SUPPLEMENTAL INFORMATION:

Participatory research to monitor lake water pollution

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Appendix A:

Supplemental Information on microbiological methods, species confirmation, participatory research and limits of detection

These details are included to provide a practical perspective on the utility and limits of detection for these microbiological methods for open science objectives, to encourage more participatory research and local water monitoring campaigns.
To note: always use sterile microbiological techniques and dispose of waste properly.

Each colony that grows (termed a 'colony forming unit' or CFU) on a microbiological plate is considered to be due to the presence of a single viable bacterial cell, that came from the inoculated sample and is able to grow under the given conditions of temperature and nutrients. This is a reason for time and temperature limits before plating, to avoid opportunities for extra growth of microbes before plating, which could artifactually increase their apparent abundance. Generally, 0.5ml of the positive control (river water) sample, with higher bacterial loads, and 4ml of the Montreux Bay samples and of the negative control (tap water or a drinking fountain water) sample were used as inocula into the Easygel media during this project. For classic agar-based

media, a 1ml inoculum (or less) is used. The chromogenic media in the context of the Easygel plates solidifies in about half an hour at 37°C and is then flipped to avoid potential negative effects from condensation (i.e. smearing of colonies).

Use of the ECA Easygel color-coded system was based primarily upon the successful use of these with high school level students (IB biology, College Champittet, 2010-2011, pers. comm. RA), and purchased from Micrology laboratories in a ready-to-use format. From their instructions :

"It contains a sugar linked to a dye which, when acted on by the enzyme β-galactosidase (produced by coliforms including E. coli), turns the colony a pink color. Similarly, there is a second sugar linked to a different dye which produces a blue-green color when acted on by the enzyme β-glucuronidase. Because E. coli produces both β-galactosidase and β-glucuronidase, E.coli colonies grow with a purple color (pink + blue). The combination of these two dyes makes possible the unique ability to use one test to differentiate and quantify coliforms and E. coli."

The provided color key from Micrology Labs identifies bacterial types as:

E .coli: dark blue/purple and larger size

Coliforms: lighter blue/gray/purple and smaller size

Aeromonas sp.: pink

Salmonella sp.: green/teal

Other (no particular color)

These microbial plates have two advantages over ordinary ones. Firstly, the chromogenic media allows one to readily distinguish at least four main classes of bacteria (*E. coli*, coliforms, *Aeromonas* and *Salmonella*). Secondly,

because the plates are based on pectin, not ordinary agarose, there is no need to heat the media. This is of particular use in the Community-Based Environmental Monitoring context (Conrad and Hilchey, 2011), and adds confidence that the data is representative of the microbes present in each water sample. Furthermore, selective bile salts in the Easygel plates and in the Levine media inhibit the growth of many bacteria (like abundant gram-positive organisms and even some pathogens that could complicate analyses in this public lab setting). To note: many environmental bacteria would not grow at 37°C, limiting observation of their growth. This higher temperature, body temperature, is used because it is of much greater interest in terms of assessing public health risks. Obtained values are certainly underestimates of actual levels of *all* bacteria in the lake water samples tested, as long as good aseptic (sterile) techniques are used. Controls, as always must be planned ahead, and used! Figure S1 shows the output of one sampling day, including various control and Easygel plates.

We were convinced to try out the Easygel 'plus' plates in the second year's sampling campaign for further confirmation of the key bioindicator species, *E. coli* by fluorescence. Indeed, this provided further confidence in our scoring, as in 2017, only very slight differences were observed between the data for CFU counted as 'Big Blue' of 'UV+', which specify the same category, the bioindicator species, *E. coli*. (This was also very well supported statistically, with a Spearman correlation coefficient of 0.99, with p-value less than 10^{-5}) As shown in Figure S2a, even *E. coli* can have different colors (ranges of blue to purple) after metabolising the Easygel chromogenic substrates. In 2020, only standard non-selective media (LB) and the classic Levine media were utilised.

Thus, in summary *E.coli*, the bioindicator for untreated sewage, was primarily distinguished as ‘Big Blue’ colonies during the 2016 campaign, as 'Big Blue' or 'UV+' CFU in 2017 and by the metallic green sheen in 2020. Cross streaks from Easygel onto Levine plates (Fig. S2b) and sequencing of colony PCR products amplified with universal 16S rDNA specific primers (see Materials and Methods) has now confirmed species identifications, as shown in Table S1. (Sequence result files also included in the Zenodo archive, doi: 10.5281/zenodo.5094576.*)*

It should be noted that fermentation differences within species can differ. For instance, classic laboratory 'cloning' strains of *E. coli*, like DH5α, do not make metallic green CFU, but the laboratory 'protein production' strain, BL21, does. In the wild, fermentation genes might vary, as well, so most microbial quantification done with these methods is again likely to underestimate the bioindicator bacteria concentrations actually present.

Finally, analyses of counts of the bacterial classes in this manuscript are simply from the initial manual counts from the plates, but these can also be based upon the images and even automated, as discussed in the methods section and depicted in Figure S3.

The wider relevance of participatory research:

How practicable was it and what lessons are there for other monitoring programmes?

With more open science community labs sharing knowledge and know-how, participatory research is a growing endeavor internationally. Limited budgets and voluntary participation means it can be difficult to set deadlines, let alone

to collect and analyse all the data, however. These challenges are worth the effort, nonetheless. Life-long learning is important, and we additionally learned as we used the Easygel plates regularly. For instance, over the course of the first summer's sampling campaign, some deterioration of plate quality (particularly of the pink reaction product for *Aeromonas* detection) was observed, most likely because the media was kept on a bench in a south-facing lab, with significant temperature increases during summer days. The media was thus kept in a cool and dark cupboard for the second summer's campaign. This highlights the importance also of the positive control samples plated from local rivers, to assess plate substrate integrity. Additionally, in some weeks we decided to reduce inocula amounts, in order to avoid difficulties in scoring amounts of each bacterial category. Even if Easygel plates were expensive from Hackuarium's non-profit perspective, their name is justified; and more ready access to these plates (and the paper alternatives Micrology Labs has been working on), would be beneficial to anyone keen to try such tests. There was no European source for the Easygel media, unfortunately, with available chromogenic media all based upon agarose. This means 1ml inocula are basically the maximum that can be used with these more available media. This affects limits of detection, as discussed further below.

Without the enthusiasm of volunteer members, none of these data would have been collected or analysed. We always say the people make the magic, and community labs should be in every town. Participants joined in for inoculations and scoring in the lab, especially in the old labs of Hackuarium, in Renens, Switzerland, before this past summer's pandemic efforts... To see the colorful

and the UV+ colonies, and also the metallic green colonies on the Levine media plates, was a big draw for some biohacker open nights! There are certain complications of the limited budget of a community lab, however, especially in terms of data management. For instance, the primary data repository in GitHub and hammerdirt sites became obselete eventually, and needed updating and archiving. For the 2016 data, a Google Fusion Table was used to collect all the data (images and scoring), but this became too slow when bringing in the 2017 data, when a kobo toolbox was instead utilised. A Hammerdirt site brought together much of the data in an interactive format, especially under its link to 'Microbiological surveys,' although this is currently defunct. Organising all the data in the Zenodo archive was a challenge. Ideally, data format, storage and analysis plans should be made very early on in such projects, and methods to ensure this are in progress (RE pers comm).

In terms of this particular study, the data from the first year are much more 'heavy' with two formats of image files from the SLR camera, in comparison to the data directly sent from a Samsung phone to the Kobo Toolbox in 2017, or aquired with an iPhone. For this reason, multiple zipped data files were necessary to archive the 2016 images. More importantly, finer levels of detail could still be obtained from the accumulated results; and we encourage further analyses, as has been done with trash data (RE, pers comm), already, also for a better understanding of the densities of the various bacterial species detected. Clearly, another round of the Montreux Clean Beach project may be of interest still to not only Hackuarium members, but international followers,

and the general public. While updates to water infrastructure are occurring locally, using these participatory research methods more regularly anywhere water quality is of concern should be beneficial for all. More sampling could still be done, and more participatory research encouraged and pursued, also for other types of water pollution, internationally.

Limits of Detection

As 4ml of sampled water was generally used as the inocula for plating on the Easygel media, and public health officials generally want to know levels of bacteria in 100ml, the number of colonies found on a plate must be normalized. When 4ml is used as the inocula, therefore, CFU values are multiplied by 25 to calculate the abundance of particular CFU in 100ml of the original sample. Thus, the 'limit of detection' on each plate is 25 CFU/100ml. For example, if 7 bioindicator CFU per 100 ml of sample on average were scored on the three plates made from the three independent samples from the particular site, that would be providing evidence for at least 175 bioindicator CFU per 100ml of that site's water samples.

In contrast, when 0.5ml of positive control river water is plated, the factor for calculation in 100ml increases to 200, and the limit of detection is increased to 200 CFU per 100ml of water, if no CFUs are observed. Thus, finding 7 bioindicator CFU on the three plates, for instance, on such plates would come to 1400 CFU per 100ml. Finally, to understand how this relates to this limit of detection: if only one plate of the three from a given site had a single bioindicator colony, this should lead to a value of 8 (25/3) on average.

If your group would like to do some local microbial monitoring, and has any questions, you are welcome to contact RA. The Hackuarium wiki pages for the project are here (Hackuarium, 2016):

http://wiki.hackuarium.ch/w/Micro_to_Macro_Water_Pollution

http://wiki.hackuarium.ch/w/CBEMresults

The general contact address for our community lab is hello@hackuarium.ch.

Appendix B:

Supplemental Information on Local Water Quality Monitoring

Swiss federal standards for water quality use microbiological indicators, like levels of *E. coli*, the bioindicator for raw sewage, and, more recently, micropollutants (FOEN, 2018), as important monitoring criteria. Not every 'beach' along the Lake Geneva shorefront is tested as a swimming beach, however, as mentioned. In particular, shoreline adjacent to river outflows is generally exempt from testing and not considered a swimming beach (Fig. 1). Local authorities responsible for water testing in Montreux are part of the Service Intercommunal de Gestion (SIGE). The water infrastructure in the region is quite complex (Fig. 2B); but Montreux Bay itself contains no official swimming beaches. For Swiss municipalities from Vevey to Veytaux, with a population of about 80,000 people, SIGE process engineers treat over 14 billion liters of wastewater each year at three sewage treatment plants (SIGE, 2018). An international commission for the protection of Lake Geneva water (CIPEL) produces an 'interactive' map of water quality of beaches around the lake, based upon local sampling results.

Local Swiss standards (Schaffner, 2013) for *E. coli* abundance in public waters are:

for drinking water - none detectable in 100ml, and

for recreational waters - less than 100 *E. coli* CFU in 100ml.

If the *E. coli* level is between 100-1000 CFU per 100ml, the Swiss standards still consider such 'B' quality water to be nothing to greatly fear. However, if the levels of Enterococcus species (termed 'other coliform' herein) also are greater than 300 CFU per 100ml, then some risk to health is assumed with

such 'C' quality water. (For more information, please see Table 3 from Shaffner, 2013.)

In contrast, 500 CFU per 100ml is allowed in recreational waters according to the EU standards. In other words, five-fold the Swiss standard. Furthermore, the European standards do not consider recreational water 'dirty' even when it has trash floating in it (Directive EU, 2006). Additionally, there are different levels of indicator species allowed by European directives, depending upon whether coastal or inland waters are being discussed (a summary in English is available (Directive EU, 2017)). For inland waters 500 *E. coli* bioindicator bacteria per 100ml is the limit - calculated from log10 CFU values as below.** These complications, in comparison to the Swiss standards, are most likely due to the need for compromise at the overall European level. Other countries have yet different standards, with the US and Canada, for instance, each including different ratios of coliform to *E. coli* in their standards, and more.

This distributed water quality sampling system on Lake Geneva is overseen by EU authorities, in particular the European Environment Agency, who noted in 2015, the first time it included Switzerland and Albania in with the other 28 member countries for this assessment, that several Lake Geneva beaches were not compliant with the EU water quality directives, and only 61% of swimming beaches of 'excellent' quality. They also remarked that complete statistics for sampling at 34 bathing areas in Switzerland were not available at that time (The Local, 2015). Public beaches around the lake are sampled at least every two years, and in the swimming season about once a month. (EEA, 2018). However, generally, results of Lake Geneva water monitoring

are satisfactory, for example in 2016, when 111 regularly-tested beaches were found to be adequate or even excellent for swimming (20minutes, 2016). Still, there are worries about many types of pollution in the lake (20minutes, 2019); and, as mentioned in the main text, the areas where regular microbial testing occurs on the lake do not include all areas where people engage in water sports.

A more recent report (EEA, 2018) conceded better status for swimming beaches in all member states, again including Swiss and Albanian beaches. It furthermore provided another reason to keep public waters clean, stating: "Bathing water quality is not only essential for public health reasons. Clean unpolluted water is necessary to improve ecosystem resilience." As 2020 was also the year for biodiversity, this provides another reason to make sure water quality management becomes a stronger priority everywhere.

** Directly from the EU directives:
Based upon percentile evaluation of the log10 normal probability density function of microbiological data acquired from the particular bathing water, the percentile value is derived as follows: (i) Take the log10 value of all bacterial enumerations in the data sequence to be evaluated. (If a zero value is obtained, take the log10 value of the minimum detection limit of the analytical method used instead.) (ii) Calculate the arithmetic mean of the log10 values (μ). (iii) Calculate the standard deviation of the log10 values (σ). The upper 90-percentile point of the data probability density function is derived from the following equation: upper 90-percentile = antilog (μ + 1,282 σ). The upper 95-percentile point of the data probability density function is derived from the following equation: upper 95-percentile = antilog (μ + 1,65 σ).

Appendix C:

Supplemental Information on sampling limitations

Representative sampling of the environment is a classic monitoring problem. Surface water samples (taken about 0.5-1 meter below the surface) were collected for this project.

Advantages of surface sampling: Simple and inexpensive, an important consideration to a program on a budget. No special sampling apparatus is required; the volunteer holds a sampling container under the surface of the water and closes the container under the water. (This avoids losing dissolved gasses, which is very important for some analyses, although not especially for this microbiology project. Because of Hackuarium projects in 2016 about biosensor bacteria for volatile pollutants, however, we followed this rule throughout.)

Disadvantages of surface sampling: Lake water has vertical gradients of temperature, oxygen, nutrients, and algae which will be missed by the surface sample. Deep lakes have a thermal gradient in the summer, and the lake will have at least two thermal regions, and, possibly, at least two chemical regions. Even in shallow, usually well-mixed lakes, chemical gradients will develop during calm periods. Gradients can even occur in the upper waters of lakes - this is especially evident for swimmers on Lake Geneva.

Also for bacteria, there are gradients of species: some might prefer surface water.

Statistical considerations

Ideally, five independent samples from each site would provide increased confidence, for averages, while at least six would be better for medians and

statistical analyses. However, limited funds and volunteers' availability to sample at least eight-week summer periods have affected the 'statistical power' of this campaign. These Easygel plates are very user friendly (no agar melting required) and sensitive (4ml inoculum possible), but so costly (especially for shipping to Switzerland) that doing 6 plates per sampling site per week (+ controls), as most desirable for better statistical analyses, would have overrun the Hackuarium budget. Each of the three lakeside sites was sampled, finally, in triplicate, allowing only the lowest threshold for averaging. At least low initial baseline levels of bacteria were detected on the Easygel plates of lake water samples all three summers, and later summer samples also generally returned to this low baseline.

There were a few other limitations of this study besides the number of plates that could be utilised. For instance, as already mentioned, fermentation differences between wild bacterial strains may be likely. (See also Appendix A, with many further details). Additionally, a much greater amount of official water sampling data to allow better comparisons to our results would have been helpful.

Choice of the sampling days was not entirely random. Initially, sampling was done on Tuesdays, as on Wednesdays were held the #OpenHackuarium evenings, when visitors thus could contribute and see the outputs from the campaign. However, we quickly realised we should be also counting and collecting images after 48h incubations, so we reassessed this choice of days for the second year's campaign, reasoning that sampling on Mondays might also reveal somewhat higher levels of bacteria than on Tuesdays, as events

of interest in the region are more highly attended on weekends than on weekdays. In fact, the second year's results revealed lower overall levels of bacteria, so the day of sampling is probably much less important than other factors (i.e. weather, transient population density). Nonetheless, the 48h plates are more impressive for the colorful colonies, on Easygel or Levine plates, if not for the Easygel Plus UV+ halos (which diffuse after 24h). For the 2020 collection dates, it depended on volunteers' availability, with at least two non-members joining in and Wednesday and Thursday being the most common sampling days.

Supplemental References

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Supplemental Figures

Figure S1:

An ‘infographic’ example of plates from the 2017 campaign, with negative (tap-water) and positive (river water) controls below, and the plates (from triplicate samples) from the 3 sites around Montreux bay stacked behind (and including a few extra control plates, above left). The positive control samples on non-selective media reveal abundant gram-positive organisms (inhibited by bile salts of the Easygel system).

Figure S2:

A: A positive control plate from a Venoges river sample, obtained the second year of sampling (19 June 2017) and imaged with bright field and UV illumination. Differences in the dark-blue/purple colours for individual UV+ CFUs may be observed, but the presence of this extra fluorescing substrate allows several to be unambiguously put in the same category.

B: A Levine media plate gridded with bacterial colonies (of defined color categories) from an Easygel plate.

Figure S3 A-E:

A: a positive control plate under bright field illumination

B: the same plate under UV illumination

C: manual annotation of the CFUs on the same plate (bright field)

D: an example of such data in the cheminfo analysis site

E: counts obtained with the cheminfo methods, with 12 coliform CFU highlighted from the counts in this example.

Figure S4. Comparisions to SIGE results

A: Swimming beaches at either end of Montreux bay include Chillon and and La Pierrier.

B: Results of official monitoring of these two beaches, screenshot provided by SIGE.

C: Correlation of results of official monitoring at these two beaches with the festival dates ('Event').

Figure S5: Some of the participatory research team after the visit to the Clarens Sewage Treatment Plant (picture by RA).

Table S1: Amplification and sequencing for molecular identification of colony species.

Colony for 16S amplification/seq	Species ID
Met green: SVT	*E. coli*
Pink: SVT 'C'	*Serratia*
Purple: SVT 'D'	*Citrobacter freundii*
Big purple with pink inside: SVT	*Kluyvera*
Pink middle giant gooey: VNX	*Enterobacter*
Purple +met green: Préverenges*	*E. coli*
Mauve +weak green sheen: Préverenges*	*Pseudomonas*
Met green ring with dark purple center: Venoges*	*E. coli*
Met green: Venoges*	*Cronobacter sakazaki* as first Blast hit, but also *E. coli*

*Préverenges is another local beach where a beach litter survey was done in 2020, and the Venoges is the local river most frequently used as the positive control for these studies.

Supplemental Figures:
Figure S1, infographic from 2017

Citizen science

Figure S2a

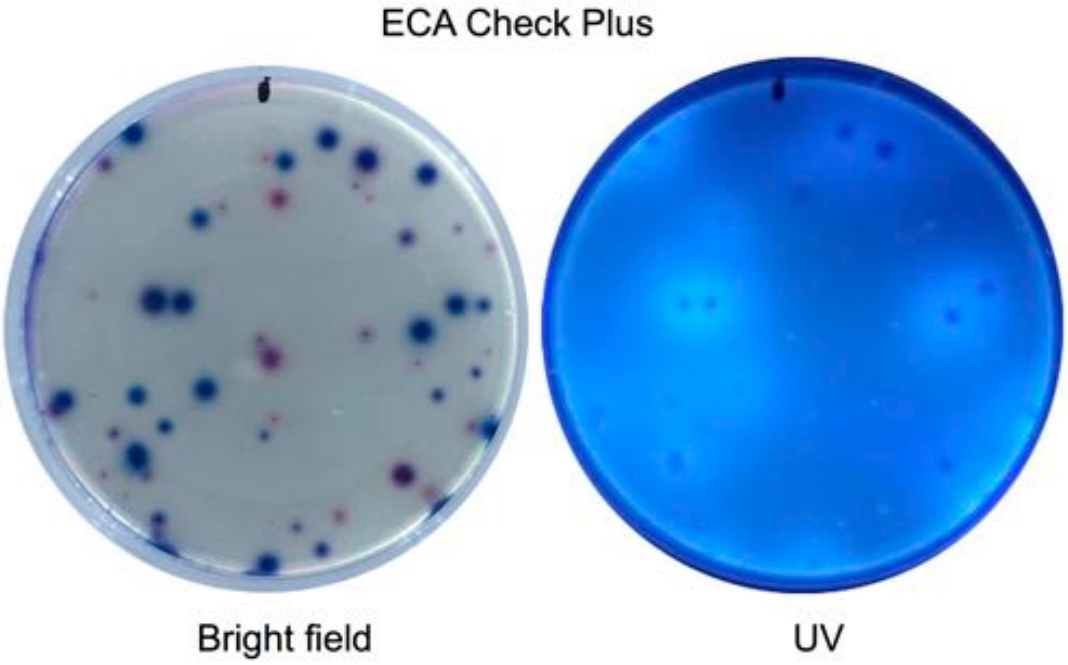


Figure S2b

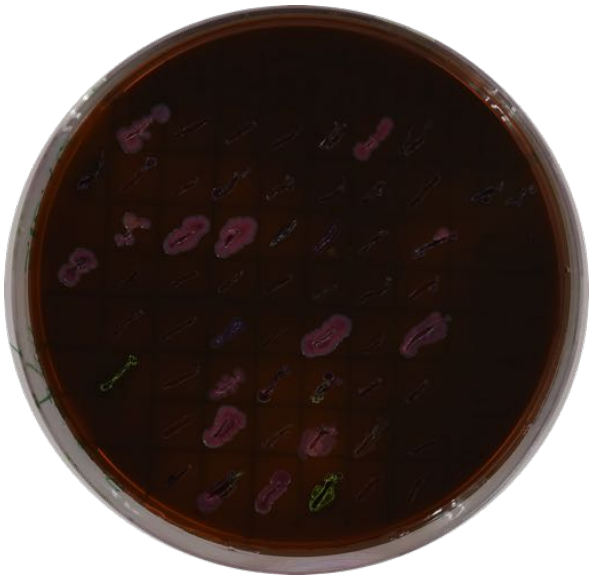


Figure S3a

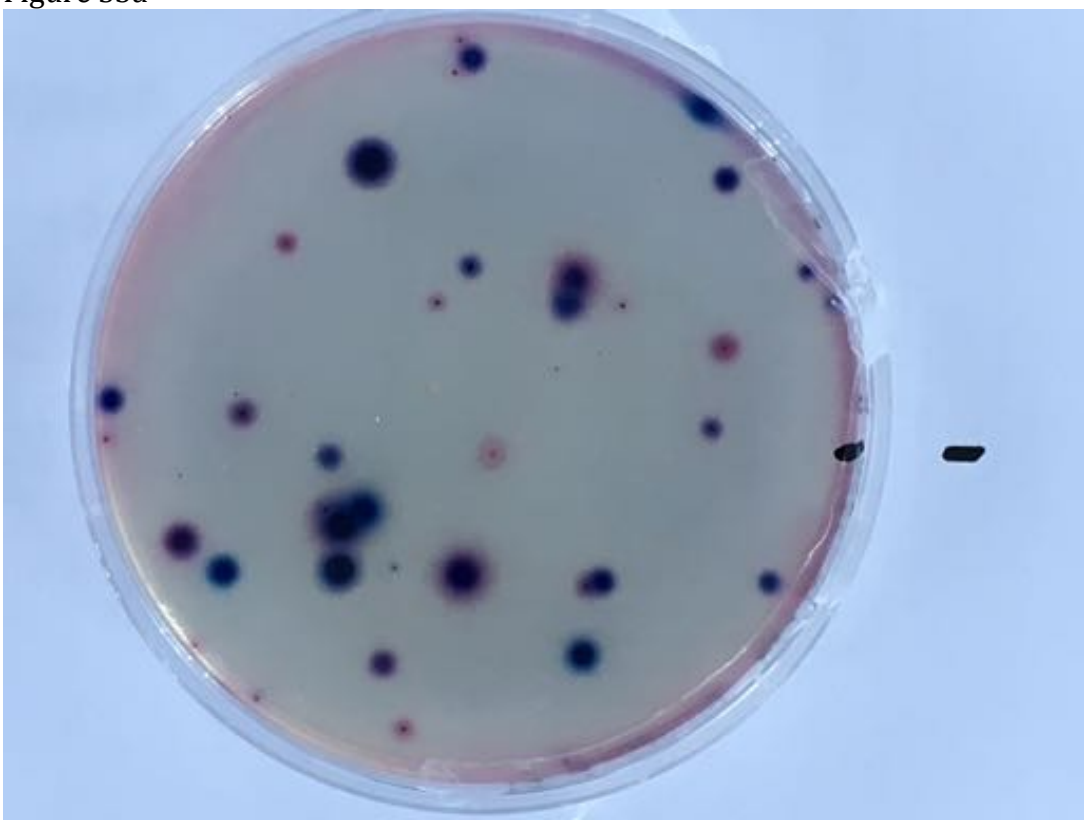


Figure S3b

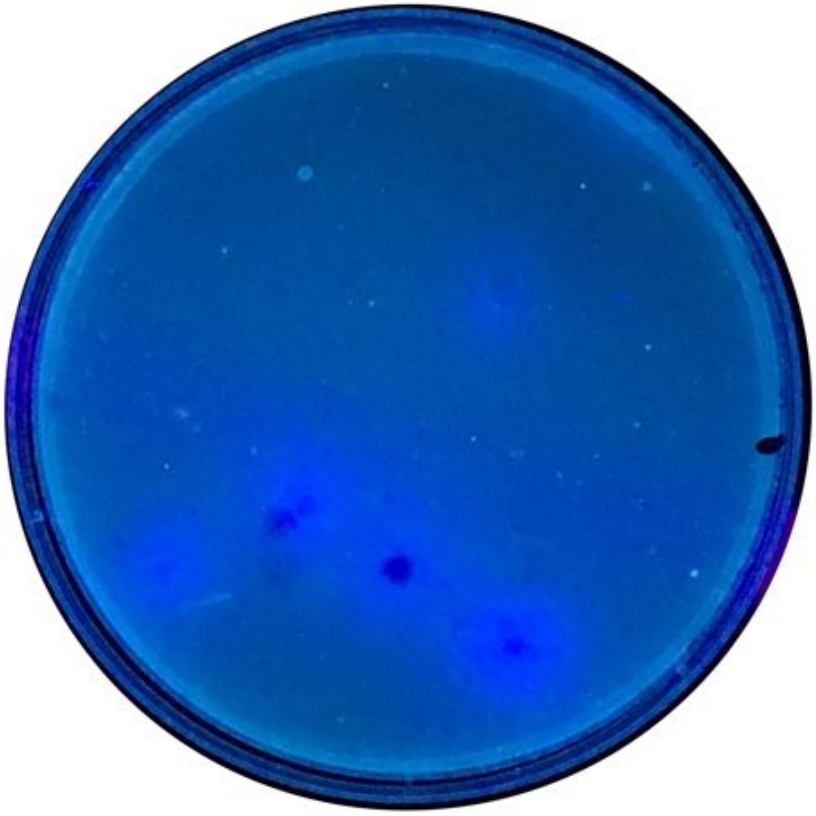


Figure S3c

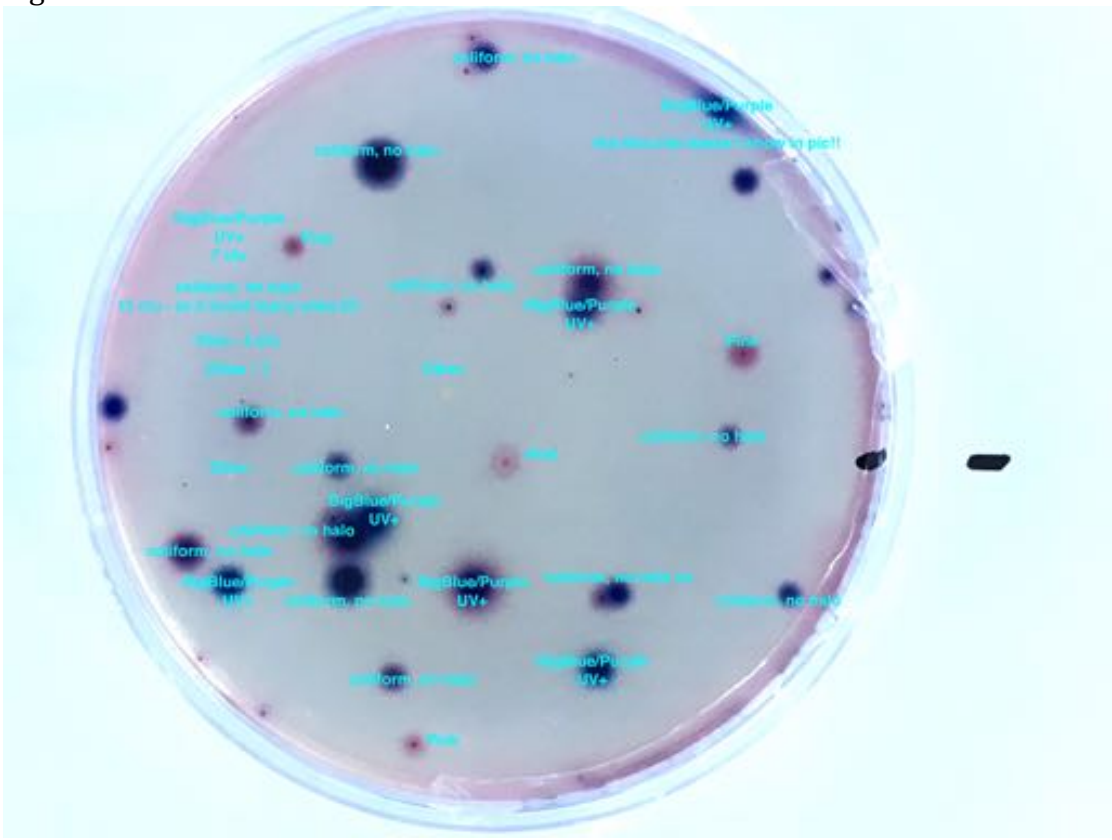


Figure S3d

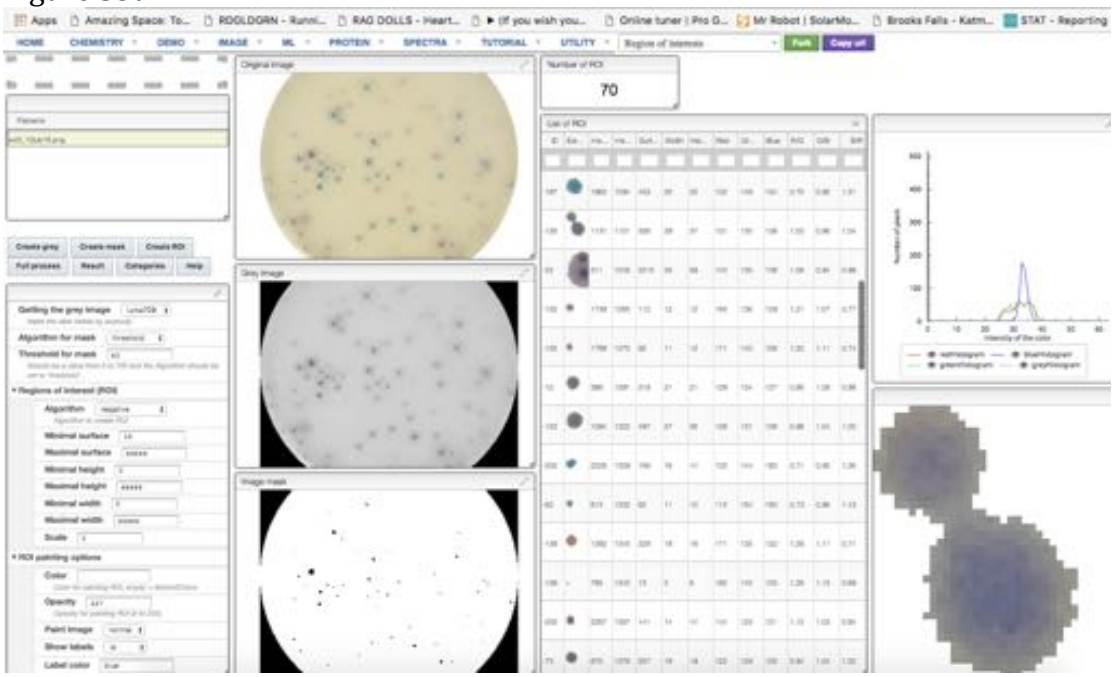


Figure S3e

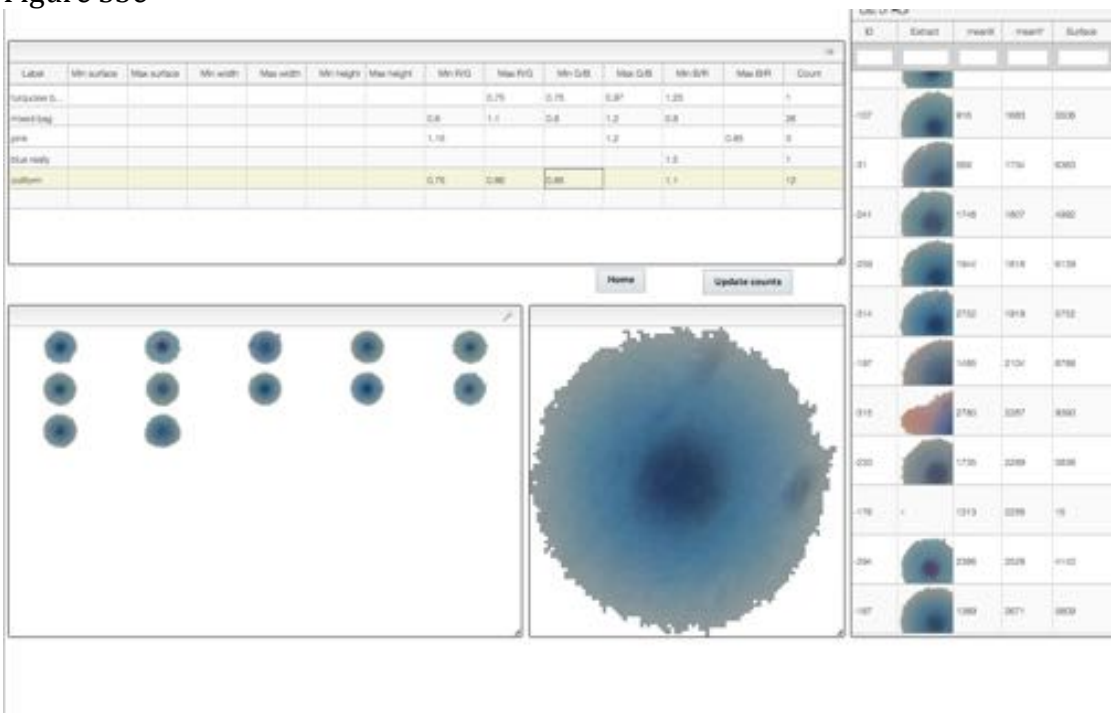
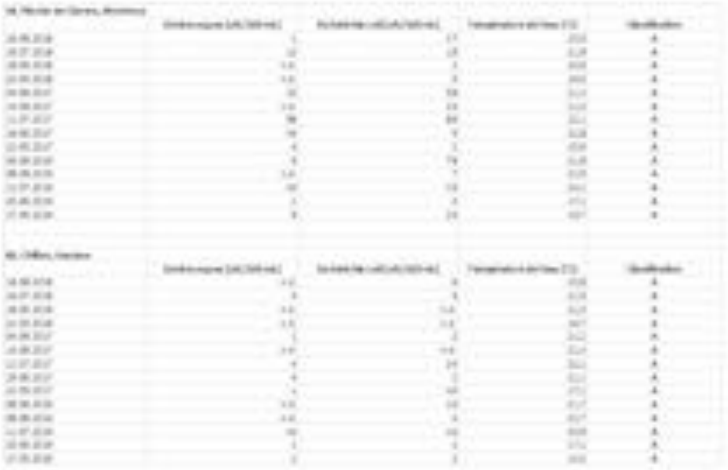


Figure S4

A

B



C

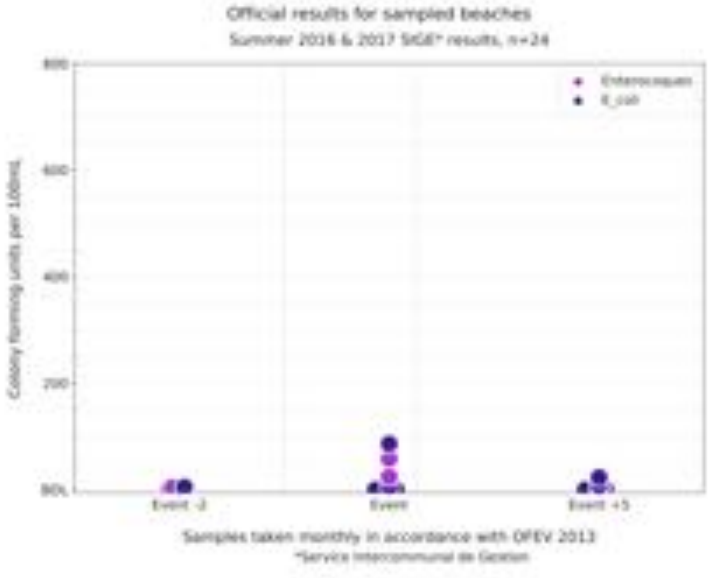


Figure S5