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# Spatial and temporal microbial pollution patterns in a tropical estuary during high and low river flow conditions

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## ABSTRACT

Spatial and temporal patterns of coastal microbial pollution are not well documented. Our study examined these patterns through measurements of fecal indicator bacteria (FIB), nutrients, and physiochemical parameters in Hilo Bay, Hawai'i, during high and low river flow. >40% of samples tested positive for the human-associated *Bacteroides* marker, with highest percentages near rivers. Other FIB were also higher near rivers, but only *Clostridium perfringens* concentrations were related to discharge. During storms, FIB concentrations were three times to an order of magnitude higher, and increased with decreasing salinity and water temperature, and increasing turbidity. These relationships and high spatial resolution data for these parameters were used to create *Enterococcus* spp. and *C. perfringens* maps that predicted exceedances with 64% and 95% accuracy, respectively. Mapping microbial pollution patterns and predicting exceedances is a valuable tool that can improve water quality monitoring and aid in visualizing FIB hotspots for management actions.

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## 1. Introduction

Microbial pollution from sewage is becoming an increasing threat to recreational water users and coastal ecosystem health as the human population expands along global coastlines. While point sources of sewage, like direct disposal from outfalls and injection wells, have been regulated in coastal waters, leaks from on-site disposal systems like cesspools and septic tanks have been more difficult to control (Boehm et al., 2002; Leonard and Gilpin, 2006; Bonkosky et al., 2009). This is particularly a problem in developing nations that often lack adequate sewage treatment infrastructure, but it is also common in developed nations in rural areas (Wear and Vega Thurber, 2015). Coastal sewage pollution is acute and especially challenging to manage

in areas that experience intense seasonal runoff and are undergoing population growth and increased development (Bonkosky et al., 2009; Futch et al., 2011). Many of these areas are located in the tropics (Corcoran et al., 2010).

Currently, the most widespread method for monitoring microbial water quality involves single-sample measurements of fecal indicator bacteria (FIB) such as *Enterococcus* spp. to assess health risks of swimmers for contracting gastrointestinal illness (Cabelli, 1983; Prüss, 1998). However, this approach is limited by a > 24-h delay in test results, in addition to costly labor-intensive methods that limit sampling frequency. Meanwhile, FIB concentrations have been found to vary greatly over space and time, on even short timescales of minutes to hours (Boehm et al., 2002; Shibata et al., 2004; Boehm, 2007). One study calculated that the percent error of beach postings based on this method could be as high as 41% due to rapid fluctuations of FIB concentrations and infrequent water sampling protocols (Kim and Grant, 2004). This single-sample method can pose a risk to recreational water users when advisories are issued late, or lead to unnecessary beach closures when the water has returned to safe swimming conditions (Hou et al., 2006), resulting in economic impacts to human health and tourism revenue (NRDC, 2014).

To overcome these challenges, research efforts have focused on better understanding FIB dynamics over space and time (Shibata et al., 2004; Boehm, 2007; Coulliette et al., 2009; Enns et al., 2012; Feng et

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al., 2015). Marine FIB concentrations can vary spatially depending on the pollution source, such as surface runoff, storm drains, beach sands, watershed land use, and physical oceanographic processes, like waves and currents (Lipp et al., 2001; Knee et al., 2008; Futch et al., 2011; Tomlinson et al., 2011). These spatial variations also change over short- and long-term time scales (Kim and Grant, 2004). Daily fluctuations can occur from solar radiation due to the inactivation of FIB cells, and/or tidal influence, if contaminated beach sands are submerged (Whitman et al., 2004; Zhu et al., 2011; Enns et al., 2012). Seasonal variation can be even greater than daily variation due to storm events, which often increase FIB concentrations due to runoff (Reeves et al., 2004; He and He, 2008; Curiel-Ayala et al., 2012).

Because FIB are so variable over space and time, the ability to establish patterns has been limited to intensive sampling regimes that are not feasible for daily monitoring practices (Boehm et al., 2002; Kim and Grant, 2004; Shibata et al., 2004). Development of real-time predictive models or high resolution spatial FIB maps from relationships with parameters that can be measured quickly with environmental sensors in high frequency are two approaches being used to overcome these limitations (i.e., Frick et al., 2008a, 2008b; He and He, 2008; Coulliette et al., 2009; Brooks and Field, 2016). Real-time statistical models, like U.S. Environmental Protection Agency's (USEPA) Virtual-Beach Model Builder, are becoming increasingly accurate in their predictions of microbial water quality (i.e., Hou et al., 2006; He and He, 2008; Zhu et al., 2011; Thoe et al., 2014; Brooks and Field, 2016). However, the majority of them do not provide high resolution spatial information regarding pollution hotspots along a beach or within a waterbody. Only recently has a real-time model been able to predict cross-shore FIB concentrations across a 1600-m beach (Feng et al., 2015). High resolution spatial FIB maps can overcome the spatial limitation of most real-time predictive FIB models by providing spatially explicit information for watershed management actions and on pollution transport patterns within a waterbody. To date, predictive FIB maps have been created for the freshwater FIB *Escherichia coli* and marine FIB *Enterococcus* spp. in a shellfish fishery zone based off of freshwater input (Coulliette et al., 2009). However, interpolated nearshore recreational water FIB maps are generally lacking.

The ability to quickly determine FIB concentration patterns and hotspots is critical at U.S. beaches, especially those in the tropics and subtropics where people recreate year-round. Hawai'i is home to many of the country's popular tropical beaches, which support 18% of the state's economy through recreation and tourism (HDLIR, 2013). Furthermore, Hawai'i's coastal waters are vital to cultural activities, coral reef ecosystems, and fisheries. However, they continuously suffer from sewage pollution due to direct disposal, accidental spills, and leaks from injection wells, cesspools, and septic tanks (Dudley and Hallacher, 1991; Hunt, 2006; Dailer et al., 2010).

Hence, assessing microbial water quality in Hawai'i is critical. However, it is challenging because the FIB used for marine recreational waters, *Enterococcus* spp., is naturally present in tropical soils and can multiply in seawater (Fujioka et al., 1999; Desmarais et al., 2002). Therefore, high concentrations of it are not necessarily indicative of sewage pollution. To overcome this issue, Hawai'i adopted a secondary FIB, *Clostridium perfringens*, that is thought to be more strongly associated with sewage pollution (Fujioka and Shizumura, 1985; Shibata et al., 2004; Curiel-Ayala et al., 2012). Additionally, microbial source tracking techniques using host-specific molecular markers for *Bacteroides* bacteria have been employed to distinguish between human and animal fecal sources in Hawaiian coastal waters and elsewhere (Betancourt and Fujioka, 2006; Vijayavel et al., 2010). While both culture- and molecular-based FIB methods have limitations (i.e., culture media specificity, matrix effects on molecular analyses, consistency of laboratory protocols, definitions, and data interpretation), the use of them in combination has been recommended for determining spatial and temporal microbial pollution patterns, and identifying pollution sources (Stewart et al., 2013).

The goal of this study was to determine spatial and temporal variability of microbial pollution in a Hawaiian estuary. This study focused on Hilo Bay, which has been recognized since the late 1970s for its high concentrations of FIB by the USEPA's 303 (d) list of impaired water bodies (HDOH, 2008). High FIB concentrations are believed, in part, to be due to limited sewage treatment infrastructure, with 60–70% of Hilo relying on cesspools and septic tanks (Silvius et al., 2005). Specifically, Hilo has 12,272 on-site sewage disposal systems, which exceeds the 16 units km<sup>-2</sup> threshold that the USEPA considers to pose a significant risk to groundwater (Yates, 1985; Whittier and El-Kadi, 2014). Our study had two components. First, spatial and temporal patterns of FIB concentrations within the Bay were examined at several stations during high and low river flow conditions, while also evaluating the source of FIB using a microbial source tracking method. Second, bay-wide spatial and temporal patterns were visualized by creating surface water maps of FIB concentrations, using relationships between FIB concentrations from grab samples and water quality parameters rapidly measured at high spatial resolution. To date, few surface water maps for marine recreational FIB concentrations have been made – two for *Enterococcus* spp. (Coulliette et al., 2009; Feng et al., 2015) and none for *C. perfringens*. As sewage pollution in coastal waters is a worldwide issue, these maps could become an important tool for natural resource managers to visualize hotspots of FIB concentrations, and identify areas in need of management actions to improve water quality.

## 2. Methods

### 2.1. Study area

Hilo Bay is located in Hilo, Hawai'i, USA, on the northeast windward side of Hawai'i Island (Fig. 1). Its watershed is the largest in the state and drains the world's tallest and most massive mountains, Mauna Kea and Mauna Loa, respectively. Hilo has a tropical climate, characterized by a wet winter season (Oct–Apr) and dry summer (May–Oct). Average annual precipitation rates near the coast are ~2700 mm and up to 7600 mm at higher elevations (Giambelluca et al., 2013). Surface freshwater inputs are dominated by two rivers, the Wailuku and Wailoa. The Wailuku is the state's largest river, originating at 3500 m on Mauna Kea. Its watershed's land use consists of conservation forest

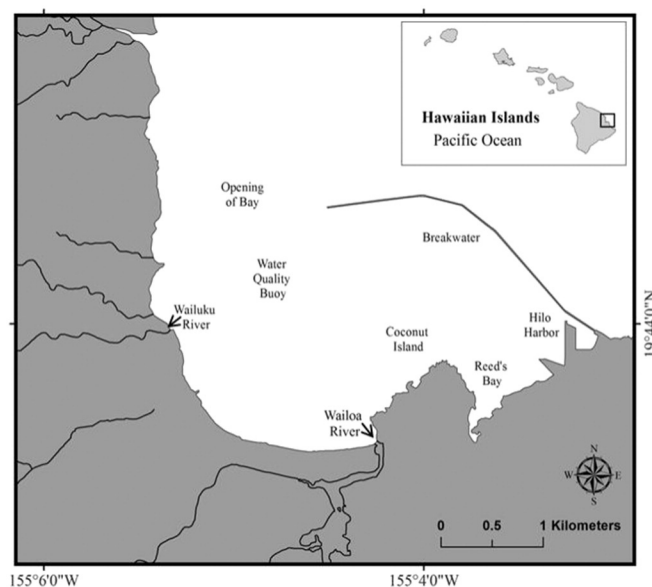


**Fig. 1.** Sampling stations in Hilo Bay, Hawai'i, USA, for examining microbial pollution patterns. Stations were selected to capture a range of different levels of freshwater input from surface runoff and groundwater discharge. Data collection was conducted on 16 occasions between October 2012 and April 2014.

(77%), agriculture (22%), and urban development (1%) (Parham et al., 2008). The Wailoa River has a smaller, less steep watershed starting at an elevation of 762 m on Mauna Loa. Its watershed's land use is similar to the Wailuku River's except for draining 8% more urban land, concentrated around its lower elevational reaches (Parham et al., 2008). East of the Wailuku River, the dominant source of freshwater to the Bay comes from large groundwater springs that discharge nearshore at a rate almost 100 times that of average annual surface runoff (M and E Pacific, Inc., 1980). Hilo Bay is ~640-ha, has depths up to 15 m, and is bounded by 9 km of coast and a 3-km long semi-permeable breakwater (M and E Pacific, Inc., 1980). A 1.5-km gap in the breakwater opens north to the Pacific Ocean allowing tides to move a distinct saltwater layer beneath outward-flowing surface freshwater with minimal mixing (M and E Pacific, Inc., 1980).

## 2.2. Study design

To determine microbial pollution patterns, this study characterized FIB concentrations over space by measuring concentrations at eight stations throughout the Bay, and over time by sampling during high and low river flow conditions. To determine if human waste was a source of FIB to Hilo Bay, *Bacteroides* in water samples were analyzed for the human-associated molecular marker (Griffith et al., 2003; Gawler et al., 2007). To evaluate predictive relationships of FIB concentrations, corresponding data were collected on physical water quality parameters, nutrient concentrations, and biological parameters. Sampling was conducted on 16 different days throughout a two-year period between October 2012 and April 2014, which were equally split between low and high river flow conditions (Fig. 2). Sampling began at sunrise to obtain the highest estimate of FIB concentrations before photo-inactivation of the FIB cells took place (Fujioka et al., 1981; Whitman et al., 2004). Eight stations were sampled to capture varying types of freshwater input and transport patterns in the Bay (Fig. 1). Stations influenced by surface runoff were the mouths of the Wailoa and Wailuku Rivers, Coconut Island, Hilo Bay Water Quality Buoy, and Opening of the Bay. Stations dominated by groundwater input were Reed's Bay, Hilo Harbor entrance, and at a station near the mid-point of the breakwater (Breakwater). At each station, three 1-L surface water grab samples were collected in acid-washed, autoclaved polypropylene bottles, and immediately stored on ice in the dark until processed in the laboratory for FIB, nutrients, and phytoplankton parameters within 6 h of collection.

During grab sample collection, high resolution spatial water quality mapping was also conducted. The mapping route first encompassed

the Bay visiting sample stations for grab sample collection, and then continued in a grid-like pattern to improve data interpolation among collection points. Data were collected with a YSI 6600 V2 data sonde, interfaced with a Garmin etrex GPS and a YSI data logger, measuring: salinity, temperature, turbidity, and chlorophyll *a* (chl *a*). The sonde was placed inside a high-volume flow cell with surface intake and output hoses connected to a marine 12-V diaphragm pump mounted on a PVC frame. Water was drawn through the flow cell at a rate of 13.2 L min<sup>-1</sup>, while measurements were logged every 3 s. Sonde data were downloaded to a computer using YSI's Ecowatch for later use in mapping software. Hilo Bay tide data were obtained for the morning of sampling ([http://tidesandcurrents.noaa.gov/tide\\_predictions.html](http://tidesandcurrents.noaa.gov/tide_predictions.html)). Meteorological data were gathered on precipitation levels at the Hilo International Airport (<http://cdo.ncdc.noaa.gov/qclcd/QCLCD>). It is the closest rainfall gauge to our stations, ~4 km from Reed's Bay (Fig. 1). River discharge rates for the Wailuku River were obtained from the US Geological Service (USGS) gauge ([http://nwis.waterdata.usgs.gov/hilo/nwis/uv?site\\_no=16704000](http://nwis.waterdata.usgs.gov/hilo/nwis/uv?site_no=16704000)). It is ~8 km upstream from the Wailuku River Mouth station (Fig. 1). Sampling of high river flow conditions was conducted after the Wailuku River discharge reached a threshold of 3.5 m<sup>3</sup> s<sup>-1</sup>, while low river flow conditions were sampled when discharge rates were <2.5 m<sup>3</sup> s<sup>-1</sup> (Fig. 2). Previous research has shown that the Wailuku River's discharge directly affects conditions in the Bay and is the only gauged river in the watershed (Wiegner et al., 2013).

## 2.3. Microbial analyses

At the laboratory, a water sample from each of the three bottles collected was analyzed for *Enterococcus* spp. and *C. perfringens*. *Enterococcus* spp. was analyzed using the Enterolert MPN method and Quanti-Tray®/2000 from IDEXX Laboratories Inc. following the manufacture's recommendations of 10 mL of sample and 90 mL of sterile water (Palmer et al., 1993). This method is used by Hawai'i's Department of Health and its analytical range is from 1 to 2419 MPN 100 mL<sup>-1</sup>. All *Enterococcus* spp. concentrations reported here were corrected for sample dilution during analysis. When no wells fluoresced blue in the QuantiTray, *Enterococcus* spp. concentrations were reported as 5 MPN 100 mL<sup>-1</sup>, one-half the detection limit of the method after correcting for sample dilution. No diluted *Enterococcus* spp. concentrations exceeded the upper detection limit of the Enterolert MPN method. *C. perfringens* was enumerated by filtering 100 mL of sample water with 0.45-μm pore size cellulose nitrate filters (Whatman) and mCP medium (Acumedia, Baltimore, MD) (Bisson and Cabelli, 1979). For some storm samples, 10 mL of water was filtered and their final concentrations were corrected for dilution.

## 2.4. Bacteroides microbial source tracking analyses

The triplicate water samples collected from each station between October 2012 and August 2013 were also evaluated for the presence of the human-associated *Bacteroides* molecular marker. Water samples (~1 L) were filtered through 0.45-μm pore size nitrocellulose membranes (Whatman® Sigma-Aldrich-Co, MO), which are chemically compatible with dilute acids. These filters were stored in 5 mL of Lysis buffer at -80 °C. For all samples and the positive (sewage from a sewage treatment plant) and negative (PCR-grade water) controls, DNA extraction methods were followed using Mo Bio's PowerLyzer powersoil DNA extraction kit with bead-beating (Mo Bio Laboratories, Inc., Carlsbad, CA) in a mini-bead beater (BioSpec Products, Inc., Bartlesville, OK) for 3 min. Positive and negative controls were used each time samples were analyzed, and all tested positive and negative, respectively. Polymerase chain reaction (PCR) was used to amplify genes from known *Bacteroides prevotella* sequences first using the General *Bacteroides* 32F/708R primer set. If positive identification of the general *Bacteroides* marker was detected, then the human-associated *Bacteroides* HF183F/708R primer set was used to identify the presence of *Bacteroides* from

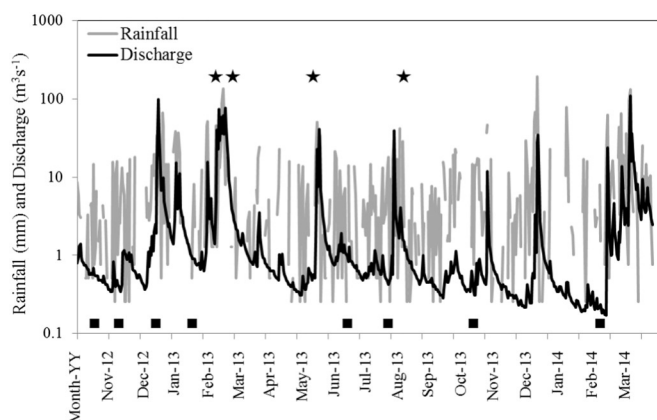


**Fig. 2.** Daily rainfall and Wailuku River discharge in Hilo Bay, Hawai'i, USA, from October 2012 to April 2014. Rainfall data were obtained from the NOAA National Climatic Data Center for the Hilo International Airport. Wailuku River discharge data were obtained from the USGS National Water Information System for gauge number 16704000 at Pi'ihonua, Hawai'i. High river flow sampling dates (Month-YY) are marked by a star (★), while low river flow sampling dates are marked by square (■).

human waste (Bernhard and Field, 2000; Dick et al., 2005; Kildare et al., 2007). PCR reactions were optimized for primer concentrations and carried out in 25  $\mu\text{L}$  total reaction volumes consisting of 3.0  $\mu\text{L}$  of template (DNA extracted from filters), 12.0  $\mu\text{L}$  2 $\times$  master mix (containing Taq DNA Polymerase, dNTPs,  $\text{MgCl}_2$  and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR (Promega, Madison, WI)), 8.0  $\mu\text{L}$  PCR-grade water, 1.0  $\mu\text{L}$  forward primer 32F or HF183, and 1.0  $\mu\text{L}$  reverse primer 708R (OLIGO, Sigma-Aldrich, MO). Thermocycler conditions for PCR amplification of the 16S rDNA gene conditions were lid 105  $^\circ\text{C}$ , 95  $^\circ\text{C}$  for 15 min, (95  $^\circ\text{C}$  for 30 s, 53–59  $^\circ\text{C}$  for 30 s, 72  $^\circ\text{C}$  for 2 min)  $\times$  30–34 cycles, 72  $^\circ\text{C}$  for 6 min, and 4  $^\circ\text{C}$  to hold. The annealing temperatures for the primers used were: Bac 32F = 56.9  $^\circ\text{C}$ , Bac 708R = 59.4  $^\circ\text{C}$ , and HF 183F = 61.1  $^\circ\text{C}$ . A 3.5  $\mu\text{L}$  volume of each PCR product was analyzed by agarose 2% gel electrophoresis with a 1 KB ladder to determine amplicon size. Bands were visualized using Lonza Gelstar<sup>TM</sup> nucleic acid dye, which gives high sensitivity for the detection of double- or single-stranded DNA (Lonza, Rockland, ME). False negatives were determined from a subset of samples seeded with a positive *Bacteroides* PCR lab control ( $10^{-3}$  dilution).

## 2.5. Nutrient analyses

The first of the three sample bottles collected from each of the eight stations was used for nutrient analyses. A known volume of water was filtered through pre-combusted (500  $^\circ\text{C}$ , 5 h) 0.7- $\mu\text{m}$  GF/F filters (Whatman@Sigma-Aldrich, MO) and stored frozen until analysis, which occurred within 2–3 mo of collection at the University of Hawai'i at Hilo Analytical Laboratory. These filters were stored frozen in the dark until analyzed for chl *a*. Nutrient samples were analyzed for concentrations of nitrate plus nitrite ( $\text{NO}_3^- + \text{NO}_2^-$ ; USEPA 353.4, Detection Limit (DL) = 0.07  $\mu\text{mol L}^{-1}$ ), ammonium ( $\text{NH}_4^+$ ; USGS I-2525, DL = 0.36  $\mu\text{mol L}^{-1}$ ), phosphate ( $\text{PO}_4^{3-}$ ; USEPA 365.5, DL = 0.03  $\mu\text{mol L}^{-1}$ ), total dissolved phosphorous (TDP; USGS I-4650-03, DL = 0.5  $\mu\text{mol L}^{-1}$ ), and silicic acid ( $\text{H}_4\text{SiO}_4$ ; USEPA 366, DL = 1.0  $\mu\text{mol L}^{-1}$ ) with a Technicon Pulse II AutoAnalyzer. Dissolved organic carbon (DOC; USEPA 415.1, DL = 10  $\mu\text{mol L}^{-1}$ ) and total dissolved nitrogen (TDN; ASTM D5176, DL = 5  $\mu\text{mol L}^{-1}$ ) were analyzed on a Shimadzu TOC-V CSH, TNM-1 analyzer (Sharp et al., 2002). Dissolved organic nitrogen (DON) was calculated from the difference between TDN and dissolved inorganic nitrogen ( $\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ ), while dissolved organic phosphorous (DOP) was calculated from the difference between TDP and  $\text{PO}_4^{3-}$ . Filters for chl *a* were analyzed on a Turner 10-AU fluorometer according to USEPA Method 445.0.

## 2.6. Statistical analyses

The arithmetic mean was used to compare FIB concentrations among stations and between river flow conditions in order to preserve sample independence among sampling events. This approach was used in contrast to the geometric mean, which is used for identifying monthly FIB concentration exceedances by assuming sample concentrations are dependent on the previous sample collected, minimizing the assessment of risk (Wade, 2007). General linear models (GLM), followed by Tukey's tests, were used to compare differences in FIB concentrations among stations and between Wailuku River flow conditions (high vs. low), with an evaluation of the interaction effect. To improve normality of the residuals, Box-Cox  $\log_{10}$  transformations were specified within the GLM. A two-way Analysis of Variance (ANOVA) with rank transformed data was used to compare differences in human-associated *Bacteroides* marker presence (present/absent) among stations and between river flow conditions (Potvin and Roff, 1993). For this analysis, each filter analyzed for the presence of the human-associated *Bacteroides* marker was considered an individual sample. Relationships between FIB concentrations (*Enterococcus* spp. and *C. perfringens*) at the Wailuku River mouth and river discharge for the day of sampling were evaluated using linear regressions. Linear regressions were also used to

examine relationships between FIB concentrations, tidal height, nutrient concentrations, chl *a* concentrations, and YSI sonde data to identify significant predictors of FIB concentrations.  $\log_{10}$  transformations were used on regression data to fulfill assumptions of normality and equal variance. These relationships then aided in building more in-depth multi-parameter regression models for *Enterococcus* spp. and *C. perfringens*. The models were assessed using a combination of best subsets and stepwise regressions to evaluate adjusted  $R^2$ ,  $R^2$  predicted, and Cp statistic, with co-linearity identified by a variance influence factor (VIF) of  $>10$  (Gonzalez and Noble, 2014). Interaction effects were included to reduce bias when reducing models (Ge and Frick, 2007). Variables were not transformed due to the large sample size of sonde mapping data ( $n \sim 2000$ ). All statistical analyses were conducted in Minitab 17, except for *Bacteroides* data which were evaluated in Systat @11 ( $\alpha = 0.05$ ).

## 2.7. Mapping microbial pollution patterns

High resolution spatial maps of predicted FIB concentrations were created using multi-parameter regression model equations for *Enterococcus* spp. and *C. perfringens* with sondes and GPS data. The ability of the models to predict FIB management decisions based on single-sample standards of 104 MPN 100  $\text{mL}^{-1}$  for *Enterococcus* spp. and the recommended monthly geometric mean of 5 CFU 100  $\text{mL}^{-1}$  for *C. perfringens* (Fujioka et al., 1997; HDOH, 2009; Fung et al., 2007), was also examined by evaluating the percent of correct decisions, percent of Type I errors (false positives), and percent of Type II errors (false negatives) (Gonzalez and Noble, 2014). Maps were created using Esri ArcGIS (10.1) software with an inverse distance weighted (IDW) method to interpolate values among collection points and create a smooth surface water map. The IDW method was chosen because it does not make predictions outside the actual sample range. Data were added to a base map of Hilo Bay with datum NAD84 and UTM Zone 5 (meters) projection.

## 3. Results

### 3.1. Spatial and temporal FIB patterns

The spatial and temporal distributions of FIB concentrations differed throughout Hilo Bay depending on the indicator bacteria (Fig. 3, Table 1). *Enterococcus* spp. concentrations were similar among stations, but different between river flow conditions ( $p < 0.001$ , Fig. 3a). Concentrations spanned two orders of magnitude, ranging from 10 to 8000 MPN 100  $\text{mL}^{-1}$  across all stations, and differed on average ( $\pm \text{SE}$ ) by an order of magnitude between high ( $1067 \pm 192$  MPN 100  $\text{mL}^{-1}$ ) and low ( $116 \pm 13$  MPN 100  $\text{mL}^{-1}$ ) river flow conditions. However, *Enterococcus* spp. concentrations at the Wailuku River mouth were not related to river discharge on the day of sampling. In contrast, *C. perfringens* differed among stations ( $p < 0.001$ ) and between river flow conditions ( $p < 0.001$ , Fig. 3b). Concentrations ranged from 0 to 35 CFU 100  $\text{mL}^{-1}$  across all stations, with the highest concentrations consistently measured at the Wailuku River mouth ( $7 \pm 2$  CFU 100  $\text{mL}^{-1}$ ), and the lowest at Reed's Bay ( $1 \pm 0$  CFU 100  $\text{mL}^{-1}$ ). During high river flow conditions, *C. perfringens* concentrations were four times higher ( $4 \pm 1$  CFU 100  $\text{mL}^{-1}$ ) than during low river flow conditions ( $1 \pm 0$  CFU 100  $\text{mL}^{-1}$ ). *C. perfringens* concentrations at the Wailuku River mouth were significantly related to river discharge on the day of sampling ( $R^2 = 0.836$ ,  $p < 0.001$ ) (Fig. 4). Lastly, there was no significant relationship between FIB concentrations and tide heights, which ranged from 0.12 to 0.79 m throughout the study period.

### 3.2. Bacteroides microbial source tracking analyses

All stations in the Bay tested positive for the human-associated *Bacteroides* marker (HF 183F). 55% of 229 samples ( $n = 127$  positives)

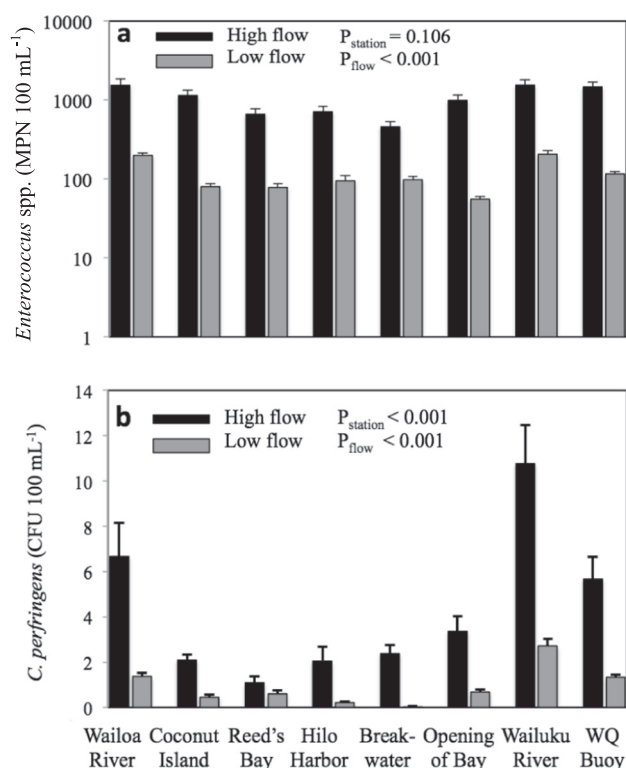


**Fig. 3.** Average  $\pm$  SE concentrations for a) *Enterococcus* spp. and b) *C. perfringens* in Hilo Bay, Hawai'i, USA, from 2012 to 2014 during high and low river flow conditions. Results from GLMs are shown on the figure ( $\alpha = 0.05$ ), and post hoc Tukey's test results are in Table 1.

collected during high and low river flow conditions tested positive for the general *Bacteroides* marker (Bac32F), and 85% of these were positive ( $n = 108$  positives) for the human-associated *Bacteroides* marker. Across the Bay, a slightly higher percentage of samples tested positive for the human-associated *Bacteroides* marker during high river flow conditions ( $48\% \pm 3$ ) compared to low flow ones ( $43\% \pm 2$ ), although

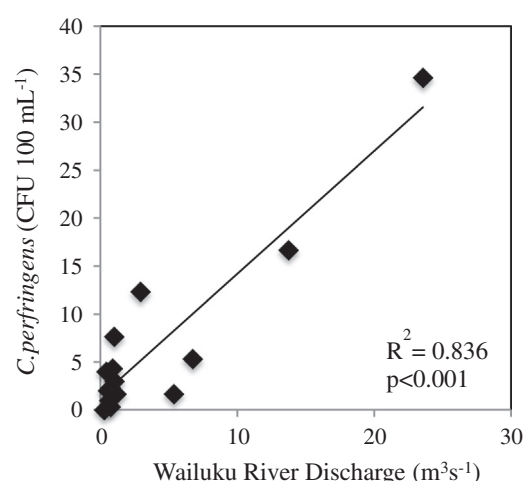


**Fig. 4.** Relationship between *Clostridium perfringens* and Wailuku River discharge in Hilo Bay, Hawai'i, USA, from 2012 to 2014. Results from regression analyses are shown on the figure ( $\alpha = 0.05$ ).

this difference was not significant. The highest percentage of samples positive for the human-associated *Bacteroides* marker were observed at the Wailoa River mouth (57%), followed by the Wailuku River mouth and the Opening of the Bay (52% each), Hilo Harbor (45%), Breakwater (43%), Coconut Island, Reed's Bay, and the Hilo Bay Water Quality Buoy (41% each); note, however, these differences were also not significant. At three of the four stations directly within the influence of the Wailuku River (Wailuku River, Opening of Bay, Breakwater), the percent of positive samples increased  $\sim 15\%$  during storms, whereas samples more influenced by groundwater inputs had similar percentage of positive samples during high and low river flow conditions (Wailoa River, Coconut Island, Reed's Bay, Hilo Harbor).

### 3.3. FIB and water quality relationships

*Enterococcus* spp. and *C. perfringens* had significant linear relationships with physical water quality parameters measured by the sonde,

**Table 1**

Average ( $\pm$  SE) and [range] of fecal indicator bacteria (FIB) concentrations and physiochemical parameter values for surface waters in Hilo Bay, Hawai'i, USA, at each station and during high and low river flow conditions between October 2012 and April 2014. Superscripts letters indicate significant groupings from post hoc Tukey's test ( $\alpha = 0.05$ ).

| Station         | <i>Enterococcus</i> spp.<br>(MPN 100 mL <sup>-1</sup> ) | <i>C. perfringens</i><br>(CFU 100 mL <sup>-1</sup> ) | Salinity                                   | Turbidity<br>(NTU)                       |
|-----------------|---------------------------------------------------------|------------------------------------------------------|--------------------------------------------|------------------------------------------|
| Wailoa River    | 871 (484)<br>[27–7998]                                  | 4 (2) <sup>ab</sup><br>[0–29]                        | 22.26 (1.90)<br>[8.95–33.47]               | 2.98 (1.07)<br>[0.50–14.70]              |
| Coconut Island  | 612 (289)<br>[17–3568]                                  | 1 (0) <sup>b</sup><br>[0–5]                          | 26.03 (1.39)<br>[12.24–32.32]              | 6.41 (1.60)<br>[0.20–25.60]              |
| Reed's Bay      | 371 (166)<br>[23–2495]                                  | 1 (0) <sup>b</sup><br>[0–5]                          | 24.85 (1.38)<br>[13.45–33.10]              | 3.76 (2.00)<br>[0.30–31.70]              |
| Hilo Harbor     | 403 (193)<br>[10–2720]                                  | 1 (1) <sup>b</sup><br>[0–12]                         | 23.91 (1.20)<br>[13.80–32.84]              | 3.61 (1.20)<br>[0.10–42.70]              |
| Breakwater      | 278 (116)<br>[17–1419]                                  | 1 (1) <sup>b</sup><br>[0–7]                          | 25.09 (13.10)<br>[11.00–31.59]             | 4.59 (3.33)<br>[0.00–52.50]              |
| Opening of Bay  | 524 (257)<br>[24–3539]                                  | 2 (1) <sup>ab</sup><br>[0–13]                        | 27.20 (1.50)<br>[10.81–31.78]              | 4.41 (2.89)<br>[0.30–45.90]              |
| Wailuku River   | 879 (385)<br>[13–4962]                                  | 7 (2) <sup>a</sup><br>[0–34]                         | 24.07 (2.33)<br>[1.97–33.38]               | 7.62 (4.02)<br>[0.60–64.00]              |
| WQ Buoy         | 792 (327)<br>[17–4274]                                  | 3 (1) <sup>ab</sup><br>[0–18]                        | 25.13 (1.75)<br>[6.69–33.40]               | 5.90 (3.47)<br>[0.50–53.70]              |
| River condition |                                                         |                                                      |                                            |                                          |
| High-flow       | 1067 (192) <sup>a</sup><br>[17–7998]                    | 4 (1) <sup>a</sup><br>[0–35]                         | 21.22 (0.85) <sup>a</sup><br>[1.97–30.89]  | 8.04 (1.83) <sup>a</sup><br>[0.20–64.00] |
| Low-flow        | 116 (13) <sup>b</sup><br>[10–420]                       | 1 (0) <sup>b</sup><br>[0–8]                          | 28.30 (0.44) <sup>b</sup><br>[18.18–33.40] | 1.09 (0.20) <sup>b</sup><br>[0.00–11.80] |

For station values: *Enterococcus* spp., salinity, and turbidity  $n = 16$ ; *C. perfringens*  $n = 15$ . For river conditions, *Enterococcus* spp., *C. perfringens*, salinity, and turbidity  $n = 64$  (except *C. perfringens* high-flow  $n = 56$ ).

the strongest of which was salinity (Fig. 5). *Enterococcus* spp. concentrations increased with decreasing salinity and water temperatures ( $R^2 = 0.318$ ,  $p < 0.001$ ;  $R^2 = 0.192$ ,  $p < 0.001$ , respectively), and increasing turbidity ( $R^2 = 0.241$ ,  $p < 0.001$ ). Similarly, *C. perfringens* concentrations increased with decreasing salinity and water temperature ( $R^2 = 0.284$ ,  $p < 0.001$ ;  $R^2 = 0.069$ ,  $p = 0.003$ , respectively), and increasing turbidity ( $R^2 = 0.254$ ,  $p < 0.001$ ). For the multi-parameter regression equations, parameters that were chosen for *Enterococcus* spp. were salinity, water temperature, and turbidity ( $R^2 = 0.444$ ,  $p < 0.001$ ), and for *C. perfringens*, they were salinity and turbidity ( $R^2 = 0.553$ ,  $p < 0.001$ ) (Table 2). A significant positive relationship was also found between *Enterococcus* spp. and *C. perfringens* ( $R^2 = 0.498$ ,  $p < 0.001$ ). There were no significant relationships between FIB concentrations and nutrient (inorganic and organic) concentrations in Hilo Bay, nor was there one with chl *a* (Table 3).

### 3.4. Mapping microbial pollution patterns

*Enterococcus* spp. and *C. perfringens* maps were created from multi-parameter regression equations (Fig. 6). The ability of the maps to predict management decisions based on recommended water quality standards (Fujioka et al., 1997; Fung et al., 2007) varied between FIB, but was overall successful (Table 4). The maps were able to predict appropriate management decisions 64% and 95% of the time for *Enterococcus* spp. and *C. perfringens*, respectively.

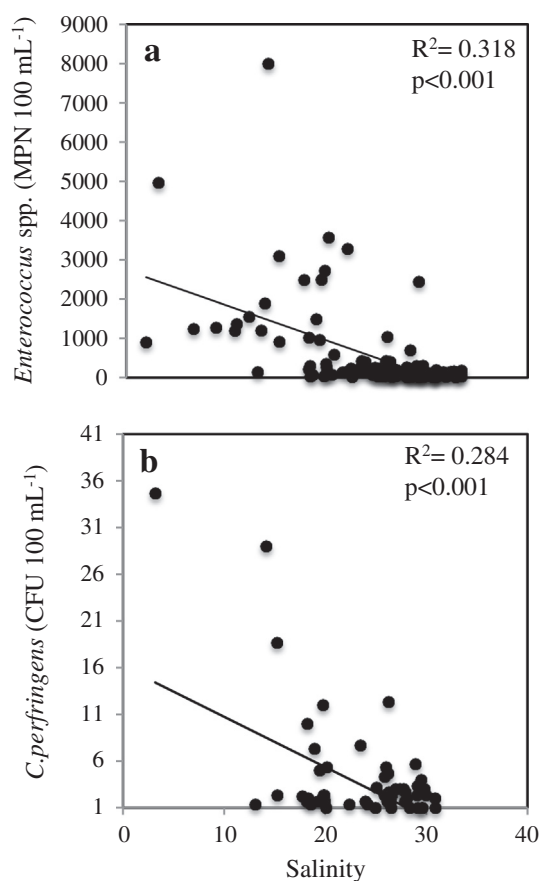


Fig. 5. Relationships of a) *Enterococcus* spp. and b) *Clostridium perfringens* with salinity in Hilo Bay, Hawai'i, USA, from 2012 to 2014. Results from regression analyses are shown on the figure ( $\alpha = 0.05$ ).

## 4. Discussion

### 4.1. Spatial microbial pollution patterns

It has long been recognized that FIB concentrations vary over space in estuaries depending on the proximity to FIB sources, runoff patterns, and physical oceanographic processes (Lipp et al., 2001; Knee et al., 2008; Coulliette et al., 2009; Futch et al., 2011; Tomlinson et al., 2011). In areas with known sewage pollution, FIB concentrations are higher nearshore and decrease with distance offshore (Lisle et al., 2004; Shibata et al., 2004; Bonkosky et al., 2009; Feng et al., 2015). However, variations in patterns across beachfronts are often site-specific and can require further investigation into additional FIB sources, such as beach sands or environmental regrowth (Shibata et al., 2004; Abdelzaher et al., 2010; Enns et al., 2012). In Hilo Bay, one source of FIB appears to be freshwater surface runoff, as *Enterococcus* spp. averages were up to two times higher at river mouths than in areas of groundwater input and the Opening of the Bay (Fig. 3, Table 1); however, these differences were not significant. The lack of significant spatial patterns may be due to the patchy nature and temporal variability of *Enterococcus* spp. (Boehm et al., 2002; Boehm, 2007), or indicative of a variety of other *Enterococcus* spp. sources that differ over space and time. Non-sewage sources of *Enterococcus* spp. include urban runoff, beach sands, animal feces, macroalgae, and tropical soils and plants (Boehm et al., 2003; Ragosta et al., 2010; Zhu et al., 2011). Microbial source tracking research using F+ coliphage, *Bacteroides* phages, *Bacteroidales*, and *Enterococcus* spp. gene in nearshore waters of Hawai'i state suggest that the source of *Enterococcus* spp. is likely from the environment or animals as the occurrence of human-associated molecular markers did not correspond with elevated levels of *Enterococcus* spp. (Knee et al., 2008; Vijayavel et al., 2010; Boehm et al., 2011; Wiegner et al., 2013).

Because patterns of *Enterococcus* spp. can be misleading about the presence of sewage in tropical estuaries, Hawai'i uses *C. perfringens* as a secondary FIB (Fujioka and Shizumura, 1985; Fujioka et al., 1997; HDOH, 2009). Based on ambient levels in coastal waters, a monthly geometric mean of 5 CFU 100 mL<sup>-1</sup> was recommended for a water quality standard (Fujioka et al., 1997). However, to assist with same-day monitoring, the Fung/Fujioka Scale was developed to classify sewage pollution risk based on single-sample concentrations (Fung et al., 2007). Based on this scale, waters with *C. perfringens* concentrations <10 CFU 100 mL<sup>-1</sup> are not contaminated with sewage, between 10 and 100 CFU 100 mL<sup>-1</sup> are contaminated by non-point source sewage pollution, and >100 CFU 100 mL<sup>-1</sup> are affected by point-source sewage pollution (Fung et al., 2007). In Hilo Bay, the average concentrations of *C. perfringens* were below the suggested geometric mean of 5 CFU 100 mL<sup>-1</sup> at each station, except at the Wailuku River mouth (7 CFU 100 mL<sup>-1</sup>). However, single-sample concentrations during high river conditions were occasionally within the range indicative of non-point source sewage contamination (10–35 CFU 100 mL<sup>-1</sup>, Table 1). This suggests that freshwater runoff carries sewage or animal feces to Hilo Bay. A slightly greater percentage of samples testing positive for the human-associated *Bacteroides* marker during high river flow conditions compared to low flow ones further supports this conclusion.

Studies have found rivers and streams carrying surface runoff can be a significant source of *C. perfringens* to the nearshore environment (Desmarais et al., 2002; Mueller-Spitz et al., 2010; Viau et al., 2011). In Hilo Bay, this is likely because *C. perfringens* concentrations were highest at both river mouths and the Water Quality Buoy, located in the Wailuku River plume. Another possible source of *C. perfringens* at river mouths can be resuspension from sediments where spores have accumulated from chronic microbial pollution (Desmarais et al., 2002; Lisle et al., 2004; Mueller-Spitz et al., 2010). This was unlikely the case for *C. perfringens* at the Wailuku River mouth, as concentrations from resuspended sediments in Lake Michigan were two to three orders of magnitude higher than the range seen in our study (Mueller-Spitz et al., 2010). *C. perfringens* has also been found to originate from groundwater

**Table 2**

Parameters used in multi-parameter regression models for *Enterococcus* spp. and *C. perfringens* evaluated by variance influence factors (VIF) and *p*-values for the purpose of mapping fecal indicator bacteria (FIB) concentrations in Hilo Bay, Hawai'i, USA, with YSI sonde data. Adjusted  $R^2$  values, Y-intercept, and *n* are also given for each model.

| Variable           | <i>Enterococcus</i> spp. |               |                 | Variable           | <i>C. perfringens</i> |               |                 |
|--------------------|--------------------------|---------------|-----------------|--------------------|-----------------------|---------------|-----------------|
|                    | Coefficient              | VIF           | <i>p</i> -Value |                    | Coefficient           | VIF           | <i>p</i> -Value |
| Salinity           | −95.6                    | 3.2           | <0.001          | Salinity           | −0.21                 | 1.5           | 0.005           |
| Water temperature  | 139.3                    | 3.6           | 0.075           | Turbidity          | 0.49                  | 6.0           | <0.001          |
| Turbidity          | 38.8                     | 1.2           | <0.001          | Salinity*          | −0.02                 | 5.0           | <0.001          |
|                    |                          |               |                 | Turbidity          |                       |               |                 |
| Y-intercept = −486 | <i>n</i> = 122           | $R^2$ = 0.444 |                 | Y-intercept = 7.11 | <i>n</i> = 114        | $R^2$ = 0.553 |                 |

All data were collected between October 2012 and April 2014. Ranges of measured values were 2–33 for salinity, 17–26 °C for water temperature, and 0–64 NTU for turbidity.

polluted by storm runoff and injection wells (Futch et al., 2011). However, in Hilo Bay, *C. perfringens* concentrations were lowest in areas of groundwater input, such as Reed's Bay and the Hilo Harbor (Table 1). Likewise, in Hanalei Bay, Kaua'i, rivers and streams were identified as the primary FIB source, not groundwater (Knee et al., 2008).

When evaluating spatial distributions of FIB in estuaries, concentrations of nutrients may help to identify pollution sources, as sewage has high total nitrogen and phosphorus concentrations (Howarth, 2000; Wiegner et al., 2016). In Hilo Bay, however, there was no significant relationship between nutrients and FIB concentrations, as they showed contrasting spatial patterns between areas of surface runoff and groundwater input (Tables 1 and 3). These results suggest that FIB and nutrients may be originating from different sources within the Hilo Bay watershed. Other studies in Hawai'i have found that high nutrient concentrations in groundwater are likely from agriculture and urban land use rather than sewage contamination (Knee et al., 2008; Wiegner et al., 2013; Wiegner et al., 2016).

#### 4.2. Temporal microbial pollution patterns

FIB concentrations can vary greatly over short-term timescales of minutes to hours and long-term timescales of years to decades (Boehm et al., 2002). Short-term variability throughout the day is largely affected by solar radiation level and tidal height (Wymer et al., 2004; Abdelzaher et al., 2010; Enns et al., 2012). FIB concentrations generally peak during the night and decrease throughout the day due to the inactivation of cells caused by solar radiation (Rozen and Belkin, 2001; Boehm et al., 2002; Enns et al., 2012). However, FIB concentrations

can increase during daytime high tide if contaminated beach sands are submerged (Abdelzaher et al., 2010; Zhu et al., 2011; Enns et al., 2012). To minimize solar radiation effects on FIB concentrations, we sampled at sunrise to achieve the highest concentrations for the day. Regression analyses also revealed that tidal height was not related with FIB concentrations in Hilo Bay. This may be due to minimal mixing between surface and bottom layers in Hilo Bay due to density stratification (Dudley and Hallacher, 1991). Also, the difference in tidal heights was fairly small throughout our study, ranging from 0.12 to 0.79 m. Tidal influence on FIB concentrations may be more important along coastal sandy beaches that can harbor *Enterococcus* spp., especially if there is a larger tidal variation, such as ~2 m seen at Huntington Beach, California (Rosenfeld et al., 2006; Enns et al., 2012).

While microbial pollution levels vary daily, large fluctuations over longer time scales occur due to seasonal changes in precipitation (Boehm et al., 2002; Mallin et al., 2002; Tomlinson et al., 2011). Storm runoff has been found to significantly increase concentrations of FIB in nearshore waters (Lipp et al., 2001; He and He, 2008; Curiel-Ayala et al., 2012; Wiegner et al., 2013), and even account for 99% of annual FIB loading in dryer climates (Reeves et al., 2004). In Hawai'i, coastal streams carry high levels of *Enterococcus* spp. and *C. perfringens* during storms leading to nearshore increases (Fujioka and Shizumura 1985, Knee et al. 2008, Boehm et al. 2011, Viau et al. 2011, Wiegner et al. 2013). In our study, *C. perfringens* concentrations at the Wailuku River mouth were significantly related to the river's discharge on the day of sampling and they were three to four times higher during high river flow events. In contrast, *Enterococcus* spp. concentrations at the Wailuku River mouth were not related to river discharge, even though

**Table 3**

Average ( $\pm$  SE) and [range] of nutrient ( $\mu\text{mol L}^{-1}$ ) and chlorophyll *a* ( $\text{chl } a$ ,  $\mu\text{g L}^{-1}$ ) concentrations for surface waters in Hilo Bay, Hawai'i, USA, at each station and during high and low river flow conditions. Superscript letters indicate significant groupings from post-hoc Tukey's test ( $\alpha = 0.05$ ).

| Station         | $\text{NO}_3^- + \text{NO}_2^-$ | $\text{NH}_4^+$           | TDN                  | DON    | $\text{PO}_4^{3-}$        | TDP                     | DOP       | $\text{H}_4\text{SiO}_4$   | DOC                   | chl <i>a</i> |
|-----------------|---------------------------------|---------------------------|----------------------|--------|---------------------------|-------------------------|-----------|----------------------------|-----------------------|--------------|
| Wailoa          | 20.48 (2.61) <sup>a</sup>       | 1.03 (0.20) <sup>a</sup>  | 27 (3) <sup>a</sup>  | 6 (1)  | 0.53 (0.08) <sup>a</sup>  | 0.7 (0.1) <sup>a</sup>  | 0.2 (0.0) | 192.4 (27.5) <sup>a</sup>  | 143 (18)              | 0.58 (0.25)  |
| River           | [2.35–36.55]                    | [0.18–3.17]               | [11–44]              | [0–18] | [0.05–1.03]               | [0.3–1.2]               | [0.0–0.5] | [43.5–440.3]               | [43–266]              | [0.00–3.94]  |
| Coconut         | 5.83 (0.82) <sup>bcd</sup>      | 0.57 (0.15) <sup>ab</sup> | 14 (1) <sup>bc</sup> | 7 (1)  | 0.16 (0.03) <sup>bc</sup> | 0.3 (0.0) <sup>b</sup>  | 0.1 (0.0) | 76.1 (9.9) <sup>b</sup>    | 239 (40)              | 0.70 (0.27)  |
| Island          | [2.62–13.98]                    | [0.18–2.10]               | [9–25]               | [2–21] | [0.02–0.44]               | [0.2–0.6]               | [0.0–0.3] | [16.8–161.3]               | [71–665]              | [0.01–4.30]  |
| Reed's          | 7.78 (1.10) <sup>bc</sup>       | 0.70 (0.19) <sup>ab</sup> | 15 (1) <sup>b</sup>  | 7 (1)  | 0.35 (0.07) <sup>ab</sup> | 0.5 (0.1) <sup>ab</sup> | 0.1 (0.0) | 120.8 (20.6) <sup>ab</sup> | 184 (57)              | 0.62 (0.26)  |
| Bay             | [1.07–15.49]                    | [0.18–2.45]               | [7–25]               | [1–10] | [0.02–0.92]               | [0.3–0.9]               | [0.0–0.4] | [8.5–303.1]                | [41–982]              | [0.02–3.83]  |
| Hilo            | 8.32 (1.14) <sup>b</sup>        | 0.65 (0.14) <sup>ab</sup> | 16 (1) <sup>b</sup>  | 7 (1)  | 0.30 (0.06) <sup>ab</sup> | 0.4 (0.1) <sup>ab</sup> | 0.2 (0.0) | 118.7 (18.4) <sup>ab</sup> | 191 (37)              | 0.59 (0.17)  |
| Harbor          | [1.95–16.34]                    | [0.18–1.92]               | [9–25]               | [1–18] | [0.05–0.76]               | [0.3–1.0]               | [0.0–0.5] | [37.7–249.3]               | [68–627]              | [0.00–1.79]  |
| Break-water     | 5.93 (0.59) <sup>bcd</sup>      | 0.64 (0.22) <sup>ab</sup> | 13 (1) <sup>bc</sup> | 6 (1)  | 0.16 (0.05) <sup>bc</sup> | 0.3 (0.1) <sup>b</sup>  | 0.2 (0.1) | 79.0 (9.0) <sup>ab</sup>   | 163 (27)              | 0.60 (0.22)  |
|                 | [2.15–10.13]                    | [0.18–3.52]               | [8–18]               | [0–12] | [0.02–0.74]               | [0.2–0.9]               | [0.0–0.7] | [33.1–144.1]               | [65–484]              | [0.00–3.31]  |
| Opening         | 2.81 (0.33) <sup>de</sup>       | 0.33 (0.04) <sup>b</sup>  | 10 (1) <sup>c</sup>  | 7 (1)  | 0.08 (0.02) <sup>c</sup>  | 0.3 (0.0) <sup>b</sup>  | 0.2 (0.0) | 52.5 (5.4) <sup>bc</sup>   | 239 (60)              | 0.46 (0.14)  |
| of Bay          | [1.09–5.71]                     | [0.18–0.52]               | [7–16]               | [4–12] | [0.02–0.30]               | [0.2–0.6]               | [0.0–0.6] | [22.7–96.8]                | [68–1090]             | [0.00–1.92]  |
| Wailuku         | 1.88 (0.46) <sup>e</sup>        | 0.86 (0.17) <sup>ab</sup> | 10 (1) <sup>c</sup>  | 7 (1)  | 0.07 (0.01) <sup>c</sup>  | 0.3 (0.0) <sup>b</sup>  | 0.2 (0.0) | 35.0 (5.0) <sup>c</sup>    | 188 (18)              | 0.49 (0.21)  |
| River           | [0.40–8.36]                     | [0.18–2.19]               | [8–18]               | [3–14] | [0.02–0.18]               | [0.3–0.8]               | [0.1–0.7] | [1.2–76.7]                 | [94–342]              | [0.00–3.43]  |
| WQ Buoy         | 4.24 (0.80) <sup>cde</sup>      | 0.82 (0.23) <sup>ab</sup> | 12 (1) <sup>bc</sup> | 7 (1)  | 0.15 (0.05) <sup>bc</sup> | 0.3 (0.0) <sup>b</sup>  | 0.2 (0.1) | 58.9 (10.6) <sup>bc</sup>  | 228 (31)              | 0.47 (0.14)  |
|                 | [0.14–12.13]                    | [0.18–3.67]               | [8–23]               | [3–12] | [0.02–0.81]               | [0.3–0.8]               | [0.0–0.4] | [2.9–173.2]                | [77–589]              | [0.03–2.08]  |
| River condition |                                 |                           |                      |        |                           |                         |           |                            |                       |              |
| High-flow       | 7.90 (0.87) <sup>a</sup>        | 0.95 (0.11) <sup>a</sup>  | 16 (1) <sup>a</sup>  | 7 (0)  | 0.27 (0.03) <sup>a</sup>  | 0.4 (0.0) <sup>a</sup>  | 0.2 (0.0) | 101.7 (9.5) <sup>a</sup>   | 162 (10) <sup>b</sup> | 0.67 (0.12)  |
|                 | [0.96–34.31]                    | [0.18–3.67]               | [7–44]               | [0–18] | [0.02–1.03]               | [0.2–1.2]               | [0.0–0.7] | [1.2–303.1]                | [41–394]              | [0.00–4.30]  |
| Low-flow        | 6.42 (0.91) <sup>b</sup>        | 0.45 (0.05) <sup>b</sup>  | 14 (1) <sup>b</sup>  | 7 (0)  | 0.18 (0.03) <sup>b</sup>  | 0.4 (0.0) <sup>b</sup>  | 0.2 (0.0) | 80.3 (9.2) <sup>b</sup>    | 232 (25) <sup>a</sup> | 0.46 (0.09)  |
|                 | [0.14–36.55]                    | [0.18–3.17]               | [7–36]               | [0–21] | [0.02–0.88]               | [0.2–1.2]               | [0.0–0.6] | [2.9–440.3]                | [65–1090]             | [0.00–3.43]  |

All data were collected between October 2012 and April 2014. For station values:  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ , TDN,  $\text{PO}_4^{3-}$ , TDP,  $\text{H}_4\text{SiO}_4$ , DOC, chl *a* *n* = 16. For river conditions:  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ , TDN,  $\text{PO}_4^{3-}$ , TDP,  $\text{H}_4\text{SiO}_4$ , DOC, chl *a* *n* = 64 for low and high-flow conditions, except for  $\text{H}_4\text{SiO}_4$  high-flow, where *n* = 63.

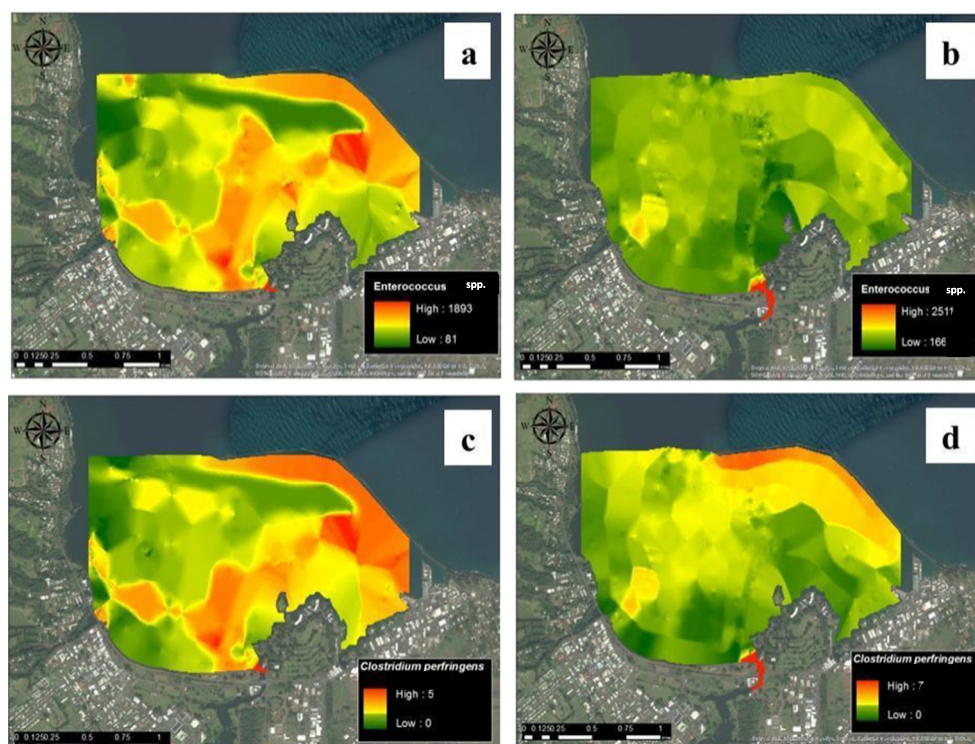


**Fig. 6.** Surface water maps of *Enterococcus* spp. (MPN 100 mL<sup>-1</sup>) and *Clostridium perfringens* (CFU 100 mL<sup>-1</sup>) hotspots in Hilo Bay, Hawai'i, USA, during high (a, c) and low (b, d) Wailuku River flow conditions, on May 30th, 2013, and Feb. 2nd, 2014, respectively. Colored legends display the range of predicted values for the date, with red indicating highest concentrations. Significant parameters used in the multi-parameter regression equations for *Enterococcus* spp. were water temperature, salinity, and turbidity ( $R^2 = 0.444$ ,  $p < 0.001$ ), and for *C. perfringens*, they were salinity and turbidity ( $R^2 = 0.553$ ,  $p < 0.001$ ).

concentrations were up to an order of magnitude higher during high river flow events throughout the Bay and a previous study found a significant relationship between *Enterococcus* spp. concentrations and 24-h rainfall (Strauch et al., 2014). The difference between *C. perfringens* and *Enterococcus* spp. at the Wailuku River mouth, suggests that *Enterococcus* spp. could have multiple non-sewage sources, such as soils and environmental regrowth, or may reach the Bay by groundwater and urban runoff (Desmarais et al., 2002; Boehm et al., 2003). The latter may be true as higher *Enterococcus* spp. concentrations have been documented in the urbanized portion of the Wailuku River compared to the upstream region dominated by native forest (Strauch et al., 2014).

#### 4.3. Mapping FIB in Hilo Bay

Creating maps of FIB hotspots could become a new visual tool to examine water quality and sewage pollution in the nearshore marine waters. Advancements in GIS software have made mapping of environmental data more available through the ability to interpolate a surface map from point data (Zambrano et al., 2009). While some studies have mapped coastal sewage inputs and overall water quality (Costanzo et al., 2001; Zambrano et al., 2009; Dailer et al., 2010), maps of FIB concentrations are generally lacking (Coulliette et al., 2009; Feng et al., 2015). Our study developed *Enterococcus* spp. and *C. perfringens* maps using predictive models with water quality parameters (salinity, turbidity, water temperature) that could be rapidly

measured with a multi-parameter data-logging device, collecting thousands of data points. We found that *Enterococcus* spp. and *C. perfringens* concentrations decreased with increasing salinity and increased with increasing turbidity levels, a pattern widely observed and explained by freshwater runoff (Lipp et al., 2001; Frick et al., 2008a, 2008b; Gonzalez et al., 2012).

Our maps can be used to display FIB concentrations and evaluate the risk of water quality exceedances. Our models' adjusted  $R^2$  values for predicting *Enterococcus* spp. and *C. perfringens* concentrations were 0.45 and 0.55, respectively. These values fall within the upper range of 0.11–0.61 reported in other modeling studies for *Enterococcus* spp. (Hou et al., 2006; Frick et al., 2008a, 2008b; Thoe et al., 2014; Feng et al., 2015). To our knowledge, no predictive models have been developed for *C. perfringens*. One potential reason for our moderate adjusted  $R^2$  values is that regression equations using salinity do not distinguish between low salinity of groundwater and that of surface runoff (McLaughlin et al., 2007). This leads to the occurrence of hotspots in areas of groundwater input, such as Reed's Bay and the Hilo Harbor, where actual FIB concentrations were low. Additionally, our maps did exceedingly well in predicting FIB management decisions. They were accurate 64% and 95% of the time for *Enterococcus* spp. and *C. perfringens*, respectively. Our predictive capability was comparable to values reported for *Enterococcus* spp. models developed for coastal waters in Louisiana, North Carolina, and Florida (Deng et al., 2014; Gonzalez and Noble, 2014; Feng et al., 2015). Our results demonstrate that the maps

**Table 4**  
Type 1 error rate, Type 2 error rate, and percent correct water quality assessment predictions for multi-parameter regression models for *Enterococcus* spp. and *C. perfringens* in Hilo Bay, Hawai'i, USA, with YSI sonde data collected from October 2012 to April 2014.

| Model                    | Threshold<br>(MPN or CFU 100 mL <sup>-1</sup> ) | Type I error (%) | Type II error (%) | Correct (%) |
|--------------------------|-------------------------------------------------|------------------|-------------------|-------------|
| <i>Enterococcus</i> spp. | 104                                             | 27               | 9                 | 64          |
| <i>C. perfringens</i>    | 5                                               | 1                | 4                 | 95          |

can be used to determine unsafe swimming conditions with reasonable accuracy.

## 5. Conclusion

The combined approach of determining spatial and temporal FIB concentrations, along with water quality relationships for FIB mapping, provided useful information on hotspot occurrence. This study demonstrated that areas of surface runoff directly affect spatial patterns of FIB and lead to dramatic increases in concentrations during high river flow conditions. Furthermore, this study confirmed that areas of high surface runoff contained sewage, suggesting that the rivers are a source of sewage to Hilo Bay. The ability to map these patterns and predict FIB exceedances provided a valuable tool that could be reproduced globally to improve the spatial and temporal extent of water quality monitoring and aid watershed managers in visualizing FIB hotspots in need of management actions. This type of tool will become especially important in assessing future impacts of increased urban development and global climate change – both of which have been shown to affect microbial pollution levels in coastal areas (Mallin et al., 1999; Mallin et al., 2001; Tiefenthaler et al., 2009; Strauch et al., 2014).

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