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CITIZEN SCIENCE

Research Article

Participatory research to monitor lake water pollution

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Abstract

1. A participatory research team, concerned about water quality around Lake Geneva, particularly in the Montreux Bay region where some were lifeguards, ran sampling campaigns to determine summertime levels of lake water pollution. The participants were brought together serendipitously through a course organized by academic researchers and 'biohackers' from the community laboratory, Hackuarium. After discussion about lifeguards' gastrointestinal and dermatological ailments each season, the decision to pursue this participatory research project was made. In order to assess water quality, thereby testing the hypothesis that unsuspected pollution enters the lake each summer season, microbiological plating of water samples was proposed.
2. Volunteers collected and analysed water samples over summer seasons (8 weeks in 2016, 2017 and 2020) from three sites around Montreux Bay, with tap and local river water samples as controls. Contamination of lake water was measured using standard microbiological methods, with growth media allowing quantitative assessment of abundance of several bacterial species. In particular, the focus was to quantify Escherichia coli, the classic bioindicator organism for raw sewage contamination.
3. These open science data reveal peaks of bioindicator and other bacterial pollution in lake water samples during all sampling years. For the initial two sampling campaigns, increased microbial burdens occurred during a popular music festival, and were not simply dependent upon rainfall. In contrast, only scattered, lower level bioindicator pollution events occurred across the sampling period during the pandemic summer of 2020, when the festival was cancelled.
4. This study confirms the power of participatory research: dedicated people on a budget can do meaningful environmental monitoring. These analyses suggest that better management, both to support water quality monitoring and for event organization, is essential, as sewage treatment facilities near many popular festivals, internationally, may need to cope with increased wastewater from visitors.

KEYWORDS
bioindicator, citizen science, Do-It-Together (DIT) research, environmental monitoring, microbiology, water sampling

1 | INTRODUCTION

Monitoring water quality through measurements of microbial abundance is crucial, to avoid risks to health, in particular, along shoreline recreational areas. Communities count on such controls, and generally trust monitoring performed by local authorities. However, governmental agencies may not always have the necessary resources to obtain sufficient temporal and spatial information for a given environment. Logistical issues, industrial processes, agricultural run-off and public events can mean that every site around a given body of water may not be as clean as one might like, particularly for fully immersive activities like swimming. This study relied upon participatory research to monitor water quality around Montreux Bay on Lake Geneva in Switzerland, and its use of classic microbiological assays provides a model for similar studies anywhere water quality is in question.

Citizen science has many facets. Sometimes, public input is viewed simply as a low-cost data collection (e.g. bird counting efforts) or useful analysis tool (e.g. gaming to help solve protein folding or understand astronomical data). Alternatively, public involvement in participatory research is possible, including participants who are actually involved in the issue being studied, for which they work to not only define the problem, but collect and analyse the data (sometimes with help of experienced scientists). Collaborative projects of 'extreme' citizen science are bottom-up and may put out data 'live' in response to crisis situations (Hacklay, 2018). PublicLab, with its network of community organizers working on low-cost solutions for environmental monitoring, starting with balloon-mapping work in response to the BP oil spill in 2010, is one example of such open science efforts. Microbiological analyses of water quality have already been academically assessed for the participatory research context, as exemplified by Water Watch (Bonney, 2009; Conrad & Hilchey, 2011; Stepenuck, 2011). Outside of academia, DIY (Do-It-Yourself) biology groups, many united by a firm code of ethics (diybio, 2011), have also been proposing Do-It-Together (DIT) research (Togetherscience, 2018), which can allow unexpected synergies. Critical to such work is open documentation of research results.

Some believe that disconnects between academic, public (governmental) and commercial worlds can be bridged via open science. The more pessimistic are certain that use of citizen science as a model providing free work, however, needs to be avoided. Furthermore, citizen science meant expressly to allow participants to 'experience' research in the field, although a worthy goal, is in some cases reduced to a marketable hobby, rather than enabling collection of useful data that might help solve some of today's problems. For such reasons, the terms and ideals of 'community science' or 'participatory research' are generally preferable. Able to help find and track pollution, all forms of participatory research can potentially provide information to guide international public policy, in particular, to better manage and prevent pollution. This is most participants' hope, especially when pursuing extreme projects as unpaid volunteers, concerned about their local environment. Such ideals also inspired participants in this study to prioritize open science documentation throughout.

Lake Geneva marks a border between France and Switzerland. Over 100 public swimming sites around the lake are regularly assayed for microbial abundance. A popular destination nestled between the shores of Lake Geneva and the first peaks of the Alps, Montreux, has about 27,000 inhabitants, and is renowned for its summer jazz music festival (the Montreux Jazz Festival; Montreuxjazzfestival, 2018), which attracts an audience of over 200,000 music fans for 2-week periods each summer. The bay of Montreux itself does not include an official swimming beach. Nonetheless, many sites in the bay are used for swimming and water sports. In fact, the lifeguard station in Montreux is located in the midst of it all, just across from the theatre where headline acts of the music festival are produced. Most lifeguards are in the water daily, already by spring, to train, as each year they must pass a series of tests to keep their license (SISL, 2018). They train, however, in water which is not monitored as a swimming beach.

Some of these lifeguards were also members of Hammerdirt, a public association that performed beach litter surveys as part of their 'Montreux Clean Beach Project' since 2014. After joining in for a biosensor course in the framework of an EU project (BRAAVOO, 2016), the founder of Hammerdirt met with members of Hackuarium, who had developed a DIYbio fluorescence detector for the biosensor course in their community laboratory association (Hirano, 2016). Hackuarium aims to democratize research and promotes open science and participatory research. Recurrent symptoms of lifeguards, in particular, skin and gastrointestinal problems, made water quality concerns seem justified. Assessment of lake water quality through participatory research thus became the focus of a collaborative project. Even though the microbial data from the first two summer seasons were available openly from the beginning (Hackuarium wiki, 2020, and see section on DATA AVAILABILITY), and presented, written about and discussed (for instance, Aronoff, 2019; Erismann, 2017), no official responses were obtained. (Some changes to the status quo have become apparent, with local water system upgrades gradually being implemented.)

Because of the current health crisis around Covid-19, the 2020 edition of the music festival in Montreux was cancelled, providing the opportunity for a further season of sampling, which would remove at least the 'festival' component from the list of the many possible variables determining levels of microbes in the water samples quantified during these sampling campaigns. Therefore, analyses for three summer seasons, the two consecutive years and including this past pandemic summer, are now formally presented. Our aims are to (1) encourage more broad use of classic monitoring methods to assess

water quality, and (2) influence water quality management decisions for a cleaner future, it is to be hoped, not only around Montreux Bay, but beyond.

2 | MATERIALS AND METHODS

2.1 | Sampling and plating for microbiological assessment

Detailed sample collection, validation experiments and analyses are accessible from Hammerdirt and Hackuarium (Hackuarium Wiki, 2016; Montreux Clean Beach Project, 2016). In brief, triplicate water samples from each of three sites were collected in three independent sterile containers at a depth of 0.5–1 m below the water surface. Samples were transported on ice in insulated containers to the Hackuarium laboratory and plated for microbiological assessment less than 6 h after collection. Bacterial colony-forming units (CFU) were counted directly from plates after incubation at 37°C and also from images, as described further below. Numbers of colonies of each bacterial class were normalized to 100 ml of water, to quantify in particular the abundance of the bioindicator for untreated wastewater, *Escherichia coli*, and other bacterial species.

Sampling was done over two 8-week periods (21 June to 9 August in 2016, and 12 June to 31 July in 2017); and 8 weeks of samples were also acquired over the course of 9 weeks in 2020 (11 June to 6 August, with no sampling the week of 30 July). Control water samples were also acquired and plated each week to ensure sterility and sensitivity of microbial plating. Ordinary drinking water, from the tap or a water fountain near the lifeguard station, provided the negative control; and river water was used as a positive control, as it reliably contains bioindicator bacteria. No bacterial growth on the negative control plates and identifiable bioindicator bacteria on the positive control plates were necessary to confirm reliable results from the sampled bay water sites. For all these participatory research water quality investigations, in the end, 213 Montreux Bay lake water samples were analysed (72 in 2016, 69 in 2017 and 72 in 2020) from the three main sites around the bay, plus at least one negative and positive control sample each week (for eight of each control type every year). In the first 2 years, there were only single samples for these negative and positive controls, but some triplicate river water samples were obtained for better quantitation in 2020. Several additional samples, for instance from other swimming beaches, particularly in 2020, were also obtained and plated.

Four kinds of microbial media were utilized in the course of these studies, including classic non-selective media and special selective media. For the 2016 campaign, ECACheck Easygel (Micrologylabs, 2016) was the special medium inoculated. The Easygel system was already approved in 1999 for the Water Watch volunteer monitoring program (e.g. Water Watch, 2018); and in 2009 its use for volunteer monitoring studies was positively evaluated (Stepenuck, 2011). Enzymes specific to each bacterial group of interest metabolize chromogenic substrates in the media to produce visible colour, allowing identification of at least three bacterial classes besides the bioindicator for raw sewage, *E .coli*: *Aeromonas* and *Salmonella* (both potential pathogens), and coliform bacteria (a general indicator of water contamination, but usually not in itself pathogenic). The media is selective, with bile salts to inhibit growth of other abundant bacteria (in particular gram-positive species), which might confound analyses. For the 2017 campaign, ECA Check Plus plates (Micrologylabs, 2017), which include an additional substrate that is metabolized to a fluorescent indicator, confirmed the presence of *E. coli*. Microbial plates made with other standard agar-based media were also used, all years, including ordinary nutrient agar to allow growth of all bacteria capable of division under these conditions (37°C aerobic) and another classic medium, Levine media (Levine, 1918), containing eosin and methylene blue, that selects for gram-negative bacteria (inhibiting gram-positive species) and allows the bioindicator *E. coli* to be distinguished from *Enterobacter aerogenes* by a metallic green sheen. (For further details, a colour key and molecular confirmation of bioindicator CFU, please see Appendix SA)

Each cultured plate was photographed after 24 and 48 h of incubation. Use of a box lined with LEDs with a black ceiling limited reflections. When reflections were needed for scoring, in particular for metallic green bioindicator colonies on Levine plates, tilting plates and various exposures were also utilized. In the first year, an SLR camera was used for imaging, but in the second year and for the most recent sampling season, telephone cameras were used. Because the fluorescence of the ECA Check Plus media diffuses rapidly, 24 h, but not 48 h, images with illumination by UV light (bulb type, Philips, TL 8V 33–640) and a 302 nm UV filter from a gel imaging system (Pharmacia Biotech's ImageMaster VDS) were acquired for each set of these plates.

For an example of output from a sampling day, which also shows controls, including growth on non-selective media for the full complement of bacteria, see Figure S1, made as an 'infographic' during the second year of the project. A short time lapse of plating study samples was used in one of the videos made for the Hackuarium crowdfunding campaign (Aronoff et al., 2017). Fluorescence halos, confirming *E. coli* CFU from a positive control plate, are shown in Figure S2a, while streaks of colonies from Easygel plates onto the Levine media are shown in Figure S2b. Note the purple/pink/metallic green observed on Levine media from lactose fermentation differences between bacteria. (Weakly or non-fermenting strains result in pink or translucent colonies.)

In addition to manual colony counts, automated scoring via a program developed at the EPFL chemistry department (cheminfo, 2016) or with ImageJ (Rasband, 1997-2019) was also utilized. In brief, threshold signals of images and colour intensities allow counts of each CFU 'spot' – with ImageJ, as greyscale regions of interest, and based on colour combinations with cheminfo. In the latter case, the image is first processed using a javascript library (image-js) designed to analyse scientific images directly in web browsers. For further detail on the cheminfo analyses: colour images are converted to greyscale using a 'luma709' algorithm, and converted to a binary image or 'mask' using the 'yen' algorithm. This binary image is processed to extract regions of interest and then adjusted, so each surface is at least 100 pixels. Mean red, blue and green colour is calculated, and the ratio of colours is plotted (as a

scatter plot). Classifications based on colour ratios and the surface are defined empirically, ultimately allowing 'automatic' counts.

In Figure S3a–c are shown examples of bright field, UV+ and annotated images of a positive control plate from 2017, whereas Figure 3d,e shows examples of 'colony counting' with the cheminfo routines.

2.2 | Validation of microbial results

Additional tests confirmed microbial identifications. For instance, Figure 2b shows growth of colonies of each colour type from an Easygel plate, gridded onto the Levine media plate. Note the purple/pink/metallic green observed on Levine media from lactose fermentation differences between bacteria. (Weakly or non-fermenting strains result in pink or translucent colonies.) Other complementary tests, for example, the gram stain on smears of well-isolated colonies or specific tests, for instance, to see whether putative *Aeromonas* colonies, pink on Easygel, and gram-negative, were also catalase negative in presence of hydrogen peroxide, were also utilized to confirm colony identification. (See also Appendix SA)

Molecular identification of colonies was furthermore pursued in 2020, taking individual colonies for PCR amplification and sequencing as described (Hackuarium wiki, 2020) using 16S rDNA primers, to confirm bioindicator and other species. A 16S 'universal' bacteria primer set was utilized:

Forward primer: 671 5′AGR GTT YGA TYM TGG CTC AG;
Reverse primer: 672 5′CCG TCA ATT CMT TTR AGT TT.

To note: 671 is called 27F, and 672 is called 907R in a classic reference (Lane et al., 1985). For additional documentation, these (27F and 907R) are also listed in table 1 from a more recent article related to FACS sorting (Rinke et al., 2014), with reference to many of the most classic papers enabling such methods. The sequenced region spans over 850 bp and gives clear identifications, as exemplified by alignments (e.g. Hackuarium wiki, 2020b).

2.3 | Physico-chemical data

Each site was assessed for pH, temperature, dissolved oxygen and turbidity at the time of sampling. For dissolved oxygen and turbidity, aquarium testing kits from JBL (Neuhofen, Germany) were used, while pH readings were simply acquired on site from paper strips (Sigma). A thermometer hanging off the SVT dock was the source for water temperature measurements.

To note: for the 2020 sampling campaign, dissolved oxygen and turbidity were not assessed.

2.4 | Analyses

Python was used for general data analyses, descriptive statistics and visualization (Hackuarium GitHub, 2021). Average CFU values were normalized to 100 ml of sample (generally based upon 4 ml inoculated for three independent plates in the Easygel media the first two summers, but only upon 1 ml on Levine or LB plates in 2020). Bioindicator abundance was the variable of greatest interest from each location and sampling week. Data were assessed with Python libraries in Jupyter notebooks (Erismann, 2018; Hunter, 2007; Pérez & Granger, 2007), in particular the SciPy package (Kokoska & Zwillinger, 2000), to identify relationships between variables from correlation coefficients and their associated *p*-values. Spearman correlation coefficients were especially useful, allowing analyses that were less dependent upon ordered data and without assumptions of normal distributions. Non-parametric permutation tests were additionally used to assess significance of average CFU abundance, using the Python library MLxtend (Raschka, 2018). Calculations were based upon direct counts of colonies from plates. For the 2017 campaign, these were input directly, using a 'Kobo toolkit' app, not scored from 'post imaging' data. Time-series correlations of bioindicator incidence in samples were also plotted from public data. For instance, rain data came from this internet source (météo, 2021), and correlations were tested with the 24, 48 and 72 h antecedent rainfall values. The 'official' microbiology results from the SIGE for their 2016/2017 data were obtained after several direct emails (Figure S4b). Further information can be found under DATA AVAILABILITY, below.

3 | RESULTS

Local swimming beaches are regularly monitored, but can be adjacent to sites deemed unsafe for swimming (Figure 1). Weekly sampling of lake water from three sites around Montreux Bay (Figure 2), which

FIGURE 1 A lakeshore 'beach' near Lausanne, looking south towards the Alps. Signs indicate areas where it is safe to swim (to the left – 'Baignade autorisé sans surveillance') and not clean enough to swim (to the right – 'Zone impropre à la baignade')

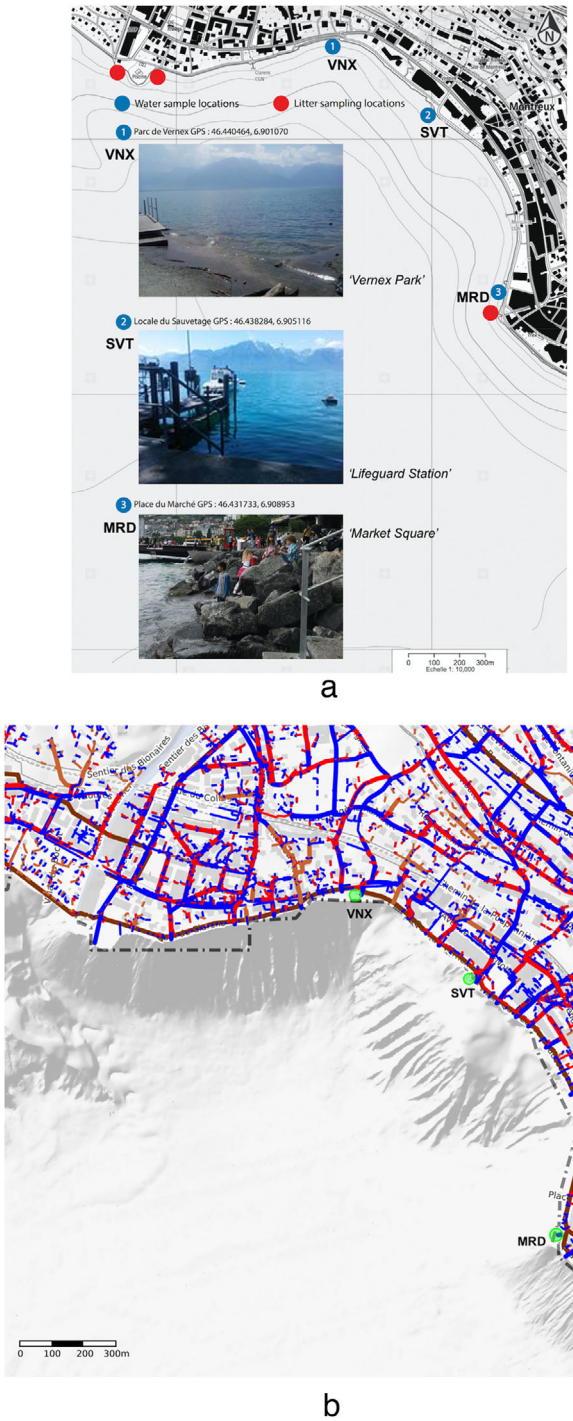

FIGURE 2 Sampling locations and water infrastructure. (a) Sampling locations around the Bay of Montreux. Blue circles denote water sampling locations, depicted in images (with the French names of the three sites* and their GPS coordinates), and red dots show sites for Hammerdirt beach litter surveys. *Translated to English, these are VNX – Vernex Park; SVT – Lifeguard Station; MRD – Market Square. (b) Water line infrastructure around Montreux Bay. Red lines carry 'dirty' used water, and blue is 'clean' rain water, although combined overflows are also evident (brown). The projection also gives the indication that the lake deepens rapidly more to the northwest, in contrast to the bay area that was sampled. To note: resolution is not high for the drawn waterlines, and this graphic reflects the situation of 2018

does not itself contain swimming beaches, namely the (1) Vernex Park, VNX, (2) Lifeguard Station, SVT, and (3) Market Square, MRD, (Figure 2a), was the basis of this participatory research study. Montreux Bay is somewhat more shallow than adjacent areas, particularly to the northwest, as shown in Figure 2b, which includes graphical details of the complex water infrastructure in the region. Results obtained from these sampling campaigns by volunteers, supplemented by data from public sources, for instance, for rainfall and numbers of visitors in the region, are described in the following sections.

To initiate this participatory research project, a 'qualification day' confirmed sampling protocols and compared bacterial colony growth on Levine media, standard LB media and the first set of Easygel plates in 2016. For the subsequent season, a kick-off event was held, including a beach litter survey in the classic Hammerdirt style (Hirano, 2017). The 2020 sampling campaign was possible, in the end, primarily because Switzerland did not have a very strict 'lockdown' due to the Covid-19 pandemic. Molecular confirmation for bioindicator bacteria (as *E. coli*) and other species identifications (Aeromonas, Pseudomonas, coliform) by 16S PCR from isolated colonies and sequencing is shown in Table S1. Control plates confirmed that results each sampling week were reliable, with negative control plates inoculated with potable water revealing no colony growth and positive control plates inoculated with river water demonstrating the presence of bioindicator bacteria. Quantitation from triplicate positive control samples from the 2020 campaign revealed from 3500 to over 15,000 bioindicator CFU on average per 100 ml river water.

Weekly bioindicator abundance and rainfall is plotted for the 2016, 2017 and 2020 campaigns in Figure 3. Montreux Bay water sampling revealed low initial baseline levels of bioindicator abundance for the first two summer campaigns at all three sites around Montreux Bay (Figure 3a,b). These consecutive summers revealed peaks of bioindicator by the fourth week of each season of sampling. The peak value in 2016 for the SVT site increased to over 800 CFU per 100 ml, eightfold higher than Swiss limit for recreational waters (100 CFU bioindicator per 100 ml); the peak for MRD goes over 400 CFU per 100 ml, about fourfold higher; and that of VNX, just at the Swiss limit of 100 CFU per 100 ml (reaching its peak of over 350 CFU per 100 ml only the following week). The peaks in the 2017 campaign were slightly less pronounced, up to 250 CFU per 100 ml at two sites, MRD and VNX, with SVT at about 100 CFU per 100 ml. For the 2020 campaign (Figure 3c), weekly averages of bioindicator revealed no readily discernible pattern, with one MRD peak the third week and few scattered sites with abundant bioindicator CFU. Although the first week, the lifeguard station site, SVT, was already at the Swiss limit for recreational waters, in subsequent weeks, decreased abundance of bioindicator was observed at this site, when abundance of bioindicator at the MRD site increased to almost threefold the Swiss limit. While both these sites were reduced again until the sixth and seventh week of sampling, and for SVT the final week of sampling, none of the peak levels reached the highest seen in 2016, even though rain was somewhat more abundant in 2020. (Note the *y*-axis scale differences.)

The main peak of bioindicator abundance in 2016 occurred on a rainy day (Figure 3a), although this was not the case in 2017 or

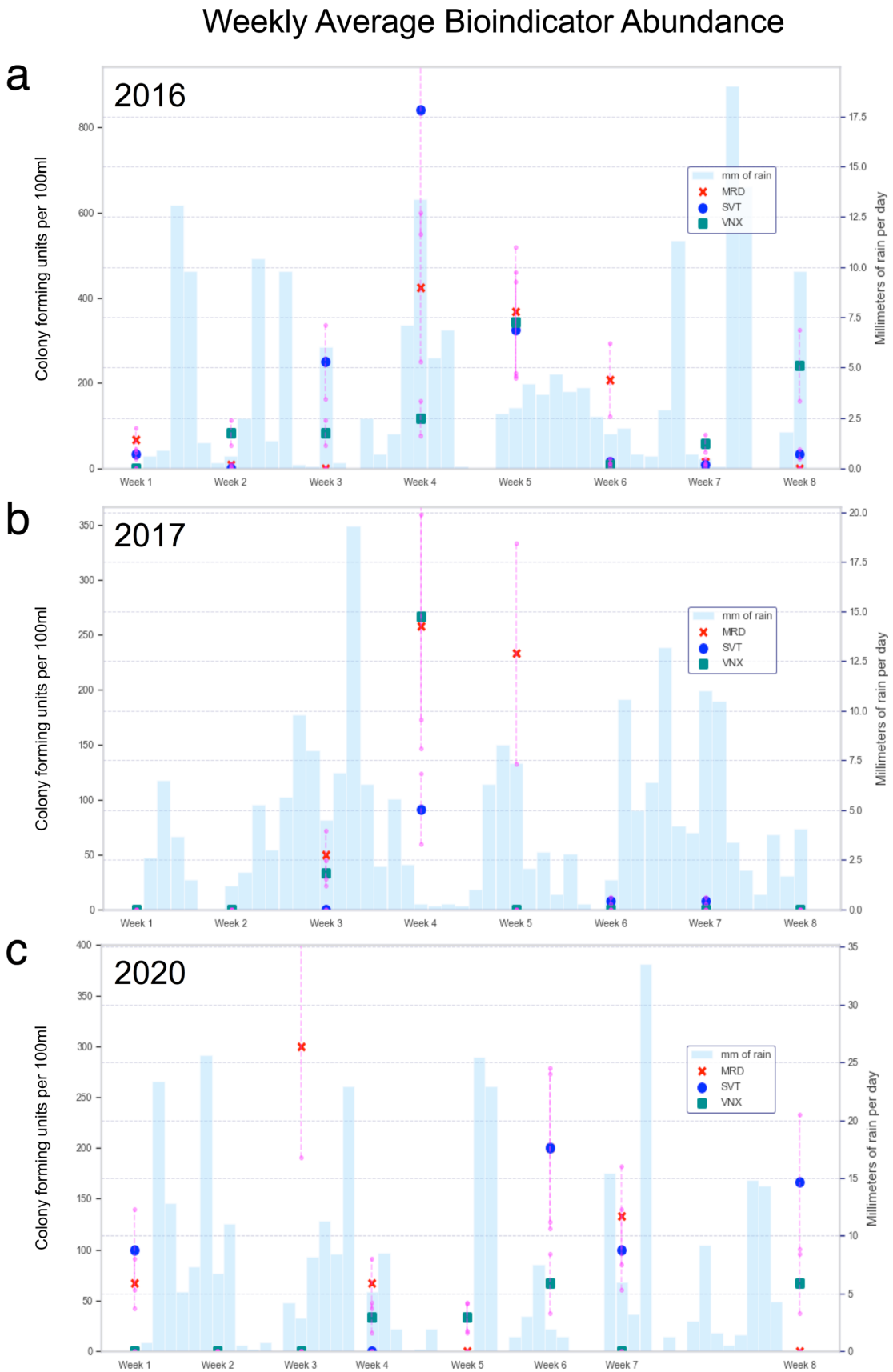

FIGURE 3 Bioindicator abundance and rain at each sampling site over time: (a) 2016, (b) 2017 and (c) 2020. Error bars for these weekly average plots were calculated from the standard deviation divided by the maximum average values

2020 (Figure 3b,c). Even though contributions to microbial abundance observed might be expected from overflow of water lines (Figure 2b), rainfall seems unlikely to simply explain these observations, as bioindicator peaks could nonetheless still occur when rainfall was low (Figure 3). To examine this association more clearly, we summed rainfall totals over 24-, 48- and 72-h periods, for all years, and plotted observed CFU values versus these summed amounts of precipitation (not shown). In support of these findings, calculated Spearman correlation coefficients for bioindicator and rain were at only −0.03 (with its associated *p*-value a bit high, however, at 0.06981). Taken together, this means (even though rain can influence maximal values, perhaps) it is very unlikely that peaks of bioindicator abundance observed are simply due to excessive rainfall.

In addition to bioindicator abundance, various bacterial species (four main colony types) are readily distinguished with the microbial plates; and each individual site varies from week to week in terms of overall abundance of these bacteria. For example data for the fourth week of sampling the first summer season are shown in Figure 4a, with *Aeromonas* abundance higher at both MRD and SVT than at VNX and *Salmonella* of most concern only at the SVT site. Figure 4b depicts results for all weeks of the 2017 sampling campaign, when rainfall was lower. Interesting trends can be observed, starting with bacterial levels mainly below detectable limits for the first weeks and changing individually over the whole summer. The first bioindicator colonies are observed already in the second week of sampling at one site (MRD), with bioindicator at all sites by the fourth week. *Aeromonas* and other coliforms come up in the third week, and later, with abundant 'other' species continuing into the sixth week, and still a few bioindicator colonies to the end of the sampling period of 2017.

The total counts obtained after adding up all these bacterial categories also vary over the 8 weeks of sampling each year, with peaks of abundance occurring especially from the fourth through sixth weeks in 2016 and especially in the fifth week in 2017. In 2020, the fifth through seventh weeks have the most total CFU of the sampling period, although for the VNX site the third week also has a peak (not shown). Of note, such total summations of the bacterial categories are much lower than actual total bacterial levels in the water samples, as many species are prevented from growing (for instance, the gram-positive organisms by bile salts, and others by the 37°C temperature).

Other factors, such as air and water temperatures, dissolved oxygen levels, water turbidity (generally low, except for the week when the MRD site could not be sampled, mentioned above) or pH, did not correlate with the increase in bioindicator observed (not shown). However, the sixth week of the first summer's sampling, an unusually high pH was measured at the SVT site; and very little bioindicator, but many 'other' CFUs were cultured from that day's sample. There could be many hidden variables that affect observed bacterial abundance, which will come up again in Section 4.

As the Montreux Jazz Music Festival attracts so many fans, it was no great surprise to find that bioindicator abundance was strongly correlated with dates of the music festival both initial years of sampling (Figure 5). More than 20 occurrences of bioindicator and coliform bacteria greater than baseline during the music festival are observed,

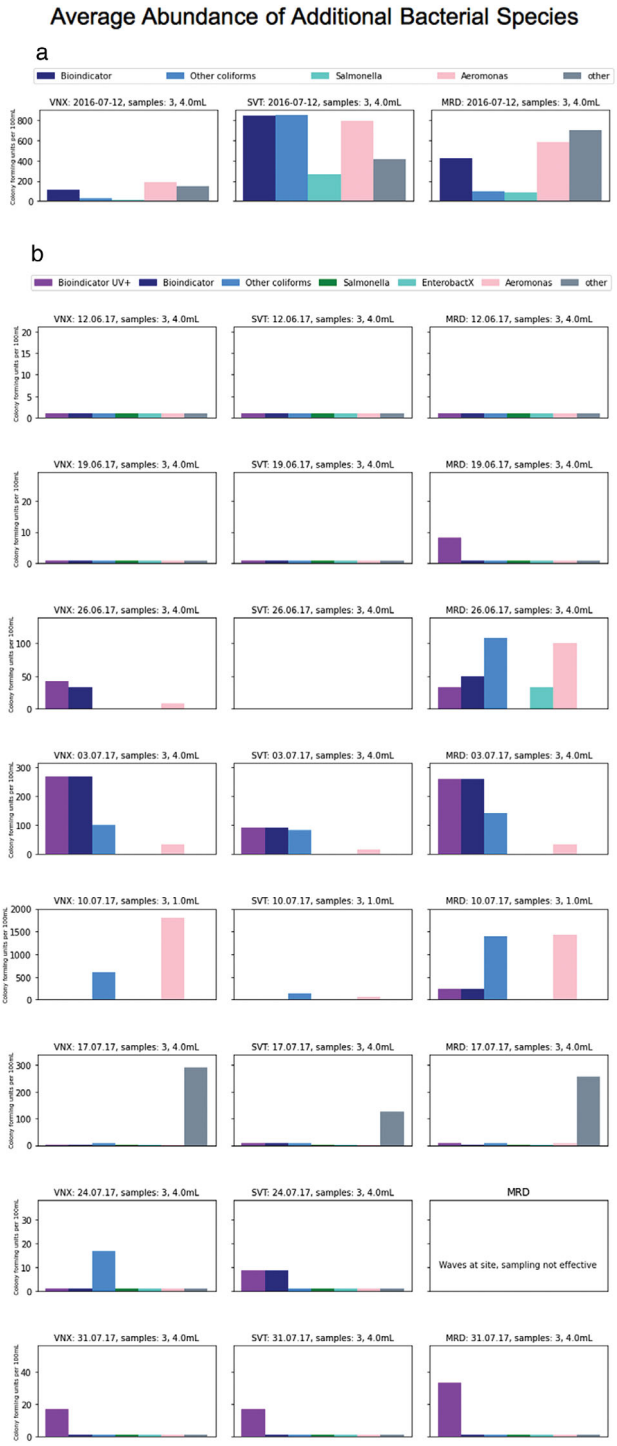

FIGURE 4 Average abundance of bacterial species. Species bar graphs with each different species color-coded as given in the legends by site (VNX, SVT, MRD) for (a) the fourth week of the 2016 sampling; and (b) the 2017 sampling season. To note: scale bars vary from week to week

in comparison to only seven in weeks before and after the event (Figure 5a). When quantification of the bioindicator bacteria is consolidated for both years to show the festival weeks overlapped (Figure 5b) for distributions of results before, during and after the event, the amount of bioindicator bacteria observed in all of the samples prior to

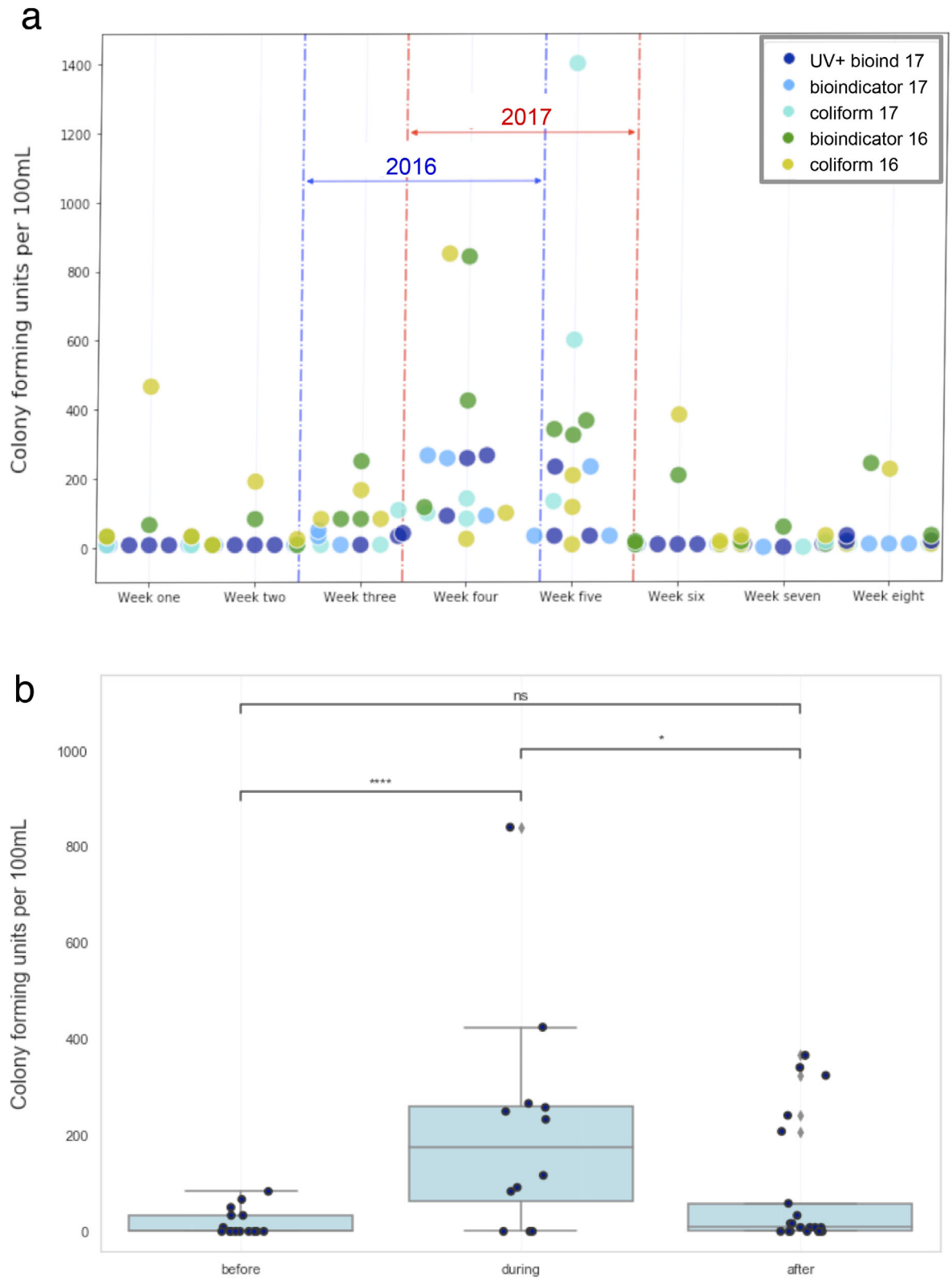

FIGURE 5 Correlation of bioindicator abundance with dates of the festival by year. (a) Higher levels of bioindicator bacteria are observed during the period when the festival occurs, in both 2016 and 2017. (b) Grouped averages of CFU per 100 ml per location from the first 2 years are plotted, with analysis of means 'before', 'during' and 'after' the festival. ns = not significant. Complete pairwise *p*-value annotations for panel (b):
ns $5.00 \times 10^{-2} < p <= 1.00$
* $1.10 \times 10^{-2} < p <= 5.00 \times 10^{-2}$
** $1.10 \times 10^{-3} < p <= 1.10 \times 10^{-2}$
*** $1.10 \times 10^{-4} < p <= 1.10 \times 10^{-3}$
**** $p \leq 1.10 \times 10^{-4}$

the event was less than the 50th percentile of the amounts obtained during the festival. After the event there are a few outliers (perhaps still due to anthropomorphic activities). Permutation tests reveal highly significant differences in the mean bioindicator values, particularly when comparing means before and during the festival ($p \leq 1.10 \times 10^{-4}$) and when comparing the 2020 summer to the levels found during the festival the first two years ($1.10 \times 10^{-3} < p \leq 1.10 \times 10^{-2}$).

Official microbial results from sampling of beaches at either end of Montreux Bay (Figure S4) are consistent with these participatory research data. For the Pierrier Beach (also shown as La Playa on Google maps, closer to the sewage treatment plant), the 11 July 2017 peak at 86 CFU/100 ml and at Chillon, 24 CFU/100 ml (Figure 4b) fits relatively well with peaks observed in the fourth week of sampling that year. There are many missing values in the official data, so a meaningful comparison is difficult. However, after these values are fit in the context of the timing of the festival (Figure S4c), these regularly sampled swimming beaches clearly have higher values during the festival than at other times, although the average values are reduced. A circular current within Montreaux Bay (a 'gyre' particularly pronounced in summertime due to stratification of water by virtue of temperature gradients) likely contributes to this effect, retaining bay water locally (Graham, 2015).

Although project information in the wiki of Hackuarium, the website of Hammerdirt and the raw open data itself were on-line throughout (see below, under DATA AVAILABILITY), not much feedbacks, let alone official responses, were obtained. Even after dissemination of 'general public' articles (Erismann, 2016, 2017), and radio and newspaper coverage about both Hammerdirt and Hackuarium efforts in this regard, impact seemed limited.

Project participants organized a guided visit of the sewage treatment facilities at Clarens and also discussed study findings with workers there. No one was allowed to share images from this visit, but clear explanations, large and noisy devices, massive circulating fermentation/holding pools, less noxious odours than expected and controls of effluents at each stage were in fact impressive, as attested by participants (Figure S5). The main surprise from the tour for a few (the engineer and the designer, in particular) was how sewage treatments include an important bacterial community component. Offers to share information with their management team on several occasions, unfortunately, elicited no response. While events with public presentations reveal general interest in this project, for instance, at Open Science Festivals, like one in Ferney-Voltaire, France (Aronoff, 2019), moving on from results of this study to action clearly requires several types of communication, especially if one wants to control pollution of public waters.

4 | DISCUSSION

Peaks of bioindicator abundance during the weeks of the festival both years were significantly different from the means before and after (Figure 5b), and indicate raw sewage flow into the lake. Neither rain nor other factors, like summer temperatures, could simply explain the increases observed. Even though higher rainfall potentially increased microbial levels, particularly in the first year, heavy rain was not correlated in general with peaks of bioindicator abundance. Instead, the festival crowds, almost 10-fold the ordinary local population density, seem the most likely source of the pollution. Then, the Covid-19 pandemic provided the opportunity for a sampling campaign during the relatively closed summer of 2020, when no music festival was held. Over the course of this later sampling campaign, only isolated peaks of bioindicator were found at various sites over the course of the sampling season, a finding highly significantly different from the earlier two sampling campaigns. Of course, the observed correlations do not prove causality, and other factors may have contributed to the observed levels of bioindicator bacteria in water samples.

In such participatory research investigations, run as community laboratory projects through volunteer efforts, there are always hopes to stimulate similar efforts internationally, in particular, in this case, as access to clean water is such a serious global issue. As highlighted in a text brought together for policymakers, who might not have the technical background to address the complexities of developing water management strategies (Jorgensen et al., 2005): 'contaminated water is still the single greatest cause of human illness and death on a global scale. Inadequate treatment of human wastes, and their subsequent discharge to receiving freshwater systems, is the primary culprit.' Therefore, even in relatively 'clean and tidy' Switzerland, it is important to disseminate these results more broadly, also in order to help more members of the public to realize that they could try some participatory research.

Overall, the microbial monitoring from this participatory research study demonstrates that the capacity for sewage treatment and storage in the area should be ameliorated (with re-design of catchments and holding tank infrastructure, as shown in Figure 2b, perhaps also helpful). There is much more discussion around this topic than can be fit in this context, like occupational hazards for lifeguards and other types of water pollution, which are well worth further thought. Everyone would like to count on having clean water. In order to make sure others are able to reproduce such studies, Appendix SA includes supplemental information on methods for participatory research. Additionally, for this discussion, a few simple yet important points should be made.

4.1 | Standards for bacterial levels in recreational waters

A distributed system oversees water monitoring on Lake Geneva, including Swiss and European authorities and an international commission. It produces an 'interactive' map of water quality of beaches around the lake (CIPEL, 2018, and Appendix SB). Local Swiss standards (Schaffner et al., 2013) for *E. coli* abundance in public waters are relatively strict at 100 Bioindicator CFU per 100 ml, in comparison to the 500 CFU/100 ml allowed by the European Union. More details about allowable levels of bioindicator in public waters internationally can be found in Appendix SB. From the work described here, peak levels of

bioindicator abundance observed at each sampled site the first two summers are at least double the value allowed by Swiss regulations, but not always up to the levels forbidden by EU regulations, particularly in the second summer of sampling. If one considers the Enterococci levels in addition, however (again, as discussed more fully in Appendix SB), it is clear that Montreux Bay during the big music festival would not be considered clean by most standards.

4.2 | Significance

This study addresses a few basic questions. Firstly, is there a measurable difference in the quantity of bioindicator bacteria at sampled sites around Montreux Bay? Secondly, is there any difference in levels of such bioindicators in a summer season with a festival (2016/2017) or without (2020)? Finally, what is the chance that these results are random, in other words, that the same results would have occurred by chance? The answer to the first two questions is 'yes' as for 2 years in a row at the height of the festival, the bacterial colonies indicating raw sewage contamination peaked in Montreux Bay sites. These data fit with the official monitoring of the swimming beaches at either side of the bay (Figure S4). The classic null hypothesis for these microbial analyses was that there would be no change in bacterial counts over the course of the sampling campaigns. The obtained data and statistical considerations from results allow overall rejection of this null hypothesis, and the permutation tests allow a conclusion that the mean differences in CFU abundance observed are not simply due to chance. A further hypothesis is that the capacity of the sewage treatment system is pushed to its limits by the music festival's denser transient population, which is supported by the finding that the 2020 summer peak values were also significantly different from the initial two summer campaigns.

Differences in total bacterial counts obtained during this study depend upon the site analysed, and it remains possible that hidden variables are also important. Certainly, different indicator bacterial species vary in abundance from site to site, each week. The species grids, as shown in Figure 4, provide evidence for dynamic differences in bacterial species abundance, which co-exist in the complex lake water environment and change over all sampling periods. These observations also help demonstrate the non-random data obtained from these assays on average. Bacterial abundance at the Vernex site in particular remained low the first year as the highest levels were reached at the other two sites on the bay. One possibility is that the quickly deepening lake at that site (as can be seen in Figure 2b) results in quicker sedimentation, thus preventing high bacterial levels from being demonstrated by the surface sampling technique. (More on this also in Appendix SC.) Another potential hypothesis is that garbage from a fast-food restaurant on the other side of the bay (at the MRD site) provides nutrients for bacterial growth, and the Vernex site contains relatively lower levels of bioindicator because it lacks this input. The most striking pH shift apparent during these summers of sampling (from whatever source it might have been: perhaps industrial processes, perhaps something added to water by regulatory agencies or just by someone cleaning something) was accompanied by decreases in the bioindicator abundance, but increases in other bacteria growing on the Easygel plates.

Unfortunately, statistical power of these analyses was limited by volunteers' time and lack of any significant budget. Median bioindicator values from six inoculated plates would have been better for statistical tests, than averaging results from only three plates, for instance. This quantitative effect, however, does not diminish the quality of these data, now further bolstered with molecular tests, confirming bioindicator species (Table S1). Other limitations to these analyses are discussed in Appendix SC. As all the data are openly available, anyone is able, and encouraged, to go back to original data (including microbial plate images) and do further analyses or to perform a meta-analysis combining this dataset with those from other studies. Comparison of original manual counts with the automatic counting techniques is still of interest, after our initial focus upon the single bioindicator species. Not only further analyses regarding bacterial classes, but also further sampling, both locally and internationally, to determine how best to prevent and clean up water pollution, will be helpful. Clearly, much more effort could be made to understand all the observations obtained from this work, and future monitoring efforts should be supported.

Of note, however, finding the bioindicator for raw sewage, *E. coli*, in lake water is not itself the most important health risk. It is all the things that can come along with the raw sewage, which are harder to detect and of much greater concern (Rodrigues & Cunha, 2017). The bioindicator bacteria, *E. coli*, after all, is a key member of our microbiome. Knowing raw sewage gets out means risks of not only encountering potentially pathogenic organisms and substances (e.g. enteroviruses, protozoa, micropollutants), but also harmful growth of organisms in the water (consider algal blooms producing toxins or depleting oxygen) are much higher. These results from summer seasons of sampling support the hypothesis that lifeguards training in such water would be at higher risk for gastrointestinal, skin and/or a variety of other possible infections or reactions.

Keeping perspective is still important. Some bioindicator levels observed during this project are, again, within European standards, if not the local Swiss requirements, but many orders of magnitude lower than what can be seen occasionally in other countries. Furthermore, river water controls often contained more *E. coli* than many of the lake water samples tested (Hackuarium Github, 2021). Levels in Swiss rivers of *E. coli* are nonetheless still not so particularly high, as, for example, a study along the Bagmati river in India found 1,000,000-fold higher concentrations of *E. coli* in some areas (Rey, 2016). Why rivers are generally dirtier than lake water could be due to a combination of continual inflow of nutrients and bacteria (from, for instance, field runoff, fertilizer and animal waste) and the highly active flow of rivers (meaning bacteria never simply settle down and always have a good supply of oxygen), in contrast with easier sedimentation and dilution effects of the larger body of water in lakes. While indirect effects from pesticides cannot be excluded, sewage treatment plants and livestock up river from the positive control sampling sites are also likely contributors.

This study should help make people more aware about the possibility for missed incidents of pollution from monitoring bodies that 'share responsibility' for safeguarding a region. Management of aquatic

resources is not simple (Jorgensen et al., 2005), but a community-based adaptive management strategy might be helpful (Habron, 2003) and foster productive discussion and action. Management of pollution around the time of big events might include both 'bottom up' community-based adaptive management via participatory research as described in this study, and additional 'top down' monitoring of effluents from the sewage treatment plant or its holding-catchments, for instance.

Encouraging more open science research for public participation in environmental management may be necessary. Both visitors coming to big events and their organizers should become aware of effects, which normal monitoring systems might not reveal, to improve the situation and help authorities keep public waters clean and safe. Perhaps this study, from a more local perspective, will also help increase the chance that renovations around Montreux Bay for its over 30-year-old sewage treatment plant at Clarens and other sewage infrastructure in the area will happen sooner rather than later. While many benefit from increases in tourism, the lake and other key tourist destinations are never likely to, unless real change happens, also internationally. General renovations, in particular for avoiding pollution with micropollutants (Canton de Vaud, 2016), are in progress and should be endorsed, but the more basic issues around raw sewage pollution of public waters, even if not at an official swimming beach, must also be addressed.

This project began about potential health consequences to local lifeguards of unsuspected pollution, but can be applied anywhere water quality is of concern. Basically, wanting to learn more is what led to these efforts to quantify microbes, in particular the bioindicator for raw sewage contamination, *E. coli*, entering Montreux Bay over the initial two consecutive summer seasons. Adding to the data, re-analysing the open data more closely, 'forking' the GitHub repository and starting a whole new set of inquiries based on this project model, all are encouraged. In conclusion, funding for participatory research studies like this one should be made a priority, particularly as local authorities everywhere cannot seem to do it all. More acknowledgement of those who make the time to do such work in their free time, as unpaid volunteers, and real support for their efforts is necessary. This study shows that participatory research can be useful for surveillance of currently unmonitored areas. We hope it will encourage others to undertake similar investigations about whatever concerns them, and that improved management policies will allow not only jazz music lovers to come without unintentionally contributing to pollution of the bay of Montreux, but international waters in general to stay cleaner.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

RE, SE and RA originally conceived and initiated the project. All three planned experiments, collected, plated, imaged and scored results, along with other Hammerdirt and Hackuarium members and public participants. AD joined the project in the second summer, plating, scoring and imaging data. Most Montreux Bay samples were collected by RE and SE, with RA collecting the positive control sample each week, and mainly responsible for the 2020 campaign. LP contributed primarily in developing, describing and sharing the cheminfo analytic pipeline, including the text used in the methods section. CVR pulled together the third control year of data and comparisons to the consecutive summer seasons, python coding data tables and statistical tests to finalize the updated GitHub repository. RA was responsible for project organization, ran the sequencing confirmation tests and did most manuscript writing, drawing on previous documents of the Montreux Clean Beach Project, with input from authors in several iterations.

DATA AVAILABILITY STATEMENT

Complete data sets for each sampling year, including plate images, sequence data and the archived GitHub repositories, are available in the Zenodo server: https://doi.org/10.5281/zenodo.5094576 (Aronoff, 2021). Plots or information that is 'not shown' in the text above, for instance, the 'Total_CFU' plots, is also available in this Zenodo dataset.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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