Influence of water hyacinth (*Eichhornia crassipes*) on concentration and distribution of *Escherichia coli* in water surrounding an informal floating community in Iquitos, Peru

Rebecca B. Neumann, Susan Paredes Fernández, Leann Andrews, Jorge A. Alarcón, InterACTION Labs Working Group

Abstract

Floating communities exist throughout the world. Many live on water with a high pathogen load due to difficulties associated with sewage management. In Claverito, an informal floating community in Iquitos, Peru, we conducted a controlled experiment to test the ability of water hyacinth (*Eichhornia crassipes*) to remove *Escherichia coli* from water. When river *E. coli* concentrations were at or below ~1500 CFU 100 mL^{-1}, water hyacinth reduced shallow concentrations (8-cm depth) down to levels deemed safe by U.S. EPA for recreational use. Above this threshold, plants were able to reduce *E. coli* levels within shallow water, but not down to "safe" levels. At deeper depths (>25 cm), there was evidence that plants increased *E. coli* concentrations. Water hyacinth removed *E. coli* from shallow water by providing a surface (i.e., submerged roots) onto which pathogens sorbed and by protecting organisms that consume *E. coli*. Unfortunately, because of root association, the total *E. coli* load within the water column was greater with water hyacinth present, and results hinted that the plants' protective environment also harbored parasites. The use of water hyacinth to keep surface water around floating communities low in *E. coli* could be beneficial as this is the water layer with which people most likely interact. Aquatic vegetation naturally proliferates in and around Claverito. While this study was based on curating aquatic plants in order to achieve a water-quality outcome, it nonetheless supports concrete actions for Claverito residents under non-curated conditions, which are outlined at the end of the manuscript.

Plain Language Summary

Globally, many people live in floating houses. Sewage treatment plants do not serve floating communities, so sewage is often dumped into surrounding water. Sewage carries pathogens that make people sick with diarrhea and others diseases. People living in floating houses get infected by these water-borne pathogens. We conducted an experiment in a floating community in Iquitos, Peru to test if a floating plant called water hyacinth could remove a pathogen called *Escherichia coli* (abbreviated *E. coli*) from water. We found that water hyacinth removed *E. coli* from near-surface water because the *E. coli* attached onto the plant roots and because organisms that eat *E. coli* congregated under the plants. Water hyacinth did not removed *E. coli* from deeper water. Also, there was a larger total number of *E. coli* in the water column when water hyacinth was present because of the number of *E. coli* associated with the plant roots. Our results indicate that water hyacinth can be used around floating houses to reduce *E. coli* concentrations in shallow water. However, it is important to know that water hyacinth does not remove *E. coli* from deeper water and the roots have a high load of *E. coli*.

Key Points

- Floating communities exist globally and are regularly exposed to water-borne pathogens; aquatic vegetation can remove pathogens from water.
- In an experiment, water hyacinth removed E. coli from shallow water; E. coli sorbed onto roots and E. coli grazers congregated under plants.

- Water hyacinth did not remove E. coli from deep water and, due to association with roots, plants increased total E. coli in water column.

Keywords
Water Quality, Aquatic Vegetation, Slum, Sanitation, Environmental Health, Public Health

1. Introduction

Despite the fact that the planned development of modern floating communities has been suggested as a novel climate adaptation strategy for coastal populations (Cusick, 2020; Revkin, 2019), floating communities already exist around the world, with some having existed for thousands of years. Well-known floating communities include: Ganvie, Benin; Ko Panyi, Thailand; Halong Bay, Vietnam; Yawnghwe, Myanmar; Tonle Sap, Cambodia; Day-asan, Philippines; Makoko, Nigeria; and Uros, Peru. However, many other less-well-known or even informal floating communities exist globally.

Delivery of clean water and management of sewage are persistent problems for floating communities due to technical challenges associated with living on water (e.g., large seasonal changes in water level, limited access to land treatment plants, etc.) and due to the fact that many floating communities are not legally recognized by local governments who adopted more static urban Western models of city planning and have limited legal frameworks for communities that live on land and water (Djonoputro et al., 2010; Pedro et al., 2020). This latter factor, in particular, limits the willingness of governments to invest in sanitation infrastructure within floating communities and, while the communities themselves often do invest in such infrastructure, their resources are limited. Without sanitation options, human waste is directly released into the water upon which the community lives. This is the same water within which people bathe, wash clothes and dishes, recreate, and sometimes obtain food and drinking water. As such, the people living within these floating communities regularly suffer from diarrheal diseases associated with pathogen exposure (Andrews, 2018; Pandey et al., 2014). Globally, diarrheal diseases associated with poor water, sanitation, and hygiene behaviors (WASH) are responsible for hundreds of thousands of deaths and tens of millions of disability-adjusted life years annually (Prüss-Ustün et al., 2019).

Since 2015, an interdisciplinary team of Peruvian and United States researchers has been working with an informal floating slum community called Claverito, located in Iquitos, Peru on the Itaya River, a tributary floodplain of the Amazon River (Figure 1). The program, called InterACTION Labs, has focused on using targeted interventions to the built environment as a way to improve One Health outcomes for the community (Alarcón et al., 2018; Andrews, 2018; Andrews et al., 2022; Bachman, 2020; Conery, 2019). Notably, the program found the pathogen burden of the water upon which the 280 community members live to be large, reaching 7700 *Escherichia coli* colony-forming units (CFU) per 100 mL of river water (Figure 7). This *E. coli* concentration indicates a substantial public health concern; for example in the United States, the Environmental Protection Agency flags measures above 126 *E. coli* CFU per 100 ml as not meeting recreational water quality standards (Environmental Protection Agency, 2012), and in Peru, waters in the natural environment are not to have greater than 3,000 most-probable-number (MPN) per 100 mL total coliforms (Ministerio del Ambiente - MINAM, 2017), of which *E. coli*

is a subset. (CFU and MPN are roughly equivalent). In addition, there is indication that residents of Claverito may be experiencing poor health outcomes related to water quality. For example, other InterACTION Labs studies examined six measures over three years, and found between 17-74% of Claverito households self-reported family members with diarrhea at any given time, including up to 1 in 3 children ages 10 and younger, and 80% of residents had a professionally diagnosed parasitic infection (Bachman, 2020).

Claverito is not recognized by the local government, and therefore has no formal access to water and sewer services. In addition, it is located immediately downstream from a much larger community of approximately 30,000 people also living in the river called Belén that lacks adequate sanitation as well. Preliminary data collected by our research team in three locations in Claverito across 6 points in time in 2017 indicated that *E. coli* counts were up to 97% lower in near-surface (8 cm) water when floating vegetation was present, particularly water hyacinth (*Eichhornia crassipes*, local name Putu-Putu) (see Supplemental Information, SI). These data indicated it might be possible to use this readily available, native, aquatic plant as a way to manage *E. coli* contamination in the water.

Aquatic vegetation is often used in treatment wetlands as a means of removing pathogens from water (Wu et al., 2016). The vegetation supports removal of pathogens from water via different mechanisms:

- The pathogens can associate with or sorb onto the plant roots, which removes them from the water but does not necessarily deactivate them (Badgley et al., 2010; Kansiime and van Bruggen, 2001; MacIntyre et al., 2006; Mathai et al., 2019; Rivera et al., 1995)..
- The plants can foster a protective environment for higher organisms like zooplankton, which eat the pathogens (Decamp and Warren, 2000; González et al., 1990; Menon et al., 2003; Song et al., 2008).
- The plant roots can trap sediment particles, including detritus from the plant, and facilitate settling of the particles out of the water column. Pathogens can associate with or sorb onto these settling particles (Boutilier et al., 2009; Jasper et al., 2013; Kansiime and van Bruggen, 2001; Quiñónez-Dìaz et al., 2001).

A non-profit called Wetlands Work! has harnessed these ideas to develop a successful sanitation system for floating communities in Cambodia called HandyPod that captures sewage within a floating container populated with water hyacinth (Wetlands Work!, 2013). Given that pathogen contamination in Claverito's water does not all originate within the community itself (i.e., Belén is a large upstream pathogen source because it did not have a functioning wastewater treatment plant), we were interested in exploring the ability of free-floating aquatic vegetation to create localized areas with minimal *E. coli* contamination for the community to access.

Toward this end, we set up a 4-month-long controlled experiment that tested the ability of water hyacinth to remove *E. coli* from water surrounding Claverito and probed the mechanisms associated with *E. coli* removal in the system. Residents of Claverito acted as partners in this study and the overall efforts of InterACTION Labs. The team sought permissions from the community, residents were informed about the study, and results and potential implications were shared through community workshops, public health fairs and handouts. Out of respect for their

livelihood and opportunities that closely revolve around water, residents were engaged in various aspects of the study alongside the academic team, including assistance with constructing the experimental frame, harvesting the plants, driving the canoes, and assisting the sampling. Further narrative of their livelihood and this engagement process can be found in the book chapter, Living on Water: Amphibious Communities in the Amazon Rainforest (Andrews et al., 2022).

2. Material & Methods

2.1. Site

The experiment was conducted in March to July during the high-river season in Claverito, an informal community located on the Itaya River, which runs along the Eastern side of Iquitos, Peru (Fig. 1). In the low-river season, houses sit on soil. In the high-river season, houses float on up to 4 meters of water. Claverito has existed for ~45 years and currently contains ~50 houses, 280 residents, and 240 domesticated animals. Most of the residents have Indigenous roots and are first or second generation migrants from rural villages in the rainforest.

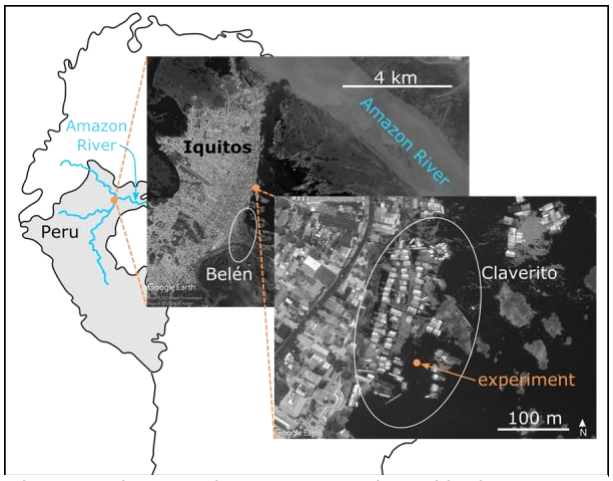

Figure 1: The experiment was conducted in the waters surrounding Claverito, an informal community located in Iquitos, Peru.

2.2. Experimental Design

To test the ability of and mechanisms associated with *E. coli* removal by floating vegetation we deployed a PVC frame that was divided into quadrants, each 3-m x 3-m, within the center of Claverito (Fig. 2). The frame was anchored in place with wood poles at the four outside corners, but it floated on the water and was able to move up and down with the water level relative to the anchors. Two of the quadrants (*A* and *C*), which were diagonal to each other, were densely packed with water hyacinth that was collected from nearby locations on the river (Fig. 2). Quadrants *B* and *D* were left unvegetated. The frame was oriented such that vegetated quadrant *A* and unvegetated quadrant *B* were upstream of unvegetated quadrant *D* and vegetated quadrant *C*, respectively (Fig. 2). However, the water flow was slow. Surface debris and plants were measured moving ~0.9 m min^{-1}, but it was not possible to determine if this movement was solely wind driven or due to river current. Therefore, we concluded that orientation of the quadrants

Figure 2: Image on the left shows the PVC frame used in the experiment along with direction of water flow; Image on the right shows *Eichhornia crassipes* and its root system.

relative to the river current was not a key factor in our study. We also note that the experiment was located in a low-traffic area of the community, however, Claverito is a living community with people swimming, fishing and boating, and with animals (domestic and wild) and humans going to the bathroom.

Quadrants were sampled six times, approximately every two weeks, between March and June 2018 for *E. coli* in water at multiple depths, *E. coli* in captured sediment, and *E. coli* on plant roots, as well as for protozoa. During sampling events, depth of the river was measured as well as water pH and total dissolved solids (TDS). Quadrants A and B were sampled in the same day and quadrants C and D sampled the following day (or as soon as possible). Given the sampling schedule, comparisons between vegetated and unvegetated treatments were made between quadrants *A* and *B*, and between quadrants *C* and *D*.

2.3. Water Sampling and Analysis

Water was collected from each quadrant at depths of 8 cm, 25 cm, 50 cm and 100 cm below the water surface using a peristaltic pump (Geotech Geopump). Tubing was disinfected prior to collecting each sample by pulling bleach solution (>10%) through the tubing for 10 minutes. The bleach solution was then kept inside the tubing as the tube was lowered to the appropriate sampling depth. Quadrant water was then pumped up through the tubing for 2 min to purge the system, with the bleach solution collected into a waste bucket. Quadrant water was then collected into sterilized 30 mL brown glass bottles. Bottles were placed in a cooler with ice packs. In addition, water was collected into small plastic cups that were used to measure pH and total dissolve solids with calibrated probes (Oakton Pocketmeters).

E. coli content of water was analyzed within the same day of collection using 3M Petrifilm *E. coli*/Coliform count plates. One mL of water was transferred from the brown glass bottles to the count plate using a sterilized pipet. Manufacturer instructions were closely followed. Plates were then incubated for 24 hours at 35 ^{o}C. Triplicate plates were incubated for all water collected from 25-cm depth (i.e., 25% of collected water samples) to gain an understanding of method variability. Available resources did not enable replicate plates for all water samples. After 24-hours, plates were removed from the incubator and *E. coli* colonies were manually counted three times for each slide and averaged. Results represent *E. coli* colony-forming units per 1 mL of water.

Coliform colonies were initially counted, but eventually it was determined that the coliform results were less reliable because coliform colonies were harder to see and to differentiate, particularly when sediment and plant samples were analyzed (described below).

2.4. Sediment Sampling and Analysis

Sediment traps were built out of 2-L plastic bottles and sterile 50-mL Falcon tubes (Fig. 3). The 2-L plastic bottle was cut roughly in half, with the top portion of the bottle (~18 cm tall) used in the sediment trap. The bottle was inverted, the top threaded portion of the bottle was placed inside a 50-mL Falcon tube, and the two were taped together with electrical tape. The open portion of the trap was 11 cm in diameter. Two traps were placed side-by-side in the middle of each quadrant with the top of the Falcon tubes placed at a depth of 70-cm below the water

surface. A brick was hung from the traps to weigh them down and keep them submerged at the appropriate depth.

Traps were deployed for a period of 15 to 21 days. At the end of the deployment period, the traps were pulled up to the surface. In quadrants with plants, the traps were moved horizontally into an unvegetated quadrant before being pulled up to the surface. Traps were then hung on wooden supports (Fig. 3) for a period of ~1.5 hours while the water in the top portion of the trap was stirred to facilitate settling of all captured material into the Falcon tubes. After all material had settled, the Falcon tubes were carefully removed, capped, and placed in coolers with ice packs.

Figure 3: Sediment traps.

In the laboratory, on the same day of collection, Falcon tubes were centrifuged at 2000 RPM for 10 minutes and river water was poured off, leaving a pellet of sediment in the tube. The sediment pellet was then resuspended in 30 mL of distilled water, using a Vortex mixer. This slurry solution was then further diluted with distilled water to 4% (1.6 mL of slurry in 40 mL of water). Three different 4% dilutions were generated. Finally, 1 mL of each dilution was transferred onto a 3M Petrifilm *E. coli*/Coliform count plate, generating three plates for each sediment sample. The sediment plates were incubated and *E. coli* colonies were counted following the same procedures as for water-sample plates. Results were transformed into *E. coli* colony-forming units (CFU) per g of sediment with the following equation:

$$\left(\frac{CFU}{1\ \mathrm{mL}_{dilut}}\right)\left(\frac{40\ \mathrm{mL}_{dilut}}{1.6\ \mathrm{mL}_{slur}}\right)\left(\frac{30\ \mathrm{mL}_{slur}}{m_{sed}}\right)$$

where *dilut* stands for the 4% dilutions, *slur* stands for the initial slurry made with distilled water, and m_{sed} is the total mass of sediment captured by the sediment traps in grams. Total mass of sediment captured in the traps was obtained by vacuum filtering all remaining sediment through pre-weighted filters that were then oven dried at 60°C for ~12 hours and re-weighed.

2.5. Plant Sampling and Analysis

During each sampling event, one plant was removed from each vegetated quadrant and placed in a large plastic bag. Back in the laboratory, on the same day of collection, plant roots were cut away from the top portion of the plant into a sterilized bucket filled with distilled water. The roots were agitated by hand to remove associated debris. The rinse solution was poured through a sterile strainer and captured roots were place in a sterile blender that was filled with distilled water. The roots were blended into a slurry. The volume of the root slurry solution was recorded and three different 4% dilutions of the slurry were generated (1.6 mL of root slurry in 40 mL of water). One mL of each dilution was transferred onto a 3M Petrifilm *E. coli*/Coliform count

plate, generating three plates for each root sample. The root plates were incubated and *E. coli* colonies were counted following the same procedures as for water-sample plates. Results were transformed into *E. coli* colony-forming units (CFU) per g of root with the following equation:

$$\left(\frac{CFU}{1\ \mathrm{mL}_{rdilut}}\right)\left(\frac{40\ \mathrm{mL}_{rdilut}}{1.6\ \mathrm{mL}_{rslur}}\right)\left(\frac{\mathrm{V}_{rslur}}{m_{root}}\right)$$

where *rdilut* stands for the 4% root dilutions, *rslur* stands for the root slurry, V_{rslur} is the measured volume of the root slurry, and m_{root} is the total mass of root contained within the slurry. Remaining root slurry was poured into pre-weighed containers that were oven dried at 60°C until dry, and re-weighed.

2.6. Organism Sampling and Analysis

Aquatic organisms from each quadrant were collected with a plankton net (Wildco 8-inch, 153 μm mesh). The net was dropped to a depth of 1 m and pulled vertically upward. In quadrants with vegetation, plants were pulled to the side during the net tow. Contents of the plankton net were rinsed off using clean water onto a mesh filter (that had a smaller pore size than the net). Contents captured by the mesh filter were then rinsed off with 20% ethanol into a 125-mL plastic bottle that was stored in a cooler with ice packs.

In the laboratory, 1 mL of the ethanol solution was transferred onto a gridded Sedgewick-Rafter counting cell. The cell had 20 rows. Two rows at the bottom, two rows in the middle, and two rows at the top of the cell were viewed under a microscope. All phytoplankton, zooplankton and unknown organisms contained within the viewed rows were counted. Organisms that were possible parasites or parasite eggs were specifically noted. The procedure was repeated two additional times, generating three independent readings of organisms in the ethanol solution. The remaining volume of ethanol was measured using a graduated cylinder.

The number of organisms per volume of water in each quadrant was estimated from the data using the following equation:

$$\left(\frac{N_{org}}{6\ \mathrm{rows}}\right)\left(\frac{20\ \mathrm{rows}}{1\mathrm{mL\ ethanol}}\right)\left(\frac{V_{ethanol}}{100\mathrm{cm}\cdot\pi\left(\frac{8\mathrm{in}}{2}\cdot\frac{2.54\mathrm{cm}}{\mathrm{in}}\right)^2}\right)$$

Where N_{org} is number of organisms counted and $V_{ethanol}$ is the measured volume of the ethanol solution. The denominator below $V_{ethanol}$ represents the volume of river sampled by the plankton net tow.

3. Results

3.1. River Height and Baseline Water Chemistry

The height of river water was ~190 cm above the river bottom at the start of the experiment and increased over the next three sampling events, reaching a maximum height of ~380 cm. It then decreased over the final two sampling events, dropping to ~180 cm above the river bottom at the end of the experiment (Fig. 4).

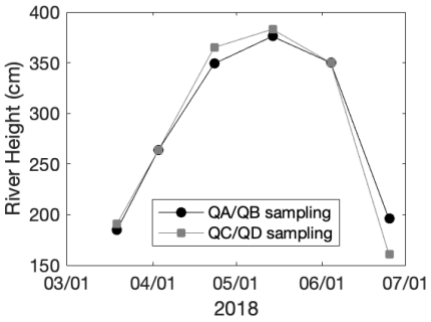

Figure 4: River height during experiment.

pH and total dissolved solids (TDS) did not notably vary across the water column or between treatments. They did however vary with time. Figure 5 shows average water-column pH and TDS versus time. In QA, QB and QC, average pH was between 6.3 to 6.4 for the first two sampling events. Average pH was lower in QD for these two events with a value of 6.2, but the standard deviation around this average value was large and overlapped with average values from the other treatments. By the third sampling event, average pH in all of the treatments jumped to ~6.8 and remained between 6.6 and 6.8 for the remainder of the experiment.

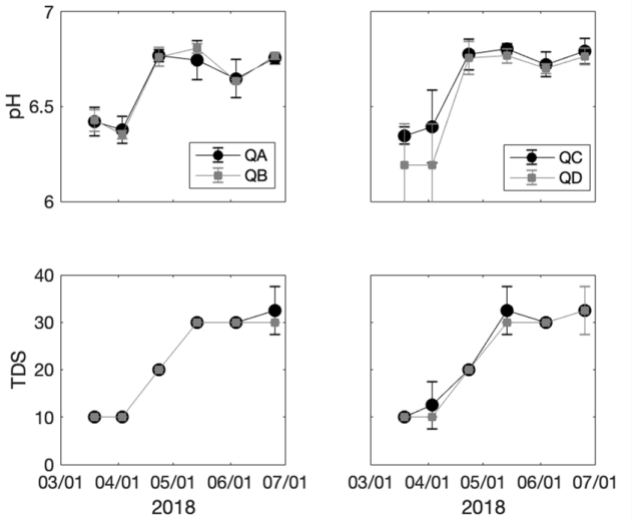

Figure 5: Average pH and TDS (in ppm) across the water column during experiment. QA and QC were vegetated. QB and QD were not vegetated.

The average concentration of total dissolved solids followed a similar pattern over time to that of pH. In all treatments, average TDS concentrations were ~10 ppm for the first two sampling events, increased to 20 ppm by the third sampling event, increased further to 30 ppm by the fourth sampling event, and remained at 40 ppm until the end of the experiment (Fig. 5).

3.2. E. coli in Water

During the experiment, the number of *E. coli* colony forming units per 100 mL of water ranged from zero up to 7700 (Fig. 6). There were no consistent trends with depth or over time across the different treatments. In QA and QB, *E. coli* counts spiked during the fourth sampling event, which was when the river height and TDS concentrations reached their maximum values (Figs. 4 and 5). However, in QC and QD, the pattern was more variable. *E. coli* counts reached a maximum during the fourth sampling event for some water depths and during the fifth sampling event for other water depths. The 100-cm depth in treatment QD experienced two peaks in *E. coli* counts, one during the second and one during the fifth sampling event.

The impact that plants had on *E. coli* counts is unclear based on the presentation of data in Figure 6. Across sampling events and water depths, *E. coli* counts were sometimes smaller and

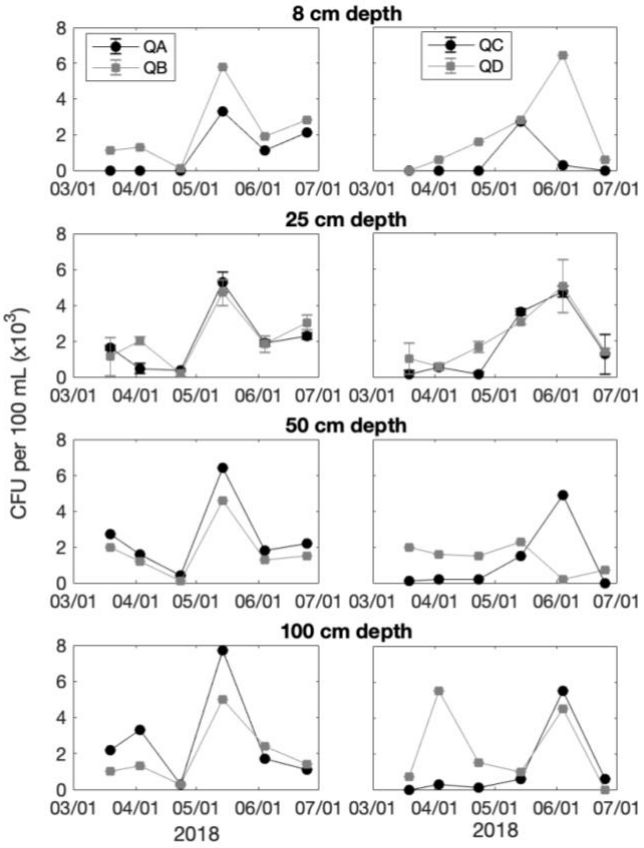

Figure 6: E. coli colony forming unites per 100 mL of water for 8-, 25-, 50- and 100-cm depth below the water surface over the experiment for treatments QA and QB (left column), and treatments QC and QD (right column). QA and QC (black symbols) were vegetated. QB and QD (grey symbols) were not vegetated. Error bars for data from the 25-cm depth represent plus and minus one standard deviation around the mean (i.e., plotted value) based on triplicate slides.

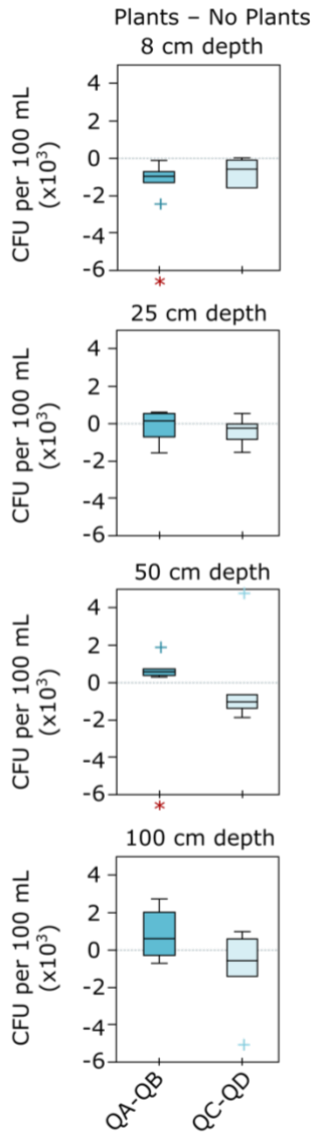

Figure 7: Distribution of differences between treatments with plants and without plants in *E. coli* CFU per 100 mL of water collected from 8-, 25-, 50- and 100-cm depths. The box tops mark the 75th percentile, the middle line marks the median, the box bottom marks the 25th percentile, and whiskers extend to the most extreme data points not consider outliers. Outliers are marked with '+' symbol and are defined as points that are greater than or less than the 75th and 25th percentile values, respectively, by an amount that exceeds 1.5x the interquartile range. Red asterisks mark distributions with medians that are statistically different than zero according to the non-parametric Rank Sum test.

sometimes larger in treatments with plants compared to treatments without plants. (Treatments QA and QC had plants while treatments QB and QD do not have plants.) Figure 7 provides a clearer understanding of the effect of plants on *E. coli* count. It presents box plots of the differences between *E. coli* counts for paired samples from treatments with and without plants for the entire experiment. The median difference in *E. coli* counts between QA and QB was –950, 117, 600 and 583 CFU per 100 mL of water for the 8-, 25-, 50- and 100-cm depths, respectively. The median difference in *E. coli* counts between QC and QD was –600, –200, –1033 and –550 CFU per 100 mL of water for the 8-, 25-, 50- and 100-cm depths, respectively. However, most of these medians were not statistically different than zero based on the non-parametric Sign Rank test (p-value ≤0.05). The only medians that were

statistically different than zero were for the QA-QB treatment pair at the 8-cm depth (–950 CFU per 100 mL) and 50-cm depth (600 CFU per 100 mL).

3.3. Sediment

The rate of sediment deposition increased and decreased over the course of the experiment (SI Fig. 1), and the temporal changes were not clearly associated with river height (Fig. 4), TDS concentration (Fig. 5), or *E. coli* CFU concentrations (Fig. 6). For all of the sampling events, the sediment deposition rate was greater in treatments with plants (QA and QC) than in treatments without plants (QB and QD) (SI Fig. 1 and Fig. 8). However, the median of the distribution of differences in deposition rates between treatments with and without plants was not statistically different than zero according to the non-parametric Sign Rank test. This non-significance is likely due to the fact that the sediment methods were not solidified by the first sampling event and therefore only five data points were available for the statistical test.

The number of *E. coli* CFU on sediment similarly had no clear trend over time or association with other measured variables (SI Fig. 1). In general, the number of *E. coli* CFU on sediment appeared greater in treatments without plants (QB and QD) compared to treatments with plants (QA and QC) (SI Fig. 1 and Fig. 8), but the median of the distribution of differences between treatments in *E. coli* CFU concentration on sediment was not statistically different than zero according to the non-parametric Sign Rank test. Multiplying the sediment deposition rate with the number of *E. coli* CFU on sediment produced the deposition rate of *E. coli* CFU due to sediment settling. This rate was both visually and statistically similar between treatments with and without plants (SI Fig. 1 and Fig. 8).

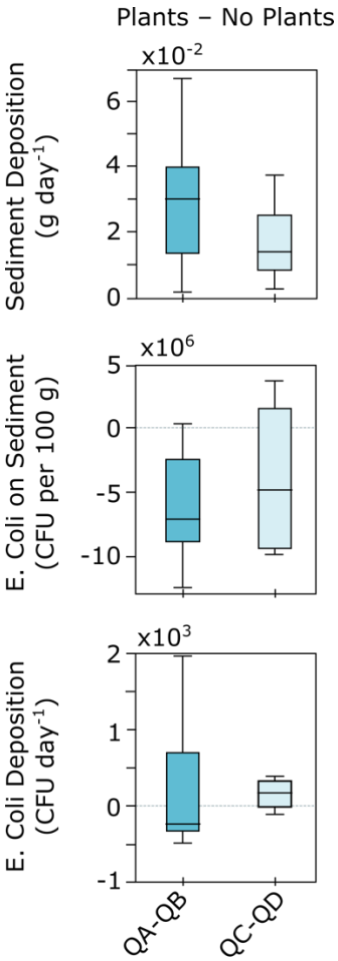

Figure 8: Distribution of differences between treatments with and without plants for sediment deposition rate (top), number of *E. coli* CFU associated with sediment (middle), and deposition rate of *E. coli* CFU due to sediment settling. Explanation of box plots is in caption of Fig. 7.

3.4. Plant Roots

In treatments with floating plants (QA and QC), *E. coli* was present on roots. The concentration of *E. coli* on the roots (CFU per root mass) was similar between the two quadrants (SI Fig. 3).

3.5. E. coli Mass Balance

We calculated the total number of *E. coli* CFU associated with each sampled substrate (water, sediment, or roots) by multiplying the measured concentrations of *E. coli* CFU with the total mass and/or volume of the substrate in each quadrant. Figure 9 shows the results. Median total *E. coli* (in CFU m^{-2}) for the four quadrants was statistically similar, according to non-parametric Wilcoxon Rank Sum test (Figure 9A). Most of this *E. coli* was associated with water; the median percentage of total CFU m^{-2} ranged between 60% and 95% for water (Figure 9B). Suspended sediment held the least amount of *E. coli* ; the median percentage of total CFU m^{-2} ranged between 0% and 10% for sediment (Figure 9C). The treatments with plants (QA and QC) had

median percentages on the lower end of both of these ranges for both water and sediment because in these treatments a notable portion of total *E. coli* was associated with roots. The median percentage of total CFU m^{-2} on roots ranged between 20% to 40% (Figure 9D). Statistically speaking, however, the median percentage of total *E. coli* CFU m^{-2} associated with water and sediment were similar for the quadrants, except for one exception. The median percentage of total *E. coli* associated with water was statistically greater in treatment QD, which lacked plants, than in treatments QA and QC, which had plants, according to non-parametric Wilcoxon Rank Sum test (Fig. 9B).

Directly comparing the paired treatments showed that plants either increased the total amount of *E. coli* present (QA-QB pair) or had no discernable impact on the total amount of *E. coli* (QC-QD pair) (Figure 9E). The paired-treatment comparison also indicated that plants did not strongly affect the total amount of *E. coli* in water or sediment. The median of the distribution of differences between treatments in the total amount of *E. coli* present in water was positive for the QA-QB pair (i.e., treatment with plants > treatment without plants) and negative for the QC-QD pair (i.e., treatment with plants < treatment without plants), but neither median was statistically different than zero, according to the non-parametric Sign Rank test (Figure 9F). For total *E. coli*

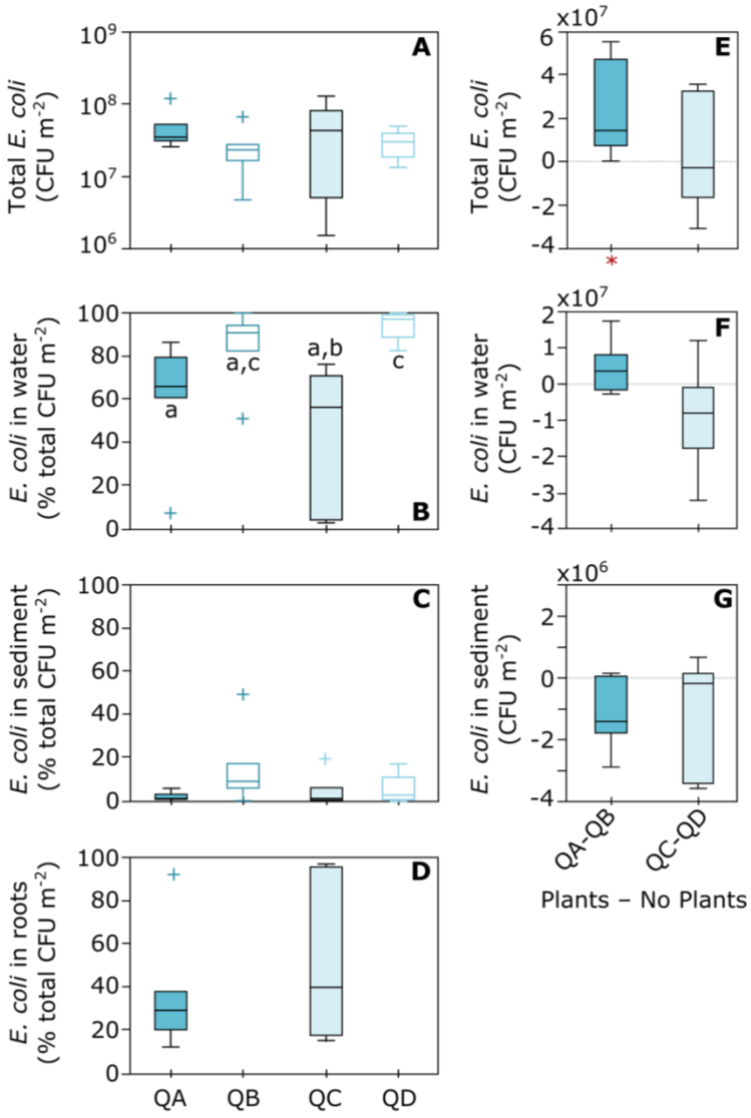

Figure 9: E. coli mass balance. Left column, top to bottom: **A.** total *E. coli* CFU per m^2, **B.** percent of total *E. coli* in water, **C.** percent of total *E. coli* in suspended sediment, and **D.** percent of total *E. coli* on plant roots in quadrants QA, QB, QC, and QD. Lower case letters indicate distributions with medians that are statistically different from each other according to non-parametric Wilcoxon Rank Sum test. Right column, top to bottom: difference between quadrants with and without plants (QA–QB and QC–QD) **E.** in total *E. coli* CFU per m^2, **F.** in *E. coli* CFU per m^2 in water, and **G.** in *E. coli* CFU per m^2 in suspended sediment. Red asterisks mark distributions with medians that are statistically different than zero according to the non-parametric Rank Sum test. Explanation of box plots is in caption of Fig. 7.

on sediment, the median of the distribution of differences between treatments was negative for both that QA-QB and QC-QD pair, and neither median was statistically different than zero, according to the non-parametric Sign Rank test (Figure 9F).

3.4. Aquatic Organisms

The number of organisms captured during the plankton-net tow per liter of water remained relatively consistent over the course of the experiment for a given organisms type (i.e., phytoplankton, zooplankton or unknown) within a given treatment (i.e., QA, QB, QC, QD) (SI Fig. 2). There was no clear connection in the temporal patterns of organism concentration with other variables, like water height (Fig. 4), water chemistry (Fig. 5), or concentration of *E. coli* CFU (Fig. 7). A majority of the collected organisms were identified as zooplankton. Those identified as phytoplankton and those which could not be identified as either zooplankton or phytoplankton (i.e., unknown organisms) had similar concentrations, with the concentration of each class of organism increasing and decreasing relative to each other over the course of the experiment.

In treatment set QA-QB, the treatment with plants (QA) had more total organisms than the treatment without plants (SI Fig. 2 and Fig. 10). The median of the distribution of differences between treatments was positive for all of the organism classes (i.e., QA > QB), but only the medians for total organisms, phytoplankton and unknown organisms were statistically different than zero based on the non-parametric Rank Sum test (Fig. 10). The median of the distribution of differences in zooplankton concentration was not statistically different than zero. In treatment set QC-QD, there was not a clear difference in organism concentrations. The median of the distribution of differences for total organisms, zooplankton and unknown organisms were positive, while the median of the distribution of differences for phytoplankton was negative. But none of these medians were statistically different than zero based on the non-parametric Rank Sum test (Fig. 10).

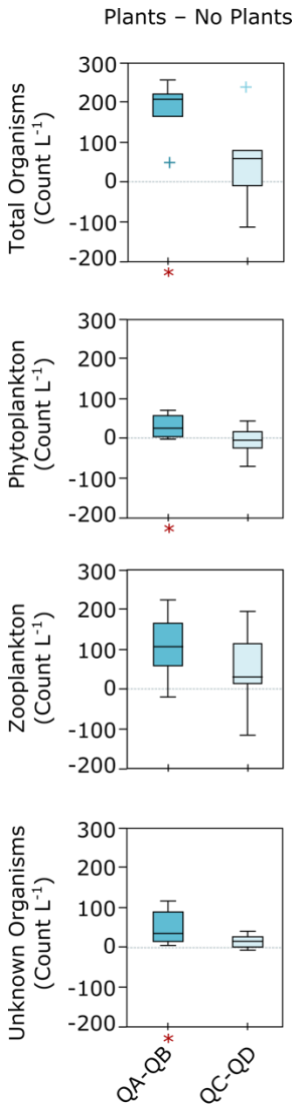

Figure 10: Distribution of differences between treatments with and without plants for total organisms (top row), phytoplankton (second row), zooplankton (third row) and other unknown aquatic organisms (bottom row) per liter of water. Red asterisks mark distributions with medians that are statistically different than zero according to the non-parametric Rank Sum test. Explanation of box plots is in caption of Fig. 7.

Table 1: Total number of potential parasites and parasite eggs identified during Sedgewick-Rafter counting

Quadrant	QA	QB	QC	QD
Plants	Yes	No	Yes	No
Count	9	2	5	1

Quadrants with plants (QA and QC) potentially harbored more parasites and parasite eggs, compared to quadrants without plants (QC and QD) (Table 1). However, the numbers in Table 1 represent *potential* parasites and parasite eggs, not confirmed organisms. Further, the numbers cannot be statistically compared to

each other as they represent a total count (the sum of identified organisms from 3 replicate samples with 6 scanned rows for each replicate).

4. Discussion

4.1. Water Height and Water Chemistry

The river water level changes (Fig. 4) matched the typical discharge pattern for the Amazon River, which peaks between May and June (Devol et al., 1995; Gibs, 1972). However, water-chemistry changes were counter to what is expected based on published relationships for the region. In our experiment, both pH and TDS increased as the river level increased. Other investigations, within the main stem of the Amazon River, found that pH and concentrations of dissolved constituents decreased as discharge increased (Devol et al., 1995; Gibs, 1972). While pH values (Fig. 5) aligned with those measured for the Amazon River near Iquitos (6.7 with a range of 5.8 to 8 (Moquet et al., 2016)), TDS concentrations (Fig. 5) were notably lower than that measured for the Amazon River (158±23 mg/L (Moquet et al., 2016)).

It is well established that the dissolved load carried by the Amazon river is due, primarily, to weathering reactions occurring in the Andes mountains (Gibs, 1967; Stallard and Edmond, 1983). Therefore, tributaries that do not originate in the Andes tend to have lower TDS concentrations. The Itaya River, along which Claverito is located, does not originate in the Andes mountains. As such, the patterns of increasing TDS with increasing river water level at Claverito (Fig. 5) can be explained by backflow of the Amazon River up into the Itaya River (Fig. 1), bringing in water with high pH and TDS concentrations.

4.2. E. coli in Water

Changes in *E. coli* water concentrations over the course of the experiment (Fig. 6) did not appear influenced by river water level (Fig. 4) or water chemistry (Fig. 5). But there was consistency across the different depths of the water column; when *E. coli* concentrations within a given quadrant increased at one sampling depth they tended to increase within that quadrant in the other depths as well. The temporal resolution of sampling was not fine enough to disentangle the factors controlling concentrations over time. It is possible that increases and decreases in *E. coli* concentrations over time were simply related to the alignment of the sampling event with upstream or nearby sewage discharge into the Itaya River.

The measured *E. coli* loads within the water near Claverito reached up to 7700 CFU mL^{-1}, which exceeded the Peruvian water standard of 3000 MPN for total coliforms (Ministerio del Ambiente - MINAM, 2017) (i.e., *E. coli* is a subset of total coliforms) and the recreational water standard in the United States of 126 *E. coli* CFU 100 mL^{-1} (Environmental Protection Agency, 2012). These elevated levels were more in line with raw municipal wastewater sampled in other studies (Ansola et al., 2003; Solano et al., 2004; Wu et al., 2016). The EPA standard is based on protecting the health of people recreating in water, with a gastrointestinal illness rate of 36 per 1000 people. In the experiment, only 17% of the collected samples (16 of 96 total) were below the EPA standard, illustrating the persistence and high load of fecal contamination within the river. (It is difficult to directly compare our *E. coli* results to the Peruvian standard since the Peruvian standard is for all coliforms and we only measured *E. coli*). In corroboration of the high fecal contamination load, some of the organisms collected with the tow net, which we assigned as ‘unknown’ in Fig. 10, appeared to be parasite eggs or larvae (Table 1, SI Fig. 3). In Claverito,

when the community is floating on water, interacting with the river is unavoidable. Therefore, it is not surprising that over 80% of adults and children in the community were diagnosed with at least one parasitic infection with 42% of these collected stools categorized as soft to diarrhea (Andrews, 2018; Bachman, 2020).

4.3. Effect of Floating Plants on E. coli in Water

The study did not find the floating water hyacinth very effective at removing *E. coli* from the water column, except at the shallowest depth sampled (8 cm) where there was a median reduction of 600 and 950 CFU 100 mL^{-1} in the two paired treatments (with the caveat that only the 950 CFU 100 mL^{-1} reduction was statistically significant) (Fig. 7). While this performance was not as effective as hypothesized at the outset of the experiment, there could, nonetheless, be a benefit associated with removing *E. coli* from the surface water layer surrounding a floating community; it is this layer of water that people mostly likely interact with while accessing and living in their homes.

During the first three sampling events (in March and April), the shallowest sampled water depth in both of the planted quadrants had zero *E. coli* CFU 100 mL^{-1} (Fig. 6) while the quadrants without plants generally had *E. coli* at concentrations exceeding the EPA recreational water quality criteria. However, in the later sampling events (May to July), *E. coli* did appear within the near-surface water layer in the planted quadrants at a concentration of ~10^3 CFU 100 mL^{-1} (Fig. 6), which is an order of magnitude above the EPA recreational water quality criteria. The data indicate that in this shallow water later, floating plants were only successful at keeping *E. coli* at acceptable levels (i.e., below 126 CFU 100 mL^{-1}) when the *E. coli* load in the shallow water layer without plants was at or below ~1500 CFU 100 mL^{-1} (Fig. 6). When *E. coli* concentrations rose above this apparent threshold, the plants were able to reduce *E. coli* levels within the near-surface water, but not down to a level that would be considered safe for human health.

At deeper depths there was some evidence that the floating plants actually increased *E. coli* concentrations in water; the median of the distribution of differences between quadrant QA (with plants) and QB (without plants) was positive for all sampled depths below 8 cm, though only the median at the 50-cm depth was statistically significantly different than zero (Fig. 7). The mass-balance calculations indicated that the presence of plants actually increased the overall *E. coli* load, on a per m^2 basis, likely due to roots harboring the pathogens (Fig. 9A,E). Within the planted quadrants, 20% to 40% of the *E. coli* was associated with plant roots (Fig. 9D). Other investigations, conducted in less-impacted water bodies, have found that plants act as a long-term reservoir for *E. coli*, harboring and protecting the pathogens from inactivation and predation (Badgley et al., 2010; Mathai et al., 2019) and increasing the overall *E. coli* load on a per area basis (Badgley et al., 2011).

It is important to note that, unlike treatment wetlands which are engineered to maximize pathogen removal, the system studied here is uncontrolled. We had no control over hydraulic regime, the length of time that water spent in contact with the plants, or chemical composition of the water, which are all variables shown to be important within treatment wetlands (Wu et al., 2016).

4.4. Investigated Mechanisms of E. coli Removal by Floating Plants

At outlined in the introduction, the experiment was set up to investigate three different mechanisms by which plants can facilitate the removal of pathogens from water: 1) pathogens sorbing onto plant roots, 2) pathogens sorbing onto particles that settle out of the water column due to the presence of plants, and 3) plants creating a protective environment for higher organisms that then graze on the pathogens.

The first mechanism did occur; *E. coli* was detected on the roots of plants within both planted quadrants (SI Fig. 3) and, as discussed in the previous section, the mass balance calculations demonstrated that a notable portion of the *E. coli* load in these quadrants was associated with roots (Fig. 9D). This association of *E. coli* with plant roots could, in part, explain the reduction in *E. coli* measured in water at the 8-cm depth (Fig. 7), as plant roots extend into and beyond this water depth. It is estimated that the thicker root section of water hyacinth extends 8 – 10 cm into the water and the thinner roots extend an addition ~15 cm, reaching a total depth of ~25 cm (Fig. 2).

In terms of the second mechanism, the presence of plants did appear to increase the rate of sediment deposition; the rate difference for each comparison between the paired planted and unplanted treatments was positive (Fig. 8). Though, there were not enough samples to get a statistically significant result. For many of the comparisons between the paired planted and unplanted treatments, the concentration of *E. coli* on the deposited sediment was greater in the unplanted quadrants than in the planted quadrants (Fig. 8). The mass-balance calculation also showed that, in general, quadrants without plants had more total *E. coli* associated with suspended sediment than quadrants with plants (Fig. 9G). Though, again, none of these differences were statistically robust. In net, the outcome was that sediment deposition removed a similar amount of *E. coli* for both planted and unplanted treatments (Fig. 8), indicating this removal mechanism was not particularly robust within the studied context.

Previous studies have shown that plants create a protected environment for aquatic organisms (Decamp and Warren, 2000; González et al., 1990; Menon et al., 2003; Song et al., 2008). In our study, the QA-QB treatment pair clearly aligned these previous findings; the total presence of organisms that could graze on *E. coli* was greater for QA, the planted quadrant, than it was for QB, the unplanted quadrant (Fig. 10). The results for the QC-QD treatment pair were less clear. The median number of organisms were greater in the planted quadrants (QC) than the unplanted quadrant (QD) but the difference was not statistically significant.

While it is not possible to isolate the exact depths within which the various organisms were residing because the net tow spanned the top 100-cm of the water column, if the organisms were congregating within the root zone, they could have contributed to the general reduction in *E. coli* concentration found in the planted treatments within the 8-cm sample depth (Fig. 7). Notably, the QA-QB treatment pair had statistically significant differences in both shallow *E. coli* concentrations (with the planted treatment having lower concentrations) and organism presence (with the planted treatment having more total organisms), while the differences between QC-QD treatment pair tended to match the behavior of the QA-QB treatment pair but had less statistical strength. This observation suggests that the extent to which the floating plants were able to successfully remove *E. coli* was connected with the presence of aquatic organisms, presumably

residing within the protected root zone. Unfortunately, it is also possible that the protected environment created by plants also harbored parasites and parasite eggs (Table 1). Though this result needs further investigation as our analysis only identified *possible* parasites and parasite eggs.

5. Conclusion

The water surrounding Claverito has a high burden of fecal contamination, which has negative impacts on the health of the community. Water hyacinth was able to keep *E. coli* concentrations at safe levels in shallow water (i.e., below the EPA recreational water threshold), but only when the overall river water had concentrations at or below ~1500 CFU mL^{-1}. When *E. coli* loads increased above this level, water hyacinth continued to reduce the presence of *E. coli* in shallow water, but not down to levels considered safe for human health in the U.S.A. It is difficult to assess how water hyacinth performed with regards to the Peruvian standard for natural water because this standard is for total coliforms and we only measured *E. coli*, which is a subset of total coliforms.

It appeared that the *E. coli* was removed from water in the presence of floating plants due to sorption onto plant roots and/or due to grazing by other organisms that congregated in greater numbers when plants were present. Unfortunately, some of these congregated organisms within the planted treatments were identified as potential parasites and parasite eggs. Sorption of *E. coli* onto plant roots did not remove *E. coli* from the system nor did it inactivate them. A notable portion of culturable *E. coli* within the water column (a median 20-40%) was associated with roots in treatments that had water hyacinth. Data indicated that due to this association of *E. coli* with roots, the presence of floating plants actually increased the total load of *E. coli*.

With the number of floating communities around the world potentially increasing due to climate change and sea level rise, and with millions already living in floating communities, many of which are informal, the design, planning, upgrade, and management of these communities can consider aquatic vegetation as a way to improve environmental quality. Other studies in the InterACTION Labs program have revealed that aquatic vegetation creates biodiversity-rich 'habitat islands' that support reptiles, amphibians, birds, and fish —important for this primarily fishing community (Andrews et al., 2022). However, the use of floating vegetation as a means to remove pathogens from water around floating communities should only be considered if there is a desire to keep the surface layer of water free from *E. coli*. It should be clearly understood that the plants do not reduce contamination within deeper water layers, and that even in the shallow water layer, the treatment does not always keep contamination at levels deemed safe. It is also possible that the plants are harboring parasites – a possibility that deserves further investigation.

6. Community Implications

Aquatic vegetation naturally proliferates in and around Claverito and is used for animal feed and as compost for hillside trees. While this study was based on the idea of intentionally placing or curating aquatic plants in order to achieve a specific water-quality outcome (i.e., low *E. coli* counts), it nonetheless supports a set of concrete actions for the residents of Claverito under natural or non-curated conditions:

- If water is going to be obtained from the river, it is best to scoop it up from the top 8 cm in areas where there are plants, but know that this water is not safe to ingest without treatment.
- Do not swim in the river, as it is not safe anywhere. If one needs to bath or swim and completely immerse oneself, do not open eyes or mouth underwater. Wash hands and face thoroughly with soap and clean water as soon as possible after submersion.
- Avoid touching submerged roots of aquatic vegetation, as they harbor active *E. coli*, and wash hands thoroughly with soap after touching or moving aquatic vegetation.
- When the water levels drops during the dry season, remove aquatic vegetation before it interacts with the soil surrounding the community. This effort will reduce the *E. coli* load delivered to the soil surface that people walk and play on. Removed vegetation can be used in gardens for fertilizer. Use gloves, a net and/or wash hands with soap after touching aquatic vegetation.
- *E. coli* can live in soil for weeks to months. The soil surface exposed during the dry season likely contains active *E. coli* that were absorbed from the overlying water and deposited by settling sediment during the flooding season. Wear closed toed shoes when walking in this exposed soil. Avoid bringing this soil into your homes by keeping shoes outside and wash hands with soap after touching the soil.

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8. Data Availability Statement

All data related to this study are available in Excel documents in HydroShare (Neumann et al., 2022). The data are organized by sampling event and include: water depth; water chemistry; *E. coli* CFU in water, sediment and plant roots; sediment captured by sediment traps; number of counted organisms from plankton tow, and biomass of sampled plants. The excel sheet references photos that were taken during the experiment and when counting organisms. These photos are available upon request due to their large number and file size. The resource is shared

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