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Immediate Impact of Hurricane Lane on Microbiological Quality of Coastal Water in Hilo Bay, Hawaii

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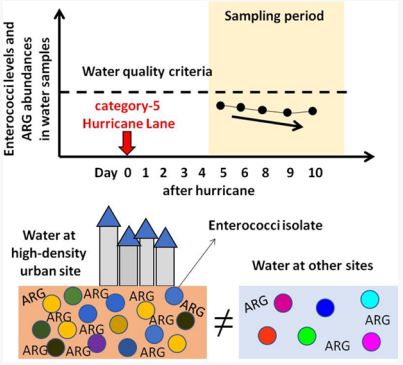
ABSTRACT: Hurricanes and associated stormwater runoff events are expected to greatly impact coastal marine water quality, yet little is known about their immediate effects on microbiological quality of near-shore water. This study sampled Hilo Bay immediately after the impact of Hurricane Lane to understand the spatial and temporal variations of the abundance and diversity of fecal indicator enterococci, common fecal pathogens, and antibiotic resistance genes (ARGs). Water samples from seven sampling sites over 7 days were collected and analyzed, which showed that the overall microbiological water quality parameters [enterococci geometric mean (GM): 6−22 cfu/100 mL] fell within water quality standards and that the temporal dynamics indicated continuing water quality recovery. However, considerable spatial variation was observed, with the most contaminated site exhibiting impaired water quality (GM = 144 cfu/100 mL). The *Enterococcus* population also showed distinct genotypic composition at the most contaminated site. Although marker genes for typical fecal pathogens (*invA* for *Salmonella*, *hipO* for *Campylobacter*, *mip* for *Legionella pneumophila*, and *eaeA* for enteropathogenic *Escherichia coli*) were not detected, various ARGs (*ermB*, *qurS*, *tetM*, *bla*TEM, and *sul*1) and integron-associated integrase *intI*1 were detected at high levels. Understanding the temporal and spatial variation of microbiological water quality at fine granularity is important for balancing economic and recreational uses of coastal water and the protection of public health post the impact of major hurricane events.



KEYWORDS: *Hurrican Lane, Hilo Bay, near-shore, water microbiological quality, spatial, temporal varations*

INTRODUCTION

Hurricane Lane was a category five hurricane that started forming on Aug 15, 2018 and eventually dissipated on Aug 29, 2018. It brought torrential rains to Hawaii islands and was the wettest tropical cyclone on record in Hawaii and the second-wettest tropical cyclone in the United States (only after Hurricane Harvey of 2017).[1] Under its impact, the City of Hilo on the Island of Hawaii experienced its wettest three-day period on record with 31.85 in. (809 mm) of precipitation, while a staggering 52.02 in. (1321 mm) of rainfall occurred in Mountain View, Hawaii. The record-breaking rainfall resulted in stormwater runoff that transformed typically tranquil streams into raging rivers and caused flooding in communities around Hilo Bay. The intense rainfall also led to rainfall-induced infiltration and inflow that overwhelmed the aging sewer infrastructures and resulted in multiple sanitary sewer overflows.

Stormwater runoff is one of the most important causes of coastal water contamination in urban areas,[2] as a large variety and high levels of contaminants can be present in urban stormwater runoff.[3] Hurricanes are expected to cause even more pronounced damage to coastal water quality and pose much elevated public health risks.[4] Several previous studies have examined the effects of hurricanes and their stormwater runoff on coastal marine or brackish water after an extended period of time had passed (e.g., 20 days after Hurricane Ivan,[5,13] 13 days after Hurricane Irma,[6] and 15 days after Hurricane Katrina[7]). However, little is known about the immediate impact of hurricane-caused stormwater runoff on coastal water quality. This is particularly important for microbiological water quality, which can change rapidly over time (e.g., within days) and have time-sensitive implications in balancing recreational activities and human health protection.

Currently, microbiological quality of coastal marine water is assessed by the density of fecal indicator enterococci,[8] which are part of normal microflora in gastrointestinal (GI) tracts of humans and warm-blooded animals but may also exist in natural environments.[9] Previous studies have shown correlation between high abundance of fecal indicator enterococci and GI illness rate in coastal marine waters impacted primarily by wastewater discharge.[10,11] Stormwater-impacted coastal

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water often reported high levels of fecal indicator bacteria (FIB)[7,12] and altered genotypic diversity of FIB population,[13] while actual public health risks of stormwater runoffs are associated with the presence of bacterial pathogens[14,15] and antibiotic resistance genes (ARGs).[16,17] Common bacterial pathogens include *Salmonella* spp., *Campylobacter* spp., *Legionella pneumophila*, and pathogenic *Escherichia coli*, which are among major causative agents of waterborne and foodborne illnesses in the United States.[18−20] Common ARGs include *ermB*, *qnrS*, *tetO*, *tetM*, *bla*TEM, *sul1*, and *vanA*, which confer resistances to the five most consumed antibiotics (macrolide, quinolone, tetracycline, ß-lactam, and sulfonamide)[21] and vancomycin as an antibiotic of last resort.[22] The class 1 integron-associated integrase (*intI*1) is associated with ARGs and considered as a proxy of anthropogenic pollution.[23]

The objective of this study was to understand the immediate impacts of Hurricane Lane on the microbiological quality of near-shore coastal water in the Hilo Bay. Water samples were collected from multiple locations within the Hilo Bay and over time immediately after the Hurricane and when field sampling became feasible. Fecal indicator enterococci was quantified and compared to water quality standards and historical data to contextualize the spatial and temporal variations of microbiological water quality. *Enterococcus* isolates were collected and DNA-fingerprinted to study their genotypic diversity. The abundance of typical fecal pathogens (including *Salmonella* spp., *Campylobacter* spp., *L. pneumophila*, and enteropathogenic *E. coli*), ARGs (*ermB*, *qurS*, *tetO*, *tetM*, *bla*TEM, *vanA*, and *sul1*), and integron-associated integrase gene (*intI*1) were determined by quantitative real-time polymerase chain reaction (qPCR).

MATERIALS AND METHODS

Study Sites. Near-shore water samples were collected from seven sites in Hilo Bay (Hawaii island, Hawaii) (Figure 1). The sites included Honolii Cove Ocean (HCO), Hilo Bay Lighthouse (HBL), Hilo Bay Canoe Beach (HBCB), Puhi Bay #3 (PB3), Onekahakaha Beach Swimming Area (OBSA), Carlsmith Beach Park (CBP), and Richardson Ocean Center (ROC). The GPS coordinates and prevalent shoreline land uses of the sampling sites are listed in Table 1. Tributary inputs to Hilo Bay include Wailuku River and Wailoa River. Land uses near the sampling sites include conservation forest, agriculture, and urban development. A wastewater treatment plant (WWTP) is located near Hilo international airport with its outfall 4400 feet out into the ocean near PB3 site.[24] In addition to the centralized wastewater treatment system, 8700 on-site wastewater units (i.e., cesspools) also exist in the Hilo Bay watershed.[25]

Table 1. Sampling Site Names, Locations, and Prevailing Shoreline Land Uses

site name	GPS coordinates	land uses[24]
HCO	19°45′18.1″N 155°05′28.3″W	intensive agriculture, low density urban, conservative
HBL	19°43′38.0″N 155°05′11.0″W	high-medium density urban
HBCB	19°43′24.7″N 155°04′33.0″W	open land, medium−high density urban
PB3	19°44′01.0″N 155°02′40.7″W	low density urban, industrial
OBSA	19°44′15.6″N 155°02′15.3″W	resort, industrial
CBP	19°44′02.1″N 155°01′41.6″W	open land, low density urban, industrial
ROC	19°44′06.7″N 155°00′50.2″W	orchard, open land, low density urban

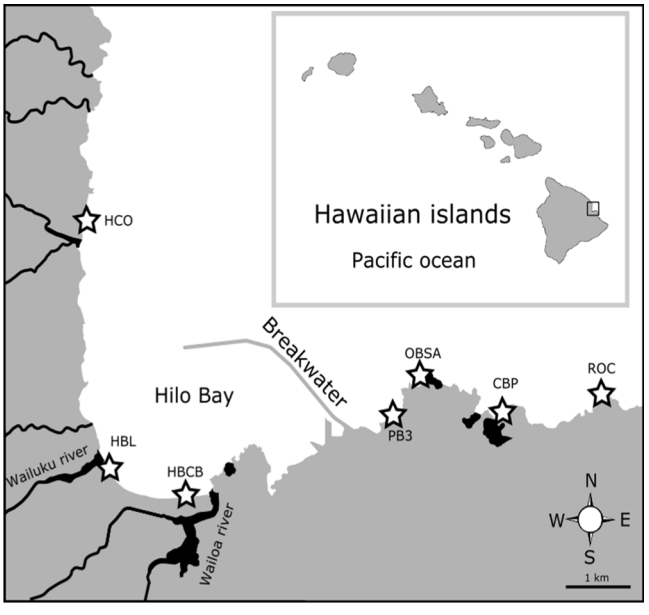

Figure 1. Coastal water sampling sites around Hilo Bay, Hawaii Island.

Sampling. The highest stream flow in the Wailuku River was recorded on August 23, 2018 (Figure 2A: day 0). Water sampling started immediately after the conditions were suitable

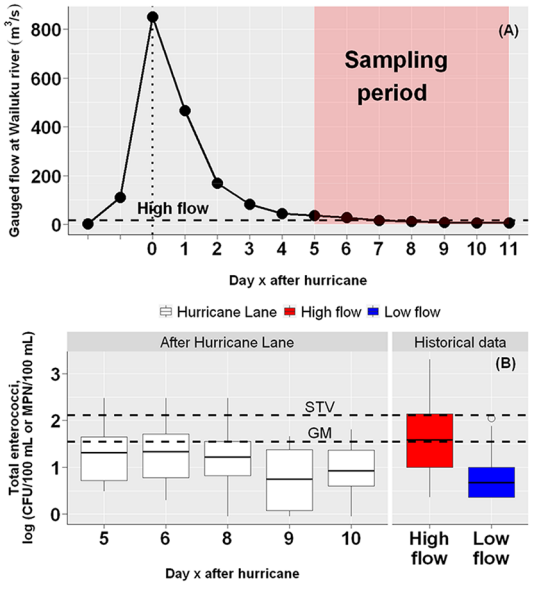

Figure 2. Water sampling period with respect to the impact of Hurricane Lane, as indicated by Wailuku River flow data (A), and enterococci density data for seven sampling sites after hurricane impact (cfu/100 mL) in reference to historical data (MPN/100 mL) (B). The dashed horizontal line in (A) indicates 95 percentile of historical flow data at the Wailuku River, while the dashed vertical line identifies the peak flow. The two horizontal dashed lines in (B) indicate the GM and STV of EPA enterococci water quality standards.

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for field sampling (i.e., August 28th 2018), which was 5 days (i.e., day 5) after the heaviest impact of Hurricane Lane. Sampling continued for 7 days, but only samples from days 5, 6, 8, 9, and 10 from the seven sampling sites were processed and analyzed because of logistic constraints. Approximately two liters of the water sample was collected at knee-depth using sterile polypropylene bottles and transported to the laboratory on ice for immediate processing (i.e., within 2 h). Water temperature and dissolved oxygen (DO) were measured on site using Ecosense DO200A Dissolved Oxygen Meter (YSI Inc., OH, USA), and pH measurement was performed in the laboratory using Oakton Ion 2700 Benchtop Meters (Cole-Pamer, IL, USA).

Enterococci Enumeration and DNA Extraction for qPCR Assay. Enterococci density in water samples was determined by following the standard membrane filtration method EPA1600.[26] In brief, 100 mL of water samples was filtrated through 0.45 μm GN-6 filter membranes (Pall Laboratory, NY, USA), and the membranes were subsequently incubated on mEI agar at 41 °C for 24 h. Duplicate analysis was conducted for each water sample, and arithmetic mean (cfu/100 mL) was reported. Additional 900 mL water samples were also filtrated as described above, and the filters were stored at −80 °C until DNA extraction. The filters were cut into pieces and extracted using an E. Z. N. A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA), and the extracted DNA samples were stored at −80 °C until qPCR assays of pathogens and ARG quantification.

Enterococci Isolation. Colonies with blue halo on the filter membranes on mEI agar were picked as presumptive enterococci isolates and streak-isolated on tryptic soy agar (Sigma-Aldrich, MO, USA) after incubation at 41 °C for 24 h. The isolates were first cultivated in a 96-well cell culture plate of Criterion Brain Heart Infusion (BHI) broth (Hardy Diagnostics, CA, USA) at 35 °C for 24 h, and the cultures were used to inoculate a new 96-well cell culture plate of BHI broth (incubated at 45 °C for 48 h) and another 96-well cell culture plate of BHI broth with 6.5% NaCl (35 °C for 48 h). A total of 206 isolates showed growth in both assays, which were confirmed as *Enterococcus* and were then cultured in BHI broth at 35 °C for 24 h prior to addition of 18% glycerol for storage at −80 °C.

BOX-PCR DNA Fingerprinting. To elucidate the spatial and temporal genotypic diversity of enterococci in Hilo Bay after the impact of Hurricane Lane, enterococci isolates (n = 117) were collected from the HBL site. A smaller number of isolates (n = 89) were also collected from other sites for comparison. A total 206 isolates were analyzed by BOX-PCR DNA fingerprinting using a BOXA2R primer by following a procedure modified from the report by Brownell et al.,[13] and a DNA template was prepared by following the protocol of Ran et al.[27] Briefly, enterococci were cultured in tryptic soy broth (Sigma-Aldrich, MO, USA) overnight at 35 °C, a 10-fold dilution of overnight culture in sterilized water was subjected to a freeze-and-thaw treatment at −20 °C, and the culture was directly used for BOXA2R-PCR. The reaction mixture contained 1× Gitschier Buffer, 10% dimethyl sulfoxide, 0.16 mg/mL bovine serum albumin, 800 mM dNTPs, 0.06 U/μL Taq polymerase, 0.5 μM primer, and 1 μL of DNA template. PCR thermocycling conditions include an initial denaturation at 95 °C for 7 min, 35 cycles of 90 °C for 30 s, 40 °C for 1 min, and 65 °C for 8 min, and final extension at 65 °C for 16 min. PCR products were separated by gel electrophoresis using 1.5% agarose gel in tris-borate−EDTA buffer at 90 V for 4 h. Gels with PCR amplicons were stained in 1× GelRed solution (Biotium, CA, USA) and visualized using a GelDoc imager (Bio-Rad, CA, USA).

The DNA fingerprints were analyzed using the BioNumerics v.7.01 (Applied Maths Inc., Sint-Martens-Latem, Belgium). The level of similarity between fingerprints was calculated using the Dice coefficient at 1.0% optimization and 1.0% band position tolerance. Bands of up to 6 kilo base pairs were included in the analysis. Dendrograms were constructed in BioNumerics using the unweighted pair group method with arithmetic mean, and the between-gel variation between the replicate runs of positive control *Enterococcus faecalis* ATCC 29212 was used to determine the cutoff threshold (89%) in genotyping. Two-hundred and six *Enterococcus* spp. isolates were clustered into 112 genotypes. A majority of the 112 genotypes were represented by one (80, 71.4%) or two (14, 12.5%) isolates (i.e., singletons and doubletons), which were not included in further analyses. Eighteen genotypes (16%) were represented by more than two isolates and were used for the comparison between the HBL site and other sites.

Real-Time PCR for Pathogens, ARGs, *intI*1, and 16S rRNA Gene. The water sample DNA extracts were used for the quantification of four pathogens [*Salmonella* (*invA*), *Campylobacter* (*hipO*), *L. pneumophila* (*mip*), and enteropathogenic *E. coli* (*eaeA*)], seven ARGs (*ermB*, *qnrS*, *tetO*, *tetM*, *bla*TEM, *sul1*, and *vanA*), and *intI*1 using qPCR. qPCR quantification of 16S rRNA gene was also performed to estimate total bacterial biomass.

The qPCR reactions (20 μL reaction volume) were run in duplicates on an ABI 7300 Real-Time PCR System (Applied Biosystems, Waltham, MA). Primers, probes, and cycling conditions for qPCR assays are provided in Supporting Information (Tables S1 and S2). Standard curves were constructed from 10-fold dilutions of synthesized DNA containing the target genes (gBlocks, IDT, Coralville, IA) ranging from 10^6 to 1 gene copies per reaction. The amplification efficiency of qPCR assays in this study ranged from 82 to 106%. Negative controls for the qPCR reaction (blank) and positive controls (10^2 gene copy of standards) were included in each qPCR run for actual water samples. The Ct value of each sample was calculated using arithmetic mean of duplicates. The concentration of target gene was calculated from the standard curve and reported as gene copies per liter (GC/L).

Environmental Monitoring Data. The available historical enterococci concentration/density at the sampling sites (April 2009 to April 2014) was obtained from the local environmental regulatory agency, which used the Idexx Enterolert method and was reported in MPN/100 mL.[28] The stream flow data of the Wailuku River were used in this study to indicate tributary flow conditions and were obtained from the USGS database, which ranged from 6.74 to 36.53 m^3/s during this study period.[29] During 2009−2014 when historical enterococci data are available, gauged water flow at the Wailuku River (site ID: USGS 16704000) ranged from 16.59 to 122.05 m^3/s during high flow and 0.087 to 0.24 m^3/s during low flow.[29] According to USGS, high flow and low flow were those higher than percentile 95th and those lower than percentile 5th of all data. The historical high-flow and low-flow ranges and corresponding enterococci data were used for comparison with data collected in this study.

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Data Analysis. The historical data of enterococci density data were subset into "high flow" and "low flow" categories accordingly to whether the Wailuku flow measurements were grouped into either high flow and low flow. For the qPCR assays, the water samples reporting no Ct values in both duplicates were recorded as 3.3 GC/L, which was the lowest detected concentration extrapolated from the standard curves. Normalization of the target genes was performed using 16S rRNA concentrations. Density data of enterococci (cfu/100 mL or MPN/100 mL) and normalized target genes were log-transformed prior to statistical analyses. The enterococci concentrations enumerated from membrane filtration (cfu/100 mL) in this study were deemed equivalent to the MPN results of historical water quality data for direct comparison.[8]

Differences of enterococci levels among various sampling days, various sampling sites, and comparison to the historical data were analyzed by the Kruskal–Wallis nonparametric test with Dunn's test for the group difference and p-values were adjusted by Benjamini and Hochberg method. Comparison of target genes and environmental parameters was performed using similar tests. Correlations of genotype relative abundance between the HBL site and other sites were determined using Pearson's correlation or Spearman's rank correlation, so were the correlations of various target genes and enterococci concentrations. The default significance level (α) was 0.05, while marginal significance level was 0.1. The nonparametric test was used, as the data set was not normally distributed accordingly to the Shapiro–Wilk test. All data analyses and plotting were performed in the R environment (version 3.6.1).

RESULTS AND DISCUSSION

Overall Microbiological Water Quality. Flow at the Wailuku River at day 5 (when field sampling became feasible after Hurricane Lane) was still above the 95 percentile of historical flow data (Figure 2A). Considerable variation in enterococci density in the water samples was observed among the sampling sites (Figure 2B). The overall enterococci abundance, as indicated by geometric mean (GM) values of the water samples from all seven sampling sites, were 21, 21, 17, 6, and 9 cfu/100 mL for days 5, 6, 8, 9, and 10 after storm, respectively, all of which were lower than the GM enterococci water quality standard (30 cfu/100 mL) and were showing gradual decrease over time, albeit no significant differences was observed due to variation between different sampling days (Dunn's test, $p = 0.06$).

When the overall density of enterococci in the Hilo Bay was compared with historical water quality data, water quality on days 5, 6, and 8 showed no significant difference from enterococci density during historical high flow conditions (Dunn's test, $p = 0.31$). In contrast, the water quality on days 9 and 10 was not significantly different from the historical low flow density (Dunn's test, $p = 0.22$). The GM density and the gradual decrease of enterococci density over time indicate that the overall microbiological water quality of Hilo Bay was no longer impaired on day 5 and was improving over time. Temperature of Hilo Bay waters varied from 21.1 to 23.6 °C. DO varied from 6.03 to 8.80 mg/L and pH varied from 6.44 to 8.09. A specific trend of temporal variation of environmental parameters was not detected. As the only detectable trend, DO levels on day 5 were higher than later days after hurricane (Dunn's test, $p = 0.005$).

Overall, the coastal water in Hilo Bay immediately after the impact of Hurricane Lane exhibited acceptable microbiological water quality, as the GM values of enterococci counts of the seven sampling sites collectively remained below the GM water quality standard and exhibited decrease over time (Figure 2B). Several factors could have contributed to this observation. First, the first flush phenomenon and the practical limitation of sampling within active hurricanes means that even the earliest efforts could still have missed the most severe pollution time points. During the first flush, stormwater runoff is expected to carry the highest load of terrestrial pollutants.[30,31] The first flush effects of stormwater runoff in the Hilo Bay watershed was previously observed, where the peak level of turbidity, which is usually positively correlated to FIB density, was observed at the beginning of storm and decreased to below embayment standard within 3 days.[32] Direct measurement on enterococci at beaches in San Diego, California, showed its density correlated to the storm hydrograph, and the recovery was observed within less than 5 days after the peak flow.[33]

The large runoff volume of Hurricane Lane and the decay reaction kinetics could have also contributed to the transport and fate of the fecal indicator enterococci in the near-shore water. At the peak of Hurricane Lane, the gauged flow rate at the Wailuku River was 849.5 m^3/s, while the highest flow rate during the historical period 2009–2014 was only 122.1 m^3/s. During the high flow period of Hurricane Lane (i.e., above the threshold Figure 1A), the cumulative flow in the Wailuku River was 1.5×10^8 m^3. Such large volumes of runoffs are expected to dilute and transport the pollutants from the first flush further away from the near-shore sampling sites. Additionally, the fecal indicator enterococci could also have experienced rapid decay in the near-shore coastal water under warm and sunny conditions. Cultivable enterococci decayed significantly faster in seawater with light ($T_{90} = 0.14$ day) than under dark conditions ($T_{90} = 0.74$ day),[34] enterococci decayed faster at shallow depths and during summer ($T_{90} = 0.15$ day),[35] and low turbidity coastal seawater with sunlight irradiation exhibited T_{90} values as low as 0.42 days.[36]

Spatial Variation of Water Quality. Because significant variation in enterococci density was observed across different sites (Kruskal Wallis test, $p < 0.001$), the data were further analyzed for the individual sites and compared with site-specific historical data (Figure 3). The mean values of enterococci density from the five different sampling days ($n = 10$) were 3 (CBP), 13 (HBCB), 144 (HBL), 26 (HCO), 4 (OBSA), 26 (PB3), and 4 cfu/100 mL (ROC) (Figure 3). Enterococci densities in HBCB, CBP, and OBSA sites were significantly lower than the historical high flow data (Dunn's test, $p = 0.03$) and showed no statistical difference from historical low flow data (Dunn's test, $p = 0.19$). Enterococci density data in ROC showed no significant difference to both historical high flow and low flow (Dunn's test, $p = 0.08$). Enterococci densities in HCO were between high flow and low flow density (Dunn's test, $p = 0.02$).

Density of enterococci in the HBL and PB3 sites showed no statistical difference from historical high flow data (Dunn's test, $p = 0.06$ and 0.29, respectively). The HBL site was the only site where the mean enterococci density exceeded the GM water quality criteria. Additionally, 60% of water samples from the HBL site exceeded the statistical threshold value (STV) water quality criteria (130 cfu/100 mL). These results indicate considerable spatial variation in microbiological water quality in Hilo Bay after the impact of Hurricane Lane, with the HBL site being the most impacted site. No specific trend of site

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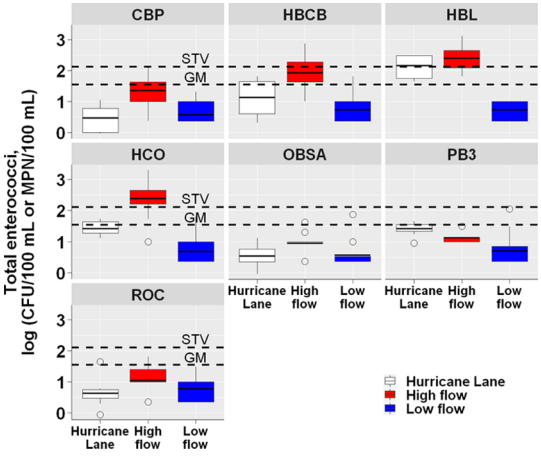

Figure 3. Boxplots of total enterococci density across 5 sampling days at each of seven sampling sites in Hilo Bay in comparison to historical high flow (red) and low flow (blue) density. The inner box line represents the mean value. The upper and lower horizontal dashed lines are STV and GM of EPA water quality criteria, respectively.

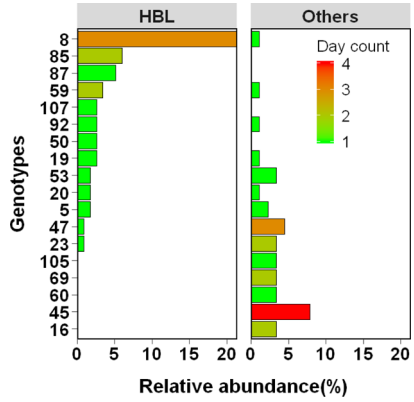

Figure 4. Distribution of isolate genotypes at the HBL site (n = 117) in comparison with other sites (n = 89). The color gradient indicates detection frequency on different sampling days.

variation of the monitored environmental parameters was observed.

Although the overall coastal water in Hilo Bay after the Hurricane Lane met the microbiological water quality standards, the large spatial variation indicated the presence of hotspot locations where increased health risks exist. Among the seven sampling sites, the HBL site was the only site with water samples showing the mean density of enterococci (144 cfu/100 mL) exceeding both STV and GM criteria. The high density of enterococci at the HBL site was probably caused by its geographic proximity to the urban core of Hilo and the Wailuku River. The Wailuku River, which receives runoff from the nearby urban area, is a recognized source of fecal contaminants and sediments in Hilo Bay.[24,37] In addition, the shorelines of the HBL site were lands with dominant use of high-density urban use, which is abundant with sewers and on-site wastewater treatment units.[24] High density of enterococci at the HBL site was previously observed through regular monitoring efforts[38] and in previous studies.[37,39]

Near-shore waves and man-made structures in the Hilo Bay could also have contributed to the observed spatial variation of microbiological water quality. Sites on the west side of the breakwater (HBCB and HBL) showed higher concentrations of enterococci than sites on the east of the breakwater (OBSA, PB3, and ROC). Waves toward the Hilo Bay shorelines are mainly driven by the trade winds from northeast direction, and shorelines west of the breakwater, where the HBL site resides, experiences low wave energy and reduced mixing.[24] In contrast, the PB3 site, which is located east of the breakwater, exhibited enterococci density higher than historical high flow data, but it was still lower than the GM water quality standard, in spite of the presence of an effluent outfall from the nearby WWTP and reported sewage spills because of the hurricane.

***Enterococcus* Genotypic Diversity.** DNA fingerprinting analysis clustered 206 *Enterococcus* isolates into 112 genotypes, 18 of which were composed of more than 2 isolates and were used for the comparison between the site HBL and others. The rank abundance plot of the genotypes at the HBL site showed that Genotype 8 had the highest relative abundance (20%), followed by genotypes 85 (5.9%), 87 (5.1%), 59 (3.4%), and other genotypes (<2.6%) (Figure 4). The distribution pattern of the genotypes at the HBL site was not observed at the other sampling sites; for example, the most abundant Genotype 8 at the HBL site was only represented by 1.1% at the other sites. Although genotypes 5, 8, 19, 20, 23, 47, 53, 59, and 92 were present at both the HBL site and the other sites, the relative abundances showed no correlation between each other (Spearman's correlation test, $\rho = -0.50$, $p = 0.17$). Furthermore, 52% of isolates at the other sites were clustered in genotypes 16, 45, 60, 69, and 105, which were not detected at the HBL site at all.

The temporal distribution of the genotypes, as indicated by their detection frequencies among the different sampling days, shows that some genotypes were detected more frequently than others. For example, Genotypes 8 and 45 were detected on three and four different days, respectively, while a majority (89/112, 79.5%) of the genotypes were only detected on one day. These data showed that the genotypic diversity at the most impacted HBL site was considerably different from other sites in the Hilo Bay, and there were substantial spatial and temporal variations in *Enterococcus* genotype diversity.

The different genotypic diversity of *Enterococcus* population between the HBL site and other sites (Figure 4) further supported the distinction between a hotspot location and other locations within the same waterbody. The observed different genotypic diversities between the HBL and other sites likely resulted from different land uses adjacent to the waterbody and source of enterococci. The HBL site is more likely to receive urban stormwater runoff and fecal contaminants than the other locations. The high genotypic diversity of the *Enterococcus* population in the water samples after the impact of Hurricane Lane was indicated by the observation that a majority of genotypes (83.9%) were represented only by 1 or 2 out of 206 isolates. Although no prior literature has reported the genotypic diversity of *Enterococcus* population after a major hurricane, higher population diversity of *Enterococcus* was observed in a Florida beach during storm runoff events.[13]

Detections of Pathogen Gene Markers and ARGs. To further evaluate immediate impact of Hurricane Lane on microbiological water quality of Hilo Bay, DNA extracted from water samples was tested for typical fecal pathogens, ARGs, and integron-associated integrase gene. The four pathogen-specific genes (*invA*, *hipO*, *mip*, and *eaeA*) and one of the ARG genes (*tetO*) were not detected in any of the water samples. Five ARGs and *intI*1 were detected in some of the water samples, and the average gene concentrations were 2.40 log

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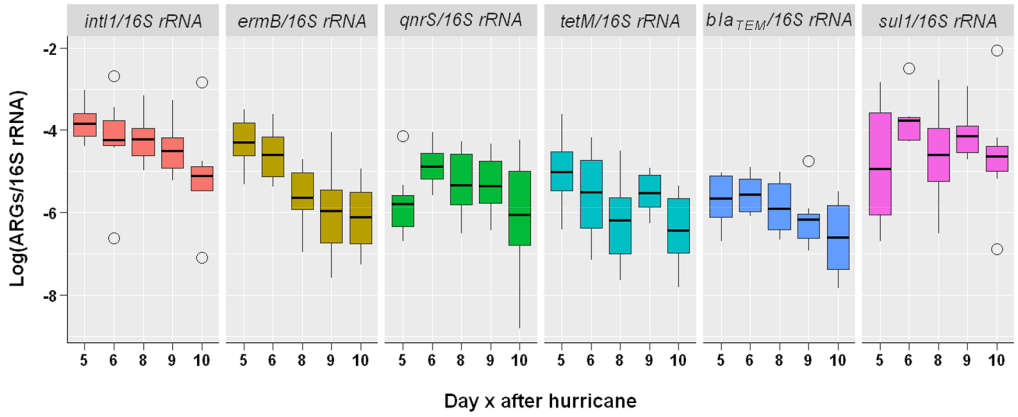

Figure 5. Boxplot of the relative abundance of *intI*1 and ARGs across sampling sites (n = 7) after the Hurricane Lane impact. The inner box line represented the mean value.

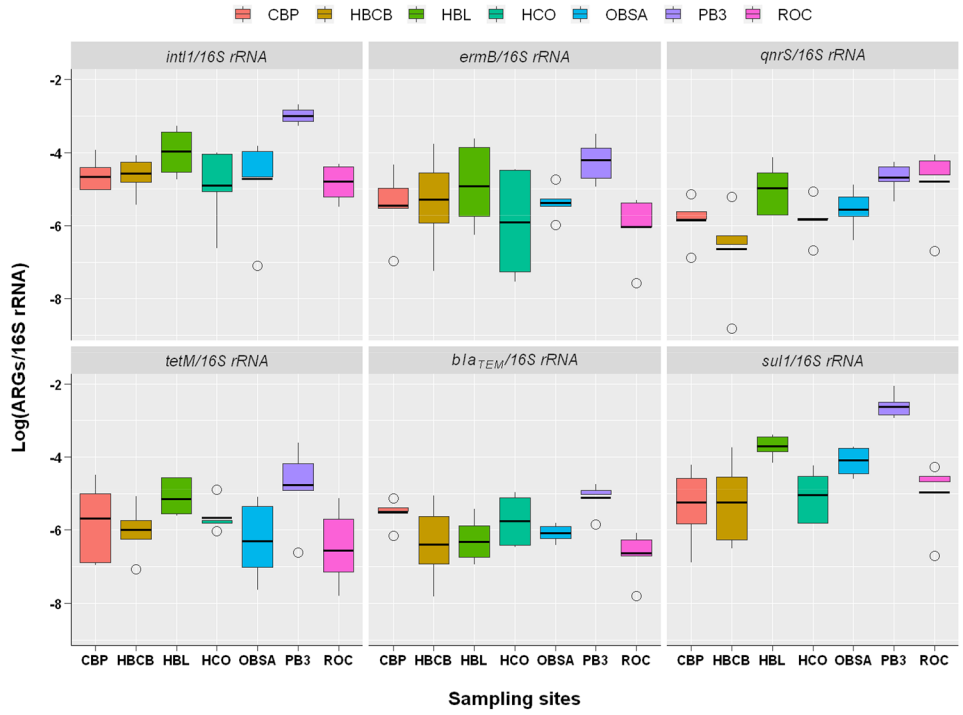

Figure 6. Boxplot of the relative abundance of *intI*1 and ARGs at the seven sampling sites in Hilo Bay. The inner line with the box represents the mean value of multiple sampling days.

GC/L for *ermB* (number of samples detected: n = 35), 2.47 log GC/L for *qnrS* (n = 26), 2.26 log GC/L for *tetM* (n = 24), 1.63 log GC/L for *bla*TEM (n = 30), 3.65 log GC/L for *sul1* (n = 28), and 3.22 log GC/L for *intI*1 (n = 33).

The ARG and *intI*1 gene concentrations were normalized by 16S rRNA gene concentrations of the same water samples to obtain relative abundances. The 16S rRNA gene concentrations ranged from 6.32 log GC/L to 9.34 log GC/L among the 35 water samples. Relative abundance of these six genes across seven sampling sites decreased over the period of five sampling days (Figure 5). Pairwise comparisons indicated that relative abundances of *ermB* at day 5 were significantly higher from those of days 8, 9, and 10 after hurricane (Dunn's test, p ranges from 0.01 to 0.03). There was approximately 42% decrease in average relative abundance of *ermB* from day 5 to day 10 after hurricane. Although no statistically significant differences in relative abundances of other six genes between different sampling days were detected (Kruskal Wallis test, p ranges from 0.06 to 0.27), 23 to 33% decreases in average relative abundances from day 5 or day 6 to day 10 were observed.

Comparison of average gene relative abundances across sampling days at the seven sampling sites also indicated high abundances at the site HBL (Figure 6). Average relative abundances of *intI*1, *ermB*, *qnrS*, and *tetM* at the site HBL were higher than those of all other sites except for the PB3 site, although no statistically significant difference between them was observed (Dunn's test, p ranged from 0.10 to 0.86). There were marginally statistically significant differences between relative abundances of *sul1* at the site HBL and the other sites including CBP, HBCB, HCO, and ROC (Dunn's test, p ranged from 0.05 to 0.07). Average relative abundance of *sul1* at the site HBL was 1.26 to 1.54 higher than the abundances of those four sites. Interestingly, the relative abundance of *intI*1 and

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ARGs at the PB3 site, which showed a low level of enterococci density, was the highest among the sampling sites.

Municipal wastewater receives antibiotic resistant bacteria developed in the guts of hospitalized patients or individuals who consume antibiotics and therefore contains diverse and abundant ARGs (e.g., as high as 10^9 GC/mL in raw wastewater influent[40]). *intI*1 encodes a class 1 integron that is commonly associated with the horizontal gene transfer of ARGs.[23] A previous study showed correlation between *intI*1 and ARG abundances in wastewaters.[41] In this study, the relative abundances of *intI*1 in the coastal water immediately after the impact of Hurricane Lane was significantly and positively correlated to *ermB*, *tetM*, and *sul1* (Spearman's correlation test, ρ ranged from 0.45 to 0.72, $p < 0.001$). During rainfall events and storms, sanitary sewer overflows contributed to dissemination of ARGs and *intI*1 in stormwater runoffs[15] and coastal waters.[42] The high relative abundances of *intI*1 and ARGs observed at the HBL site were consistent with shoreline characteristics of high-density urban, prevalence of sewer networks, cesspools, and proximity to the polluted river, which also corroborated with results of enterococci concentrations and genotype diversity. For example, the relative abundance of *ermB* significantly correlated with enterococci concentrations (Spearman's correlation test, $\rho = 0.52$, $p = 0.001$). The observation of the highest relative abundance of *intI*1 and ARGs at the site PB3 is likely due to its proximity to the effluent outfall of nearby WWTP. Secondary treated and disinfected effluents can still contain high levels of ARGs (e.g., $10^{6.96}$ GC/L *tetO* and $10^{6.71}$ GC/L *tetW*[43]).

This study, for the first time, collected data to investigate the immediate impacts of a hurricane on the spatial and temporal variations of fecal indicator enterococci density, *Enterococcus* genotypic diversity, and the presence of common bacterial pathogens and ARGs in near-shore coastal marine water. Understanding the temporal and spatial variation of microbiological water quality after hurricane impact is important to balancing economic and recreational uses of coastal water and the protection of public health. For example, during Hurricane Lane, regulatory water quality monitoring at Hilo Bay was suspended for 3 weeks, while recreational water use resumed much more quickly as swimmers and surfers were observed at the onset of the sampling efforts. The rapid field response implemented in this study provided a rare opportunity to understand the temporal dynamics of water microbiological quality, which is time-sensitive and may not be able to extrapolate from studies on less severe types of stormwater runoff. The observations of overall microbiological water quality parameters and continuing water quality recovery (as indicated by enterococci density) were different from common perceptions, further highlighting the importance of the temporal factor in water quality. On the other hand, the presence of a contaminated HBL site indicate that spatial variation in water quality after the Hurricane Lane impact was also an important factor driving the water quality and potential health risks. This spatial variation was corroborated by the geographic distribution of *Enterococcus* genotypes among the sampling sites and the high relative abundance of ARGs and *intI*1 at the HBL and PB3 sites, further demonstrating the potential health risks and suggesting impacts from man-made coastal infrastructures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c07082.

qPCR assays targeting total bacteria and bacterial pathogens and qPCR assays targeting ARGs and integron class 1 (PDF)

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Notes

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