Spatiotemporal Analysis of Distribution Patterns of the Pathogen *E. Coli* in an Urban Wetland

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We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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절대 포기하지 말라고 가르쳐 주신 할머니 감사합니다,
나는 당신을 사랑하고 당신이 너무 보고싶어요.

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ABSTRACT

Escherichia coli (*E. coli*) is a naturally occurring group of bacteria that is ubiquitous throughout aquatic environments and tropical climates. Despite the multitude of natural sources, *E. coli* may also originate from wastewater, and in high concentrations can lead to severe health issues. This study set out to monitor *E. coli* concentrations across a wetland that is surrounded by urban land uses, including wastewater infrastructure, cemented stream beds capturing runoff, parking lots, and a highway. The approach to understanding the distribution patterns of *E. Coli* over space and time was to collect monthly water samples at 20 different locations across the wetland. I hypothesized that *E. coli* distributions would vary according to proximity to wastewater infrastructure, runoff from parking lots and roads, and springs delivering groundwater to the wetland. On the temporal scale, the hypotheses explored *E. coli* surges in relation to rainfall events linked to surface runoff, or alternatively, to higher temperatures relating to stagnant water bodies on the wetland. All water samples were processed and analyzed using an IDEXX Colilert 100 mL format system for quantification according to the manufacturer's instructions. In contrast to predictions, the primary determinant of *E. coli* concentrations was spatial, with the highest concentrations along the eastern sector, perhaps associated with the indirect connection to an outflow. There was no correlation found with rainfall although there were no major precipitation events during the course of study.

Keywords: *E. coli*, Fecal Bacteria, Urban Wetland

LIST OF ABBREVIATIONS

Abbreviations	Definitions
E. coli	*Escherichia coli*
GIS	GIS Geospatial Information Systems
mm	Millimeters
M	Meters
ºC	Celsius
mL	Milliliters
μS/cm	Siemens per centimeter
MPN	Most Probably Number
FIB	Fecal Indicator Bacteria
CFU	Coliform Forming Units
CSV	Comma Separated Values
KMZ	Keyhole Markup Language, Zipped
YSI	Yellow Springs Instruments
EPA	Environmental Protection Agency
U.S.	United States of America
USDA	United States Department of Agriculture

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 MOTIVATION

E. coli is a naturally occurring Fecal Indicator Bacteria (FIB) that lives in the intestines of warm-blooded organisms and can be found in soils and aqueous environments across tropical climates. In fact, it has been shown that FIB levels in all streams on the island of Oahu exceed EPA hygienic recreational water quality standards (Fujioka et al., 1988). But because some strains of FIB can be harmful to human health EPA and USDA monitor its presence and assume that if FIB is present then the water body is polluted by sewage (Fujioka et al., 1988). This approach ignores the fact that, due in part to climate and humidity, the soil in Hawai‘i is a natural habitat for FIB, and rain and surface runoff can readily transport it into water bodies (Fujioka et al., 1988). Building on these findings, this study sets out to investigate the distribution of *E. coli* in a wetland where multiple potential vectors of *E. coli* transfer exist.

1.2 SCIENTIFIC SIGNIFICANCE STATEMENT

One way to differentiate *E. coli* transport mechanisms is to explore its temporal and spatial distribution with respect to the location of potential sources and temporal triggers. A coastal wetland fed by groundwater springs but also surrounded by urbanized landscapes including wastewater infrastructure, parking lots, highways, and restaurants in close proximity is a potential testing site for *E. coli* patterns (Figure 1). The hope is to discern sewage vs soil runoff sources based on spatial pattern analysis and also to shed light on transport mechanisms such as surface runoff following rainfall events.

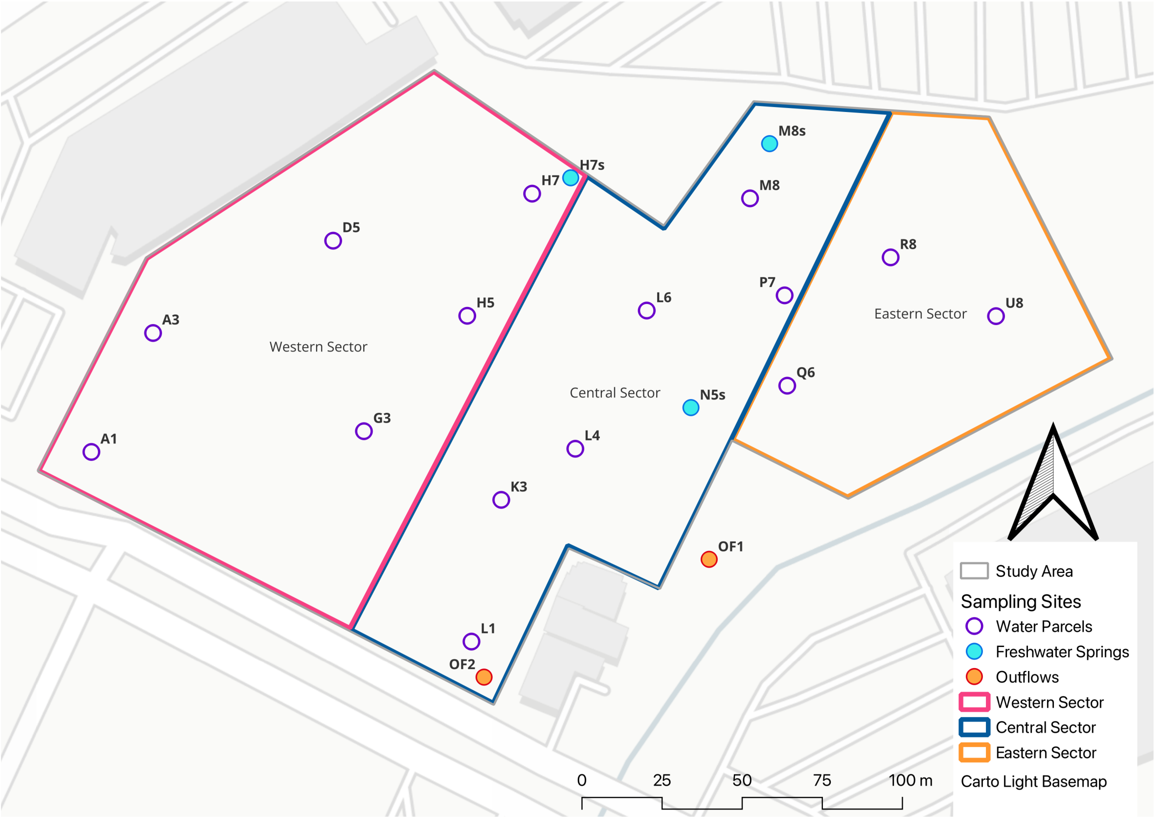

Figure 1: Aerial map view of the study site with symbols indicating the 20 sampling locations for *E. coli* analysis. The sampling locations are represented by purple circles for parcels of water with vegetation, blue circles for locations of groundwater discharge, and orange circles are the locations of the outflow catchments. The three polygons are reference sections of the study area indicated by the western, central, and eastern sectors.

1.3 ESCHERICHIA COLI

E. coli is a bacterium that is naturally found in the intestines of animals and humans. While most strains of *E. coli* are harmless, some strains may cause serious illnesses (Ishii & Sadowsky, 2008). Some factors that can affect *E. coli* concentrations include soil characteristics, deposition of fecal matter, and environmental and water quality parameters. In some areas *E. coli* can become naturalized, where its presence is found in sand and soil (Ishii & Sadowsky, 2008). This becomes an issue as *E. coli* is associated with fecal contamination. Most commonly *E. coli* is used to determine wastewater contamination by sewage infiltration in freshwater systems (Fujioka et al., 2015). When analyzing for *E. coli* or other FIB in water, it is becasue they are correlated with health risks. Monitoring water sources such as groundwater, recreational water, and drinking water for fecal bacteria is mandated to prevent implications to public health (DeViliss et al., 2021). *E. coli* is often used as the fecal indicator organism because it is easily quantifiable with inexpensive methods. It's important to note that while *E. coli* can cause illnesses, it also holds a prominent part in the natural ecosystem of the human gut. It helps to facilitate the digestive process and to produce certain vitamins such as K2 and B vitamins (LeBlanc et al., 2013; Park et al., 2020).

1.4 ESCHERICHIA COLI IN HAWAIʻI

E. coli has been prevalent throughout Hawaiʻi. As it is a naturally occurring bacterium, *E. coli* has been recorded in coastal and terrestrial environments across Hawaiʻi (Fujioka et al., 2015; Oshiro & Fujioka, 1995; Kirs et al., 2017; Kirs et al., 2020). Due to the distinct climate of the Hawaiian Islands, *E. coli* is present in multiple mediums such as soil, sand, and water with sources that include birds, mongoose, and other warm-blooded mammals (Fujioka, 2015; Oshiro & Fujioka, 1995). In other cases, *E. coli* can be transported through groundwater from sewer and stormwater infrastructure during heavy rainfall events (Kirs et al., 2017).

Soil can also be an environmental source of contamination of *E. coli* which can be transferred to streams during rain events (Fujioka et al., 2015). A study near the Mānoa Stream analyzed the concentrations and sources of *E. coli* and determined that soil is a likely source of the indicator bacteria in the freshwater streams of Hawaiʻi (Hardina & Fujioka, 1991). Soil samples obtained near the stream bank and across the University of Hawaiʻi at Mānoa campus were determined to contain high levels of *E. coli* (5.4×10^4 MPN Index/100 grams of soil) (Hardina & Fujioka, 1991). The warmer temperature, relative to its surroundings, and the high nutrients in soil create favorable environments for *E. coli* to multiply. During rain events, the *E. coli* in the soil can be transported into streams, which can explain the high concentrations of fecal bacteria in Hawaiʻi's freshwater streams.

In coastal settings, *E. coli* can be present in sand from potential sources such as warm-blooded animals. A study done at Hanauma Bay found that pigeons and mongooses in Hawaiʻi can be a vector for *E. coli* contamination in beach sand (Oshiro & Fujioka, 1994). The fecal matter of each animal was analyzed for *E. coli* per gram, pigeon feces contained 1.7×10^8, and mongooses contained 9.2×10^8. Both animals were observed in the study area where they would defecate on the sand. The results of the study provided evidence that warm-blooded animals in coastal regions can be sources of *E. coli*. Furthermore, the study at Hanauma Bay indicated that the samples collected inland from the shoreline where the moisture of sand decreased, and soil content increased had higher levels of *E. coli* compared to those at the shoreline.

Due to Hawaiʻi's natural climate and humidity, the levels of *E. coli* outweigh those of other regions of the world. The EPA standards for measuring *E. coli* are difficult to follow in Hawai‘i since there seem to be independent populations of *E. coli* that can thrive outside of their hosts in the natural environment, this means there is a naturally high background population of *E. coli* thriving in the environment. The EPA makes two assumptions: the disregard towards Hawai‘i’s environmental resources and that fecal bacteria can not multiply in the environment under natural conditions (Fujioka et al., 1988). Because of these assumptions established by the EPA, the water quality standards are not applicable to Hawai‘i since all stream waters will exceed that standard (Fujioka et al., 1988).

2.0 METHODS

2.1 STUDY AREA

The study focused on a wetland located in the Kalauao ahupuaʻa, on the moku of Ewa, Hawaiʻi. The wetland area (Figure 1) consists of 116 parcels covering 4521 m^2, fed by more than 5 major fresh- and brackish water springs collectively called the Kalauao Spring complex. The entirely spring-fed wetland drains through two outfalls and is tidally connected to Puʻuloa (Pearl Harbor). The periphery of the wetland is 869 m out of which 204 m borders a highway, 363 m is a parking lot, and 212 m is along a stream. Sewer lines lay along the whole section of the highway downstream of the wetland (363 m) and along the berm on the northwest side of the wetland with a restaurant bordering the periphery (60 m). Along the west, northwest, and northeast boundaries, about 5-10 m wide vegetation-covered soil with trees, shrubs, and kalo plants surround the wetland.

2.2 SAMPLE COLLECTION

Water samples were collected at 20 locations across the wetland, each sampling was spaced out roughly 30 days over a period of 11 months. The collection sites included 15 water parcels, three freshwater springs, and two outflow canals. We followed a sampling technique outlined in the instruction manual for a Colilert kit manufactured by IDEXX, which is a U.S. EPA-approved method included in Standard Methods for Examination of Water and Wastewater (Standard Methods, 2017). We used a 36-inch push-point Field Investigation Sampler manufactured by MHE Products, which was positioned within the water column while not disturbing or retrieving bottom sediments. A Tygon tubing was attached to the sampler and a Global Water SP200 Variable Speed, Peristaltic, Fluid Sampling Pump, which drew water at a controlled speed into 125-mL clean sterilized polypropylene bottles. We filled the bottles exactly with 100 mL of water. The bottles were cleaned by soaking in 10% bleach overnight followed by tap water rinse and air drying. Prior to sample collection, the containers were rinsed three times using the sampled water and when filled, they were placed in a chilled cooler until processing at the Kapiʻolani Community College Kokiʻo Lab. During sample collection, water quality parameters, temperature, specific conductance, and salinity were also measured using a handheld multiparameter sonde (Professional Plus Manufactured by YSI). For consistency of repeat samplings, each sampling location was confirmed by corresponding GPS coordinates.

2.3 ENUMERATION OF E. COLI IN WATER SAMPLES

A container with 100 mL sample water was used to determine the *E. coli* concentrations using Colilert®-18 kits (IDEXX Laboratories, Inc.; Westbrook, ME) following the manufacturer's instructions. This procedure included the addition of a proprietary Defined Substrate Technology substrate into the sample, which was then poured into the Quanti-Tray 2000®, The Quanti-tray was sealed and incubated at 35 ^{o}C for 24 hours. After incubation, the color of the wells was read using an ultraviolet lamp and the Most Probable Number (MPN) per 100 mL was generated using the table for the Quanti-Tray 2000®. Because this study focuses on spatial and temporal trends, all numbers are reported as using the Z-score method which standardizes values. The raw values of *E. coli* are transformed using this method of standardization by which all values are subjected to the following formula: Z-score($\log_{10}$(MPN+1)). Moreover, the Z-score equation uses the mean and standard deviation of the $\log_{10}$(MPN+1) to calculate a normalized distribution of the *E. coli* values. Where the $\log_{10}$ MPN+1 value is subtracted by the mean and divided by the standard deviation. This method uses these standardized values to analyze the spatial and temporal distribution.

2.4 MULTIVARIATE ANALYSIS USING PYTHON

Multivariate analysis is a statistical study that evaluates multiple variables to identify any possible correlations between variables. It measures the strength of association between multiple variables, providing insights into the degree by which they co-vary. By exploring the correlative patterns of different variables, we can gain a deeper understanding of the complex structures underlying datasets and identify important relationships that may not be evident from univariate analyses. The measured data was organized based on location and time sampled to perform a multivariate analysis using Python (version 3.9.7), using packages numpy, pandas, seaborn, matplotlib.pyplot, and scipy.stats. The following variables were used for correlation analysis: precipitation data from the National Weather Service, YSI water quality parameters (temperature and specific conductance,), and *E. coli* MPN.

2.5 GEOSPATIAL PATTERN ANALYSIS

The open-source Quantum Geospatial Information System (QGIS) software (version 3.30.0 - Hertogenbosch) was used to analyze the spatial distribution of the normalized *E. coli* concentrations. Using Google Earth Pro (version 7.3.6.9345 (64-bit)), place markers were created at each sampling location and a polygon was created to represent the study area. The point and polygon files were then exported as a KMZ file. Using Microsoft Excel (version 16.71), the normalized *E. coli* data was compiled for each sampling site along with latitude and longitude. The excel format was then converted to a CSV format.

In QGIS the study area KMZ was dragged into the layers panel. Using the Add Delimited Text Layer, the CSV data was imported into QGIS. With the processing tools, an Inverse Distance Weighted (IDW) raster was calculated for the wet (November-March) and dry (April-October) season sampling times. The symbology render type was changed from the default single band gray to single band pseudocolor. The minimum was adjusted to -2 and the maximum was adjusted to 1 to reflect the range of standardized z-score values. After the interpolated surface was generated and the values were adjusted to reflect the data values, I clipped the layer using the study area KMZ. This technique was also applied to the measured water temperature and specific conductance measurements.

3.0 RESULTS

3.1 E. COLI CONCENTRATIONS

Throughout the sampling period, *E. coli* values appeared within the measurable IDEXX detectable range in 117 out of the total of 220 samples collected monthly between Jan 2022-Nov 2022 (Table 1). From the 15 parcel sites sampled, all 15 showed *E. coli* levels at least once over the 11 months, and 7 had *E. coli* consistently present every month. From the springs, none had positive detections year-round, and observed detectable levels only in August out of the 11 months of sampling, respectively. In comparison to the parcels, the springs had much lower levels of *E. coli* year-round (Figure 3). The two outflow canals had *E. coli* present year-round with a single exception: March at OF1. Looking at the averaged values of *E. coli* concentrations during the sampling period the eastern plots had the highest levels of *E. coli* across the wetland, this includes plots Q6-U8 and both outflows.

Table 1: Normalized *E. coli* concentrations over 11 months of sampling.

Sample	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
A1	0.21725173	-0.8887738	-0.4212169	-0.1948122	0.53379177	-0.183485	-0.8340493	0.88172863	-0.4047579	0.24994513	-0.0479688
A3	-0.8887738	-0.7437309	-0.9986294	-0.4973061	-0.4313718	-0.4818468	0.71569445	0.36915843	1.69872764	-0.5646974	-0.8340493
D5	-1.3465929	-0.6023338	-2.0200067	-0.0153986	-0.2915124	0.34050409	-0.0564709	0.74260453	0.77001412	0.63482531	1.69872764
G3	-1.0042776	-0.2430175	-0.7119015	-0.1323868	1.4423562	-0.2678621	0.79763105	0.68914201	0.90838295	0.90838295	0.01643079
H5	-0.9505958	-1.0712651	0.34050409	-0.4780589	0.27783996	0.36012273	0.05990658	0.06295231	1.09812959	0.47405587	-0.8543615
H7	-0.459558	1.18058935	-2.0200067	-0.2488166	-0.6378912	0.28207892	-0.3398163	0.36915843	-0.5469367	-0.3384063	-0.7570818
H7s	-1.6891922	-2.0200067	-1.0712651	-1.6891922	-1.0099934	-1.2424282	0.42547862	0.57373172	-1.4956782	-1.6891922	0.66327407
K3	-0.2183065	-1.1492143	-0.361481	-0.1397579	0.1764429	-0.4487922	0.35488162	1.15275983	0.93577737	-0.4875855	-0.9455454
L1	-0.2864351	-0.4875855	-0.4780589	0.55352816	0.9901889	0.11141202	0.23281608	1.69872764	0.50845258	1.5394921	0.79763105
L4	-0.6458679	-0.2915124	-0.1714272	0.39102646	0.77001412	0.45814124	1.04410569	0.85314948	0.39467668	1.04410569	0.77001412
L6	-0.7921906	0.42377105	-0.2915124	-1.0042776	-0.0343749	-0.2801634	-0.8461326	1.69872764	0.10976344	0.16284747	-0.4112735
M8	-1.3465929	-1.0042776	-0.297936	0.88085412	-0.0479688	-0.2316264	-0.6431942	0.59411331	1.69872764	-0.0564709	0.74260453
M8s	-2.0200067	-2.0200067	-2.0200067	-2.0200067	-2.0200067	-2.0200067	-1.6891922	0.0980611	0.90838295	-1.6891922	-2.0200067
N5s	-2.0200067	-2.0200067	-2.0200067	-2.0200067	-2.0200067	-2.0200067	-1.6891922	0.35488162	-1.3465929	-1.4956782	-1.6891922
P7	-0.1251279	0.31182464	-0.3935652	0.02571123	-0.0299283	0.59411331	1.12530901	1.40229262	0.47661493	0.42377105	0.68914201
Q6	-0.0878971	-0.667815	1.40229262	1.04410569	0.82533749	1.05660847	1.15275983	0.37456208	1.20892533	1.09812959	0.40988602
R8	-1.4956782	1.4423562	-0.051043	-0.5072288	1.48724282	1.12530901	0.79763105	1.15275983	1.69872764	1.69872764	0.35488162
U8	0.39102646	1.09812959	0.74260453	0.351908	0.31862492	0.88085412	0.9901889	1.26785788	0.90838295	1.5394921	0.37927973
OF1	0.20388211	0.72397358	-1.6891922	0.42205735	1.26785788	1.23795639	0.67956338	1.0710744	1.20892533	1.36554131	0.65771836
OF2	0.00285194	0.76835503	0.14986668	0.23281608	0.85314948	0.65368487	0.51438435	1.60459273	0.68914201	1.09812959	0.13704311

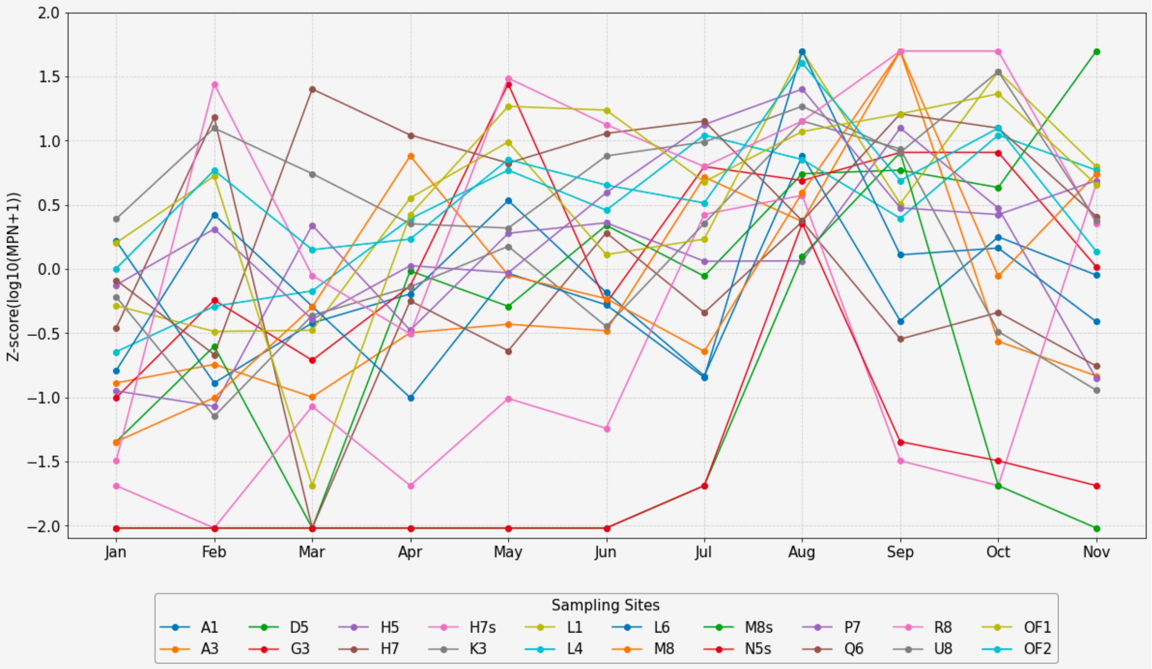

Figure 2: Standardized *E. coli* concentrations at each of the 20 sampling locations between January-November 2022.

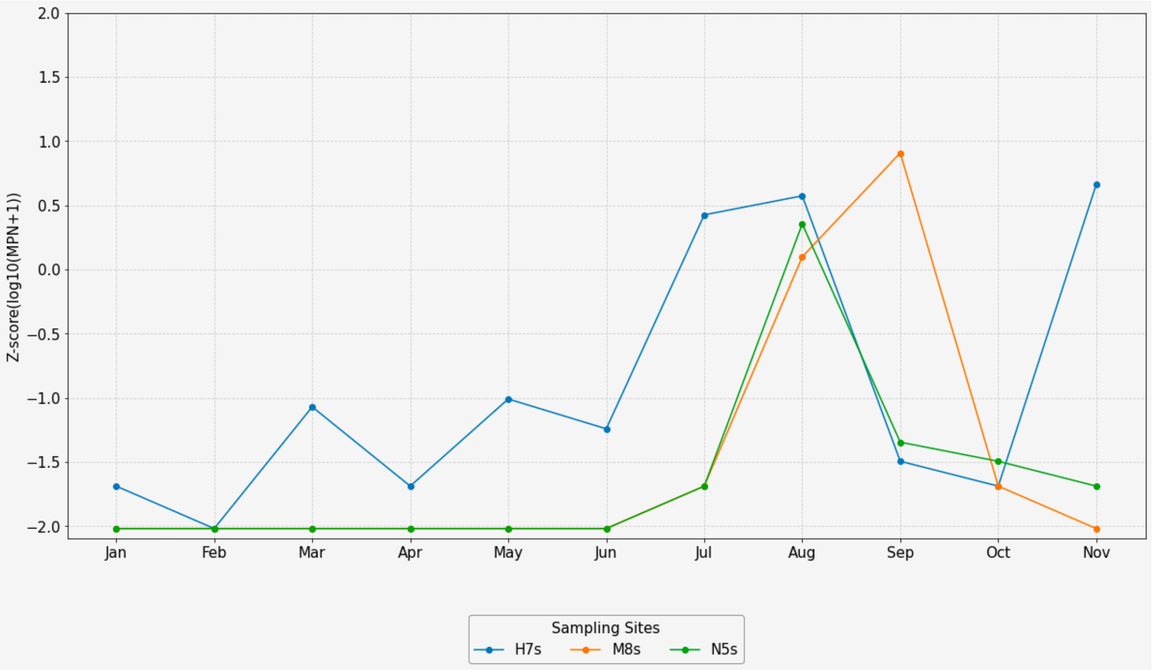

Figure 3: Standardized *E. coli* concentrations at the freshwater springs. All values above the zero Z-score value reside above the mean while the negative values are below the

mean. Almost all freshwater spring values are well below the mean, with the exception of some values from July through September.

During the months of November through March (wet season), the parcels in the eastern sector (Figure 1) have some of the highest values of *E. coli* concentrations (Figure 4). Parcel U8 had the highest *E. coli* concentrations during the wet season (Figure 4). Moreover, during the months of April through October (dry season), the eastern sector had the highest values of *E. coli* concentrations (Figure 5). Comparing the two heat maps generated using QGIS, the eastern sector showed to have the highest measurements of *E. coli* concentrations.

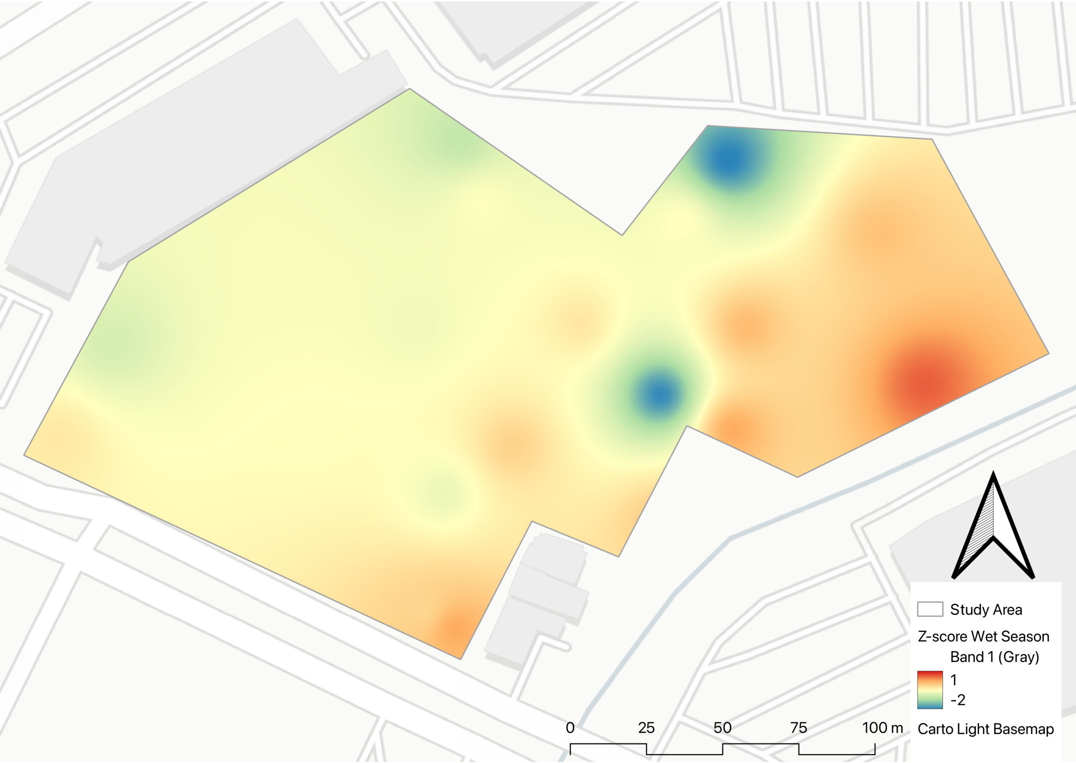

Figure 4: Wet Season (Nov-Mar) Standardized *E. coli* Heat Map using QGIS.

Figure 5: Dry Season (Apr-Oct) Standardized *E. coli* Heat Map using QGIS.

3.2 ENVIRONMENTAL PARAMETERS

Rainfall events recorded at the Honolulu International Airport located 6 km from the study site (NOAA), were downloaded as daily averages. For our purposes, we used the sum from 5 days including the day of and prior to sampling for the correlation analysis (Figure 6). Daily precipitation over the study period ranged from 0 to 0.94 mm, with an average of 0.003 mm over the dry season and 0.013 mm over the wet season. 5-day sums capturing the period before sampling ranged between 0 to 84.58 mm.

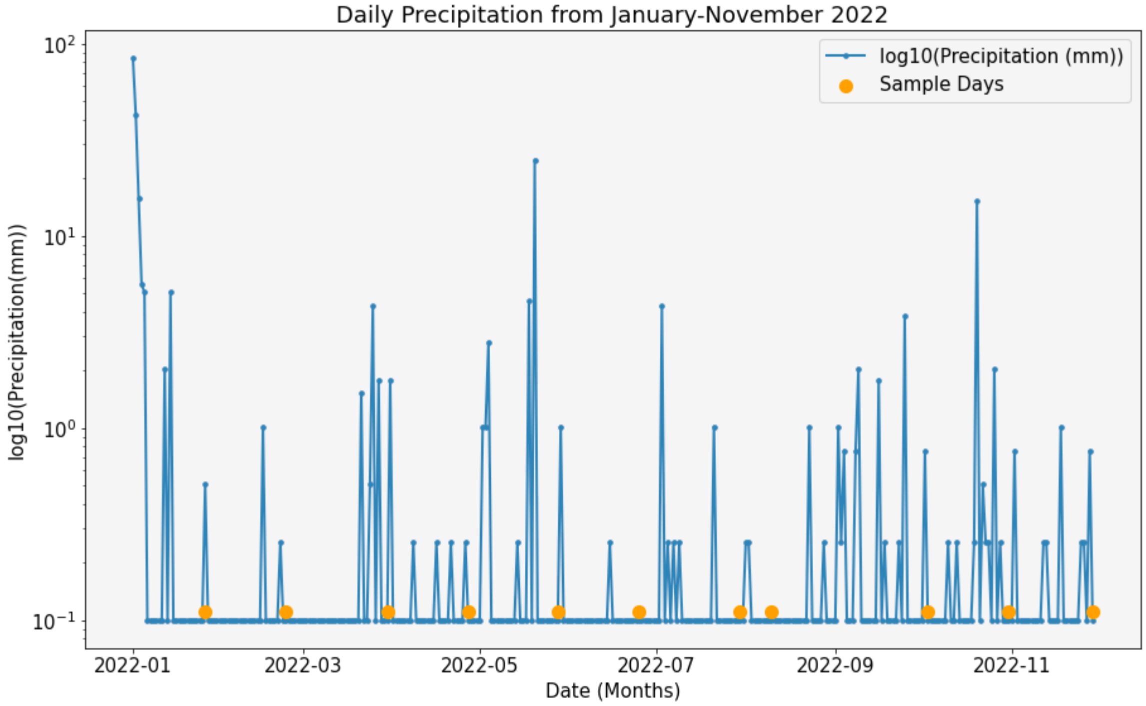

Figure 6: Precipitation data collected from the Hawai‘i Climate Data Portal Station USW00022521; the orange dots indicate times of field collection.

3.3 WATER QUALITY PARAMETERS

The collected water quality parameters obtained from the YSI sensor include water temperature and specific conductance. Water temperature across the wetland had a range between 20.3-28.4 ºC (Table 2). Of the 15 parcels many experienced high variabilities throughout the sampling period (Figure 7). In comparison, the springs had the lowest and relatively consistent temperatures between 20.3-23.2 ºC (Table 2).

Table 2: Water temperature [ºC] at each sampling location.

Sample	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
A1	23.6	23.8	25.7	24.4	22.2	24.2	23.7	24.9	25.7	25.6	24.4
A3	24.2	23.2	24.6	24.6	22.2	23.6	23.2	23.7	26	24.7	23.7
D5	22	22.7	22.9	21.1	23	22.1	22.8	22.1	23.9	26.4	23.2
G3	21.1	23.1	23.9	21.4	22.4	21.5	23.4	23.7	24.9	25.6	23.3
H5	21.1	21.7	25.7	22.3	21.2	23.7	23.7	21.3	27	25.8	22
H7	22	22.9	25.2	23.1	21.9	21.7	23.9	22.9	22	24.5	21.8
H7s	22.1	21.8	23.2	22.8	22.1	21.8	23	22.1	21.8	22.1	22.8
K3	22.5	22.1	23.8	22.7	21.6	22.4	23.5	24	23.8	25.4	22.8
L1	21.9	22.6	24.2	21.9	23	22.3	25.4	24.3	23.8	24.6	23
L4	21.6	22.6	24	21.9	23.5	22.3	25.2	25.3	23.6	23.9	22.9
L6	21.8	21.7	22.6	21.3	21.7	21.3	22.1	25.1	22.3	22.4	21.4
M8	20.9	21.2	21.6	21.2	20.6	21.2	22.1	23.4	21.6	21.5	21.3
M8s	20.6	20.3	20.6	20.4	20.5	20.3	20.4	20.3	20.4	20.4	20.4
N5s	21.3	21	20.8	20.6	20.7	20.9	20.5	20.5	20.6	20.5	20.5
P7	22.8	22.8	24.2	24.7	21.7	24.3	25.3	24.3	23.4	24.1	24.6
Q6	24.3	23.8	25.3	23.6	21.8	23.3	28.3	24.2	26.9	24.6	24
R8	21	23.6	22.6	21.5	20.9	24.01	25.6	21.8	27	22.7	22.3
U8	22.7	24.8	24.8	23.9	22	24.5	25.4	22.3	28.4	23.4	21.9
OF1	22.8	23.4	23.7	21.8	23	24.5	25.8	23.4	25.2	22.8	23
OF2	22.8	24	24.3	23.2	22.9	23.2	26.7	24.5	25.4	25	23.4

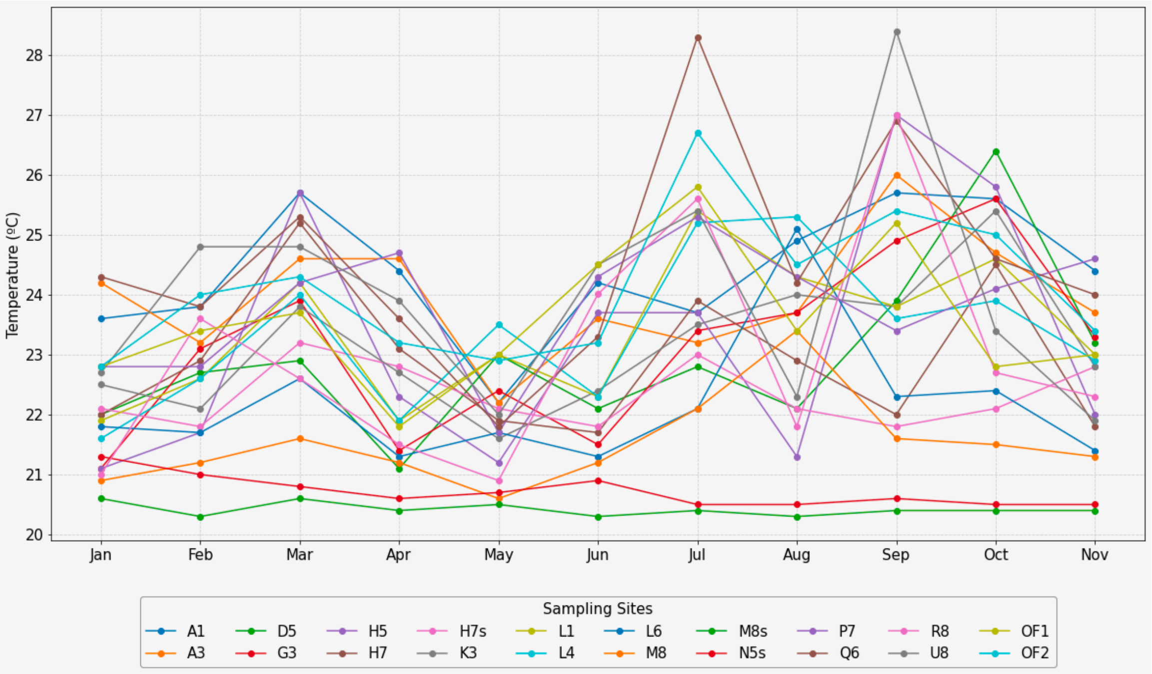

Figure 7: Water temperature at each sampling location from January-November 2022.

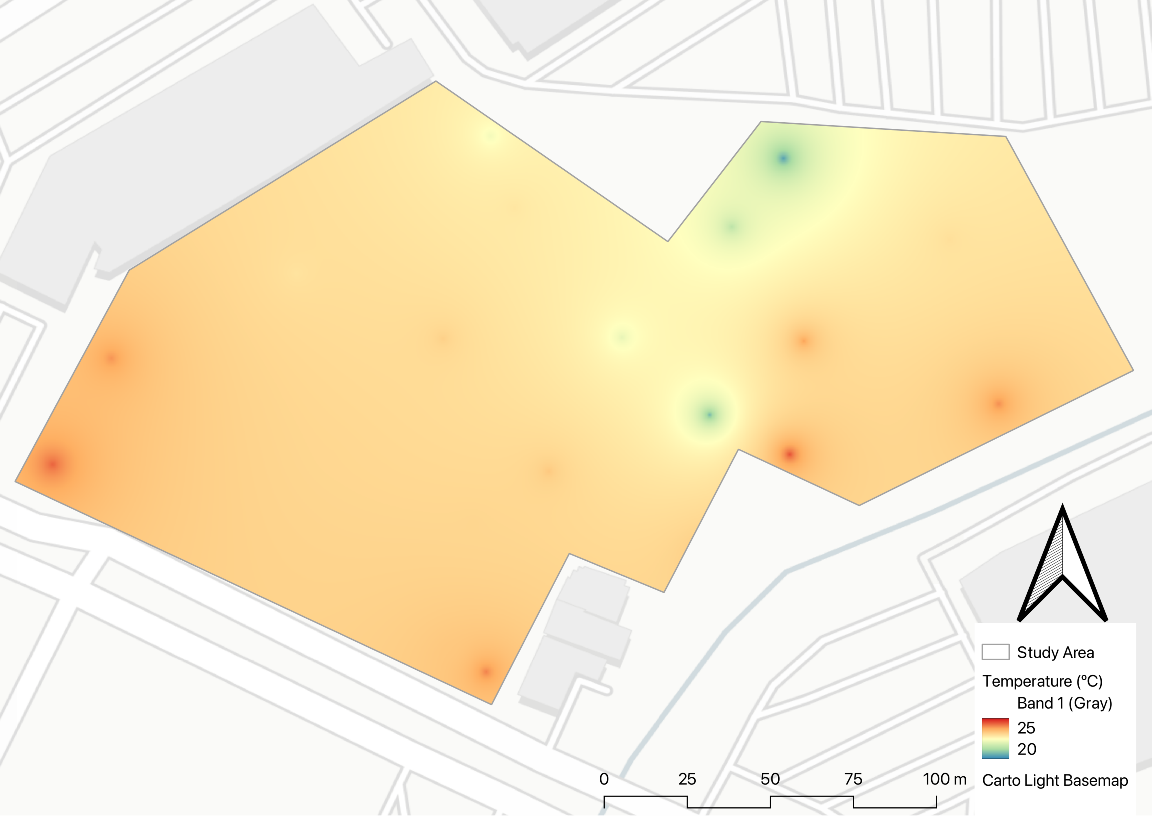

Figure 8: Heat map of the averaged measured temperature across the study area.

Measurements of specific conductance ranged from 0.2-3,160 µS/cm. The majority of the sampling locations had a large variation in specific conductance. Specifically, OF2 had the greatest variance (Figure 11).

Table 3: Specific conductance [µS/cm] of each location during sampling.

Sample	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
A1	783	832	784	860	819	963	836	563	820	863	745
A3	710	775	601	418.6	818	1086	871	423	820	567	349.5
D5	804	766	817.5	926	937	1075	699	433	373.9	917	178.2
G3	1550	626	1142.5	1322	1602	1484	1606	1680	1249	1026	535
H5	513	544	671	771	714	626	934	1081	892	647	642
H7	526	572	573.5	578	600	565	557	376	298.8	614	560
H7s	1762	631	676	662	443.3	686	690	663	645	666	611
K3	3050	1808	1316.5	2070	2376	1609	2513	2610	2626	2325	2507
L1	2027	1978	1968	2184	2151	2045	2360	1811	1693	2386	1660
L4	670	1813	1743.5	951	1995	84.7	423	3160	1016	886	1437
L6	593	396.6	517	528	549	413	485	614	364.3	507	322.2
M8	1426	575	473.5	528	522	71.3	449	1153	771	548	96.7
M8s	525	488.3	522.6	251	527	496	547	504	199	487	466.7
N5s	2035	2130	2060.5	2246	2177	2088	2163	2121	2085	2082	255.5
P7	1400	1457	1119.5	658	1392	1475	1387	1334	1280	1232	132.9
Q6	1099	0.2	892	609	1283	486.9	1165	422	1164	834	968
R8	489.7	582	607.5	608	558	457.6	556	607	644	576	406.9
U8	304.6	48	1645.5	586	538	672	606	594	726	477	646
OF1	989	925	1667	2204	613	981	1008	1781	1025	1915	1481
OF2	2129	2110	2131	2170	2128	2374	2341	2283	2133	2259	2229

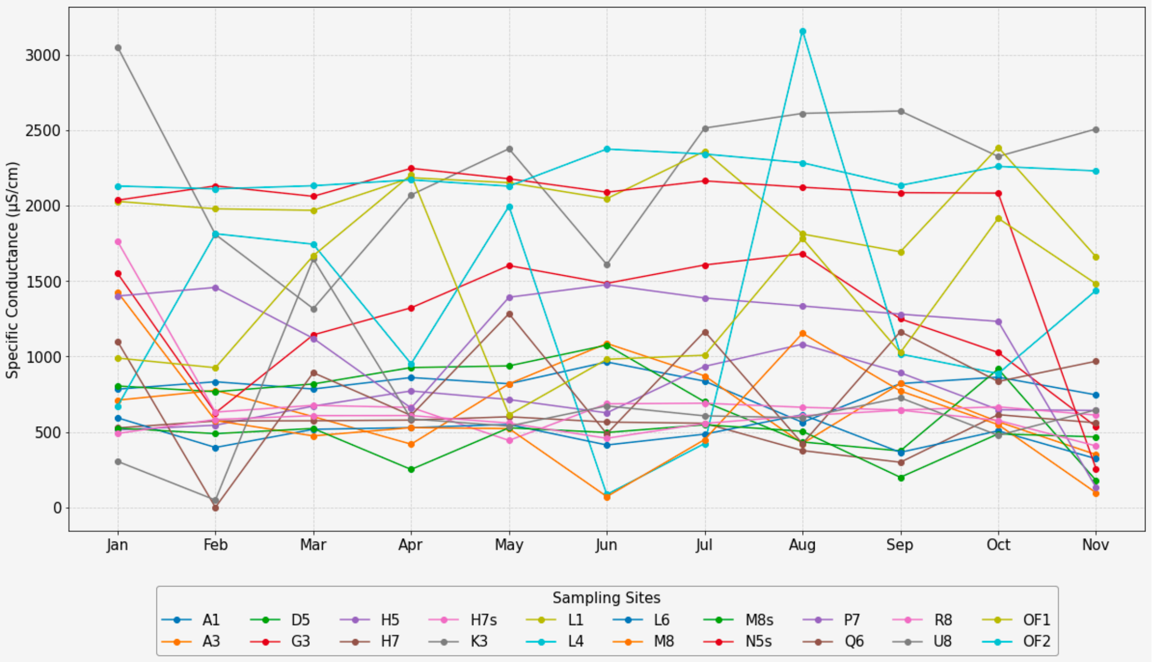

Figure 9: Specific conductance at each sampling location from January-November 2022.

Figure 10: Heat map of the averaged specific conductance across the study area.

4.0 DISCUSSION

4.1 SPATIAL PATTERNS OF E. COLI

The enumeration of *E. coli* showed to be positive at each location throughout or at least once during the sampling period. In contrast, the springs where groundwater discharge occurs showed to have the lowest concentrations of *E. coli*. This was reassuring as this allowed groundwater contamination as a source of *E. coli* to be ruled out (Figure 3). Confirming the cleanliness of the freshwater springs that feed the wetland was crucial.

Among the 20 sampled sites, field data show that the concentrations of *E. coli* are consistently higher in the eastern sector, specifically at sites R8-U8 (-1.5-1.7). In contrast, the measurements of *E. coli* have lower concentrations in the western sector (0.03-0.21). This pattern was consistent between wet and dry seasons (Figures 3 and 4). The locations of groundwater discharge, H7s, M8s, and N5s were clearly observed to have the lowest concentrations of *E. coli* throughout the entire sampling period (Figure 3 and 4). In contrast, the two outfall locations, OF1 and OF2 experienced relatively high concentrations when comparing the wet and dry season heat maps (Figure 3 and 4).

The eastern sector of the wetland proved to have the highest overall concentrations of *E. coli* (Figure 3 and Figure 4). Speculations can be made that the shape of the eastern sector can be the cause of the higher abundance of *E. coli*, due to the one degree slope of the study area the flow of water is deflected off the southern retaining wall of the eastern sector, and travels along the adjacent perimeter following the natural flow of water until reaching OF2 (Figure 4). It is important to conduct monitoring of the wetland to recognize areas where *E. coli* was present. With the given analysis (Figure 5,

Figure 7, and Figure 9), the environmental and water quality correlations did not point to any relative influences in *E. coli* concentrations.

4.2 ENVIRONMENTAL AND WATER QUALITY INFLUENCES

The relationship between precipitation and water column *E. coli* concentrations was explored to answer the hypothesis that rain related surface runoff would transport *E. coli* into the wetland. We can conclude that overall, the *E. coli* concentrations with respect to precipitation did not have a significant correlation. However, the overall precipitation during 2022 was relatively low compared to other years. Rainfall being the ultimate source of stream water can be an effective transport mechanism for *E. coli* concentrations (Fujioka et al., 2015) but it did not turn out to be the driving factor in this case. I investigated the impact of precipitation on *E. coli* levels by analyzing the correlation between total precipitation in the month preceding sampling and standardized MPN. I discovered that a second-order polynomial provided the best fit for the data (p = 0.0032 for the squared term, p = 0.43 for the linear term). This correlation accounted for approximately 20% of the variability in E. coli concentrations (R2=0.20).

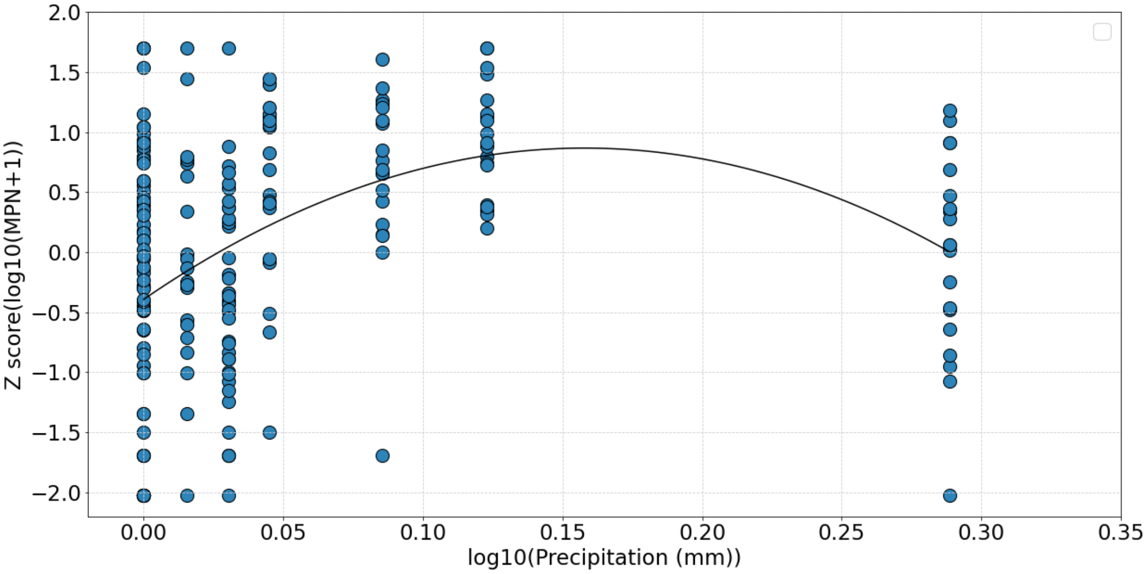

Figure 11: Standardized *E. coli* concentrations plotted against increasing ($\log_{10}$(Precipitation (mm)) during the sampling period.

Past studies showed that *E. coli* and temperature are linked, if this relationship is relevant to the temperature scales of this study, then there will be a correlation between *E. coli* and temperature. Water temperature can have a significant effect on the concentration of coliforms including *E. coli* (Pachepsky et al., 2014). The optimal growth temperature for *E. coli* is between 20-37 ºC (Pachepsky et al., 2014). During the field collection period, the observed temperature in each sampling site was within a reasonable range to expect that the microbes would tolerate. Moreover, I discovered a positive linear correlation between temperature and normalized MPN, which was relatively strong (F=80.06, df=217) and highly significant ($p < 0.0001$). This correlation accounted for approximately 27% of the variability in E. coli concentrations (R2=0.27). On the other hand, I did not find any significant relationship between specific conductivity and normalized MPN ($p = 0.71$).

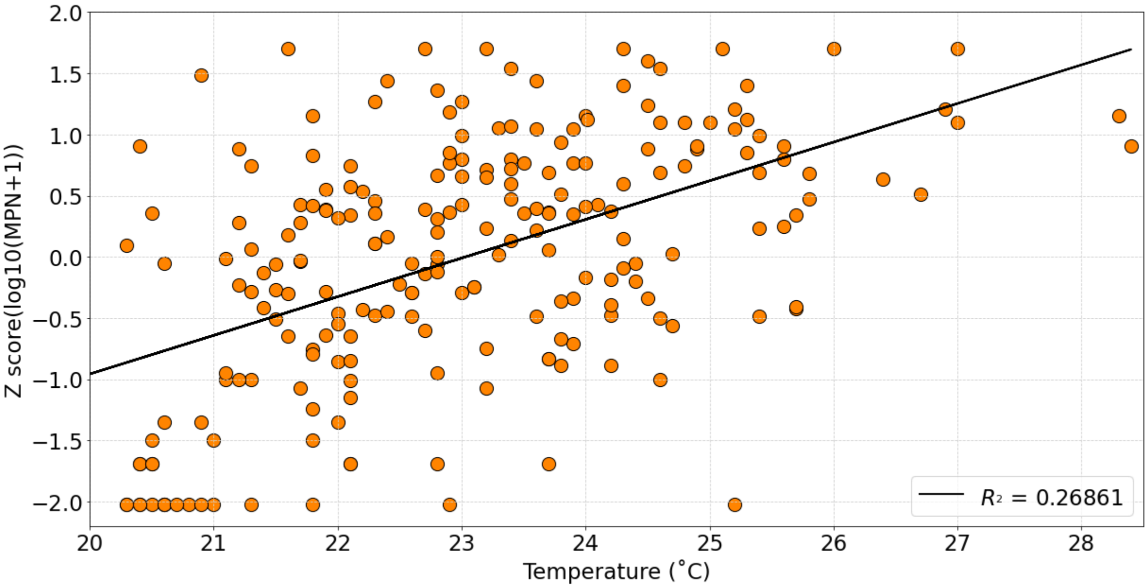

Figure 12: Standardized *E. coli* concentrations plotted against increasing temperature during the sampling period.

The salinization of freshwater in the range of 13 to 350 µS/cm increases the survival of *E. coli* (DeVilbiss et al., 2009). However, in concentrations above 1500 µS/cm, the abundance of *E. coli* decreased which may be due to a decrease in survival rate (DeVilbiss et al., 2009). As a result, the survival rate of *E. coli* is shown to not be dependent on specific conductance. Moreover, the type of cation in salt ionic compounds can substantially affect the concentration of *E. coli*. Salinized water containing chlorinated salts with magnesium had the greatest survivorship compared to salinized water with sodium, calcium, or potassium as the base cation (DeVilbiss et al., 2009).

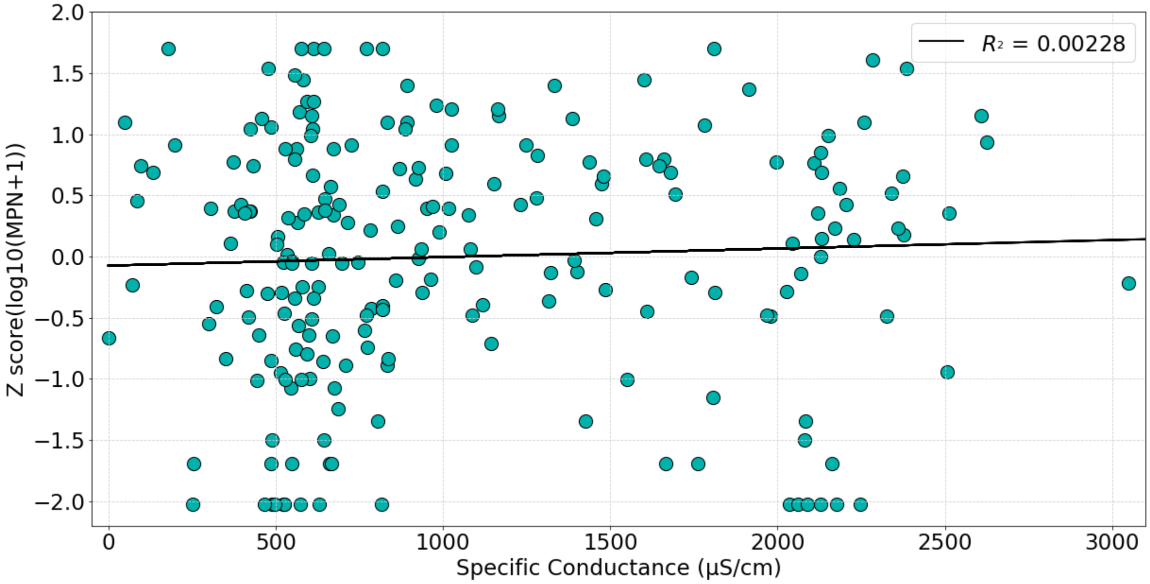

Figure 13: Standardized *E. coli* concentrations plotted against increasing specific conductance during the sampling period.

To summarize the findings, *E. coli* was present in the wetland year-round, but it did not originate in the groundwater springs that are sources of freshwater. Based on the results of the *E. coli* spatial analysis (Figure 3 and 4) the freshwater springs proved to not be a significant source of *E. coli* entering the study area. *E. coli* was more persistent in the eastern sector of the wetland, which does not spatially match the location of any sewer lines.

The average temperature of each sampling site ranged between 20.4-24.6 ºC. This range is within the optimal growth temperature of *E. coli* production with the maximum being 50 ºC, at this point *E. coli* concentrations will begin to decrease (Papchepsky et al., 2014). The lowest temperature occurred at the springs, while the highest temperatures occurred in the eastern sector in parcels such as Q6 and U8.

The average specific conductance of each sampling site ranged between 456-2256 µS/cm. The lowest averaged specific conductance was measured in the eastern and western sectors of the study area, while the central sector including the K through L and P conduits measured about 1100-2256 µS/cm. Both the H7s and M8s springs were under 800 µS/cm, except N5s which measured almost 200 µS/cm. M8s had the lowest average specific conductance, this may have been due to being one of the only sampling locations that is under a tree canopy, thus reducing evaporation.

4.3 PROPOSED FUTURE RESEARCH DIRECTION

Future direction to the research scope, building on these findings, could focus on further refining the sources of *E. coli*. It is unknown why the eastern sector had a higher concentration of the bacteria than other parcels. During the sampling time, no significant rain events occurred. Having increased sampling frequency would create better interpretations of the effects precipitation has on *E. coli* concentrations. I would propose to include event-triggered sampling - after large rain events. This would provide some clarity to the spatiotemporal distribution of *E. coli* concentrations throughout the wetland, as precipitation is a driver for *E. coli* concentrations (Kirs et al., 2017). Additionally, the sampling period of this study occurred in 11 months, while increasing the sampling or comparing to this study can provide a comparison to how *E. coli* concentrations change over time.

Another method to determine sources of bacterial contamination is using rapid QPCR (Converse et al., 2009). However, the technique used would have to be modified as the rapid QPCR is limited by the inability to distinguish between human and non-human sources. This was adjusted by targeting certain rRNA genes (Converse et al., 2009). This method could help to exclude potential sources in future studies.

5.0 CONCLUSION

Streams on Hawaiʻi consistently exceed the EPA standards of FIB in recreational waters (Fujioka et al., 1988). Since *E. coli* is ubiquitous throughout tropical climates, this study sought to explore the correlations between *E. coli* concentrations and water quality and environmental parameters and its proximity to urban landscapes. Multiple studies suggest that soil and warm-blooded animals are the source of the indicator bacteria (Fujioka et al., 1988; Hardina & Fujioka, 1991; Oshiro & Fujioka, 1995; Ishii & Fujioka, 2008; Strauch et al., 2014; Kirs et al., 2020). Moreover, the environmental and water quality parameters that are present in the Hawaiian climate have an influence on the concentration of *E. coli* (Byappanahalli et al., 2012; Strauch et al., 2014; Kirs et al., 2016; Kirs et al., 2020; DeVilbiss et al., 2021). Water temperature can cause stagnant water bodies which do not facilitate the flushing of water. Despite past studies that proved that precipitation could cause surges of *E. coli*, results of the precipitation that occurred during the sampling period express there was no consistent correlation to the concentrations of *E. coli* (Strauch et al., 2014). I conducted a fully factorial linear regression to examine how temperature, specific conductance, and precipitation interacted with *E. coli* concentrations. However, only temperature was found to be statistically significant in the final model, and adding the other factors did not result in any improvement in explanatory power or significance compared to a model that only included temperature. Therefore, we concluded that temperature is the most reliable predictor of *E. coli* concentrations among these three parameters.

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