). This higher %N in the macroalgal tissue reflects a plant's exposure to higher N loadings (Barr et al., 2013, Amato et al., 2016). Like $\delta^{15}N$, %N values are generally higher along the shoreline or near offshore pollution sources (García-Sanz et al., 2010; García-Sanz et al., 2011; Barr et al., 2013; Amato et al., 2016).

In our study, $\delta^{15}N$ macroalgal values in shoreline and offshore surface and benthic waters fell within the range for soil, seawater, and low elevation groundwater NO_3^-. This assessment agrees with the $\delta^{15}N$ range reported for macroalgae exposed to N from fertilizer/natural/mixed N sources (Amato et al., 2016). In a concurrent study (Abaya et al., 2018), six out of 16 shoreline stations in Puakō had macroalgal $\delta^{15}N$ within the sewage range; note, our five stations in this study are included in this number, and two of them were previously reported to be in the sewage range. Additionally, our shoreline *U. fasciata* $\delta^{15}N$ values may be underestimated by up to ~6‰ due to the high NO_3^- concentrations (> 10 μmol/L). Under these conditions, macroalgae discriminate more between ^{15}N and ^{14}N during nutrient uptake (Swart et al., 2014). If this occurred in our study, then all of our shoreline $\delta^{15}N$ macroalgal values fall within sewage range determined for Puakō (Abaya et al., 2018). Dye tracer studies at Puakō have confirmed the presence of sewage at several locations along the shoreline (Abaya et al., 2018, Colbert et al. unpubl. data), including one station in this current study. Thus, our shoreline $\delta^{15}N$ macroalgal tissue values likely reflect sewage contamination. Unfortunately, the relative percent contribution of sewage to the shoreline N pollution load at Puakō cannot be determined from macroalgal $\delta^{15}N$ measurements alone, and this information is crucial for assessing current and future N inputs from sewage, especially following a sewage collection and treatment upgrade project. To obtain this information, a study measuring $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- in coastal waters and N sources using a mixing model to partition out N sources' contributions is needed (Xue et al., 2009; Wiegner et al., 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.

4.5. 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^+, and 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 × 3) + ($\delta^{15}N$ macroalgae level × 3) + (*Enterococcus* spp. level × 2) + ($NO_3^- + NO_2^-$ level × 1) + (NH_4^+ level × 1) + (TDP level × 1). Sewage pollution score categories were: "low" = 11–17, "medium" = 18–25, and "high" = 26–33.

The shoreline stations had medium and low sewage pollution scores, with stations 1 and 3 being potential hotspots with the highest scores (Fig. 8). These stations were previously identified as hotspots in Abaya et al., 2018, with station 3 as a known location of a leaking OSDS. Offshore, the majority of the stations had low pollution scores, with only station 1 possibly being contaminated with sewage (Fig. 8). Future studies need to combine sewage pollution scores with coral health indices in order to establish stronger links between sewage pollution and coral health.

5. Conclusion

Our study used a multi-technique approach and pollution scoring system to document the offshore spatial extent of sewage pollution in surface and benthic waters of a Hawaiian coral reef ecosystem. We found that sewage was largely concentrated along the shoreline. However, daily tidal groundwater pulses to the benthos were detected, which may be delivering sewage and other land-based pollutants to the reef. The negative correlations between coral cover with FIB, macroalgal $\delta^{15}N$, and nutrient concentrations support this supposition, and suggest that sewage may be contributing to the reef's declining condition. Overall, our study demonstrates that measurements of multiple sewage indicators, as well as physical characterization of benthic water properties are necessary for assessing sewage impacts to coral reefs. Our successful approach may help researchers and natural resource managers in other locations better assess the spatial impacts of sewage to their reef habitats.

Acknowledgements

We are grateful to J. Panelo, D. Aguiar, C. Wung, B. Tonga, L. Economy, L. Muehlstein and B. Velez-Gamez for their assistance in the

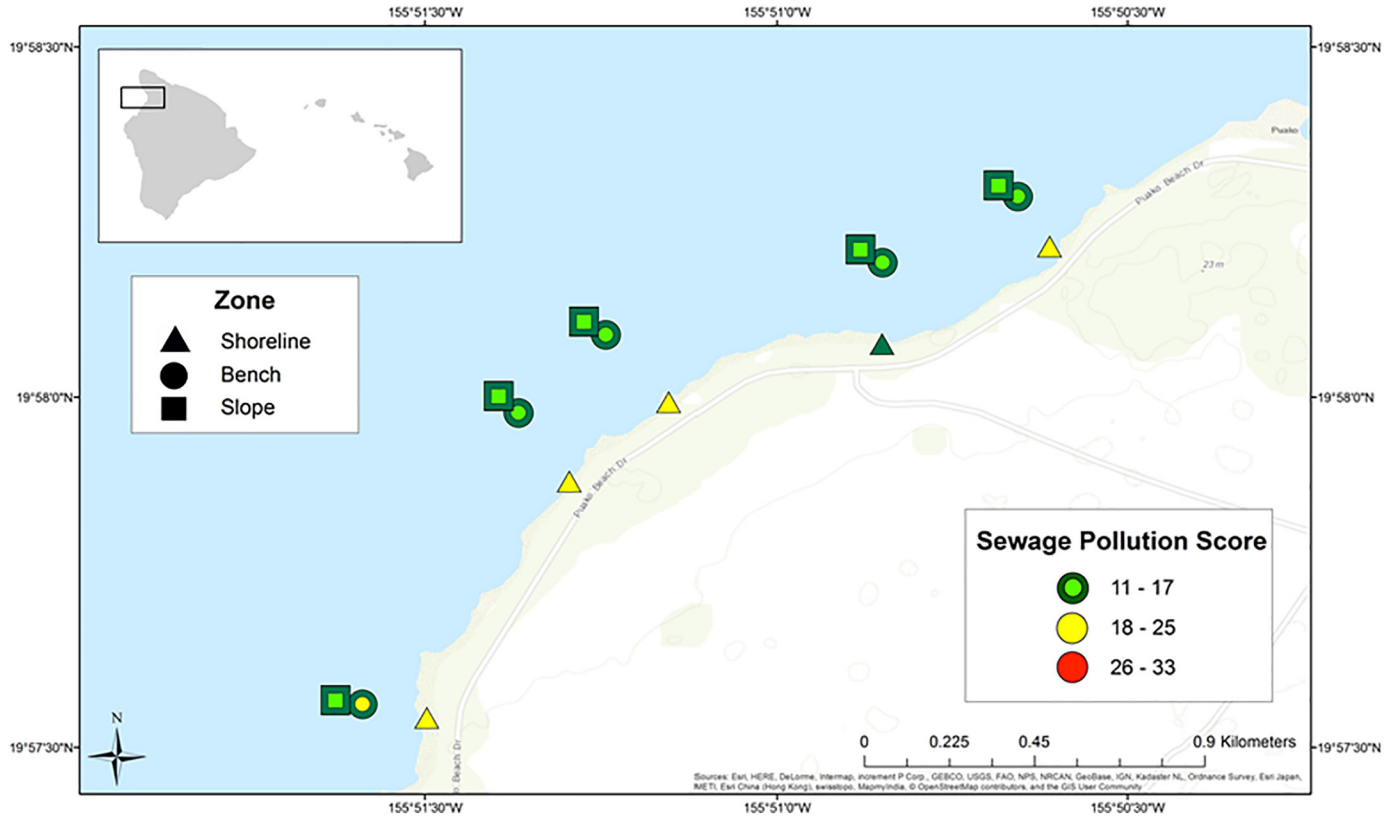

Fig. 8. Map of sampling stations at Puakō, Hawai'i, with their sewage pollution scores. Smaller shapes represent surface stations and larger ones benthic stations. Different shapes represent different zones. Scores were calculated using established and recommended water quality standards and literature 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

field and laboratory. M. Bell, S. Annandale, K. Pascoe, A. Pugh, T. Phelps, K. Chikasuye, K. Brown, J. Rose, and J. Stewart for diving and boat support. K. McDermid, J. Awaya, and an anonymous reviewer for their reviews of this manuscript, and Puakō community members, P. Hackstedde, K. Anderson, and G. Robertson, for logistical support and lodging. This paper is funded by a grant/cooperative agreement from the National Oceanic and Atmospheric Administration (NOAA), Project No. NA14NOS4820087. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. Undergraduate research assistants' support was provided by UH Hilo's Pacific Internships Program for Exploring Science (PIPES, NSF Grant No. 1005186, 1461301), and the UH Hilo Marine Science Department. Graduate student support was provided by the Puakō Community Association and Kamehameha Schools.

References

Abaya, L.M., Wiegner, T.N., Colbert, S.L., Beets, J., Carlson, K.M., Kramer, K.L., Most, R., Couch, C., 2018. A multi-indicator approach for identifying shoreline sewage pollution hotspots adjacent to coral reefs. Mar. Pollut. Bull. 129, 70–80.

Abbott, I.A., Huisman, J.M., 2004. Marine Green and Brown Algae of the Hawaiian Islands. Honolulu Abu, Hawai'i. Bishop Museum Press, Honolulu, Hawai'i.

Amato, D.W., Bishop, J.M., Glenn, C.R., Dulai, H., Smith, C.M., 2016. Impact of submarine groundwater discharge on marine water quality and reef biota of Maui. PLoS One 11 (11), e0165825.

Aqua Engineering, 2015. Puakō Hawai'i Community Feasibility Study and Preliminary Engineering Report. (Prepared for the Coral Reef Alliance).

Atkinson, M.S., Smith, S.V., 1983. C:N:P ratios of benthic marine plants. Limnol. Oceanogr. 28, 568–574.

Baker, D.M., Jordán-Dahlgren, E., Maldonado, M.A., Harvell, C.D., 2010. Sea fan corals provide a stable isotope baseline for assessing sewage pollution in the Mexican Caribbean. Limnol. Oceanogr. 55 (5), 2139–2149.

Barr, N.G., Dudley, B.D., Rogers, K.M., Cornelisen, C.D., 2013. Broad-scale patterns of tissue-δ^{15}N and tissue-N indices in frondose *Ulva* spp.; developing a national baseline indicator of nitrogen-loading for coastal New Zealand. Mar. Pollut. Bull. 67 (1), 203–216.

Bird, C., 2001. Photo grid benthic image analysis software website. Available at: http://www.photogrid.netfirms.com/, Accessed date: 15 April 2017.

Bisson, J.W., Cabelli, V.J., 1979. Membrane filter enumeration method for *Clostridium* membrane filter enumeration method for *Clostridium perfringens*. Appl. Environ. Microbiol. 37, 55–66.

Bonkosky, M., Hernandez-Delgado, E.A., Sandoz, B., Robledo, I.E., Norat-Ramrez, J., Mattei, H., 2009. Detection of spatial fluctuations of non-point source fecal pollution in coral reef surrounding waters in southwestern Puerto Rico using PCR-based assays. Mar. Pollut. Bull. 58, 45–54.

Brown, E., Minton, D., Daniel, R., Klasner, F., Basch, L., Snyder, A., Craig, P., Dicus, G., DeVerse, K., Jones, T., 2011. Pacific Island Network Benthic Marine Community Monitoring Protocol: Version 2.0. Natural Resource Report NPS/PACN/NRTR—2011/339. National Park Service, Fort Collins, Colorado.

Cabelli, V.J., 1983. Health effects criteria for marine recreational waters. U.S. Environmental Protection Agency EPA-600/1-80-031. Cincinnati, OH. http://www.epa.gov/microbes/online.htm.

Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A new approach for detecting and mapping sewage effects. Mar. Pollut. Bull. 42, 149–156.

Costanzo, S.D., Udy, J., Longstaff, B., Jones, A., 2005. Using nitrogen stable isotope ratios (δ^{15}N) of macroalgae to determine the effectiveness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. Mar. Pollut. Bull. 51, 212–217.

Couch, C.S., Garriques, J.D., Barnett, C., Preskitt, L., Cotton, S., Giddens, J., Walsh, W., 2014a. Spatial and temporal patterns of coral health and disease along leeward Hawai'i Island. Coral Reefs 33, 693–704.

Couch, C.S., Most, R., Wiggins, C., Minton, D., Conklin, E., Sziklay, J., Amimoto, R., Pollock, K., Caldwell, Z., 2014b. Understanding the Consequences of Land-based Pollutants on Coral Health in South Kohala. Final Report to Hawai'i Division of Aquatic Resources.

Dailer, M.L., Knox, R.S., Smith, J.E., Napier, M., Smith, C.M., 2010. Using δ^{15}N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Mar. Pollut. Bull. 60, 655–671.

Dailer, M.L., Ramey, H.L., Saephan, S., Smith, C.M., 2012. Algal δ^{15}N values detect a wastewater effluent plume in nearshore and offshore surface waters and three-dimensionally model the plume across a coral reef on Maui, Hawai'i, USA. Mar. Pollut. Bull. 64, 207–213.

Derse, E., Knee, K., Wankel, S., Kendall, C., Paytan, A., 2007. Identifying sources of nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae.

Environ. Sci. Technol. 41, 5217–5223.
Dudley, B., Barr, N., Shima, J.S., 2010. The influence of light intensity and nutrient source on $\delta^{13}C$ and $\delta^{15}N$ signatures in *Ulva pertusa*. Aquat. Biol. 9, 85–93.
Fujioka, R.S., Shizumura, L.K., 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J. Water Pollut. Control Fed. 57, 986–992.
Fujioka, R.S., Hashimoto, H.H., Siwak, E.B., Young, R.H., 1981. Effect of sunlight on survival of indicator bacteria in seawater. Appl. Environ. Microbiol. 41 (3), 690–696.
Fujioka, R., Roll, B., Byappanahalli, M., 1997. Appropriate recreational water quality standards for Hawai'i and other tropical regions based on concentrations of *Clostridium perfringens*. Proc. Water Environ. Fed. 4, 405–411.
Fujioka, R.S., Solo-Gabriele, H.M., Byappanahalli, M.N., Kirs, M., 2015. US recreational water quality criteria: a vision for the future. Int. J. Environ. Res. Public Health 12 (7), 7752–7776.
Fung, D.Y.C., Fujioka, R., Vijayavel, K., Sato, D., Bishop, D., 2007. Evaluation of Fung double tube test for *Clostridium perfringens* and easyphage test for f-specific RNA coliphages as rapid screening tests for fecal contamination in recreational waters of Hawai'i. J. Rapid Methods Autom. Microbiol. 15, 217–229.
García-Sanz, T., Ruiz-Fernández, J.M., Ruiz, M., García, R., González, M.N., Pérez, M., 2010. An evaluation of a macroalgal bioassay tool for assessing the spatial extent of nutrient release from offshore fish farms. Mar. Environ. Res. 70, 189–200.
García-Sanz, T., Ruiz, J., Perez, M., Ruiz, M., 2011. Assessment of dissolved nutrients dispersal derived from offshore fish-farm using nitrogen stable isotope ratios ($\delta^{15}N$) in macroalgal bioassays. Estuar. Coast. Shelf Sci. 91, 361–370.
Griffin, D.W., Gibson III, C.J., Lipp, E.K., Riley, K., Paul III, J.H., Rose, J.B., 1999. Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of the Florida Keys. Appl. Environ. Microbiol. 65, 4118–4125.
Grossman, E.E., Logan, J.B., Presto, M.K., Storlazzi, C.D., 2010. Submarine Groundwater Discharge and Fate Along the Coast of Kaloko-Honokōhau National Historical Park, Island of Hawai'i; Part 3, Spatial and Temporal Patterns in Nearshore Waters and Coastal Groundwater Plumes. December 2003–April 2006: U.S. Geological Survey Scientific Investigations Report 2010 5081 (76 p).
Harinda, C., Fujioka, R., 1991. Soil: The environmental source of *Escherichia coli* and *Enterococci* in Hawaii's streams. Environ. Toxicol. Water Qual. Ann. Int. J. 6, 185–195.
Hayes, T., Hourigan, T., Jazwinski, S., Johnson, S., Parrish, J., Walsh, D., 1982. The Coastal Resources, Fisheries and Fishery, Ecology of Puakō, West Hawai'i. 81–1. Hawai'i Cooperative Fishery Research Unit, pp. 1–245.
HDAR, 2013. South Kohala Reefs Are in Dire Straits. Public Handout.
Hughes, T.P., 1994. Catastrophes, phase shifts, and large scale degradation of a Caribbean coral reef. Science 265, 1547–1551.
Hunter, C.L., Evans, C.W., 1995. Coral reefs in Kaneohe Bay, Hawai'i: two centuries of western influence and two decades of data. Bull. Mar. Sci. 57, 501–515.
Johannes, R.E., 1980. Ecological significance of the submarine discharge of groundwater. Mar. Ecol. Prog. Ser. 3 (4), 365–373.
Johannes, R.E., Hearn, C.J., 1985. The effect of submarine groundwater discharge on nutrient and salinity regimes in a coastal lagoon off Perth, Western Australia. Estuar. Coast. Shelf Sci. 21 (6), 789–800.
Jones, N.L., Lowe, R.J., Pawlak, G., Fong, D.A., Monismith, S.G., 2008. Plume dispersion on a fringing coral reef system. Limnol. Oceanogr. 53, 2273–2286.
Kaczmarsky, L., Richardson, L.L., 2011. Do elevated nutrients and organic carbon on Philippine reefs increase the prevalence of coral disease? Coral Reefs 30 (1), 253–257.
Kaldy, J., 2011. Using a macroalgal $\delta^{15}N$ bioassay to detect cruise ship waste water effluent inputs. Mar. Pollut. Bull. 62, 1762–1771.
Knee, K.L., Layton, B.A., Street, J.H., Boehm, A.B., Paytan, A., 2008. Sources of nutrients and fecal indicator bacteria to nearshore waters on the north shore of Kaua'i (Hawai'i, USA). Estuar. Coasts 31, 607–622.
Knee, K., Street, J.H., Grossman, E.G., Paytan, A., 2010. Nutrient inputs to the coastal ocean from submarine groundwater discharge in a groundwater-dominated system: relation to land use (Kona coast, Hawai'i, U.S.A.). Limnol. Oceanogr. 55, 1105–1122.
Kramer, K.L., Cotton, S.P., Lamson, M.R., Walsh, W.J., 2016. Bleaching and Catastrophic Mortality of Reef-building Corals Along West Hawai'i Island: Findings and Future Directions. Proc. 13th Int. Coral Reef Symposium, Honolulupp. 219–230.
Kuta, K.G., Richardson, L.L., 2002. Ecological aspects of black band disease of corals: relationships between disease incidence and environmental factors. Coral Reefs 21, 393–398.
Lapointe, B.E., Barile, P.J., Littler, M.M., Littler, D.S., 2005. Macroalgal blooms on southeast Florida coral reefs: II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen. Harmful Algae 4 (6), 1106–1122.
Lesser, M.P., Jarett, J.K., 2014. Culture-dependent and culture-independent analyses reveal no prokaryotic community shifts or recovery of *Serratia marcescens* in *Acropora palmata* with white pox disease. FEMS Microbiol. Ecol. 88 (3), 457–467.
Lin, H.J., Wu, C.Y., Kao, S.J., Kao, W.Y., Meng, P.J., 2007. Mapping anthropogenic nitrogen through point sources in coral reefs using $\delta^{15}N$ in macroalgae. Mar. Ecol. Prog. Ser. 335, 95–109.
Lisle, J.T., Smith, J.J., Edwards, D.D., McFeters, G.A., 2004. Occurrence of microbial indicators and *Clostridium perfringens* in wastewater, water column samples, sediments, drinking water, and Weddell seal feces collected at McMurdo Station, Antarctica. Appl. Environ. Microbiol. 70, 7269–7276.
Littler, M.M., Littler, D.S., 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. Am. Nat. 116, 25–44.
Minton, D., Conklin, E., Weiant, P., Wiggins, C., 2012. 40 years of decline on Puakō's coral reefs - a review of historical and current data (1970–2010). Nature Conserv. 1–140.
Moynihan, M.A., Baker, D.M., Mmochi, A.J., 2012. Isotopic and microbial indicators of sewage pollution from Stone Town, Zanzibar, Tanzania. Mar. Pollut. Bull. 64, 1348–1355.
Nelson, C., Donahue, M., Dulaiova, H., Goldberg, S., La Valle, F., Lubarsky, K., Miyano, J., Richardson, C., Silbiger, N., Thomas, F., 2015. Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. Mar. Chem. 177, 232–243.
Ochoa-Izaguirre, M.J., Soto-Jiménez, M.F., 2015. Variability in nitrogen stable isotope ratios of macroalgae: consequences for the identification of nitrogen sources. J. Phycol. 51 (1), 46–65.
Parsons, M.L., Walsh, W.J., Settlemier, C.J., White, D.J., Ballauer, J.M., Ayotte, P.M., Osada, K.M., Carman, B., 2008. A multivariate assessment of the coral ecosystem health of two embayments on the lee of the island of Hawai'i. Mar. Pollut. Bull. 56, 1138–1149.
Pastorok, R.A., Bilyard, G.R., 1985. Effects of sewage pollution on coral-reef communities. Mar. Ecol. Prog. Ser. 10, 175–189.
Paul, J.H., Rose, J.B., Jiang, S., Kellogg, C., Shinn, E.A., 1995. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Appl. Environ. Microbiol. 61, 2235–2241.
Paytan, A., Shellenbarger, G.G., Street, J.H., Gonneea, M.E., Davis, K., Moore, W.S., Young, M.B., 2006. Submarine groundwater discharge: an important source of new inorganic nitrogen to coral reef ecosystems. Limnol. Oceanogr. 51, 343–348.
Pinto, B., Pierotti, R., Canale, G., Reali, D., 1999. Characterization of "faecal streptococci" as indicators of faecal pollution and distribution in the environment. Lett. Appl. Microbiol. 29, 258–263.
Potvin, C., Roff, D., 1993. Distribution-free and robust statistical methods: viable alternatives to parametric statistics. Ecology 74, 1617–1628.
Prüss, A., 1998. Review of epidemiological studies on health effects from exposure to recreational water. Int. J. Epidemiol. 27, 1–9.
Redding, J.E., Myers-Miller, R.L., Baker, D.M., Fogel, M., Raymundo, L.J., Kim, K., 2013. Link between sewage-derived nitrogen pollution and coral disease severity in Guam. Mar. Pollut. Bull. 73 (1), 57–63.
Reopanichkul, P., Schlacher, T.A., Carter, R.W., Worachananant, S., 2009. Sewage impacts coral reefs at multiple levels of ecological organization. Mar. Pollut. Bull. 58, 1356–1362.
Rodgers, K.S., Jokiel, P.L., Brown, E.K., Hau, S., Sparks, R., 2015. Over a decade of change in spatial and temporal dynamics of Hawaiian coral reef communities. Pac. Sci. 69 (1), 1–13.
Rogers, C.S., Miller, J., 2006. Permanent 'phase shifts' or reversible declines in coral cover? Lack of recovery of two coral reefs in St. John, US Virgin Islands. Mar. Ecol. Prog. Ser. 306, 103–114.
Rogers, J.S., Monismith, S.G., Dunbar, R.B., Koweek, D., 2015. Field observations of wave-driven circulation over spur and groove formations on a coral reef. J. Geophys. Res. Oceans Atmos. 120, 145–160.
Savage, C., 2005. Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. Ambio 34 (2), 145–150.
Shibata, T., Solo-Gabriele, H.M., Fleming, L.E., Elmir, S., 2004. Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. Water Res. 38, 3119–3131.
Simpson, J.H., Brown, J., Matthews, J., Allen, G., 1990. Tidal straining, density currents, and stirring in the control of estuarine stratification. Estuar. Coasts 13, 125–132.
Street, J.H., Knee, K.L., Grossman, E.E., Paytan, A., 2008. Submarine groundwater discharge and nutrient addition to the coastal zone and coral reefs of leeward Hawai'i. Mar. Chem. 109 (3), 355–376.
Sutherland, K.P., Porter, J.W., Turner, J.W., Thomas, B.J., Looney, E.E., Luna, T.P., Meyers, M.K., Futch, J.C., Lipp, E.K., 2010. Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, *Acropora palmata*. Environ. Microbiol. 12 (5), 1122–1131.
Swart, P.K., Evans, S., Capo, T., Altabet, M.A., 2014. The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate. Biogeosciences 11, 6147–6157.
Umezawa, Y., Miyajima, T., Yamamuro, M., Kayanne, H., Koike, I., 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by $\delta^{15}N$ in macroalgae. Limnol. Oceanogr. 47, 1405–1416.
Vega Thurber, R.L., Burkepile, D.E., Fuchs, C., Shantz, A.A., Mcminds, R., Zaneveld, J.R., 2014. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob. Chang. Biol. 20, 544–554.
Wang, Y., Wilson, J.M., Vanbriesen, J.M., 2015. The effect of sampling strategies on assessment of water quality criteria attainment. J. Environ. Manag. 154, 33–39.
Wear, S.L., Vega Thurber, R., 2015. Sewage pollution: mitigation is key for coral reef stewardship. Ann. N. Y. Acad. Sci. 1355, 15–30.
Wei, P., Huang, L., 2010. Water quality and eutrophication in the Guangzhou Sea Zone of the Pearl River estuary. Chin. J. Oceanol. Limnol. 28 (1), 113–121.
Whittier, R., El-Kadi, A., 2014. Human health and environmental risk ranking of on-site sewage disposal systems for the Hawaiian islands of Kauai, Molokai, Maui, and Hawai'i. http://health.hawaii.gov/wastewater/files/2015/09/OSDS_NI.pdf.
Wiegner, T.N., Mead, L.H., Molloy, S.L., 2013. A comparison of water quality between low-and high-flow river conditions in a tropical estuary, Hilo Bay, Hawai'i. Estuar. Coasts 36 (2), 319–333.
Wiegner, T.N., Mokiao-Lee, A.U., Johnson, E.E., 2016. Identifying nitrogen sources to thermal tide pools in Kapoho, Hawai'i, U.S.A, using a multi-stable isotope approach. Mar. Pollut. Bull. 103, 63–71.
Wiegner, T.N., Edens, C.J., Abaya, L.M., Carlson, K.M., Lyon-Colbert, A., Molloy, S.L., 2017. Spatial and temporal microbial pollution patterns in a tropical estuary during high and low river flow conditions. Mar. Pollut. Bull. 114 (2), 952–961.
Xue, D., Botte, J., De Baets, B., Accoe, F., Nestler, A., Taylor, P., Cleemput, O., Berglund, M., Boeckx, P., 2009. Present limitations and future prospects of stable isotope

methods for nitrate source identification in surface- and groundwater. Water Res. 43, 1159–1170.
Yoshioka, R., Kim, C.J.S., Tracey, A., Most, R., Harvell, C.D., 2016. Linking sewage pollution and water quality to spatial patterns of *Porites lobata* growth anomalies in Puakō, Hawai'i. Mar. Pollut. Bull. 104, 313–321.
Zambrano, L., Contreras, V., Mazari-Hiriart, M., Zarco-Arista, A., 2009. Spatial heterogeneity of water quality in a highly degraded tropical freshwater ecosystem. Environ. Manag. 43, 249–263.