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Towards real-time wastewater pollution tracking: Evaluating the performance of instrumentation-based wastewater pollution proxies in an urban estuary

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TITLE

Towards real-time wastewater pollution tracking: Evaluating the performance of instrumentation-based wastewater pollution proxies in an urban estuary

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Author contribution statement

C.C. wrote the main manuscript text, collected data, analyzed data, and prepared all figures. D.A., P.F., H.M, N.S., and S.W. collected data. C.C., D.A., M.D., C.N., and M.M secured funding to support the research. C.N. assisted with data analysis. M.M. supervised the work. All authors reviewed the manuscript.

ABSTRACT

Wastewater pollution poses risks to both ecosystem and public health via the introduction of pathogens, excessive nutrients, and chemicals of emerging concern to the aquatic environment. Typical monitoring to alert the public of elevated fecal bacteria levels and potential wastewater pollution relies on discrete water samples and 24-hr culturing of indicator bacteria, which leads to alerts being posted no earlier than 1-2 days after pollution impacts. Discrete sampling can also miss acute wastewater impacts from storms or spills. Therefore, there is interest in real-time proxies to provide earlier public alerts and track wastewater pollution inputs to water bodies at a finer time scale. Emerging proxies include real-time monitoring of dissolved organic fluorescence, particularly tracking fluorescence wavelengths similar to optical brighteners (a component of laundry detergent) and tryptophan (an amino acid) that have been demonstrated to be enriched in wastewater. In this study, we examined the ability of in situ, real-time-capable sensors to predict wastewater-associated fecal indicator bacteria concentrations in the Ala Wai Harbor, Honolulu, HI. A fluorometer measuring "optical brightener" fluorescence and "tryptophan" fluorescence was deployed alongside an existing long-term monitoring sensor package measuring temperature, salinity, turbidity, and chlorophyll-a fluorescence. Discrete bottle samples were analyzed for fecal indicator bacteria, sucralose (a conservative wastewater tracer), nutrients, and 3-D scans of fluorescent dissolved organic matter. A positive correlation was observed between culture-based counts of the fecal indicator bacteria *Enterococcus spp.* and sucralose, which is found only in human waste, indicating consistent wastewater contamination in the Ala Wai Harbor. Dissolved fluorescence measurements in the wavelengths associated with optical brighteners were found to be the best instrumentation-based predictor of *Enterococcus* spp. concentrations, alone or in combination with other parameters (R^2=0.71 - 0.72). The best-specified categorical models to predict *Enterococcus spp.* exceedances of the Hawai‘i state threshold of 130 colony forming units (CFU) /100 mL included both optical brightener fluorescence and salinity with >95% sensitivity and ~75% specificity. The addition of the optical brightener fluorescence channel doubled the explained variance in fecal indicator bacteria by real-time sensors when compared to the existing suite of temperature, salinity, and turbidity (R^2 =0.36-0.38). Our results demonstrate that real-time measurements of optical brightener fluorescence could provide instantaneous warnings of wastewater spills and elevated fecal bacteria levels in waterways at risk of wastewater pollution, and this data stream could be integrated into existing outflow plume models and forecasts to provide better real-time estimates of water safety for ocean users in the surrounding marine areas.

1. INTRODUCTION

1.1 Wastewater pollution in the coastal environment

Wastewater pollution in coastal systems is a global water quality concern for both human and ecosystem health. Globally, it is estimated that 104 out of 112 territories, states, and

countries with coral reefs have documented sewage contamination issues in their coastal waters (Wear & Thurber, 2015). Wastewater pollution contains nutrients, endocrine disruptors, solids, metals, and toxins that can all have negative impacts on marine organisms and ecosystems, including reduced reproductive success in corals, increased disease and mortality, increased algae overgrowth of corals, reduced coral calcification and increased erosion, reduced reef net primary productivity, and reduced fish biomass (Foo et al., 2021; Reopanichkul et al., 2009; Silbiger et al., 2018; Wear & Thurber, 2015; Yoshioka et al., 2016). Sewage also can contain many human pathogens (Olaolu & Co, 2014), and sewage pollution into coastal waters has been estimated to cause up to 120 million cases of gastrointestinal disease and 50 million cases of respiratory disease globally each year (Shuval, 2003). Detecting wastewater pollution in coastal and recreational waters is typically accomplished by culturing fecal indicator bacteria such as *Escherichia coli* and *Enterococcus* spp., because elevated levels of these bacteria have been linked to increased risk of gastroenteritis in swimmers in certain studies where wastewater contamination was expected (Cabelli et al., 1982). In the United States each state sets water quality criteria and thresholds for public health risk for these bacteria per the Beaches Environmental Assessment and Coastal Health Act (BEACH Act) (Beaches Environmental Assessment and Coastal Health Act of 2000, 2000; Hannides et al., 2021).

1.2 Challenges of using fecal indicator bacteria for wastewater pollution alerts

Standard culture-based methods for measuring fecal indicator bacteria abundances require 24 h of incubation, which means that critical public health information about high bacteria levels and hazardous swimming conditions may only be available 1-2 days after an acute pollution event. Discrete water samples are commonly taken at weekly to monthly intervals for routine monitoring (Hannides et al., 2021) and therefore they may miss fluctuations in fecal indicator bacteria due to storms and acute wastewater spills. As climate change brings changes in rainfall intensity patterns (Fandrich et al., 2022; Giambelluca & Beamer, 2025) and sea level rise leads to inundation of sewers and individual wastewater systems (McKenzie et al., 2021; Yamamoto et al., 2026), there is an urgent interest in real-time, *in situ* monitoring for wastewater pollution in coastal waters to protect public health and track both baseline pollution and acute events over time.

E. coli and *Enterococcus* spp. are not specific to humans and are found in the feces of other mammals, reptiles and birds (Byappanahalli, Nevers, et al., 2012; Mundt, 1963), which poses an additional challenge in interpreting fecal indicator bacteria as a wastewater indicator. *Enterococcus* spp. populations can also be found in tropical soils (Byappanahalli, Roll, et al., 2012) and in beach sand (Cui et al., 2013; Steadmon et al., 2024). Regulatory thresholds are based on fecal indicator bacteria and therefore continued monitoring is important, but the lack of source-specificity leaves a gap in identifying wastewater pollution to coastal waters. Because of the lack of source-specificity to wastewater, especially in tropical environments, the presence of *Enterococcus* spp. may not always be an indicator of sewage pollution and harmful pathogens (Boehm & Sassoubre, 2014).

1.3 Identifying wastewater pollution

There are several methods to determine whether elevated fecal indicator bacteria are related to the presence of wastewater pollution. Microbial source tracking can identify human-specific Bacteroides gut bacteria, and microbial community analysis can reveal multiple wastewater-associated taxa (García-Aljaro et al., 2019). Pharmaceuticals (Styszko et al., 2021; Vidal-Dorsch et al., 2012) and the artificial sweetener sucralose (Berg et al., 2023; Fu et al., 2020; Oppenheimer et al., 2011) are examples of chemicals of emerging concern found in wastewater. Of these chemical tracers, sucralose is more commonly ingested by humans than prescription medication and is therefore the most likely to appear in wastewater streams, and it is a well-established and conservative wastewater tracer (Mawhinney et al., 2011). Although these human-specific methods may provide more conclusive evidence of anthropogenic pollution, they require a significant amount of time and equipment that is not yet capable of producing real-time data integration with public alerting systems.

1.4 Instrumentation-based pollution monitoring

In situ instrumentation can provide real-time data on various parameters such as temperature, salinity, turbidity, and various types of fluorescent dissolved organic matter (fDOM). Many of these parameters are related to land-based inputs to coastal systems and can be evaluated as potential proxies for fecal indicator bacteria and wastewater pollution. Temperature and salinity are commonly measured by traditional oceanographic instruments, providing insight into freshwater input to estuarine and coastal areas. In chronically polluted areas, higher freshwater input may correlate with higher wastewater input. Turbidity is an optical property determined by measuring light scatter, and it is an indicator of water clarity and related to suspended particles (Lawler, 2016). Turbidity can fluctuate due to many factors including sediment and stormwater runoff, wastewater input, resuspension of particulates from wave action, and biological processes. Chlorophyll *a* fluorescence increases in coastal waters when phytoplankton grow in response to nutrient input or retention, and it can be measured *in situ* using excitation wavelengths from 455-470 nm (blue light) range. Excess nutrients in coastal systems can come from wastewater pollution but can also originate from fertilizer or animal waste.

1.5 Emerging instrumentation-based proxies for wastewater

Certain types of fDOM are related to wastewater pollution more closely. Tryptophan is an amino acid that fluoresces at an excitation (ex) of ~275 nm and emits (em) at 340 to 350 nm (Coble, 1996; Sorensen et al., 2018)). Optical brighteners are fluorescent compounds that are used in laundry detergents to brighten and whiten clothing (Balcers & Teteris, 2006), with excitation at UV wavelengths and emission in the 400-550 nm range (Mustalish, 2000). Both tryptophan-like and optical brightener-like fluorescence channels are commonly used in industrial wastewater applications, and they have shown promise in environmental wastewater

monitoring in a variety of environments (Finegan & Hasenmueller, 2023; Knopp et al., 2026; Mladenov et al., 2024; Spong et al., 1995; Tavares et al., 2008). However, other non-wastewater associated parameters may also fluoresce at the same excitation/emission wavelengths, so any instrumentation-based parameter considered here should be considered as a proxy with low specificity and not a direct wastewater measurement.

This study investigated the effectiveness of instrumentation-based parameters (temperature, salinity, turbidity, tryptophan-like fluorescence, and optical brightener-like fluorescence) in predicting *Enterococcus* spp. concentrations in an urban estuary, the Ala Wai Harbor, Oʻahu, Hawaiʻi. We additionally measured sucralose, nutrients, and fluorescent dissolved organic matter from discrete bottle samples to investigate the relationship between wastewater proxies (salinity, nutrients, turbidity, fluorescence, fecal indicator bacteria) and a conservative wastewater tracer (sucralose).

2. METHODS

2.1 Ala Wai Deployments

The Ala Wai Harbor is located in an urban estuary at the mouth of the Ala Wai Canal in Honolulu, Hawaiʻi (Figure 1). The canal is fed by the Mānoa, Pālolo, and Makiki streams in the Ala Wai watershed, and is a popular area for recreational use including boating, fishing, canoe paddling, and surfing. The three streams that feed into the canal are impacted by multiple pollution sources, including ~500 cesspools, feral animal waste, urban stormwater runoff, and upland erosion and sedimentation that is exacerbated by invasive plant and animal species in the upper watersheds. The canal itself is impacted by sea level rise-driven inundation of sewer systems, which allows wastewater extrusion from the sewer lines and into groundwater and coastal waters (McKenzie et al. 2021).

A Turner C3 fluorometer was deployed in the Ala Wai Harbor during April 2023, November-December 2024, and February-May 2026 (Figure 1). This fluorometer was equipped with sensors to measure the hypothesized wastewater indicators “tryptophan-like” fluorescence (ex 275 nm / em 325-375 nm) and “optical brightener-like fluorescence” (ex 365 nm / em 437.5-452.5 nm), as well as chlorophyll-a fluorescence (ex 460 nm / em 674-718 nm), which indicates the presence of phytoplankton growth. The sensor measured fluorescence every 4 minutes during these deployment periods. The Turner C3 was additionally deployed from February 2026 onwards, sampling at a 30-minute interval. The sensor was serviced every 2-4 weeks to remove biofouling, charge the battery, and replace anti-biofouling copper tape and wire. Solid standards were provided by Turner and were checked before each re-deployment to confirm sensor responsiveness.

The Turner C3 fluorometer was deployed adjacent to and slightly shallower than the Pacific Islands Ocean Observing System (PacIOOS) Nearshore Sensor 2 (NS02). NS02 is a Sea-Bird Instruments 16plus V2 conductivity-temperature-depth sensor with an ECO-FLNTU

chlorophyll-a and turbidity sensor. This sensor package has been deployed continuously since 2008 except for maintenance periods and measures all parameters at 4-minute intervals.

During each deployment period, bottle samples were collected between 6-7:30 AM next to the Turner C3 and NS02 sensors. During the 2023 deployment, 16 bottle samples were collected on 16 separate days. During the 2024 deployment, 38 samples were collected on 19 separate days. 2023 and 2024 samples were analyzed for *Enterococcus* spp., inorganic nutrients, sucralose, and fluorescent dissolved organic matter (fDOM). During 2026, bottle samples were collected opportunistically and analyzed for *Enterococcus* spp and fDOM analysis, but this is a partial dataset and does not include nutrients or sucralose. A total of 47 (n_{2023}=16; n_{2024}=20, n_{2026}=11) water samples with time-synced, quality-controlled instrumentation data were included in the modeling portion of this study.

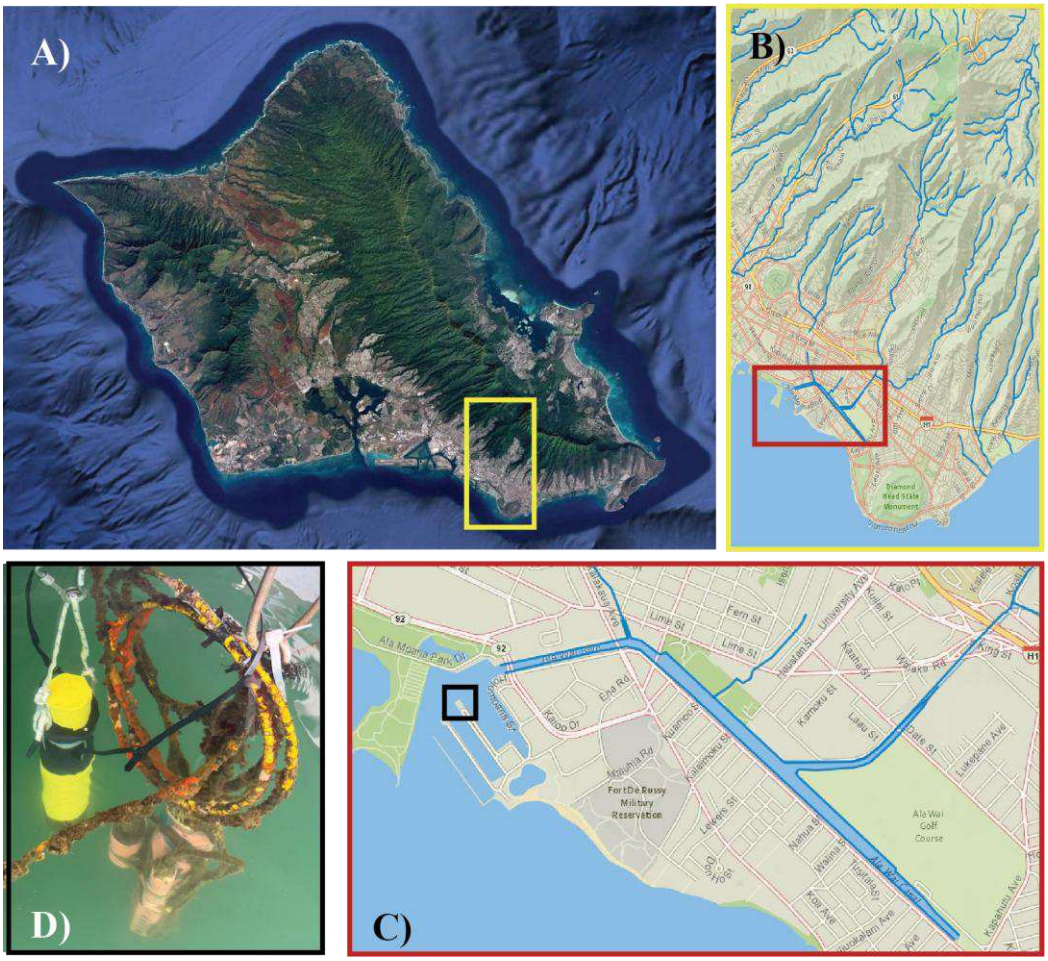


Figure 1. Map of the study site. A) Island of Oʻahu, Hawaiʻi. B) Watersheds of Makiki, Mānoa, and Pālolo. C) Ala Wai Canal and Harbor showing location of the Turner C3 fluorometer and the NS02 CTD and turbidity/chlorophyll-a sensors in black. D) Photo of the deployed sensors.

2.2 Water sample processing and analysis

Enterococcus spp.

Unfiltered water samples were stored on ice and analyzed for *Enterococcus* spp. concentrations within 6 hours of collection following the IDEXX *Enterolert* method (ASTM Method D6503-19). Sample water was diluted with MilliQ water in a 10:1 ratio. Laboratory blanks were analyzed using 100 mL of MilliQ water with the Enterolert reagent. The limit of detection using the IDEXX method at 10x dilution is 10 colony forming units (CFU)/100 mL; non-detects are reported as 5 CFU/100 mL.

Sucralose

A 100 mL portion of unfiltered sample water was frozen for Sucralose concentration analysis by online SPE HPLC - High Resolution Mass Spectrometry, which was performed by the Environmental Analysis Research Laboratory at Florida International University. The method detection limit is 12.1 ng/L (Batchu et al., 2013). Confirmation of sucralose was based on multiple criteria in addition to signal intensity: mass accuracy within +/- 5 ppm, retention time agreement with isotopically labeled internal sucralose standard, and the presence of a chlorine isotopic pattern (since sucralose contains chlorine). Quality control practices included reagent blanks run every 8 samples, continuing calibration calibration injections, and matrix spikes and duplicates. Non-detects are reported as zero.

Nutrients

A 60-100 ml aliquot of sample water filtered through a 0.22 um filter was frozen for nutrient analysis. Samples were analyzed at the SOEST Laboratory for Analytical Biogeochemistry (SLAB) for nitrate + nitrite (Armstrong et al., 1967; Grasshoff K., Ehrhardt M., and Kremling K., 1983), phosphate (Murphy & Riley, 1962), ammonium (Kérouel & Aminot, 1997), silicate (Grasshoff K., Ehrhardt M., and Kremling K., 1983), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) using a Seal Analytical AA3 HR Nutrient Autoanalyzer. Detailed methods for each measurement are available on the SLAB website (https://www.soest.hawaii.edu/S-LAB/equipment/slab_autoanalyzer.htm). Dissolved organic nitrogen (DON) was calculated by subtracting ammonium and nitrate+nitrite concentrations from the TDN measurement.

Fluorescent dissolved organic matter

A 20 mL aliquot of filtered sample water (0.22 um filter) was stored in the refrigerator in an amber glass bottle for benchtop scanning fluorescence analysis. Fluorescence of filtered water samples was measured using a Horiba Aqualog scanning fluorometer. Excitation-Emission Matrices (EEMS) were generated with excitation values ranging from 240 nm to 500 nm and emission values were measured ranging from 250 nm to 825 nm. Excitation wavelengths were increased at 5 nm increments and measurements were integrated over 4 s at each increment. Inner filter correction was applied to account for fluorescence quenching by absorbance

(Kothawala et al., 2013). Fluorescence values were scaled to Raman Units by dividing by the integrated emission range of 381 nm to 426 nm at an excitation of 350 nm in averaged deionized water (DIW) blanks (Lawaetz & Stedmon, 2009). The average EEMs from DIW blanks run immediately before and after each set of samples were subtracted from the sample EEMs.

Two indices were calculated to match the excitation and emission wavelengths measured by the Turner C3 fluorometer in the field. The first index was calculated to match the "tryptophan" channel (ex 275 nm / em 350 nm) and the second index matched the "optical brightener" channel (ex 325 / em 445). Additional calculated indices included "M:C" (ex 310 / Em 510 divided by ex 345 / em 445) which reflects the ratio of marine to terrigenous-derived DOM (Burdige et al., 2004); "BIX," autochthonous biological activity index (ex 308 / em 380 to ex308 / em 430), with values >1 indicating more recent biological production (Huguet et al., 2009); "FI," the fulvic acid index (the integrated emission fluorescence from 450 - 500 nm, excitation of 370 nm); and "HIX," humification index (integrated fluorescence from ex 254 / em 434-480 to integrated fluorescence from ex 254 / em 300-346), with lower values indicating increasingly autochthonous origin of humic acids (Zsolnay et al., 1999).

2.3 Quality Control of in situ instrumentation data

Data collected by *in situ* instrumentation may be influenced by extraneous factors such as biofouling, mechanical issues (i.e. a component of the instrument such as a bio-wiper becoming stuck over an optical lens), entrapment of debris or biota within the sampling volume or in the path of an optical beam, battery and power supply issues, and drift over time. The data records from each instrument were examined for quality control issues.

First, all instrumentation data were re-timed using timepoint bin syncing to produce data series with equivalent timestamps. A piece of debris impacted the NS02 sensor from November 9 - November 12, 2024, and a similar interference with the optical signal of the tryptophan channel was observed from November 29 - December 4, 2024. These points with physical interference were removed. The ECO-FLNTU sensor experienced drift from November 27 - December 4, 2024, resulting in a downward trend in the chlorophyll and turbidity measurements. Since both the ECO-FLNTU and C3 sensors had chlorophyll fluorescence channels, the two data series were plotted against each other to locate areas of disagreement. The chlorophyll sensor on the ECO-FLNTU had a decreased response intensity compared to the C3 sensor from Nov 27-Dec 4, and the ECO-FLNTU data (turbidity and chlorophyll channels) were removed from analysis.

The optical brightener and tryptophan sensor results were covaried with the lab-derived fDOM data by averaging for 30 minutes surrounding the time of discrete sampling. The Turner C3 data from November 6-7, 2024, were removed from correlation analysis due to stratification resulting in a lack of congruence between the bottle sample data and instrument data.

2.4 Environmental Data

Rain and wind data were extracted from the NOAA Local Climatological Data for Honolulu International Airport (Data). Continuous stream discharge data were downloaded for the Mānoa-Pālolo Stream, which drains to the Ala Wai Canal (USGS, Data). Rain data were extracted from the Mānoa monitoring station hosted by the University of Hawai‘i Atmospheric Sciences (Data).

2.5 Data analysis

Time-synced bottle and instrumentation data

Correlation analysis was performed for bottle sample parameters and time-synced instrument data and evaluated by Pearson correlation coefficient (r) value. Some parameters were log-transformed to approximate a normal distribution: turbidity, *Enterococcus* spp., and nutrients. A subset of bottle sample and instrumentation data (temperature, salinity, turbidity, optical brightener-like fluorescence, tryptophan-like fluorescence, *Enterococcus*, sucralose, nutrients, and several fDOM indices BIX, HIX, FI, and M:C ratio) were analyzed using principal component analysis (PCA). Clustering was examined by applying hierarchical partitioning, using the “average” clustering method.

Model selection

Linear regression models were constructed to determine the best instrumentation-based predictors for a standard and widely used fecal indicator bacteria (*Enterococcus* spp.). Linear models were constructed using the following predictors: salinity, turbidity, temperature, chlorophyll *a*, tryptophan-like fluorescence and optical brightener-like fluorescence. To meet the assumption of a normal distribution of residuals, *Enterococcus* spp., turbidity, and all fluorescence channels were log-transformed using the natural log, and salinity was transformed by the “exp” function. All possible predictor combinations that were analyzed using the “dredge” function in the “mumin” package for R (Barton, 2015), which ranked models using Akaike Information Criterion value with a correction for small sample sizes (AICc). Models were fitted with main effects only and no interaction effects. The best-fit models were selected by the lowest AICc scores (within $\Delta 2$ AICc).

Model validation

Instrumentation-based parameters were also used to construct categorical models with two outcomes: *Enterococcus* spp. concentration above and below the Hawai‘i state “beach action value” threshold of 130 CFU/100 mL, associated with an illness rate of 3.6%. These models were constructed as generalized linear models (GLM) with the “binomial” family link function and Firth’s bias correction using the “brglm2” package (Kosmidis, 2026). All possible model combinations were compared using the “dredge” function in the “mumin” package for R (Barton, 2015). AICc scores were used to assess the best-fit models. Models were assessed for

performance using leave-one-out cross-validation (LOOCV) and confusion matrices in the "caret" package (Kuhn, 2008).

Code availability

All custom code and data used in analysis (correlations, PCA, and models) is available at https://github.com/ccomfort-coastaloceans/alawai.

3. RESULTS

3.1 In situ fluorometer deployments - environmental context

The first *in situ* fluorometer deployment (April 3 - April 28, 2023) was preceded by a large rain event April 2-3rd (Rain #1) that caused elevated discharge in the Mānoa-Pālolo stream reaching upwards of 30 m^3 sec^{-1} (Figure 2, Figure S1). Additional rainfall occurred from April 18 - April 20, 2023 (Rain #2), which caused a small increase in stream discharge from near zero to about 4 m^3 min in the Mānoa-Pālolo stream (Figure 2, Figure S1).

The second deployment (November 6, 2024 - December 12, 2024) was again preceded by a rain event, variable winds and elevated stream flow (up to 11 m^3 sec^{-1}) from November 3-6 (Rain #3) (Figure S1). Rain #3 occurred most heavily on November 5, 2024 (Figure 2). There were two additional instances of elevated stream flow between November 12-14 (Figure S1).

During the 2026 deployment, several rain events occurred, including an extreme rain event in March (Rain #4) (Figure 2, Figure S1). Very high stream flows were observed during these rain events. The fluorometer recorded data normally during Rain #4 and subsequent rain events, but the NS02 sensor experienced damage due to the storm, and data collection was limited after Rain #4. Samples that were collected opportunistically during this longer deployment were incorporated into the modeling portion of this study (sections 2.5, 3.5).

3.2 Time-series data - instrumentation and bottle samples

The time-series data for all measured parameters showed that extremes of variability in many parameters were associated with rain events. During each of the rain events observed in the study, we observed spikes in *Enterococcus* spp., sucralose, nutrients, and key fDOM parameters of interest (optical brightener and tryptophan fluorescence) in discrete bottle samples (Figure 2). During Rain #1 and #2 in 2023, and rain #4 in 2026, instrumentation revealed freshwater discharge, elevated turbidity, and elevated optical brightener-like and tryptophan-like fluorescence. During Rain #3 in 2024 the freshwater plume was not detected by the instrumentation, with salinity and temperature remaining at typical marine levels, likely due to stratification.

Several of the instrumentation-based parameters were correlated. Optical brightener fluorescence was positively correlated with tryptophan-like fluorescence ($r = 0.267$, $p < 0.0001$) and turbidity ($r = 0.417$, $p < 0.0001$) and negatively correlated with chlorophyll a fluorescence

(r=-0.078, p <0.0001) (Figure 3A-3C). Tryptophan fluorescence and chlorophyll *a* fluorescence were positively correlated (r = 0.459, p<0.0001) (Figure 3D).

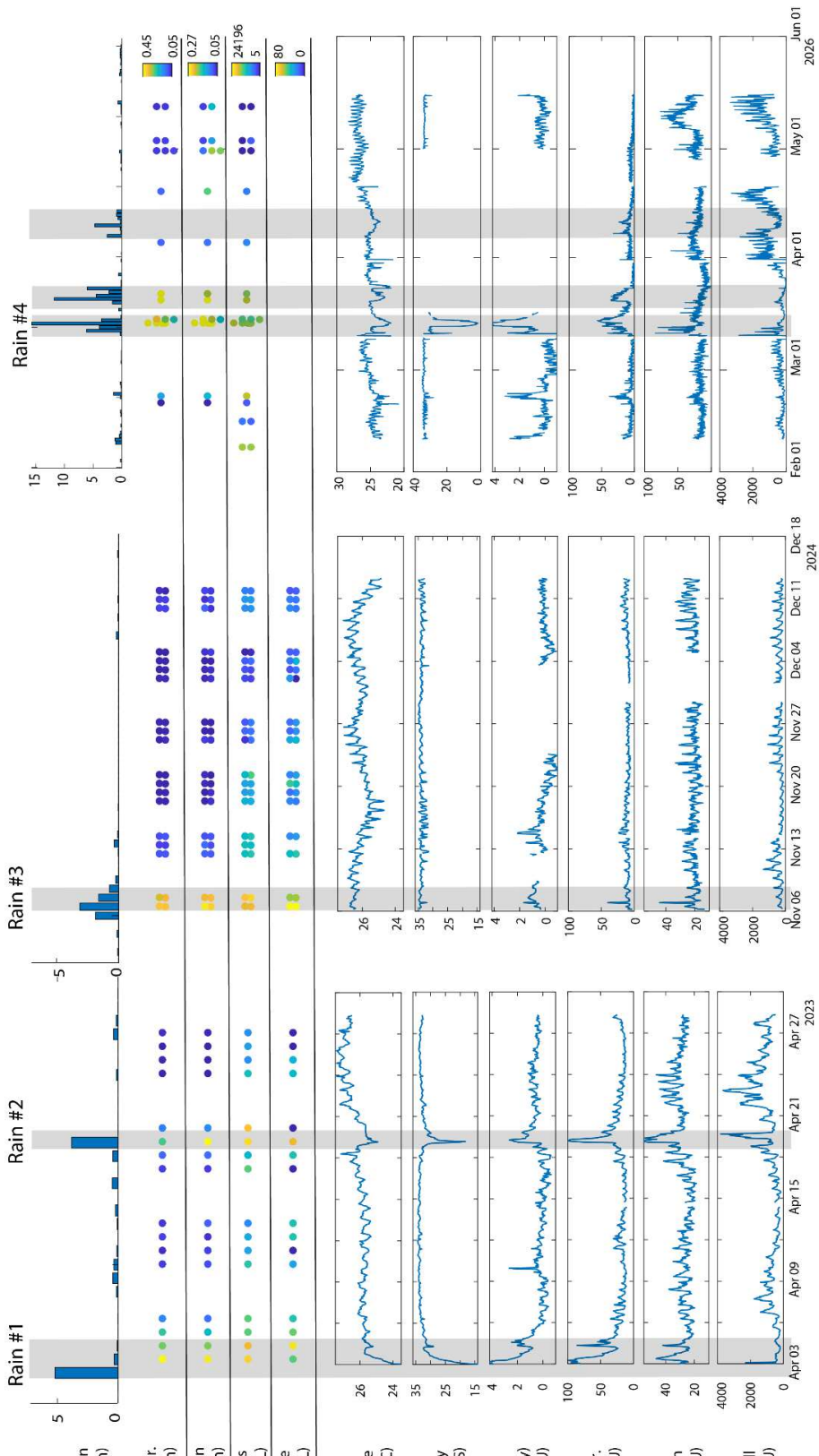


Figure 2. Time series of instrumentation and discrete bottle sample data for the two primary deployments in 2023 and 2024 and the additional deployments in 2026. Measurements from discrete bottle samples are indicated by circles. Yellow indicates higher readings and blue indicates lower readings. Instrumentation-derived data is represented by blue lines. Grey boxes represent rain events.

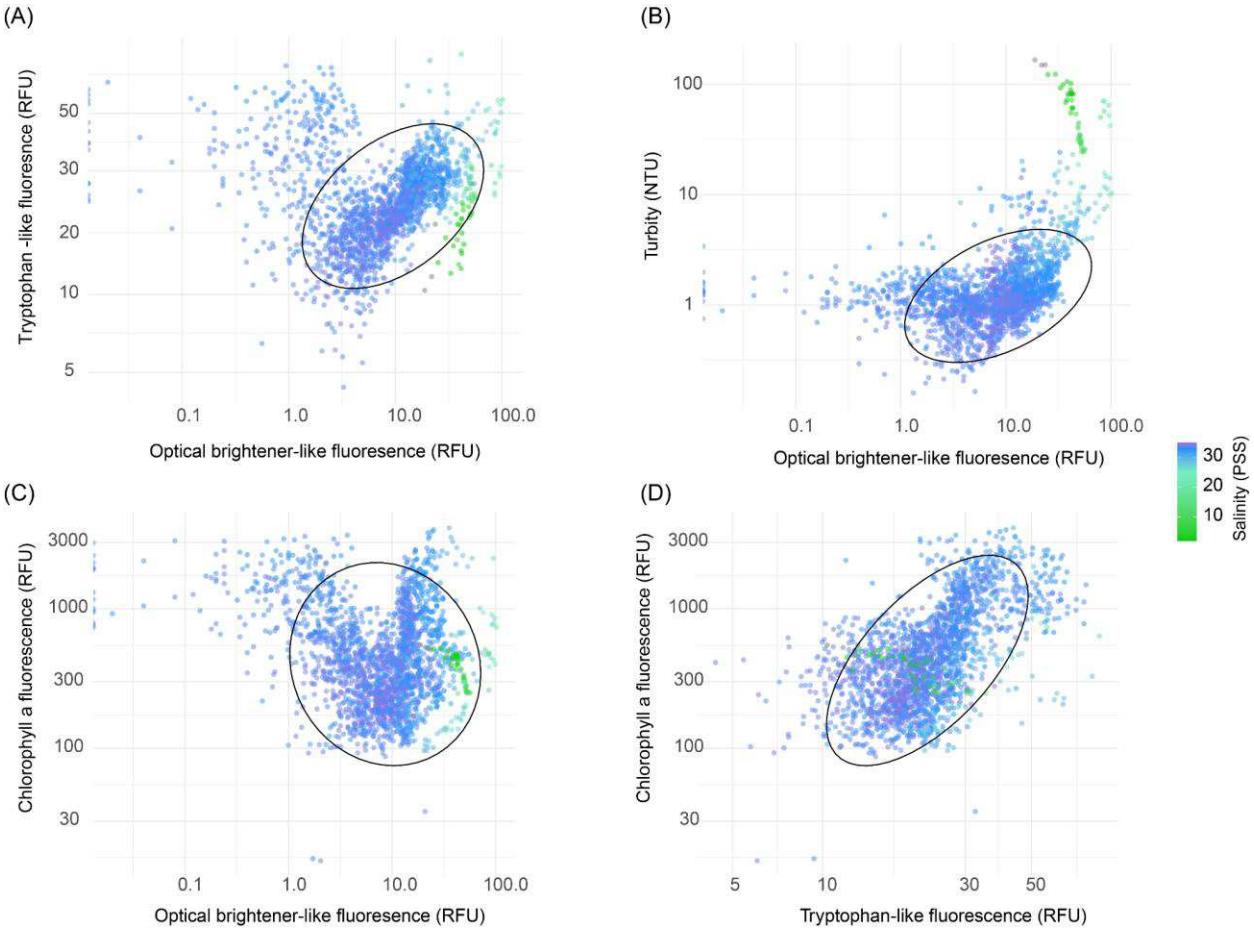


Figure 3. Significant correlations between selected instrumentation parameters. Correlations between optical brightener fluorescence, tryptophan fluorescence, turbidity, and chlorophyll-a are shown with salinity on the color axis. A) Optical brightener-like fluorescence vs tryptophan-like fluorescence; B) Optical brightener-like fluorescence vs turbidity; C) Optical brightener-like fluorescence vs chlorophyll a fluorescence; and D) Tryptophan-like fluorescence vs chlorophyll a fluorescence.

3.3 In situ Turner C3 performance versus laboratory analysis

Two fDOM channels from the Turner C3 fluorometer (optical brightener, ex 325 / em 445 and tryptophan, ex 275 nm / em 350 nm) were compared to measurements conducted in the laboratory using samples from 2023, 2024, and 2026 deployments, excluding samples where stratification caused decoupling between instruments and bottle samples. The same excitation and emission wavelengths were measured, but the samples in the laboratory were filtered (0.22 µm) before assessment. Since filtration is not practical for *in situ* instrumentation, field measurements may suffer from interference from particulates. The *in situ* optical brightener-like fluorescence was positively correlated with the laboratory measurements of the same wavelengths ($r = 0.798$, $p = 3.67^{-14}$, $n=59$) (Figure 4a), while the tryptophan sensor was less tightly coupled to the benchtop readings ($r = 0.244$, $p=0.079$, $n=53$) (Figure 4b).

3.4 Correlations between physical parameters, biogeochemical parameters and wastewater indicators

Enterococcus spp. and sucralose were positively correlated ($r = 0.493$, $p = 0.0002$, n=55 samples) (Figure 4c). Since sucralose is a more conservative wastewater tracer than *Enterococcus* spp., it is expected that some samples with *Enterococcus* spp. detections would not also have sucralose detections. *Enterococcus* spp. was detected in all 55 samples, and sucralose was detected in 49 of 55 samples. Enterococcus spp. was also significantly correlated with *in situ* optical brightener fluorescence in the 2023, 2024, and 2026 deployments (Figure 4c; $r=0.837$, $p = 2.2^{-16}$).

Additional correlations between parameters are shown in a multivariate correlation matrix (Figure S2). This multivariate correlation analysis was conducted using bottle samples and quality-controlled, time-synced instrumentation data from the 2023 and 2024 deployments (n=36 samples). The wastewater indicators *Enterococcus* spp. and sucralose were positively correlated with nutrients, turbidity, sucralose, HIX (humification index), optical brightener fluorescence, and tryptophan fluorescence. These indicators were both negatively correlated with salinity and various fDOM indices including the FI index (fulvic acids), the M:C ratio (marine to terrigenous humic acids), and BIX (autochthonous DOM origin) (Figure S2)

Principal component analysis with hierarchical clustering partitioned samples into four groups (Figure 6, Figure S3). Group A samples had low salinity, high *Enterococcus* spp., high sucralose, and high nutrients (Figure 5, Table 1). Group B samples (n=4) were a small cluster driven by high temperature and high BIX fluorescence index. Group C comprised samples with lower *Enterococcus* (median = 167 CFU/ 100 mL; this group included all samples with *Enterococcus* below the 130 CFU/100 mL regulatory threshold). These samples had high salinity and M:C fluorescence index, indicating more influence of marine waters. Group D samples had intermediate values between groups A and C (Figure 5, Table 1).

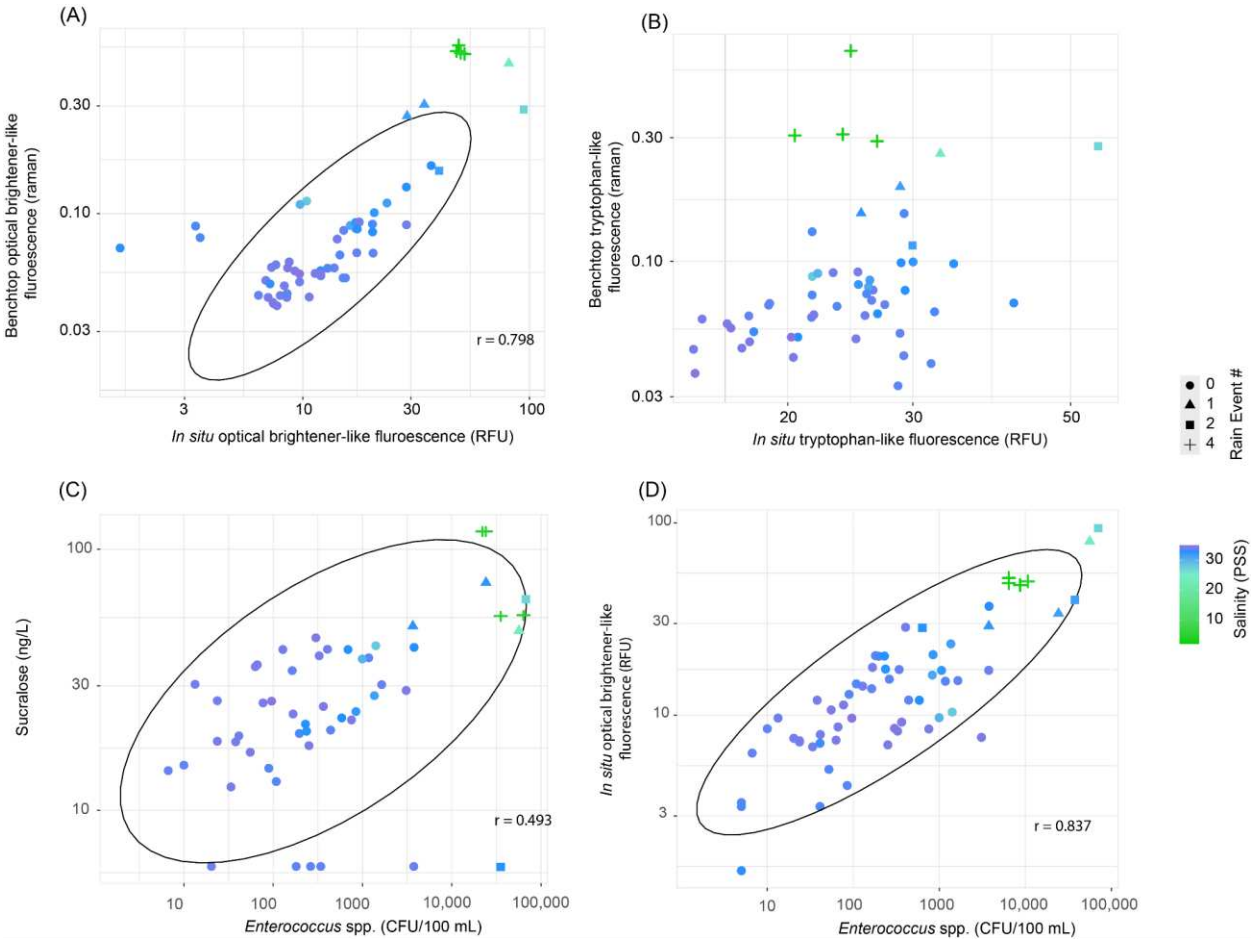


Figure 4. Selected correlations of fluorescent, microbial, and chemical measurements from the Ala Wai Harbor study site. A) *in situ* and filtered laboratory measurements of optical brightener-like fluorescence and B) tryptophan-like fluorescence. C) Correlation of *Enterococcus* spp. and sucralose concentrations. D) Correlation of Enterococcus spp. and *in situ* optical brightener-like fluorescence. Significant correlations are indicated by an ellipse (CI = 95%) and Pearson correlation coefficient r.

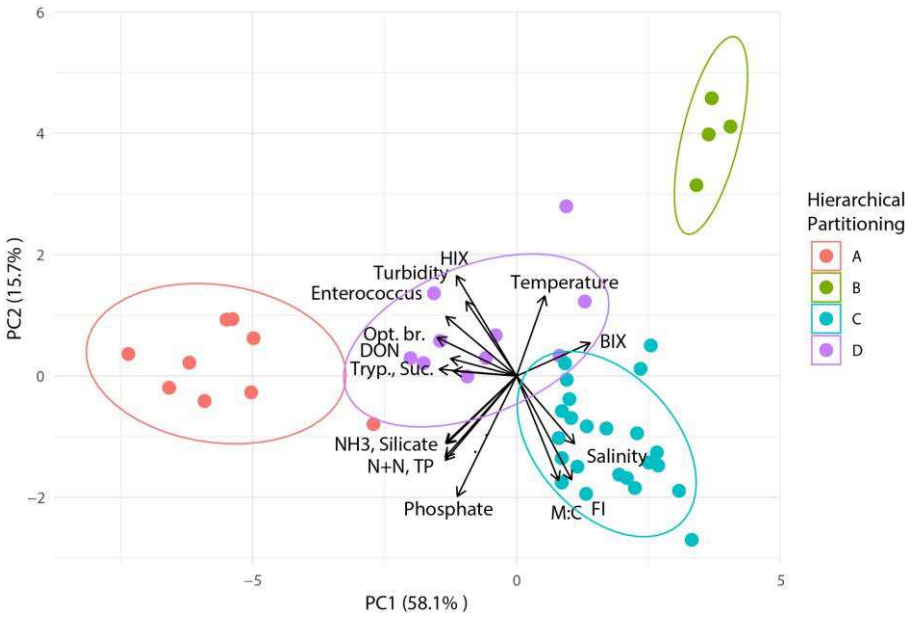


Figure 5. Principal component analysis of bottle sample data and time-synced instrumentation data. Ellipses and colors represent groups determined by hierarchical partitioning.

Table 1. Microbial and chemical characteristics of PCA clusters in Figure 5. Summary of groupings from hierarchical partitioning of PCA analysis including group median and range for wastewater-associated parameters *Enterococcus* spp., sucralose, nitrate+nitrite, and salinity.

		***Enterococcus* spp. (CFU/100 mL)**	**Sucralose (ng/L)**	**Nitrate + nitrite (µmol/L)**	**Salinity (PSS)**	**Characterization**
A	**Geomean**	22862	65.3	20.7	19.3	Highest *Enterococcus*, sucralose, and nutrients; lowest salinity
n=9	**Range**	3768-70123	42.1-117	13.2-30.36	10-33.1	
B	**Geomean**	309	14.8	1.14	33.7	Moderate *Enterococcus*, lower sucralose, lowest nutrients, high salinity
n=4	**Range**	163-1175	8-38.4	0.48-1.77	33.6-33.7	
C	**Geomean**	121	20.7	5.51	33.9	Lowest *Enterococcus*, moderate sucralose, low nutrients, high salinity
n=23	**Range**	6.7-3088	0-45.8	2.36-10.02	32.7-34.5	
D	**Geomean**	1423	20.4	6.15	32.1	High *Enterococcus*; moderate sucralose, nutrients, and salinity
n=10	**Range**	341-36167	0-42.7	2.81-14.32	28-34.1	

3.5 Linear and categorical models

Models included all samples from 2023, 2024, and 2026 deployments where the salinity of the discrete water samples measured in the lab was within 4 ppt of the salinity measured at the

NS02 sensor at the same time. Samples were excluded if any of the instrumentation channels (optical brightener, tryptophan, temperature, salinity, turbidity) showed evidence of optical window blockages, sensor drift, or fouling. In total, this resulted in 16 samples from 2023, 20 samples from 2024, and 11 samples from 2026 for a total of 47 samples. Using all instrumentation-based parameters, the best performing linear model was the following (R^2 =0.7, $p=2.95^{-13}$, AICc=161.5) (Table S1)

log(*Enterococcus* spp.) ~ lm(log(optical brightener-like fluorescence))

Adding additional parameters (temperature, turbidity, tryptophan-like fluorescence, chlorophyll a fluorescence, or salinity) resulted in similarly performing models but with higher complexity (Table S1). This simple model also outperformed the best-fit linear model among those evaluated from the existing real-time NS02 parameters of salinity, turbidity, chlorophyll a fluorescence, and temperature (R^2 = 0.38, $p= 8.90^{-6}$, AICc = 198.5).

log(*Enterococcus* spp.) ~ lm(log(turbidity) + log chlorophyll)

Categorical models to assess predictions of *Enterococcus* spp. concentrations over or under the Beach Action Value threshold of 130 CFU/100mL were fit using the following instrumentation parameters: temperature, salinity, turbidity, chlorophyll-a fluorescence, optical brightener fluorescence, and tryptophan fluorescence. The best-fit GLMs (family = binomial, ΔAICc < 2) are listed in Table S2. All included optical brightener-like fluorescence, which was the sole significant predictor, and additionally included non-significant predictors salinity, temperature, tryptophan-like fluorescence and/or turbidity. The model using only optical brightener-like fluorescence (ΔAICc = 2.3) was also included in model performance analysis. Model performance is reported by the cross-validated accuracy and kappa (Table S2) and rates of misclassification (Figure 6).

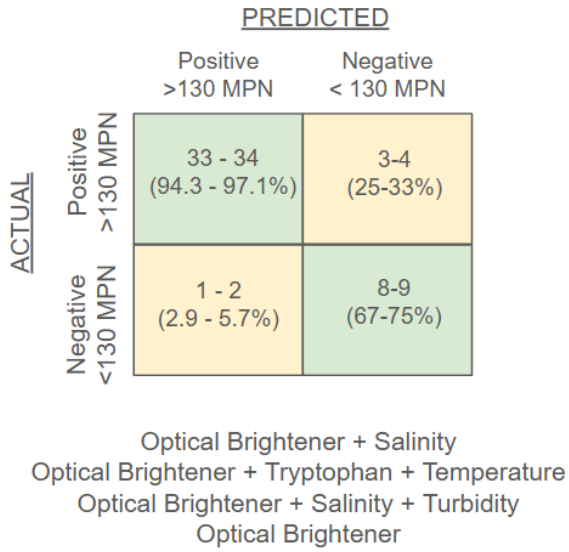


Figure 6. Performance of categorial prediction models for *Enterococcus* spp. regulatory exceedances. Confusion matrices for the best-specified categorical models predicting 130 CFU/100 mL threshold exceedances for *Enterococcus* spp..

4. DISCUSSION

4.1 Chronic wastewater pollution in the Ala Wai Harbor

Wastewater pollution to waterways and coastal areas is a concern throughout Hawai'i due to aging sewer pipes, tens of thousands of cesspools that dispose of household wastewater without any treatment, and sea level rise, flooding, and storm surge can infiltrate aging coastal infrastructure and cause exfiltration of wastewater to the environment (Mezzacapo et al., 2020). The Ala Wai canal and boat harbor are known to receive wastewater contamination through groundwater inundation of sewer infrastructure and wastewater exfiltration (McKenzie et al., 2021; Spengler & Heskett, 2022), leaky sewer pipes that pollute streams that feed into the canal (Kirs et al., 2017), and sewage spills (Fujioka, 2008; Venzon, 2007). There are also about 500 cesspools in the watersheds of the Makiki, Mānoa, and Pālolo streams that may also contribute wastewater pollution to the Ala Wai canal (Mezzacapo & Shuler, 2022).

While regulatory water quality monitoring in marine areas relies on *Enterococcus* spp. concentration thresholds due to the established positive association between gastrointestinal illness and enterococci concentrations in recreational waters (Beaches Environmental Assessment and Coastal Health Act of 2000, 2000; Cabelli et al., 1982), enterococci are not a source-specific indicator of wastewater contamination. Enterococci are also found as autochthonous members of the microbial community in sediments and beach sand and are generally found in high concentrations in Hawai'i streams regardless of wastewater contamination (Byappanahalli, Nevers, et al., 2012; Byappanahalli, Roll, et al., 2012; Cui et al., 2013; Hardina & Fujioka, 1991; Steadmon et al., 2024). In the Ala Wai Harbor, however, we

found that across the full range of observed *Enterococcus* spp. concentrations, there was a positive correlation with the conservative wastewater tracer, sucralose in 89% of samples. Alongside previous work that highlights wastewater intrusion to the Ala Wai Canal and its source streams, this relationship indicates that the observed *Enterococcus* spp. concentrations were at least in part caused by wastewater pollution to the canal and harbor on most occasions.

4.2 Predicting Enterococcus *spp. concentrations with* in situ *instrumentation*

The optical brightener fluorescence channel on the Turner C3 fluorometer (ex 325/ em 445) was the best predictor of *Enterococcus* spp. concentrations in the Ala Wai Harbor among all available instrumentation parameters, including the tryptophan fluorescence channel (ex 275 / em 350), turbidity, salinity, and temperature. Previous studies have shown promise in using optical brightener fluorescence as a marker of wastewater pollution in stream environments (Finegan & Hasenmueller, 2023; Tavares et al., 2008). Since the *Enterococcus* spp. concentrations in the Ala Wai Harbor are associated with wastewater pollution, the optical brightener fluorescence signal should capture fabric brighteners as a component of wastewater.

A categorical binomial model with optical brightener fluorescence and optional additional parameters (salinity, turbidity, and/or tryptophan) was able to correctly predict exceedances of Hawai'i's 130 CFU/100 mL Beach Action Value for recreational waters >94% of the time (i.e., the model predicted an exceedance and the bacterial culture also showed an exceedance). The models performed less well for correctly identifying bacteria counts below the BAV, with a negative prediction matching negative culture results 67-75% of the time. Since the Ala Wai Harbor is chronically polluted, there were relatively few samples that met recreational water quality standards for *Enterococcus* spp. The instrumentation and model performance at low concentrations of wastewater-derived *Enterococcus* spp. should be further assessed.

The predictive ability of the optical brightener channel for *Enterococcus* spp. concentrations shown in this study is similar to that of tryptophan-fluorescence based sensors used to predict *E. coli* threshold exceedances (Bedell et al., 2022; Knopp et al., 2026). A tryptophan-based sensor was able to achieve >90% prediction for a binary classification threshold in an experiment in the Seine River in Paris, France, and good correlations in other locations on multiple continents (Knopp et al., 2026). The tryptophan and optical brightener wavelengths in this study had a similar correlation to *Enterococcus* spp. in filtered benchtop samples, but the relationship between tryptophan and *Enterococcus* spp. concentration was weaker in the field, perhaps due to interference from turbidity.

Other studies have also found that a ratio of tryptophan fluorescence to colored dissolved organic matter (CDOM, ex 325 / em 470) can provide good estimates for fecal indicator bacteria concentrations in stream and estuary settings. In Alvarado Creek, California, the ratio of tryptophan fluorescence:CDOM fluorescence was found to be a useful real-time indicator of a wastewater spill, and the fluorescence data correlated strongly with both *Enterococcus* spp. and *E. coli* concentrations as well as wastewater-specific metrics such as caffeine (Mendoza et al., 2020). In an estuary environment near the mouth of Tijuana River, the tryptophan:CDOM ratio

was also found to be a good indicator of intermittent repeated sewage flows at the monitoring site. Inner filter effects were observed during events that lead to very high FIB concentrations, such as storms and heavy rains, which reduced the performance of the *in situ* sensors in predicting FIB concentrations in the Tijuana estuary. In that study, the benchtop fluorescence data performed more consistently than *in situ* data (Mladenov et al., 2024), which was also observed in the tryptophan fluorescence channel in this study.

4.3 Considerations when using fluorescence to track fabric brighteners in the environment

In streams where *Enterococcus* spp. concentrations are driven by animal feces or autochthonous populations in sediments rather than wastewater pollution, high fecal bacteria counts may not correlate with fabric brighteners that indicate wastewater. However, the excitation and emission peak for the optical brightener whitening agents are likely to overlap with other fluorescent dissolved organic matter, which could confound source tracking. The fluorescent indices "Coble C" and "Coble A," representing terrestrially derived humic-like DOM, fluoresce at ex 345 / em 445 and ex 320 / em 450 respectively (Coble, 1996, 2007), and these wavelengths are similar to the optical brightener sensor used in this study (ex 365 / em 437.5-452.5 nm). Since the Ala Wai canal and boat harbor have chronic wastewater pollution, the observed optical brightener fluorescence readings are likely to reflect both terrestrial humic acid input and fabric brighteners in laundry detergent in wastewater pollution.

Optical brightener compounds degrade when directly exposed to UV light, so measuring fluorescence at the peak excitation and emission frequencies for brighteners before and after the sample has been irradiated with UV can distinguish between fabric brighteners and humic acids (Hartel et al., 2007; Iyer et al., 2005). In this study, we sampled early in the morning, minimizing UV degradation effects of both *Enterococcus* spp. and optical brightener compounds. This study was aimed at assessing the ability of *in situ* instrumentation to predict *Enterococcus spp.* concentrations, and we did not have a way to separate humic acids from optical brighteners in the field. However, an assessment of the proportion of the optical brightener fluorescence signal that is due to brighteners versus humic acid is a useful next step to understanding what compounds in the water the instrument is truly measuring.

4.4 Towards real-time alerts and wastewater pollution forecasting

Measurements of optical brightener fluorescence significantly improved estimates of fecal indicator bacteria in the Ala Wai Harbor compared to the existing real-time parameters that are currently included on the PacIOOS NS02 sensor. The Ala Wai Turbidity Plume Model provides a 3-day hindcast and 2-day forecast of the turbidity plume from the Ala Wai Harbor using real-time turbidity and stream gauge data paired with data predicting currents from the Regional Ocean Modeling System (ROMS) (http://www.pacioos.hawaii.edu/water/model-plume-alawai/). *Enterococcus* spp. concentrations in the plume have been previously modeled based on discrete bottle samples from 5 rain events spanning 2006 to 2009 (Johnson et al., 2013). This model included the ROMS current predictions and stream gauge readings as well as UV decay,

light attenuation and variable *Enterococcus* spp. survival at depth, and the model predicted observed concentrations of *Enterococcus* spp. during the rain events well (Johnson et al. 2013). If a fluorometer is configured to transmit real-time data, the optical brightener fluorescence channel could be used to predict *Enterococcus* spp. concentrations, and these estimates could be integrated into this sophisticated pre-existing plume model to forecast wastewater pollution trajectories for nearby surf breaks, paddling routes, and beaches. Also, real-time alerts could be implemented to alert ocean users when optical brightener fluorescence predicts exceedances of Department of Health *Enterococcus* spp. thresholds, improving public health outreach and hopefully preventing wastewater-associated illnesses and infections.

In addition to public health, coral reef health is significantly impacted by wastewater pollution (Wear et al. 2015). Elevated nutrients from wastewater pollution or other land-based sources can interrupt key processes such as calcification and primary production and can shift reefs towards erosion rather than accretion (Silbiger et al. 2018). Wastewater pollution can also increase the proportion of benthic algae relative to coral and lead to phase shifts towards algal dominated ecosystems (Adam et al., 2021; Lesser, 2021). Wastewater pollution has also been identified as a key factor that negatively impacts coral reef resilience to increasing thermal stress and other climate change impacts (Gove et al., 2023). Real-time tracking of wastewater spills and spikes in pollution can help to improve quantification of the overall wastewater impact on coastal and reef ecosystems. Traditional bottle sampling conducted at weekly to monthly intervals may struggle to quantify the impacts of storms, wastewater spills, large wave events, and other extreme or unusual weather patterns. A high-resolution time-series of estimated wastewater pollution may help managers to better estimate the cumulative loading of pollutants to sensitive environments and help with decisions and prioritization of watershed rehabilitation actions to improve coastal ecosystem health.

5. CONCLUSIONS

We assessed the effectiveness of various instrumentation-based parameters to predict fecal indicator bacteria concentrations in the Ala Wai Harbor, which is known to be regularly polluted with wastewater based on previous studies and sucralose concentrations observed in this study. Existing long-term monitoring systems at the sampling site measure temperature, salinity, turbidity, and chlorophyll-a fluorescence, and an experimental sensor in this study measured fluorescent dissolved organic matter at two excitation/emission wavelength sets - one corresponding to optical brightener compounds in laundry detergent, and one corresponding to tryptophan, which is often correlated with *E. coli* and *Enterococcus* fecal bacteria. Optical brightener fluorescence was found to be the best predictor of *Enterococcus* spp. concentrations, outperforming standard parameters (temperature, salinity, and turbidity) and tryptophan fluorescence. Categorical models using optical brightener fluorescence as the dominant predictor were able to predict exceedances of Hawai‘i Department of Health 130 CFU/100 mL Beach Action Value for *Enterococcus* spp. with >95% sensitivity and 75% specificity. In the future,

optical brightener fluorescence could be integrated into existing Ala Wai Canal plume models to predict BAV exceedances and improve public health alerts.

Instrumentation improves upon monitoring using discrete bottle samples, since instruments can provide real-time data as well as a much finer sampling interval (minutes vs days-weeks). However, instrument placement is important. In this study, rain events resulted in spikes in fecal bacteria, sucralose, nutrients, turbidity, and fDOM. One of these rain events resulted in a shallow stratified plume, and the instruments were located deeper in the water column and did not effectively measure the pollution signal. Also, all the parameters measured by instruments in this study are proxies rather than direct measurements of wastewater pollution. The best-performing instrumentation proxy for fecal bacteria, optical brightener fluorescence, likely reflects a mixture of fabric brightener chemicals in wastewater and terrestrial humic acids due to similarities in the excitation/emission wavelengths. Further work is needed to separate the signals.

The Ala Wai Canal drains three watersheds in urban Honolulu, and the resulting freshwater plume into the nearshore marine environment can impact recreational waters. Real-time detection of wastewater pollution spikes and BAV exceedances in the Ala Wai and other pollution-prone coastal waterways would improve public health communication and waterborne illness prevention efforts. Instrumentation-based monitoring can also help to better understand the total loading of wastewater pollution to the nearshore environment by dramatically increasing the sampling rate. Fluorescence-based wastewater pollution indications, especially optical brightener-like fluorescence, is likely to be transferable to other urban watersheds and areas with aging or insufficient wastewater infrastructure and widespread use of fabric brighteners in detergent, including other areas in Hawai‘i, the Pacific islands, and coastal communities. To reduce wastewater pollution at the source, infrastructure improvements such as upgrading cesspools to appropriate wastewater treatment systems and continued repairs of sewer pipe infrastructure are needed, especially in the face of rising sea levels and increasing sewer line inundation.

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