# RAINFALL DRIVEN SHIFTS IN *STAPHYLOCOCCUS AUREUS* AND FECAL INDICATOR BACTERIA IN THE HILO BAY WATERSHED

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#### Abstract

Bacteria in nearshore waters can threaten coastal communities, as some land-based bacteria can be pathogenic. The transport of these pathogens to the ocean is largely regulated by rainfall and stream flow patterns. Climate change will impact weather patterns, altering dynamics of pathogen loading to coastal waters. To assess the impact of pathogen loading to nearshore waters under varying weather conditions, a study was conducted in the Hilo Bay watershed in Hilo, Hawai'i, USA. Staphylococcus aureus, Methicillin-Resistant S. aureus (MRSA), and fecal indicator bacteria (FIB, Enterococcus spp. and Clostridium perfringens) were quantified within Hilo Bay and its watershed streams, and within potential landscape sources (road runoff, sewage, and soil), using culture-based methods. Bacteria concentrations were compared to rainfall and stream flow data from pre-existing gauges. S. aureus and FIB were found across all Hilo Bay stations, and within streams, road runoff, sewage, and soil. MRSA was also found within Hilo Bay stations close to shore, and within streams and road runoff, but was less prevalent. S. aureus and FIB were significantly related to rainfall across stations in Hilo Bay. Additionally, S. aureus and FIB concentrations had positive relationships with peak stream discharge, as well as the ratio of current stream flow and the 7-d moving average. These data suggest that more intense storms, and increased dry periods between storms, will increase S. aureus and FIB loads to Hilo Bay. The patterns and models developed in this study will be useful for informing the public of impaired water quality conditions in Hawai'i, and across the wet tropics.

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#### 1. Introduction

The presence of bacteria in nearshore waters is an unseen health threat to coastal communities worldwide, as certain bacteria are agents of disease. One bacteria that is a health concern in coastal waters is Staphylococcus aureus, a pathogen that is becoming increasingly prevalent in the environment (Zetola et al., 2005). Though S. aureus was originally limited as a threat within healthcare settings, community-acquired infections are on the rise, and have been associated with marine water use (Charoenca and Fujioka, 1993; Zetola et al., 2005; Soge et al., 2009). One study found that beachgoers were four times more likely to develop S. aureus infections compared to non-beach users (Charoenca and Fujioka, 1995). These community-acquired infections can be quite severe, with even previously healthy individuals contracting symptoms such as skin rashes, painful abscesses, necrotizing fasciitis (flesh-eating disease), and scaled skin syndrome (Archer, 1998; Chambers, 2001). Additionally, some strains exhibit antibiotic resistance, including Methicillin-Resistant S. aureus (MRSA), which can be difficult to treat (Zetola et al., 2005). As S. aureus and MRSA have been found in coastal waters across the globe (Papadakis et al., 1997; Goodwin and Pobuda, 2009; Esiobu et al., 2013), users of these aquatic systems face serious health risks.

The presence of *S. aureus* in marine waters has been traditionally attributed to the shedding of *S. aureus* from human skin during bathing (Elmir et al., 2007); however, recent studies have shown that watershed sources can contain *S. aureus* and MRSA (Viau et al., 2011a). *S. aureus* and MRSA have been found in streams (Viau et al., 2011a), in soils near pig farms (Schulz et al., 2012), and on wildlife (Lewis et al., 2008; Porrero et al., 2013). These bacteria have also been found in urban runoff, with higher concentrations in high-density residential areas compared to low-density residential and commercial regions (Olivieri et al., 1978; Selvakumar

and Borst, 2006). Additionally, *S. aureus* and MRSA have been found in sewage across the United States and in Europe (Börjesson et al., 2009; Rosenberg Goldstein et al., 2012), and these pathogens can be leaked into the groundwater in areas with onsite sewage disposal systems (OSDS) (Whittier and El-Kadi, 2014). As *S. aureus* and MRSA are present in the watershed, the transport of these pathogens to near-shore waters may result in increased human threats of *S. aureus* and MRSA infection.

To assess human health risks associated with sewage contamination in nearshore waters, the United States Environmental Protection Agency (USEPA) uses fecal indicator bacteria (FIB). These indicators are used as proxies for pathogens in sewage, and standards have been established that relate bacteria concentrations to health risks for water users (USEPA, 2012a). The federal FIB for marine recreational water is *Enterococcus* spp.; however, these bacteria can reproduce in soil and may not be indicative of sewage pollution (Hardina and Fujioka, 1991; Fujioka et al., 1999; Fujioka et al., 2015). Hence, in tropical regions such as Hawai'i, C. perfringens is used as a secondary indicator (Fujioka et al., 2015). C. perfringens does not reproduce in the environment, and is spore forming and persistent, making it useful to indicate past pollution (Bisson and Cabelli, 1979; Wright, 1989; Desmarais et al., 2002). Though these FIB are used to assess public waters with the intention of screening for human sewage, they have also been found within animal wastes, as *Enterococcus* spp. is naturally present within the gut of warm-blooded animals, and C. perfringens within carnivorous mammals (USEPA, 2009; Vierheilig et al., 2013). As FIB is not only present within OSDS and sewer systems, but also on the landscape, these bacteria can be moved into nearshore waters via rainfall, and subsequent runoff and stream flow.

Climatic changes will likely impact the movement of bacteria from the landscape to nearshore waters, as pathogen transport from land to coast is largely influenced by precipitation patterns (Strauch et al., 2014; ASCE Foundation, 2017). It is predicted that climatic changes will result in an increase in the intensity and frequency of precipitation events for the majority of regions across the globe (Ruosteenoja et al., 2003; IPCC, 2014). Heavy precipitation events have been shown to increase pathogen transport (Boxall et al., 2009), leading to increased human health risks (Curriero et al., 2001; Kistemann et al., 2002). Drought conditions are also expected to impact many regions, though there is lower confidence with this prediction (IPCC, 2014). Under drought conditions, pathogens may not persist in the environment due to high temperatures and low soil moisture. However, such soils may become unable to hold water, leading to more runoff and expedited transport of pathogens when precipitation does occur (Boxall et al., 2009). Additionally, bacterial concentrations in dry soils may increase in response to infrequent rainfall (Fierer et al., 2003; Strauch et al., 2014). In the tropics, this may lead to increased bacterial loads being transported to coastal waters during storms (Strauch et al., 2014), due to the coupling of an overall reduction in rainfall and increased storm frequency in some areas (Palmer and Räisänen, 2002; Chu et al., 2010; Elison Timm et al., 2011). Due to these changing rainfall patterns and rising air temperatures, it is also predicted that river flow will exhibit more extreme high and low flow conditions (Falloon and Betts, 2006). These changes are likely to impact the mechanisms, and amount, of pathogen loading during high precipitation events under projected climate scenarios for wet tropical regions.

The transport of bacteria into nearshore waters represents health risks for community users engaged in recreation, subsistence, and cultural activities. Such risks will become be influenced by climatic changes. To investigate how pathogen loading is impacted by climate

patterns in the wet tropics, this study assessed the loading of *S. aureus*, MRSA, and FIB to nearshore waters by quantifying these bacteria under various rainfall and stream flow conditions. The Hilo Bay watershed on Hawai'i Island was chosen as a study site because it is a tropical region that receives high and variable rainfall and stream flow (Engott, 2011; Giambelluca et al., 2013). It is also used by the community year-round, making it an ideal location for this project. This project had three goals: 1) to identify the presence of *S. aureus*, MRSA, and FIB within coastal waters, streams, and landscape sources (runoff, sewage, and soils) of the Hilo Bay watershed, 2) to assess relationships between *S. aureus*/FIB and rainfall, stream flow metrics, and water quality parameters, and 3) to develop predictive models that could be used to infer water quality risks in Hilo Bay and make inferences regarding climate driven patterns. Results from this project will improve our understanding of water quality health risks related to a changing climate. Additionally, the patterns in this study provide foundations for adaptive strategies to protect the health of water users in coastal communities in Hawai'i, and elsewhere in the wet tropics.

#### 2. Methods

#### 2.1 Study site

The Hilo Bay watershed is located on the east side of Hawai'i Island, in Hilo, Hawai'i, USA (Fig. 1). This windward facing shore receives annual precipitation rates between ~3050 mm near the coast and up to ~7000 mm at higher elevations, which is one of the highest precipitation rates in the United States (Giambelluca et al., 2013). Precipitation patterns are characterized by wet winter (Oct - Apr) and dry summer seasons (May - Oct). Surface waters enter Hilo Bay from the Wailuku and Wailoa rivers. The Wailuku is the State of Hawai'i's largest river in terms of flow, draining over a third of the Hilo Bay watershed (Engott, 2011). The Wailuku River watershed

contains conservation forest (77%), agriculture (22%), and low intensity urban development (1%) (Parham et al., 2008). The Wailoa River has a smaller watershed, and its land use is similar to that of the Wailuku River, except for draining 8% more urban land, which is concentrated around its lower elevation reaches (Parham et al., 2008). Hilo Bay receives majority of its freshwater input through groundwater seeps, which is estimated to be 100 times greater than the average value for annual surface runoff (M & E Pacific Incorporated, 1980). A 2.9-km breakwater bounds Hilo Bay, increasing the residence time of water within the bay (M & E Pacific Incorporated, 1980).

#### 2.2 Study design

Bacteria (*S. aureus*, MRSA, and FIB), physiochemical water quality parameters, and nutrient concentrations were analyzed through surface water sample collection in the Hilo Bay watershed over a range of rainfall and stream flow conditions. Sampling began at sunrise to obtain the highest estimate of bacteria concentrations before photo-inactivation of the cells occurred (Fujioka et al., 1981). Sampling in Hilo Bay was conducted on 20 different days from March 2014 through March 2017. Stations included Reed's Bay, the Wailoa River mouth, the Wailuku River mouth, and the Hilo Bay water quality buoy (Fig. 1). Within the bay, the Reed's Bay station is dominated by groundwater input, while the Wailuku River mouth, Wailoa River mouth, and Hilo Bay water quality buoy stations are impacted by surface water inputs. Water was also collected at two upstream stations, one in the Wailuku River (elevation: ~245-m) and one in the Wailoa River (elevation: ~0-m). Rivers were sampled over ten different days from June 2016 to March 2017. At all stations, three 1-L replicate samples were collected. During collection of marine and stream water samples, physiochemical parameters were measured with a YSI Pro 2030 multi-parameter probe (salinity, temperature, and dissolved oxygen [% saturation and

concentration]). Turbidity was measured in the laboratory using a turbidimeter (HACH 2100P). Both instruments were calibrated before each use.

Samples were also collected from potential landscape sources of bacteria, including road runoff, sewage, and soils. Road runoff was collected at two locations within downtown Hilo during a single sampling event in July 2017. Influent and effluent from the Hilo Wastewater Treatment Plant (HWWTP) was sampled once in August 2017. Sewage was sampled from the HWWTP because this site was easier to access than cesspools, which have openings that are often sealed and buried, and the influent is likely similar in composition. Soils from the Pu'u Makala Natural Area Reserve System (NARS) (19.51731<sup>°</sup>N, 155.28514 <sup>°</sup>W) were also sampled in triplicate at sampling locations. Though this area is not within the Hilo Bay watershed, it was used to assess impacts of feral ungulate presence on soil bacteria concentrations, and to assess bacteria concentrations within native forest soils. Permitted soil collection was conducted in three locations within an ungulate-free site (fenced for over 10 years at the time of sampling), and an ungulate-invaded site (fencing was not complete), over three sampling days from June – July 2017 (Appx. I). All samples were collected in sterile, acid-washed, polypropylene bottles, immediately placed on ice, and processed within 4 – 8-h of collection.

Rainfall data were obtained from the National Oceanic and Atmospheric Administration (NOAA)/National Weather Service (NWS) Hilo International Airport rainfall gauge (http://www.prh.noaa.gov/hnl/ hydro/ pages/rra\_graphs.php?station = HTO&mo=032015), and from the NOAA/NWS Pi`ihonua rainfall gauge (HI91) (archived data requested). Rainfall data from the National Climatic Data Center's (NCDC) daily climate normals, which is collected from the NOAA/NWS Hilo International Airport, were also used as thresholds to compare bacteria and nutrient concentrations, between high and low rainfall conditions at the Wailoa

River and Reed's Bay stations. The Hilo International Airport rainfall gauge is close to the Reed's Bay and Wailoa River mouth stations (~4-km), so these data were only used to analyze relationships at these locations. There were no climate normals data available for the Pi`ihonua rainfall gauge. Data from the Pi`ihonua rainfall gauge, located on the Wailuku River, were used to analyze trends within the Wailuku River mouth and Hilo Bay water quality buoy stations. River discharge data for the Wailuku River were gathered from the US Geological Service (USGS) gauge (16704000) (http://waterdata.usgs.gov/usa/nwis/ uv?16704000). This gauge is located ~8-km upstream from the Wailuku River mouth station. The Wailuku River is the only gauged river in the Hilo Bay watershed.

#### 2.3 Bacteria analyses

In the laboratory, water from each replicate sample collected was analyzed for *S. aureus*, MRSA, and FIB (*Enterococcus* spp., and *C. perfringens*). *S. aureus* and MRSA were enumerated by filtering sample water through 0.45-µm pore size cellulose nitrate filters (Whatman), and culturing on BBL CHROMagar *S. aureus* and BBL MRSA II *Select* media, respectively (Becton, Dickinson Co., MD) (Goodwin and Pobuda, 2009; Viau et al., 2011a). The cultures were incubated at 37°C for 24-h, and confirmed morphologies were counted as *S. aureus* or MRSA. To confirm morphologies, putative colonies were re-streaked on their respective media types, and incubated again for 24 - 48-h. A subset of 87 colonies was analyzed through gram staining methods, and 21 of those colonies were identified as gram positive, cocci clusters under a microscope (1000X magnification). These colonies were analyzed using 24-h tube coagulase tests (BD BBL Coagulase Plasma, Rabbit with EDTA, BD Diagnostics, Franklin Lakes, NJ, USA), and 14 yielded a positive coagulase result. Only morphologies similar to these 14 colonies were used as confirmed morphologies in the *S. aureus* or MRSA plate counts. *Enterococcus* spp.

was analyzed using the Enterolert MPN method and QuantiTray®/2000 from IDEXX Laboratories Incorporated, following manufacture recommendations (10-mL sample and 90-mL of sterile water) (Palmer et al., 1993). All *Enterococcus* spp. concentrations reported were corrected for sample dilution. *C. perfringens* were enumerated by filtering water through 0.45µm pore size cellulose nitrate filters (Whatman) and mCP medium (Acumedia, Baltimore, MD) (Bisson and Cabelli, 1979). Methods for *Enterococcus* spp. and *C. perfringens* quantification used in this study are also used by the Hawai`i Department of Health (HDOH). Road runoff and sewage samples were diluted with autoclaved, deionized water up to a volume of 100-mL for vacuum filtration. Soil samples were processed by methods described in Myers et al. (2007) and Strauch et al. (2016). Briefly, 10-g of soil from each sample were shaken with 200-mL of 0.15 M NaCL for 45-min at 100 rpm, left to settle for ~5-min, and an aliquot was removed from the top and transferred into a sterile bottle with autoclaved, deionized water for vacuum filtration.

#### 2.4 Water quality analyses

A portion of each water sample was filtered for nutrient analyses through pre-combusted (500°C, 5 h) 0.7- $\mu$ m GF/F filters (Whatman) and stored frozen until chlorophyll *a* (Chl *a*) analysis. Nutrient samples were analyzed for concentrations of nitrate plus nitrite (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>; USEPA 353.4, Detection Limit (DL) = 0.07  $\mu$ mol L<sup>-1</sup>), ammonium (NH<sub>4</sub><sup>+</sup>; USGS I-2525, DL = 0.36  $\mu$ mol L<sup>-1</sup>), phosphate (PO<sub>4</sub><sup>3-</sup>; USEPA 365.5, DL = 0.03  $\mu$ mol L<sup>-1</sup>), total dissolved phosphorous (TDP; USGS I-4650-03, DL = 0.5  $\mu$ mol L<sup>-1</sup>), and silicic acid (H<sub>4</sub>SiO<sub>4</sub>; USEPA 366, DL = 1.0  $\mu$ mol L<sup>-1</sup>) with a Technicon Pulse II AutoAnalyzer using standard methods and reference materials (NIST; HACH307-49, 153-49, 14242-32, 194-49). Dissolved organic carbon (DOC; USEPA 415.1, DL = 10  $\mu$ mol L<sup>-1</sup>) and total dissolved nitrogen (TDN; ASTM D5176, DL = 5  $\mu$ mol L<sup>-1</sup>) were analyzed on a Shimazdu 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 (DIN =  $NO_3^- + NO_2^- + NH_4^+$ ), while dissolved organic phosphorous (DOP) was calculated from the difference between TDP and  $PO_4^{3-}$ . Filters for Chl *a* were analyzed on a Turner 10-AU fluorometer according to USEPA method 445.0. All nutrient analyses were conducted at the University of Hawai'i at Hilo Analytical Laboratory within three months of collection.

#### 2.5 Statistical analyses

Generalized linear mixed models, under negative binomial and gamma distributions, were used to compare differences in bacteria concentrations among stations. General linear mixed models under normal distributions were used to test relationships between bacteria concentrations and cumulative 24-h rainfall, peak stream flow, the ratio of stream flow to the 7-d moving average, and the relative change in stream flow. When normal distributions did not fit the data, generalized linear mixed models under negative binomial distributions were used. These analyses included relationships between cumulative 24-h rainfall and C. perfringens concentrations at the Wailuku River mouth station, and cumulative 24-h rainfall and all bacteria concentrations at the Hilo Bay water quality buoy station. MRSA was absent from most of the samples collected; therefore, binary (presence/absence) logistic regressions using generalized linear mixed models were used to assess associations between the likelihood of MRSA presence and rainfall, and peak stream flow. General linear mixed models (normal distributions) were also used to examine relationships among S. aureus, Enterococcus spp., and C. perfringens. Model selection using general linear mixed global models and Akaike Information Criterions were used to assess relationships among bacteria (S. aureus, Enterococcus spp., and C. perfringens) and all physiochemical parameters and nutrients. Comparisons between high and low rain and river flow conditions for physiochemical parameters and nutrient concentrations were analyzed with

general linear mixed models, and generalized linear mixed models under gamma distributions, depending on the spread of the data. Assumptions of independent observations and linearity between the explanatory variable and the link function for generalized linear mixed models were assessed. For general linear mixed models, response and explanatory variables were log<sub>10</sub> or square root transformed to meet model assumptions of normal distributions for variables and residuals. In each model, date (month/year) was taken out as a random effect to reduce the variability due repeated measures (sampling the same space over time). In models analyzing data from multiple stations against the same parameter, station was removed as a random effect to reduce to reduce variability due to spatial distribution. Analyses were conducted in the program R (R Core Team, 2017), using the R packages 'lme4' (Bates et al., 2015), 'lmerTest' (Kuznetsova et al., 2016), and MuMIn (Barton, 2016) (α=0.05).

#### 3. Results

#### 3.1 Spatial distribution of bacteria concentrations in nearshore waters

*S. aureus*, MRSA, and FIB were present at all stations within Hilo Bay, except at the Hilo Bay water quality buoy, where MRSA was not observed (Figs. 2 and 3, Table 1). *S. aureus* concentrations differed among stations (p < 0.001, Fig. 2a), and ranged from 0 - 687 CFU 100/mL across all marine water stations. The highest (mean ± SE) concentration was found at the Wailuku River mouth station ( $94 \pm 35$  CFU/100 mL) and the lowest one was at Reed's Bay ( $30 \pm 8$  CFU/100 mL). MRSA was absent from majority of samples, but was sometimes present in marine waters at low concentrations ranging from 0 - 13 CFU/100 mL. MRSA was most frequently observed at the Wailuku River mouth station (30% of sample events), and not detected at the Hilo Bay water quality buoy station (0%) (Fig. 3). *Enterococcus* spp. concentrations also differed among stations (p < 0.001) (Fig. 2b). Values ranged by orders of

magnitude (10 - 10,670 MPN/100 mL). The highest concentrations for *Enterococcus* spp. in nearshore waters were found at the Wailoa River mouth (1337  $\pm$  632 MPN/100 mL), and the lowest were at Reed's Bay (234  $\pm$  121 MPN/100 mL). *C. perfringens* concentrations also differed among stations (p < 0.001), with concentrations ranging from 0 - 45 CFU/100 mL within nearshore waters (Fig. 2c). *C. perfringens* concentrations in nearshore waters were highest at the Wailuku River mouth station (12  $\pm$  3 CFU/100 mL), and the lowest at the Reed's Bay station (3  $\pm$  1 CFU/100 mL).

#### 3.2 Bacteria concentrations in streams

*S. aureus, MRSA* and FIB were identified in both the Wailuku and Wailoa streams. *S. aureus,* MRSA, *Enterococcus* spp., and *C. perfringens* were higher at the upstream Wailoa River station, and there were significant differences between *S. aureus* and *Enterococcus* spp. concentrations (Fig. 2 and Table 1). MRSA was not frequently detected, and thus, not included in these analyses. Across all marine and freshwater stations, the upstream Wailoa River station had the highest concentrations of bacteria with means for *S. aureus* at  $256 \pm 108$  CFU/100 mL, *Enterococcus* spp. at  $2484 \pm 1693$  MPN/100 mL, and *C. perfringens* at  $17 \pm 7$  CFU/100 mL). The lowest concentrations for *Enterococcus* spp. across all marine and freshwater stations were at the upstream Wailuku River station ( $230 \pm 122$  MPN/100 mL) (Fig. 2b).

#### 3.3 Bacteria concentrations in potential watershed sources

*S. aureus* and FIB were identified in road runoff, sewage, and forest soil, while MRSA was identified in all of these sources, except forest soil. Road runoff also contained high concentrations of bacteria with high variability between the two samples (Table 2). A single sampling of sewage yielded higher bacteria concentrations in the influent, and lower concentrations in the effluent, except for *S. aureus*, where the opposite pattern was observed

(Table 2). Effluent samples were just exposed to chlorination when collected, suggesting reported bacteria concentrations will further decrease with longer exposure. Forest soil samples collected had low concentrations of *S. aureus* and FIB, and MRSA was not present (Table 2). There were no significant differences in concentrations for any of the bacteria between ungulate and ungulate-free native forest plots (p > 0.05).

#### 3.4 Relationships between rainfall and bacteria concentrations

*S. aureus, Enterococcus* spp., and *C. perfringens* concentrations had significant and positive relationships with cumulative 24-h rainfall measured at the NWS Hilo International Airport rainfall gauge for the Wailoa River mouth and Reed's Bay stations (Fig. 4), as well as at the NWS Pi'ihonua rainfall gauge for the Wailuku River mouth and Hilo Bay water quality buoy stations (Fig. 5, Table 3, and Appx. II.1). MRSA was absent from most of the samples collected (Fig. 3). No relationships between rainfall and the likelihood of MRSA being present at Wailoa River mouth or Reed's Bay (p = 0.335, z = 0.963), or the Wailuku River mouth (p = 0.322, z = 0.989) were found (Appx. III.1).

#### 3.5 Relationships between stream flow metrics and bacteria concentrations

Significant relationships were found between stream flow metrics and *S. aureus, Enterococcus* spp., and *C. perfringens* concentrations at the Wailuku River mouth station in Hilo Bay (Figs. 6-8, Table 4, and Appx. II.2). Concentrations of *S. aureus* (p = 0.033, t = 2.317), *Enterococcus* spp. (p = 0.008, t = 3.011), and *C. perfringens* (p = 0.001, t = 3.897) were found to significantly increase with higher peak 24-h stream flow (Fig. 6). No relationship between peak flow and the likelihood of MRSA being present at the Wailuku River mouth was found (p = 0.335, z = 0.964; Appx. III.2). Significant and positive relationships were found between the ratio of stream flow at the time of sampling and the 7-d moving average ( $Q/Q_{7dm}$ ) with *S. aureus* (p < 0.001, t =

5.246), *Enterococcus* spp. (p = 0.006, t = 3.215), and *C. perfringens* (p = 0.001, t = 3.954) concentrations at the Wailuku River Mouth (Fig. 7). Significant and negative relationships were also found between the relative change in stream flow for descending hydrograph conditions ( $\Delta Q = Q_t/Q_{t-1}$  for  $\Delta Q \le 1.0$ ) and *Enterococcus* spp. (p < 0.001, t = -7.012), and *C. perfringens* (p = 0.002, t = -3.847) concentrations at the Wailuku River mouth station, while no relationship was observed between this parameter and *S. aureus* (p > 0.05) (Fig. 8).

#### 3.6 Relationships among bacteria, physiochemical parameters, and nutrients

No significant relationships were found among *S. aureus, Enterococcus* spp., and *C. perfringens* concentrations under low rainfall conditions as determined by the NCDC climate normals at the Wailoa River mouth and Reed's Bay stations (Fig. 9). When both high and low rainfall conditions (all rainfall values) were included in the analyses, significant relationships were found between *S. aureus* and *C. perfringens* (p = 0.001, t = 3.574) concentrations, and *Enterococcus* spp. and *C. perfringens* (p = 0.001, t = 4.391) concentrations. No significant relationship between *S. aureus* and *Enterococcus* spp. concentrations (p > 0.05) was observed at these stations (Fig. 9).

Model selection analyses were used to determine which parameters (physiochemical and nutrients) were best associated with bacteria (Table 5). The two top models for *S. aureus* included turbidity (AIC = 118.9), and turbidity and salinity (AIC = 119.2, null model AIC = 138.0). These two models were averaged to get a model predicting the *S. aureus* pathogen concentrations in Hilo Bay (Fig. 10 and Table 5). Turbidity was the best parameter to model *Enterococcus* spp. (AIC = 109.9). However, the AIC for this model was not significantly different from the model without any added parameters (AIC = 111.9); therefore, none of the models were adequate to predict *Enterococcus* spp. (null model AIC = 163.9) (Table 5). The top

model for *C. perfringens* also only included turbidity (AIC = 51.6, null model AIC = 99.1) (Fig. 11 and Table 5).

#### 4. Discussion

#### 4.1 Spatial distribution of bacteria concentrations in nearshore waters

The presence of human pathogens within nearshore waters is a public health concern, as ocean users may contract infections following water contact (Charoenca and Fujioka, 1995). This study corroborates prior findings that S. aureus and MRSA are present in nearshore waters (Charoenca and Fujioka, 1993; Papadakis et al., 1997; Yamahara et al., 2012; Esiobu et al., 2013; Plano et al., 2013), as S. aureus was identified at all four stations across Hilo Bay (Fig. 2A), while MRSA was only found at three (Fig. 3). Concentrations of S. aureus and MRSA were variable in Hilo Bay (Table 1), but were within the ranges of concentrations found in studies from Florida and Mexico (Curiel-Ayala et al., 2012; Esiobu et al., 2013). However, ranges in this study encompassed values that were an order of magnitude higher than those found in a California study (Goodwin et al., 2012). This pattern could be due to climatic differences, as the former study sites are more similar to Hawai'i, with higher annual rainfall averages and warmer air temperatures. MRSA was not as widespread as S. aureus in Hilo Bay, and no concentrations could be calculated as MRSA was absent from the majority of water samples collected (Appx. 1). MRSA was also not found at the offshore water quality buoy station, suggesting that it is sourced from the landscape, and may be diluted in seawater. FIB were also found at all stations in Hilo Bay, and concentrations were within the range of previous studies in the area (Wiegner et al., 2013, 2017). Concentrations of FIB regularly exceeded standards used by the HDOH, including the statistical threshold value of 130 CFU/100 mL for Enterococcus spp., and the suggested geometric mean of 5 CFU/100 mL for C. perfringens (Fujioka and Byappanahalli,

1996; Fung et al., 2007; USEPA, 2012a) (Fig. 2). These patterns suggest that *S. aureus*, MRSA, and FIB are present within tropical estuaries, and their presence, or the presence of pathogens associated with them, may pose a risk to the health of ocean users.

Spatial distribution patterns of FIB in coastal waters have shown that concentrations are generally highest at river mouths (Fujioka et al., 1999; Wiegner et al., 2017), suggesting that these bacteria have watershed sources. These patterns were observed in this study, as median S. aureus and FIB concentrations were highest within coastal waters at the mouth of the Wailuku and Wailoa rivers (Fig. 2). This further suggests that the majority of S. aureus and FIB introduced into Hilo Bay are from the landscape and are moved downstream by surface flow. This differs from major paradigms, as S. aureus is mainly though to enter the environment through direct removal from human skin (Charoenca and Fujioka, 1993; Elmir et al., 2007), and the main source of C. perfringens is the gastrointestinal tract of humans and warm-blooded animals (Vijayavel et al., 2009). Enterococcus spp. is used as a FIB by the USEPA to detect sewage in recreational waters (USEPA, 2012a); however, this bacteria has been found to be prevalent within the tropical soils, making it unreliable as a FIB (Hardina and Fujioka, 1991). In this study, S. aureus and C. perfringens had the same spatial patterns as Enterococcus spp., implying that they may also be prevalent within the watershed and/or have shared means of transport to coastal waters. Therefore, both FIB may not be reliable indicators for human sewage. 4.2 Bacteria concentrations in streams

Bacteria, including *S. aureus* and FIB, have been identified in tropical streams (Roll and Fujioka, 1997; Viau et al., 2011a; Strauch et al., 2014). In our study, direct sampling in upstream segments of the Wailuku and the Wailoa rivers verified the presence of *S. aureus*, MRSA, and FIB, confirming that these bacteria are present within the upper Hilo Bay watershed.

Concentrations of *Enterococcus* spp. significantly differed between the upstream and river mouth stations of the Wailuku River (p < 0.05) (Fig. 2). The higher concentrations of *Enterococcus* spp. at the Wailuku River mouth could be due to the cumulative impact of different landscape sources converging at this lower point in the watershed. There were no other significant differences in concentrations of *S. aureus* and FIB between upstream and the mouths of the Wailuku River or the Wailoa River. However, at the upstream Wailuku River station, bacteria concentrations had the lowest variability, suggesting a relatively stable supply of bacteria at this station. The upstream station of the Wailoa River had the highest median value of *S. aureus* and FIB across all stream and bay stations (Fig. 2), and had high amounts of variability. This station is at a much lower elevation and is located within an urbanized area of Hilo; therefore, concentrations may be influenced by surrounding land cover, and direct inputs of bacteria from storm drains and runoff. This is supported, as other studies have found the presence of *S. aureus*, and higher concentrations of FIB, are associated with more urbanized areas (Mallin et al., 2001; Viau et al., 2011a; Walters et al., 2011; Strauch et al., 2014).

#### 4.3 Potential watershed sources of bacteria

Urban runoff and sewage have been identified as non-point sources of bacteria to streams and coastal waters (Olivieri et al., 1978; Mallin et al., 2001; Selvakumar and Borst, 2006; Börjesson et al., 2009; Rosenberg Goldstein et al., 2012). Our study found *S. aureus*, MRSA, and FIB within road runoff in Hilo, supporting these findings (Table 2). The high amounts of FIB observed are likely due to animal feces, as domesticated and urban animals such as cats, dogs, birds, and rats can contribute significant bacterial loads to the landscape in urban areas (Young and Thackston, 1999; Selvakumar and Borst, 2006; Vierheilig et al., 2013). *S. aureus* can enter into the environment from shedding from human carriers during contact with surfaces (Boyce et

al., 1997; Huang et al., 2006; Miller and Diep, 2008; Otter et al., 2011), and studies have found that *S. aureus* and MRSA can persist within homes and on household pets, and transportation surfaces, such as buses and trains (Otter and French, 2009; Davis et al., 2012; Iwao et al., 2012; Roberts et al., 2013). Concentrated bacteria on the developed landscape may be mobilized in runoff during rainfall events, and moved into the lower watershed (Mallin et al., 2000; Noble et al., 2003). OSDS leakage and faulty sewer lines can result in sewage contaminating groundwater, which can leach into streams and coastal waters (Dudley and Hallacher, 1991). Cesspools are widespread in Hilo, and it is estimated that 21,198 m<sup>3</sup> of effluent is discharged into Hilo Bay each day (HDOH, 2018). This study analyzed sewage from the HWWTP and found *S. aureus* and FIB present (Table 2). As seepage from cesspools and septic tanks occurs underground, these sources may represent a consistent source of pollution to groundwater, and groundwater discharge could increase during heavy rainfall due to the porous, basalt substrate of the Hilo Bay watershed. The transport of these land-based bacteria to coastal waters could increase health risks for water users (Viau et al., 2011b).

*S. aureus* and FIB may have additional shared reservoirs within the landscape, such as soils (Hardina and Fujioka, 1991; Roll and Fujioka, 1997; Schulz et al., 2012). FIB have been observed to reproduce in tropical soils, and provide a consistent source of bacteria to streams (Hardina and Fujioka, 1991; Fujioka et al., 1999). This study found *S. aureus* and FIB, but not MRSA, within native forest soils (Table 2). Additionally, there was no difference found between *S. aureus* and FIB soil concentrations within forests with and without feral ungulates, although the sample size for this comparison was small. Strauch et al. (2016) found lower FIB concentrations within soils of forests where ungulates were excluded by fences, compared to unfenced plots, suggesting that forests with ungulates could have higher bacteria loads. The

plethora of landscape sources of *S. aureus* and FIB suggest that these bacteria are quite widespread and can be quickly transported to nearshore waters after precipitation events. A reduction in their presence in coastal waters may be dependent on watershed-wide management strategies, including constructed wetlands, enhanced riparian buffers, and storm-water infrastructure (Mallin et al., 2000; Walters et al., 2011).

#### 4.4 Rainfall and river driven shifts in bacteria concentrations

The transport of land-based pathogens to coastal waters via surface runoff and stream flow can lead to rapid contamination following heavy rainfall and flow events (Fujioka et al., 1999; Morrison et al., 2003; Muirhead et al., 2006). This study suggests that S. aureus is one such landbased contaminant, as heavier rainfall events in the Hilo Bay watershed led to significant increases in S. aureus concentrations (Fig. 4A and D, Fig. 5A and D). S. aureus and FIB concentrations also significantly increased within Hilo Bay following higher maximum stream flows of the Wailuku River (Figs. 6 and 7). These trends were not observed for MRSA (Appx. III), suggesting that this pathogen is not as prevalent on the landscape compared to the other bacteria investigated. The pattern between S. aureus and rainfall has been previously observed in a tropical coastal system in Florida (Curiel-Ayala et al., 2012). Additionally, similar patterns between rainfall and FIB were found in this study (Fig. 4B, C, E, F and Fig. 5B, C, E, F), and have been documented elsewhere (Walters et al., 2011; Curiel-Ayala et al., 2012; Strauch et al., 2014). Relationships between bacteria measured in this study and stream flow were enhanced when heavy flow events occurred after dry periods, specifically when stream flow was high relative to the stream's 7-d moving average (Fig. 7). This can be explained by the wash-out effect. Tropical soil moisture is ideal for bacterial growth (Fujioka et al., 1999), and when a pulse rain event occurs, bacteria are flushed out of the soil and into stream water (Strauch et al., 2014;

Tryland et al., 2011). Maximum stream flow itself, is therefore not the best indicator of bacterial loading, as previous heavy flow events may have already flushed out bacteria within the watershed (Strauch et al. 2014). Additionally, the reduced soil moisture due to antecedent dry periods can also reduce the connectivity of soil particles, allowing soils to be more vulnerable to transport during heavy rain events, and facilitating the conveyance of solids (Holz et al., 2015; Strauch et al., 2018), and likely bacteria. Heavy stream flow following dry conditions, therefore, increases potential pathogen and bacteria loading to coastal waters, including *S. aureus* and FIB.

The flashiness of many tropical river systems may put downstream waters at a high risk for water quality impairment (Augustijn et al., 2011; Strauch et al., 2014). Storm events introduce energy to the system, allowing for the rapid movement of sediment, soil leachate, and bacteria downstream (Wiegner et al., 2013; Blaustein et al., 2016). A recent study found high bacterial concentrations during ascending flows, compared to descending flows (Strauch, 2017). Our study only examined bacterial concentrations in relation to descending flows, as dates with ascending flow were low in sample size. Higher FIB concentrations were found during steeper descending flows, compared to descending flows with a smaller rate of change (this was not observed for *S. aureus*) (Fig. 8). This is likely an artifact of sampling during stormy days, with stream systems quickly moving out of ascending flows and into descending flows, as antecedent conditions, land use, and discharge rates can interact to result in a lag in the impacts of storm events (Gellis, 2013). This suggests that when stream flow conditions are unstable, even when the stream flow is decreasing, pathogen loading into nearshore waters can be increased.

The increases in *S. aureus* and FIB concentrations within Hilo Bay following heavier rainfall and stream flow events implies that these bacteria are moved to coastal waters through analogous pathways. Continued development will likely increase runoff, strengthening the

relationship between rainfall and bacterial loads (Mallin et al., 2001; Strauch et al., 2014). Vegetation removal and construction of impermeable surfaces will result in increases to the volume of runoff and rates of overland transport (Booth, 1991), leaving urban microbes more vulnerable to mobilization by rainfall, and causing an increase in bacterial loads in streams and coastal waters (Mallin et al., 2001). Therefore, rainfall and stream flow metrics may be good indicators that could be used to assess and predict pathogen loads and water quality health risks for the public.

#### 4.5 Indicators for water quality

Tropical watersheds are prone to rapid changes in water quality following heavier rain and stream flow events (Augustijn et al., 2011; Wiegner et al., 2013). This rapid change was observed in this study, with significant changes in bacterial concentrations in Hilo Bay following peak 24-h rains and stream flow (Figs. 4 and 5). Direct sampling of waters immediately following storms is not always feasible, and sample processing can take 7 - 24-h to complete, inhibiting rapid public notification of water quality conditions (Griffith and Weisberg, 2011). The models created in this study are a way to overcome these limitations and could be used to predict S. aureus and FIB concentrations in Hilo Bay using real-time data from rainfall and stream gauges (Tables 3 and 4). As water quality measurements including temperature, salinity, and turbidity have also been suggested as measures that could predict FIB, real-time data from the Hilo Bay water quality buoy could also be used for predictive modeling (USEPA, 2010; Walters et al., 2011; Gonzalez et al., 2012). This study found that turbidity and salinity had relationships with S. aureus and FIB, and therefore would be the best water quality measures for predicting these bacteria (Figs. 10 and 11). Such relationships with FIB were found previously (Wiegner et al., 2017). This study found also found significant relationships between FIB and S.

*aureus*; however, such relationships were not present during periods of low rainfall (Fig. 9). FIB, therefore, cannot be used as an indicator for *S. aureus* during dry periods. These data suggest that rainfall is driving the relationships between FIB and *S. aureus*, and that rainfall may be better for predicting *S. aureus* concentrations. Rainfall and high stream flow seem to be driving majority of the changes in water quality (Appx. IV.2 and IV.3), suggesting that precipitation patterns have the strongest influence on water quality conditions in Hilo Bay. As technology for rapid public notification of water quality hazards is still under development (USEPA, 2010; Griffith and Weisberg, 2011), and water quality impairment will likely be exacerbated by climatic changes (Strauch et al., 2014), the models produced in this study could allow rainfall, stream flow and/or turbidity and salinity to be used in simple, baseline assessments of public health risks to ocean users in the tropics.

#### 4.6 Climate change impacts on bacteria transport to nearshore waters

As climate change predictions suggest there will be an increase in the frequency and intensity of precipitation events for most of the globe (Ruosteenoja et al., 2003; IPCC, 2014), this may have synergistic effects with continued land development, leading to increased bacterial loading to coastal waters (Mallin et al., 2001). As this study found higher concentrations of *S. aureus* and FIB across Hilo Bay following heavier rainfall and stream flow events (Figs. 4 - 7), transport and subsequent exposure to *S. aureus* in coastal waters could be an evolving climate change risk. Predictions for the tropics also suggest an increase in dry periods throughout the year (IPCC, 2014), but more intense and variable rainfall events (Easterling et al., 2000). This could exaggerate the impacts of bacteria loading due to pulse events, when more intense rains and stream flow conditions affect areas that have been dry (Hathaway et al., 2010; Tryland et al., 2011), and lead to increased water quality impairment (Strauch et al., 2014). There is also a

relatively high degree of temporal variability in precipitation patterns in the tropics (Wohl et al., 2012), and this instability may yield even more dislodging of pathogens which can make their way into coastal water systems. In addition, climatic changes including rainfall, UV radiation, and cloud cover could impact the persistence of bacteria in the environment, result in ever-changing human health risks (Deller et al., 2006; Boxall et al., 2009).

#### 5. Conclusion

*S. aureus* and FIB are widespread pathogens in nearshore waters, while MRSA was less prevalent. These bacteria were also found within the upper reaches of streams, and within landscape reservoirs, including road runoff, sewage, and soils. The transport of *S. aureus* and FIB from the landscape to nearshore waters is impacted by rainfall and stream flow, with heavier events leading to higher concentrations in coastal waters. Heavy stream flow after dry periods, and unstable stream flow conditions, such as rapidly descending flows, result in increased bacterial concentrations in nearshore waters. These conditions are likely to become enhanced with climatic changes, resulting in greater public health risks with regards to water use. Rainfall and stream flow metrics may be good indicators for predicting unsafe pathogen loads in coastal waters. Additionally, turbidity and salinity could be used to predict certain bacteria concentrations in estuaries. The observed patterns and the models developed in this study could be used to inform the public of potential water quality hazards in Hawai'i, as well as across the wet tropics.

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## Tables

**Table 1 -** Average (±SE) and [range] of bacteria concentrations for surface waters in the Hilo watershed in Hilo, Hawai`i, USA. All data were collected between March 2014 and March 2017. Sample size ranged from 7 - 20 depending on station and bacteria type.

Station	<i>S. aureus</i> (CFU/100 mL)	MRSA (CFU/100 mL)	Enterococcus spp. (MPN/100 mL)	C. perfringens (CFU/100 mL)
Wailoa River	67 (16)	2 (1)	1,337 (632)	10(3)
Mouth	[0-270]	[0-13]	[24-10,670]	[0-40]
Reed's Bay	30 (8)	1 (0)	234 (121)	3(1)
	[0-147]	[0-3]	[10-2,495]	[0-11]
Wailuku River	94 (35)	2 (2)	1,278 (477)	12 (3)
Mouth	[0-687]	[0-17]	[63-7,135]	[0-45]
Water Quality	38 (16)	0 (0)	587 (260)	6(2)
Buoy	[2-223]	[0-0]	[10-3,663]	[0-22]
Upstream	53 (15)	1(1)	230 (122)	8 (3)
Wailuku River	[20-160]	[0-7]	[23-1,251]	[0-24]
Upstream	256 (108)	5 (5)	2,484 (1,693)	17(7)
Wailoa River	[0-927]	[0-53]	[27-17,329]	[0-46]

Source	S. aureus	MRSA	Enterococcus spp.	C. perfringens					
		CFU or MPN/100 mL, Single Samples							
Road Runoff									
Site 1	5,000	500	12,997	>50					
Site 2	8,000	700	>24,196	>50					
Sewage									
Influent	1,600	NC	99,315	>50					
Effluent	2,900	NC	300	>50					
	CI	FU or MPN/g dry w	rt. soil, Median (95% CI	)					
Forest Soil									
Ungulate	0.03 (0.002, 0.3)	0.0 (0.0, 0.0)	1.0 (0.3, 1.6)	0.0 (0.0, 0.7)					
Ungulate Free	0.2 (0.03, 1.0)	0.0 (0.0, 0.0)	0.6 (0.3, 1.2)	0.0 (0.0, 4.6)					
*NC: Not counted									

Table 2 -	Concentrations	of bacteria	found in	n potential	landscape	sources	near Hilo,	Hawai`i,
USA.								

Response Variable	Explanatory Variable	Equation	Station
S. aureus (SA)			
Log <sub>10</sub> (1 + SA)	Log <sub>10</sub> (1 + Airport Rain)	y = 0.9897x + 1.1843	Wailoa River Mouth
Log <sub>10</sub> (1 + SA)	Log <sub>10</sub> (1 + Airport Rain)	y = 0.9528x + 0.8209	Reed's Bay
Log <sub>10</sub> (1 + SA)	Log <sub>10</sub> (1 + Pi'ihonua Rain)	y = 1.5329x + 1.1817	Wailuku River Mouth
(1 + Rounded SA)	Log <sub>10</sub> (1 + Pi'ihonua Rain)	y = 2.9969x + 2.2426	Hilo Water Quality Buoy
Enterococcus spp. (ENT)			
Log <sub>10</sub> (ENT)	Log <sub>10</sub> (1 + Airport Rain)	y = 1.3901x + 1.9206	Wailoa River Mouth
Log10 (ENT)	Log10 (1 + Airport Rain)	y = 0.6986x + 1.6571	Reed's Bay
Log <sub>10</sub> (1 + ENT)	(1 + Pi'ihonua Rain)	y = 0.0699x + 2.3400	Wailuku River Mouth
(1 + ENT)	Log <sub>10</sub> (1 + Pi'ihonua Rain)	y = 2.4231x + 4.0569	Hilo Water Quality Buoy
C. perfringens (CP)			
Sqrt. (1 + CP)	Log <sub>10</sub> (1 + Airport Rain)	y = 4.1320x + 1.3820	Wailoa River Mouth
Log <sub>10</sub> (1 + CP)	Log10 (1 + Airport Rain)	y = 0.6295x + 0.2147	Reed's Bay
(1 + Rounded CP)	Pi'ihonua Rain	y = 0.2589x + 1.3100	Wailuku River Mouth
(1 + Rounded CP)	Log10 (1 + Pi'ihonua Rain)	y = 2.0715x + 0.8106	Hilo Water Quality Buoy

**Table 3** - Simplified, linear models using only fixed effects for bacteria concentrations and cumulative rainfall within the past 24-h for each station in Hilo, Hawai'i. Rainfall data are from the Hilo International Airport rainfall gauge (Airport Rain) or the USGS Pi'ihonua rainfall gauge (Pi'ihonua Rain).

Response Variable	Explanatory Variable	Equation			
S. aureus (SA)					
Log <sub>10</sub> (1 + SA)	Log <sub>10</sub> (1 + Max Flow)	y = 0.4412x + 1.1042			
Log <sub>10</sub> (1 + SA)	Log <sub>10</sub> (1 + Q/7dma)	y = 17.8894x + 0.7345			
Log <sub>10</sub> (2 + SA)	$Log_{10} (1 + \Delta Q)$	Not significant			
Enterococcus spp. (ENT)					
Log <sub>10</sub> (ENT)	Log <sub>10</sub> (1 + Max Flow)	y = 0.4874x + 2.0649			
Log <sub>10</sub> (ENT)	Log <sub>10</sub> (1 + Q/7dma)	y = 1.0327x + 2.1477			
Log <sub>10</sub> (ENT)	$Log_{10} (1 + \Delta Q)$	Y = -45.2600x + 15.7650			
C. perfringens (CP)					
СР	Log <sub>10</sub> (1 + Max Flow)	y = 14.5050x - 3.6380			
$Log_{10}(1+CP)$	Log <sub>10</sub> (1 + Q/7dma)	y = 1.0448x + 0.3476			
Log <sub>10</sub> (1 + CP)	$Log_{10} (1 + \Delta Q)$	y = -34.6370 + 10.8830			

**Table 4 -** Simplified, linear models using only fixed effects for bacteria concentrations and stream flow metrics for the Wailuku River mouth station in Hilo, Hawai'i. Stream flow data are from the USGS stream flow gauge at Pi'ihonua.

\*Max Flow: Maximum stream flow discharge 24-h prior to sampling; Q/7dma: Stream discharge 30 min prior to sampling over the 7-d moving average;  $\Delta Q$ : The flow rate at the time of sampling over the discharge rate 1-h prior to sampling for descending flows.

Response Variable	Parameters	df	Log Likelihood	AICe	ΔΑΙC	Weight
Log <sub>10</sub> S. aureus			00000000000000000000000000000000000000			
	Log Turbidity + (Date) + (Station)	5	-53.982	118.860	0.000	0.369
	Log Turbidity + Salinity + (Date) + (Station)	6	-52.946	119.165	0.305	0.317
	(Date) + (Station)	4	-56.582	121.655	2.892	0.087
Log <sub>10</sub> Enterococcus spp						
Tokli musi sestens obbi	Log Turbidity + (Date) + (Station)	5	-49.518	109.880	0.000	0.461
	(Date) + (Station)	4	-51.316	111.187	1.307	0.240
I og C perfringens						
Log <sub>10</sub> c. perfringens	Log Turbidity + (Date) + (Station)	5	-20.227	51.453	0	.901
	Log Turbidity + Water Temperature + (Date) + (Station)	6	-21.367	56.158	4.704	0.086
	(Date) + (Station)	4	-29.0393	66.734	15.281	0.000

**Table 5** – Model selection results for bacteria concentrations and water quality parameters measured in Hilo Bay, Hawai'i. Two top models and models without fixed effects are presented, and parameters in parentheses are random effects included in the model.

\*df: Degrees of freedom; AICc: Corrected Akaike's Information Criterion; ∆AIC: Change in AICc from top model.

**Table 6** - Average ( $\pm$ SE) and [range] of physiochemical parameters measured in surface waters in the Hilo Bay watershed in Hilo, Hawai'i, USA. All data were collected between March 2014 and March 2017. For the Wailoa River mouth, Reed's Bay, Wailuku River mouth stations, n=20. For the Hilo Bay water quality buoy station, n=17. For upstream stations, n=10.

Station	Salinity (ppt)	Temperature (`C)	Turbidity (NTU)
Wailoa River	13.27 (1.56)	21.98 (0.26)	2.51 (0.58)
Mouth	[3.67-26.50]	[20.47-24.50]	[0.36-09.28]
Reed's Bay	21.86 (1.03)	22.61 (0.24)	3.40 (1.18)
10	[10.20-30.40]	[20.11-24.57]	[0.33-21.30]
Wailuku River	19.29 (1.62)	22.90 (0.31)	7.49 (2.32)
Mouth	[3.19-31.00]	[20.10-24.97]	[0.35-45.17]
Water Quality	24.02 (1.50)	23.48 (0.33)	3.66 (0.89)
Buoy	[12.63-33.70]	[21.57-26.20]	[0.46-14.20]
Upstream	0.02 (0.00)	18.88 (0.43)	3.95 (0.65)
Wailuku River	[0.02-0.03]	[16.83-20.97]	[1.37-7.19]
Upstream	0.30 (0.07)	21.44 (0.26)	4.08 (2.17)
Wailoa River	[0.10-0.70]	[20.50-23.13]	[0.40-23.17]

#### Figures



**Fig. 1** - Stations in the Hilo Bay watershed in Hilo, Hawai'i, USA, that were sampled to examine climate driven patterns in bacteria concentrations. Stations within the bay were selected to capture variable impacts from rainfall and stream flow conditions. Upstream stations were selected to confirm presence of bacteria within the watershed, and to compare to coastal water conditions. Sample collection occurred between March 2014 and March 2017 (n = 20; n = 10 for upstream stations).



**Fig. 2** - Median (±quartiles and range) of concentrations for a) *S. aureus*, b) *Enterococcus* spp. and c) *C. perfringens* in the Hilo Bay watershed, Hawai'i, USA, from March 2014 to March 2017. Solid lines separate marine and fresh water stations. Dashed lines on fecal indicator bacteria panels indicate the Hawai'i Department of Health statistical threshold values for a single sample. Results from generalized linear mixed models, with negative binomial (a and b) or gamma distributions (c) are shown in the figure, and letters indicate differences among stations found through paired statistical analyses with the same tests ( $\alpha = 0.05$ ).



**Fig. 3** - Percent of samples with MRSA present across all stations sampled in the Hilo Bay watershed, Hawai'i, USA. Data were collected from March 2016 to March 2017.

#### Wailoa River Mouth



Fig. 4 - Relationships between cumulative rainfall 24-h prior to sampling and a) *S. aureus*, b) *Enterococcus* spp., and c) *C. perfringens* concentrations at the Wailoa River mouth, and d) *S. aureus*, e) *Enterococcus* spp., and f) *C. perfringens* concentrations at Reed's Bay, in Hilo Bay, Hawai'i. Data were collected from March 2014 to March 2017, and rainfall data are from the NWS Hilo International Airport rainfall gauge. Results from general linear mixed models are shown in the figure ( $\alpha = 0.05$ ).

#### Wailuku River Mouth



**Fig. 5** - Relationships between cumulative rainfall 24-h prior to sampling and a) *S. aureus*, b) *Enterococcus* spp., and c) *C. perfringens* concentrations at the Wailuku River mouth, and d) *S. aureus*, e) *Enterococcus* spp., and f) *C. perfringens* concentrations at the water quality buoy, in Hilo Bay, Hawai'i. Data were collected from March 2014 to March 2017, and rainfall data are from the Pi'ihonua rainfall gauge. Results from general linear mixed models under a normal distribution or generalized linear mixed models under a negative binomial distribution are shown in the figure ( $\alpha = 0.05$ ).



**Fig. 6** - Relationships between maximum stream flow 24-h prior to sampling and a) *S. aureus*, b) *Enterococcus* spp., and c) *C. perfringens* in the Wailuku River mouth in Hilo Bay, Hawai'i, USA. Data were collected from March 2014 to March 2017. Results from general linear mixed models are shown in the figure ( $\alpha = 0.05$ ).



**Fig. 7** - Relationship between the ratio of stream flow and the 7-d moving average (Q/7-d moving average) and a) *S. aureus*, b) *Enterococcus* spp., and c) *C. perfringens* in the Wailuku River in Hilo, Hawai'i, USA. X-values  $\leq 0.301$  are indicative of dry sampling dates preceded by higher flow levels, while x-values > 0.301 indicate wet sampling days preceded by dry periods, and dashed lines separate these sampling dates. Data were collected from March 2014 to March 2017. Results from general linear mixed models are shown in the figure ( $\alpha = 0.05$ ).



**Fig. 8** - Relationships between the relative change in descending stream flow ( $\Delta Q = Q_t/Q_{t-1}$  for  $\Delta Q \le 1.0$ ) and a) *S. aureus*, b) *Enterococcus* spp., and c) C. *perfringens* and in the Wailuku River in Hilo, Hawai'i, USA. Data were collected from March 2014 to March 2017. Results from general linear mixed models are shown in the figure ( $\alpha = 0.05$ ).





**Fig. 9** - Relationships between a) *S. aureus* and *Enterococcus* spp., b) *S. aureus* and *C. perfringens*, and c) *Enterococcus* spp. and *C. perfringens* during low rainfall conditions as determined by the NCDC climate normals, and d) *S. aureus* and *Enterococcus* spp., e) *S. aureus* and *C. perfringens*, and f) *Enterococcus* spp. and *C. perfringens* during all rainfall conditions, in the Wailoa River mouth and Reed's Bay stations in Hilo, Hawai'i, USA. Data were collected from March 2014 to March 2017. Results from general linear mixed models are shown in the figure ( $\alpha = 0.05$ ).



**Fig. 10** - Relationships between *S. aureus* and a) salinity and b) turbidity and in Hilo Bay, Hawai`i, USA. Data were collected from March 2014 to March 2017. Significant relationships were determined by model selection using Akaike's Information Criterion.



**Fig. 11 -** Relationships between *C. perfringens* and turbidity and in Hilo Bay, Hawai'i, USA. Data were collected from March 2014 to March 2017. Significant relationships were determined by model selection using Akaike's Information Criterion.

## Appendix

Appendix I: Soil sampling at Pu`u Maka`ala Natural Area Reserve



**Appx. I -** Location of soils sample collection sites at Pu'u Maka'ala Natural Area Reserve, Volcano, Hawai'i, USA.

## Appendix II: Statistics for rainfall and stream flow models

**Appx. II.1** – Statistics for models analyzing relationships between bacteria concentrations and cumulative 24-h rainfall prior to sampling, in Hilo, HI. Rainfall data are from the Hilo International Airport rainfall gauge (Airport Rain) or the USGS Pi`ihonua rainfall gauge (Pi`ihonua Rain).

Response Variable	Explanatory Variable	Parameter Estimate	Standard Error	T-Value* or Z-Value	P-Value	Station
S. aureus (SA)						
Log <sub>10</sub> (1 + SA)	Log10 (1 + Airport Rain)	0.9897	0.2505	3.951*	0.001	Wailoa River Mouth
Log10 (1 + SA)	Log10 (1 + Airport Rain)	0.9528	0.2656	3.587*	0.004	Reed's Bay
Log10 (1+SA)	Log10 (1 + Airport Rain)	1.5329	0.4036	3.798*	0.003	Wailuku River Mouth Hilo Bay
(1 + Rounded SA)	Logio (1 + Airport Rain)	2.9969	1.0942	2.739	0.006	Water Quality Buoy
Enterococcus spp. (ENT)						
Logio (ENT)	Log10 (1 + Airport Rain)	1.3901	0.2344	5.93*	<0.001	Wailoa River Mouth
Log10 (ENT)	Log10 (1 + Airport Rain)	0.6986	0.2702	2.586*	0.019	Reed's Bay
Log10 (1 + ENT)	(1 + Pi'ihonua Rain)	6.99 x 10 <sup>-2</sup>	3.13 x 10 <sup>5</sup>	2234.6	<0.001	Wailuku River Mouth Hilo Bay
(1 + ENT)	Log10 (1 + Pi'ihonua Rain)	2.4231	0.7581	3.169	<0.001	Water Quality Buoy
C. perfringens (CP)						
Sqrt. (1 + CP)	Log10 (1 + Airport Rain)	4.132	2.46 x 10 <sup>-4</sup>	16792.7*	<0.001	Wailoa River Mouth
$Log_{10} (1 + CP)$	Log10 (1 + Airport)	0.630	5.58 x 10 <sup>-4</sup>	1076.2*	< 0.001	Reed's Bay
(I + Rounded CP)	Pi'ihonua Rain	0.259	0.069	3.753	<0.001	Wailuku River Mouth Hilo Bay
(1 + Rounded CP)	Log <sub>10</sub> (1 + Pi`ihonua Rain)	2.0701	0.743	3.635	<0.001	Water Quality Buoy

Response Variable	Explanatory Variable	Parameter Estimate	Standard Error	T-Value	P-Value
S. aureus (SA)					
Log <sub>10</sub> (1 + SA)	Log <sub>10</sub> (1 + Max Flow)	0.4412	0.1833	2.407	0.028
Log <sub>10</sub> (1 + SA)	$Log_{10} (1 + Q/7 dma)$	17.8894	3.4101	5.246	< 0.001
Log <sub>10</sub> (2 + SA)	$Log_{10} (1 + \Delta Q)$	-49.105	8.481	-5.790	0.091
Enterococcus spp. (ENT)					
Log (ENT)	Log <sub>10</sub> (1 + Max Flow)	0.4874	0.1690	2.884	0.010
Log <sub>10</sub> (ENT)	$Log_{10} (1 + Q/7 dma)$	1.0327	0.3212	3.215	0.006
Log <sub>10</sub> (ENT)	$Log_{10} (1 + \Delta Q)$	-45.260	6.455	-7.012	< 0.001
C. perfringens (CP)					
СР	Log <sub>10</sub> (1 + Max Flow)	14.505	3.280	4.423	0.006
Log <sub>10</sub> (1 + CP)	Log <sub>10</sub> (1 + Q/7dma)	1.0448	0.2642	3.954	0.001
Log <sub>10</sub> (1 + CP)	$Log_{10} (1 + \Delta Q)$	-34.637	9.004	-3.847	0.002

**Appx. II.2** – Statistics for models analyzing relationships between bacteria concentrations and stream flow metrics at the Wailuku River mouth station in Hilo, HI. Stream flow data are from the USGS stream flow gauge at Pi`ihonua.

\*Max Flow: Maximum stream flow discharge 24-h prior to sampling; Q/7dma: Stream discharge 30 minutes prior to sampling over the 7-d moving average;  $\Delta Q$ : The flow rate at the time of sampling over the flow rate 1-h prior to sampling for descending flows.

Appendix III: MRSA analyses



**Appx III.1** - Logistic curve showing the predicted probability and 95% CI of MRSA being present within the a) Wailoa River mouth and Reed's Bay stations, and b) the Wailuku River mouth in Hilo Bay, Hawai'i, USA, under varying cumulative rainfall. Data were collected from March 2016 to March 2017. Rainfall data are from a) NWS Hilo International Airport rainfall gauge, and b) NWS Pi'ihonua rainfall gauge. Results from generalized linear mixed models under a binomial distribution are shown in the figure ( $\alpha = 0.05$ ).



**Appx. III.2** - Logistic curve showing the predicted probability and 95% CI of MRSA being present (present = 1, absent = 0) in the Wailuku River mouth in Hilo Bay, Hawai'i, USA, under varying maximum stream flow conditions. Data were collected from March 2016 to March 2017. Results from a generalized linear mixed model under a binomial distribution are shown in the figure ( $\alpha = 0.05$ ).

#### Appendix IV: Water quality and nutrient patterns

Physiochemical parameters, nutrients and Chl a varied across marine and freshwater stations within the Hilo Bay watershed (Table 3 and Appx IV.1). When comparing high and low rainfall (thresholds determined by NDCD climate normals) for Wailoa River mouth and Reed's Bay stations, salinity (p = 0.011, t = 2.748) and DOC (p = 0.005, t = 2.961) were significantly lower during high rainfall conditions (Appx. IV.2). No differences were found between the other parameters (Appx. IV.2). These analyses only used data from the Wailoa River mouth and the Reed's Bay stations, as these are adjacent to the rainfall gauge collecting NDCD climate normal data. No climate normal data were available for the Pi'ihonua rainfall gauge, which is upstream of the Wailuku River. Comparisons of high  $(>3.5 \text{ m}^3 \text{ s}^{-1})$  and low  $(<2.5 \text{ m}^3 \text{ s}^{-1})$  river flow conditions (thresholds used in Wiegner et al., 2017) yielded significant differences in the Wailuku River mouth station (Appx. IV.3). All physiochemical water quality parameters differed significantly between high and low river flow (p < 0.05), with salinity and temperature (C) being higher during low flow, and dissolved oxygen (%) and turbidity (NTU) higher during high river flow conditions (Appx. IV.3). Such significant differences were also found for nutrients, with  $NO_3^{-}+NO_2^{-}$ , TDN, and DON being higher at high river flow, and DOC lower at high river flow conditions (p < 0.05) (Appx. IV.3).

**Appx. IV.1** - Average ( $\pm$ SE) and [range] of nutrients (µmol L<sup>-1</sup>) and chlorophyll *a* (µg L<sup>-1</sup>) concentrations for surface waters in the Hilo Bay watershed in Hilo, Hawai'i, USA. All data were collected between March 2014 and March 2017. For the Wailoa River mouth, Reed's Bay, and Wailuku River mouth stations, n=20 for nutrients, and n=19 for chlorophyll *a*. For the Hilo Bay water quality buoy Station n=17 for nutrients, and n=16 for chlorophyll *a*. For upstream stations n=10 for nutrients and chlorophyll *a*.

Station	NO <sub>3</sub> <sup>-</sup> +NO <sub>2</sub> <sup>-</sup>	NH4 <sup>+</sup>	TDN	DON	PO43-	TDP	DOP	H <sub>4</sub> SiO <sub>4</sub>	DOC	Chl a
Wailoa River	24.76 (1.21)	1.57 (0.21)	96 (27)	70 (28)	0.69 (0.04)	0.9 (0.1)	0.2 (0.1)	214.9 (14.0)	167 (28)	0.63 (0.20)
Mouth	[14.37-35.89]	[0.00-3.56]	[23-422]	[0-394]	[0.38-1.03]	[0.5-1.5]	[0.0-0.8]	[100.7-328.7]	[32-482]	[0.06-3.94]
Reed's Bay	15.63 (1.19)	1.73 (0.20)	119 (43)	102 (43)	0.72 (0.05)	0.9 (0.1)	0.2 (0.1)	153.2 (17.5)	225 (46)	0.49 (0.09)
31	[5.64-28.01]	[0.18-3.72]	[10-671]	[0-653]	[0.38-1.22]	[0.0-1.8]	[0.0-0.6]	[50.4-341.3]	[23-798]	[0.12-1.90]
Wailuku River	3.15 (0.41)	1.70 (0.23)	172 (64)	167 (64)	0.08 (0.01)	0.2 (0.1)	0.1 (0.1)	49.0 (4.0)	235 (40)	0.93 (0.35)
Mouth	[1.10-8.36]	[0.00-3.93]	[7-744]	[2-741]	[0.00-0.19]	[0.0-0.8]	[0.0-0.7]	[19.6-86.4]	[12-528]	[0.16-7.10]
Water Quality	5.27 (1.06)	1.55 (0.22)	151 (70)	144 (70)	0.20 (0.05)	0.2 (0.1)	0.0 (0.1)	63.7 (12.9)	300 (65)	0.48 (0.12)
Buoy	[0.81-17.43]	[0.00-3.61]	[7-836]	[3-829]	[0.00-0.81]	[0.0-0.9]	[0.0-0.8]	[11.1-220.6]	[12-981]	[0.00-1.47]
Upstream	4.77 (0.64)	0.98 (0.32)	192 (64)	187 (65)	0.16 (0.02)	0.2 (0.1)	0.0 (0.1)	15.4 (8.6)	319 (123)	0.34 (0.08)
Ŵailuku River	[2.28-9.65]	[0.00-3.26]	[10-487]	[6-482]	[0.09-0.23]	[0.0-0.8]	[0.0-0.7]	[0.0-89.7]	[8-1132]	[0.11-0.95]
Upstream	26.54 (2.58)	1.21 (0.30)	90 (32)	62 (34)	0.30 (0.04)	0.4 (0.1)	0.1 (0.1)	51.9 (10.2)	262 (94)	0.52 (0.21)
Wailoa River	[14.52-36.55)	[0.00-3.06]	[25-362]	[1-347]	[0.18-0.53]	[0.0-1.2]	[0.0-0.9]	[0.0-85.6]	[25-777]	[0.05-2.29]

**Appx. IV.2** – Statistical output from comparing high and low rainfall conditions for physiochemical parameters, nutrients ( $\mu$ mol L<sup>-1</sup>) and chlorophyll *a* ( $\mu$ g L<sup>-1</sup>) concentrations for surface waters in the Wailoa River mouth and Reed's Bay stations in Hilo, Hawai'i, USA. Data were analyzed with a general linear mixed model under a normal distribution, unless indicated ( $\alpha$ =0.05). All data were collected between March 2014 and March 2017. For nutrients, n=40, and for chlorophyll *a*, n=38. Rainfall thresholds are based on the National Climate Data Center's daily normal values for the Hilo International Airport rainfall gauge.

Parameter	Parameter Estimate	Standard Error	T-value	<b>P-value</b> 0.01*	
Salinity	5.36	1.95	2.75		
Temp (C)	0.54	0.37	1.45	0.16	
DO (%)	0.80	2.78	0.29	0.78	
Turbidity (NTU)	-0.01 *	0.06	-0.09	0.93	
NO <sub>3</sub> <sup>+</sup> HO <sub>2</sub> <sup>-</sup>	1.28	1.95	0.66	0.52	
PO43-	-0.01	0.68	-0.74	0.94	
H <sub>4</sub> SiO <sub>4</sub>	6.95	25.77	0.27	0.79	
NH <sub>4</sub> <sup>+</sup>	-0.51	0.31	-1.64	0.12	
TDP	-0.05	0.12	-0.43	0.67	
TDN	0.02 ab	0.05	0.33	0.74	
DOC	0.40*	0.14	2.96	0.01*	
DON	0.21 ab	0.21	1.02	0.31	
DOP	0.08 ab	0.14	0.59	0.55	
Chl a	0.07*	0.05	1.42	0.17	

\*Response variable was log10 transformed

Analyzed with a generalized linear mixed model under a gamma distribution

**Appx. IV.3** – Statistical output from comparing high (>3.5 m<sup>3</sup> s<sup>-1</sup>) and low (<2.5 m<sup>3</sup> s<sup>-1</sup>) river flow conditions for physiochemical parameters, nutrients ( $\mu$ mol L<sup>-1</sup>) and chlorophyll *a* ( $\mu$ g L<sup>-1</sup>) concentrations for surface waters in the Wailuku River mouth station in Hilo, Hawai'i, USA. Data were analyzed with a general linear mixed model under a normal distribution, unless indicated ( $\alpha$ =0.05). All data were collected between March 2014 and March 2017. For nutrients, n=20, and for chlorophyll *a*, n=18.

Parameter 1	Parameter Estimate	Standard Error	T-value	P-value
Salinity	12.04	2.37	5.08	< 0.01*
Temp (C)	1.73	0.54	3.19	0.01*
DO (%)	-12.58	0.44	-28.55	0.03*
Turbidity (NT	U) -0.49 *	0.14	-3.49	< 0.01*
NO3 <sup>+</sup> +NO2 <sup>-</sup>	-0.19*	0.07	-2.78	0.01*
PO43-	0.01 <sup>a</sup>	0.01	0.93	0.39
H <sub>4</sub> SiO <sub>4</sub>	10.61	2.38	4.46	0.11
NH4 <sup>+</sup>	0.13 ª	0.07	1.92	0.07
TDP	0.03 ª	0.02	1.29	0.22
TDN	0.13 ab	0.01	9.48	< 0.01*
DOC	93.77	1.63	57.49	< 0.01*
DON	-0.14 ab	0.02	-6.97	< 0.01*
DOP	0.03 *	0.04	0.97	0.35
Chl a	0.04 ª	0.07	0.55	0.62

\*Response variable was log10 transformed

<sup>b</sup>Analyzed with a generalized linear mixed model under a gamma distribution

#### **Appendix V: Study's drawbacks and future work**

As *S. aureus*, MRSA and FIB are present within tropical watersheds, they are a potential health risk to users of aquatic systems. This study provided foundational information on these bacteria in a tropical watershed; however, more work must be done to find direct connections between the links between the presence of these pathogens and morbidities. The actual risk that these environmental microbes present to humans must be assessed through epidemiological studies. Such a study has been done on O'ahu, HI (Charoenca and Fujioka, 1995); however, more studies are needed with the advance of screening techniques and increased threats due to climate change.

Additionally, the work done in this study used the CHROMagar and MRSA *Select* II to distinguish *S. aureus* and MRSA. These media were designed for use in medicinal fields, and therefore are not as selective when used to distinguish a single species of bacteria from all the bacteria within the ocean. Putative positive colonies ranged in color from light pink to dark mauve, making enumeration difficult. Cultures had to be confirmed with gram-staining and coagulase tests, and therefore the analyses were time-consuming. Future analyses of *S. aureus* and MRSA should be undertaken with genetic screening, such as quantitative PCR. Though such techniques are expensive, they would yield results with return times that would be quicker, and more useful for educating the public about unsafe water quality conditions. These types of analyses are already underway to assess water quality indicators, such as *Enterococcus* spp., and can yield results within 3 – 4-h (Fujioka and Byappanahalli, 1996; USEPA, 2012b). Such methods would also permit more sampling days, and more robust predictive models.