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# Accurate rapid in-situ measurement of the microbiological impact of pollution sources

Dan Angelescu, Andreas Hausot, Joyce Wong, Vaizanne Huynh

Fluidion R&D division

# Fluidion

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- A small company with an international footprint
- Specialized in novel water quality sensor and their use in complex studies
- Unique in-situ instruments:  
microbiology (*E.coli*), nutrients, oceanography
- Installations all over the world:
- USA, France, Germany, UK, Belgium,  
Italy, Portugal, Greece, Romania, S.Africa
- ETV-certified sampling technology  
(VN20180030) 
- Water quality aquatic drones

Paris



Los Angeles



# Context

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**Goal: quantify the in-situ impact of microbiological pollution and mitigation actions**

- Models can be over-simplified and often lack adequate input data
- Pollution may be generated in random patterns, with many confounding variables
- Traditional point-sampling methods not representative
- Bacterial measurement methods inadequate for long-term in-situ monitoring

## Case studies:

- A. Impact of untreated boat sewage on a receiving water body (Marne river, France)**
- B. 3-year continuous monitoring of the Seine river (Paris, France)**
- C. Measuring efficiency of mitigation actions: pump-out (Lake Chelan, WA, USA)**
- D. Impact of sewage discharges in small rivers and streams (Tijuana river, CA, USA)**

## Instrumentation used

1. **FLUIDION® DRONE** for water quality, equipped with RS-14V ETV-certified sampler
2. **FLUIDION® ALERT LAB** portable *E.coli* analyzer
3. **FLUIDION® ALERT SYSTEM** in-situ *E.coli* analyzer
4. Aerial drone with camera
5. Fluorescence lamp and filtered camera for time-lapse fluorometry



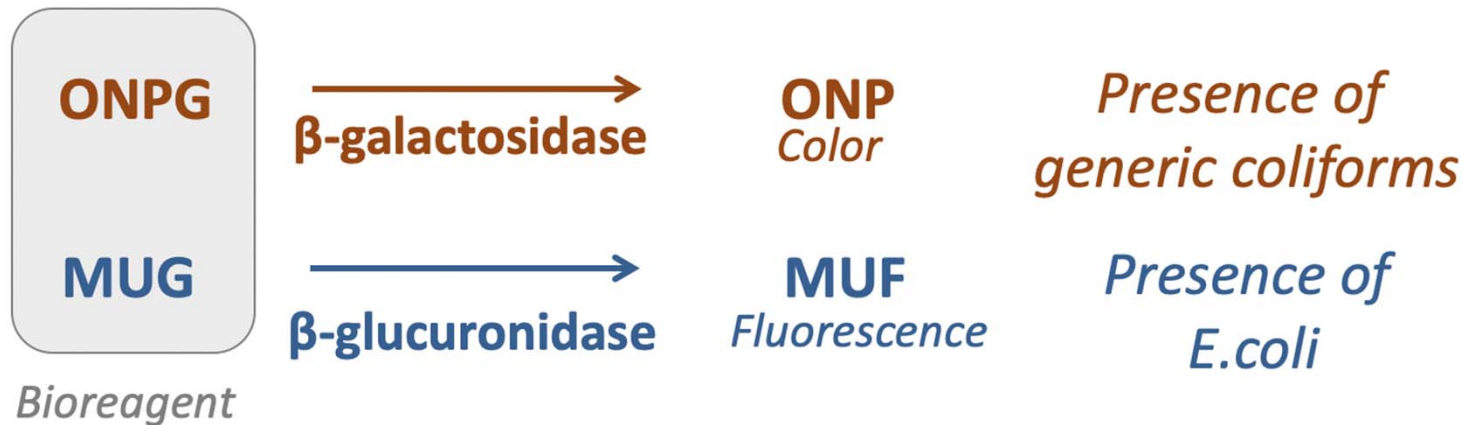
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## *Optimized liquid bioreagent (growth medium and indicator)*

- ❖ Rapid culture-based method
- ❖ Enzymatic reaction with fluorogenic substrate
- ❖ Time-resolved optical detection (absorbance and fluorescence)



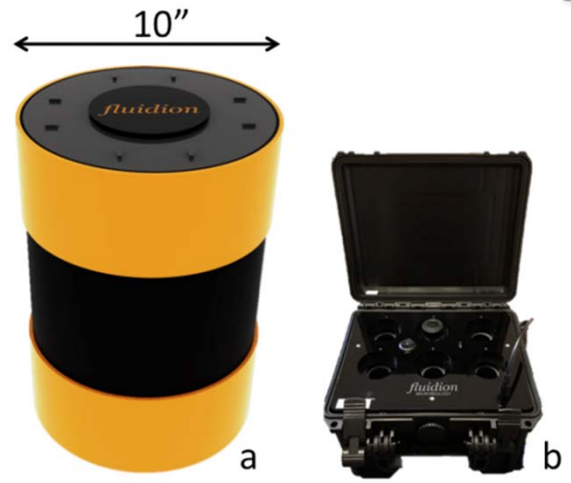
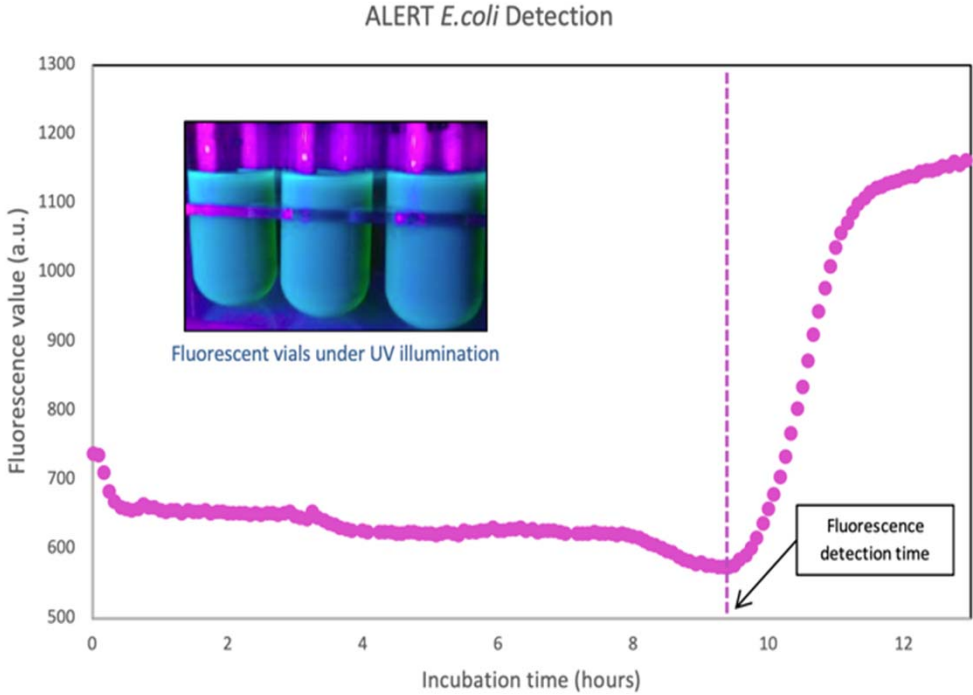
*ONPG: ortho-nitrophenyl- $\beta$ -galactoside*  
*MUG: 4-methylumbelliferyl-beta-D-glucuronide*

*ONP: ortho-nitrophenol*  
*MUF: 4-methylumbelliferyl*

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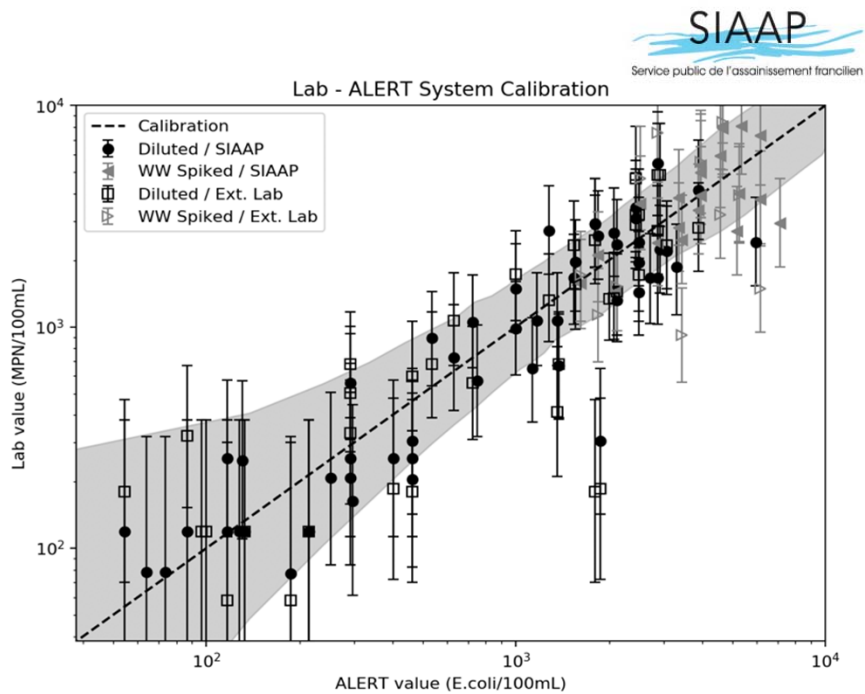
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# E.coli measurements in surface waters

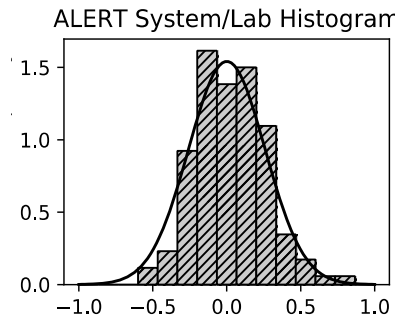
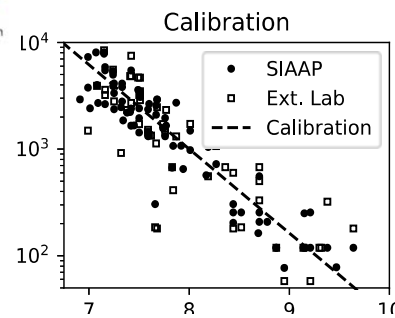
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Surface water calibration (Seine / Marne)

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## Journal of Applied Microbiology

### Autonomous system for rapid field quantification of *E. coli* in surface waters

Running Head: Rapid autonomous system for *E. coli* quantification

Dan E. Angelescu<sup>1</sup>, Valzanne Hayn<sup>1</sup>, Andreas Hauso<sup>1</sup>, Gizem Yalcin<sup>1</sup>, Victor Pfl<sup>1</sup>, Jean-Marie Mouchel<sup>2</sup>, Sabrina Guérin-Rechdaoui<sup>3</sup>, Sam Azimi<sup>1</sup>, Vincent Rocher<sup>1</sup>

<sup>1</sup>Fluidion SAS, R&D Division, 231 Rue St. Honoré, 75001 Paris, France  
<sup>2</sup>Sorbonne Université, CNRS, EPHE, UMR 7619 Meta, F-75005, Paris, France  
<sup>3</sup>SIAAP, Direction Innovation Environnement, 82 Avenue Kléber, 92700 Colombes, France

**Abstract**  
 Aims: The purpose of this work is to present and evaluate the performance of a novel Automatic Lab-in-vial *E. coli* Remote Tracking (ALERT) technology based on an automated real-time defined substrate approach, and implemented in both portable and in-situ instruments.

**Methods and Results:** We present the fresh-water calibration procedure, and assess performance using side-by-side comparison with most-probable-number (MPN) approaches in terms of accuracy, reproducibility and capability to correctly generate early-warning alerts. Long-term data from an operational in-situ deployment at a potential bathing site is presented as well.

**Conclusions:** ALERT technology is shown to be an accurate and rapid bacterial quantification technology, capable of autonomous in-situ measurements with metrological capabilities comparable to those of an approved laboratory using MPN microplate techniques.

**Significance and Impact of the Study:** Rapid quantification of bacterial pollution is a requirement in water quality applications ranging from recreational water use, agriculture and aquaculture to drinking and wastewater treatment. The method and instruments presented in this work should enable fast and accurate bacterial concentration measurements to be performed in a portable or in-situ manner, thus simplifying operational logistics, reducing time-to-result delays, and eliminating sample transportation constraints associated with traditional techniques.

**Keywords:** Water quality; *E. coli* (all potentially pathogenic types); Environmental/recreational water; Rapid quantification; Surface waters; Wastewater; Rapid test

#### Introduction

Surface water quality (in lakes, rivers, and coastal areas) has a direct impact on the safety of recreational water users, and on ensuring safe supply for drinking, aquaculture and agriculture use. Microbiological pollution with human and animal waste pathogens can have a variety of sources. In urban areas, bacteria can enter waterways through effluent from wastewater plants, amplified during heavy rain episodes by sewer and sanitary overflow phenomena (McLellan et al. 2007), and in specific cases by boat sewage. In rural settings, pollution is generally associated with agricultural run-off (e.g. from livestock operations) or natural presence of birds and other warm-blooded animals. Exposure to fecal pathogens via contaminated water is a major health risk, causing a wide variety of illnesses and infections, with potentially fatal consequences in vulnerable populations with weaker immune systems, such as children and the elderly. Microbiological pollution also has a major economic impact. In Los Angeles and Orange Counties alone, 600,000 to 1.5 million gastrointestinal illness cases are caused by contamination of recreational water annually, with healthcare costs in excess of \$21 million (Given et al. 2006). Additionally, beach access closures due to water quality issues impact local tourist-based economies and property prices.

The use of natural waters for recreational activities requires regular quality control checks to ensure public safety (European Council 2006; U.S. EPA 2012). The concentration of *Escherichia coli* (*E. coli*) bacteria shows direct correlation with gastrointestinal illness rates associated with recreational use and is generally accepted as a suitable indicator of overall fecal contamination. There is a positive correlation between the number of *E. coli* bacteria and the occurrence of gastrointestinal illness.



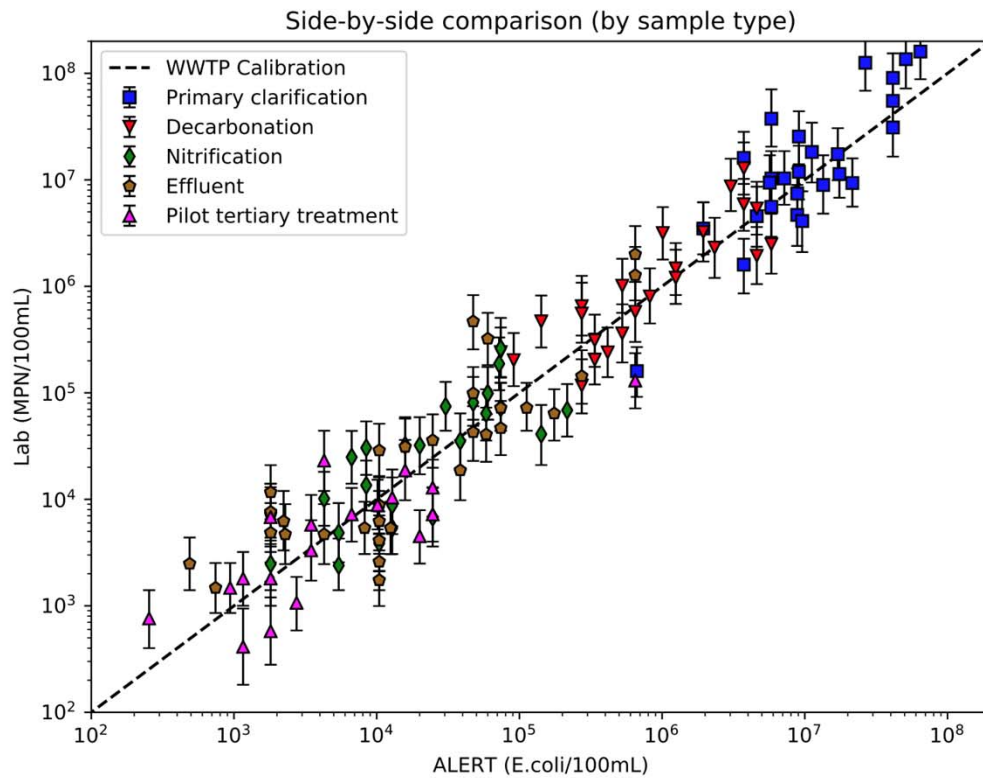
Angelescu, D. E. et al. (2018). Autonomous system for rapid field quantification of *Escherichia coli* in surface waters. *Journal of Applied Microbiology*, 126, 332–343.



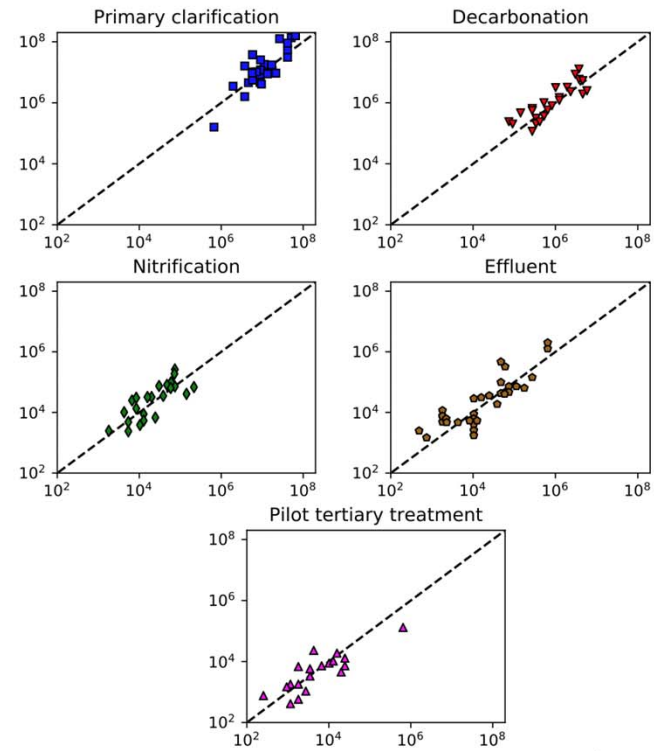
# E.coli measurements in wastewater (raw and treated)

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Soon to appear in:



# Houseboats, barges and packetboats

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Early Days of Rapid Transit / Edward Lamson Henry

<https://depositphotos.com/123024826/stock-photo-amsterdam-canal-singel-with-dutch.html>



<https://harborcottagehouseboats.com/galleries/>



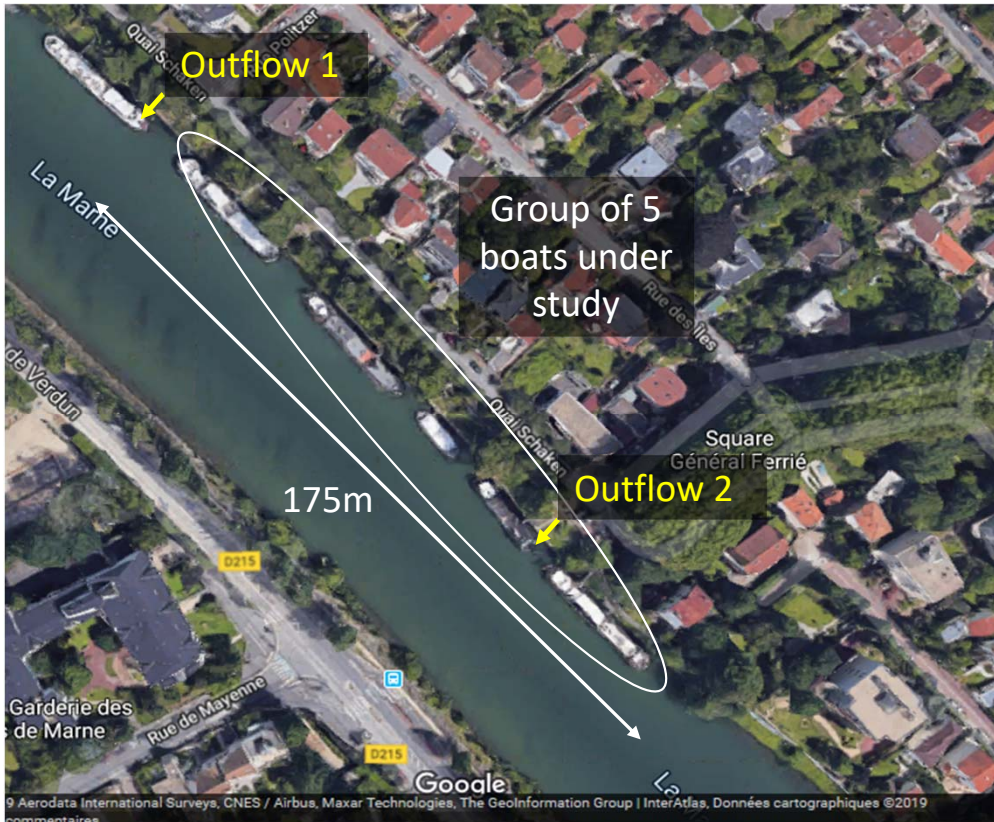
[https://commons.wikimedia.org/wiki/File:France,\\_Paris,\\_des\\_p%C3%A9niches\\_sur\\_la\\_Seine\\_entre\\_le\\_Pont\\_des\\_Arts\\_et\\_le\\_Pont-Neuf.jpg](https://commons.wikimedia.org/wiki/File:France,_Paris,_des_p%C3%A9niches_sur_la_Seine_entre_le_Pont_des_Arts_et_le_Pont-Neuf.jpg)



une célébration d'une vie

Preparing for the  
2024 summer  
Olympic games:  
A major river bacterial  
reduction action  
throughout Paris region

# Setup and methodology



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## Three experimental stages:

1. High-frequency impact measurements during synchronized pollution (in-situ dispersion study)
1. Greywater measurements by source of water (shower, laundry, dishwasher) and by boat
2. Long-term in-situ *E.coli* time-series and differential microbiology between upstream and downstream locations

# In-situ dispersion study

## Objectives :

1. Measure pollution travel time
2. Identify extent of lateral pollution
3. Measure downstream dilution factors
4. Measure downstream pollution duration

## Techniques :

1. Simultaneous flushing
2. Time-lapse fluorescein tracing
3. 3-camera video recording
4. Downstream sampling (manual/drone)
5. Fluorescence measurements (comparator)
6. *E.coli* measurements

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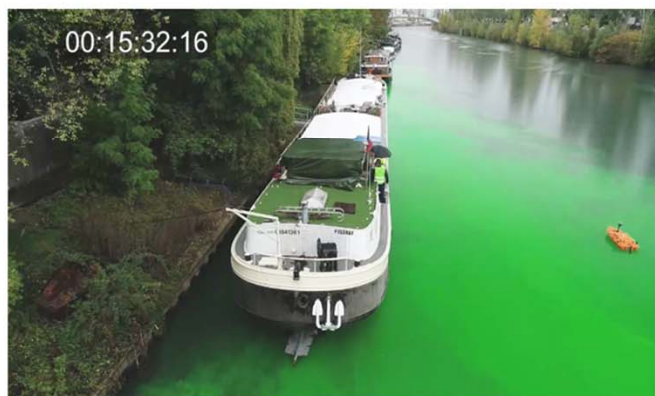
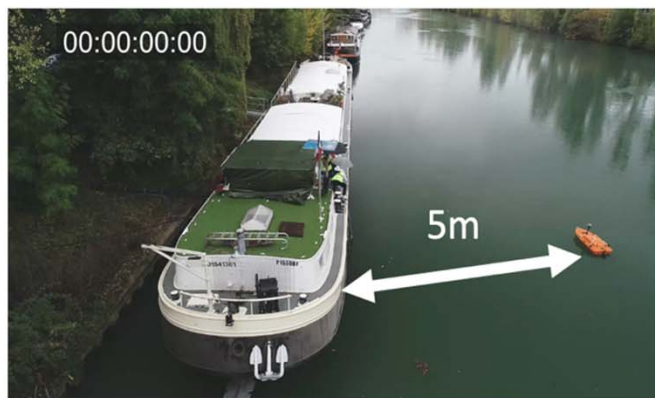
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# Time sequence of synchronized pollution

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# Lateral extent of downstream pollution

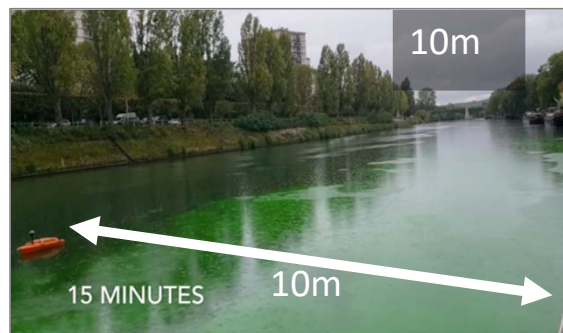
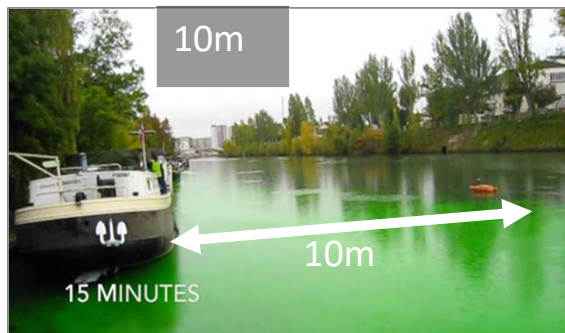
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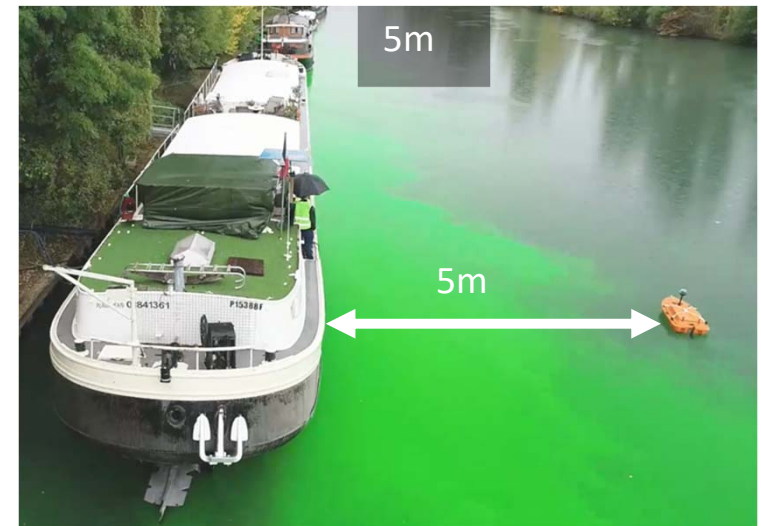
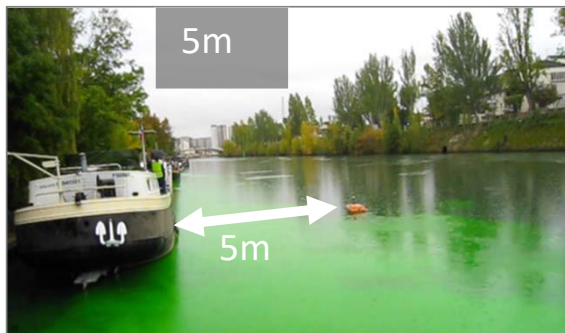
Maximum lateral impact: at 15' (fixed camera and drone images)

Upstream

Downstream



Lateral extent of pollution  
11m maximum, observed at 15'



# Dilution factor determination ●

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## Real river matrix (Marne) fluorescence comparator

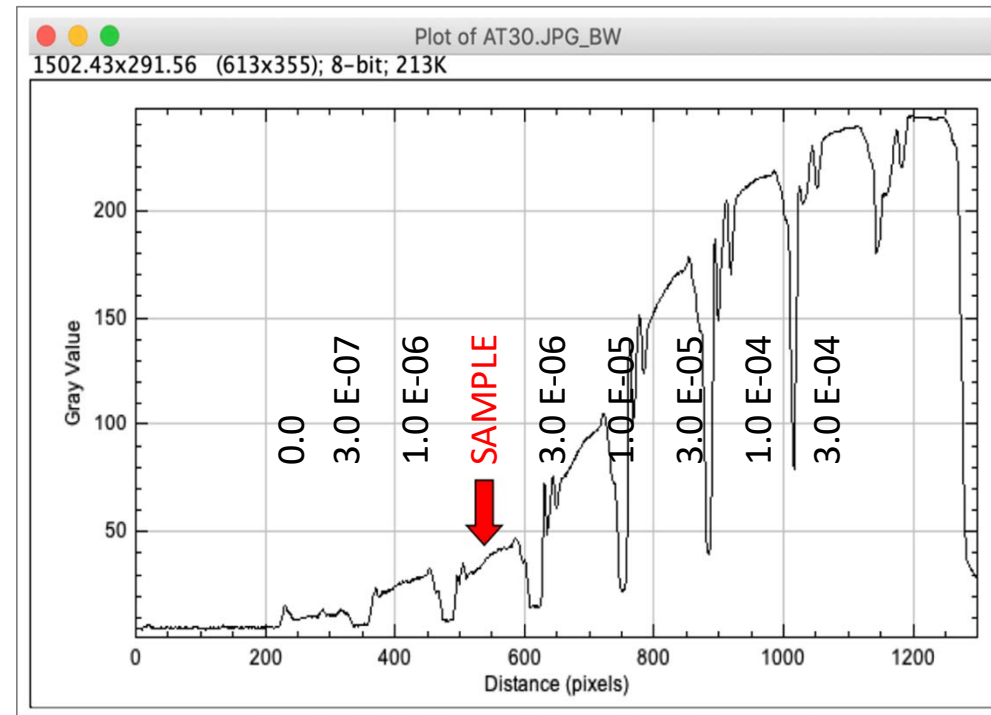
Fluorescein solution 300g/L (Fluotechnik)

Linear interpolation between closest standards

Standard range: 0.0 – 3.0E-04

Reliable quantification range: 1.0E-07 - 1.0E-04

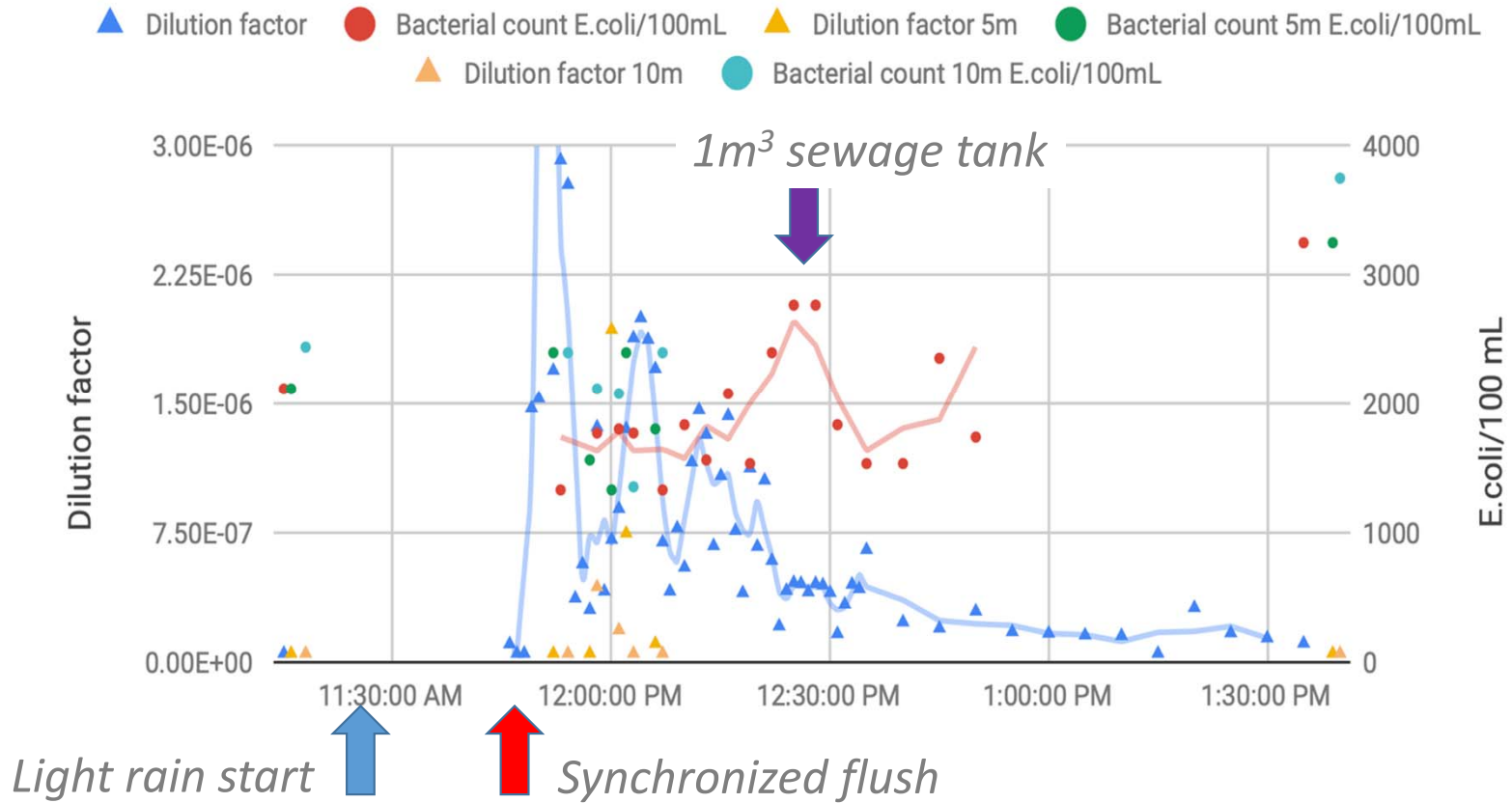
Quantification error: larger of 10% or 1.0E-07



# In-situ dispersion results

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## Gray water and raw sewage measurements

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Showers: High variability, between 0 and  $5,0 \times 10^5$  E.coli / 100mL

Dishwasher : High variability, between 0 and  $2,5 \times 10^4$  E.coli / 100mL

Laundry: between 0 and  $1,9 \times 10^3$  E.coli / 100mL

1m<sup>3</sup> sewage tank:  $7,5E+05 - 1,0E+06$  E.coli / 100mL

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### Consistency check for 1m<sup>3</sup> sewage tank:

2L Fluoresceine solution in 1000L : initial in-tank dilution  $2,0E-03$

Downstream measured dilution factor: between  $3,0E-07$  and  $1,0E-06$



Actual sewage dilution factor:

between  $1,5E-04$  and  $5,0E-04$

Downstream calculated E.coli contribution : 112 – 500 E.coli / 100mL

Downstream measured E.coli contribution : < 1000 E.coli / 100mL

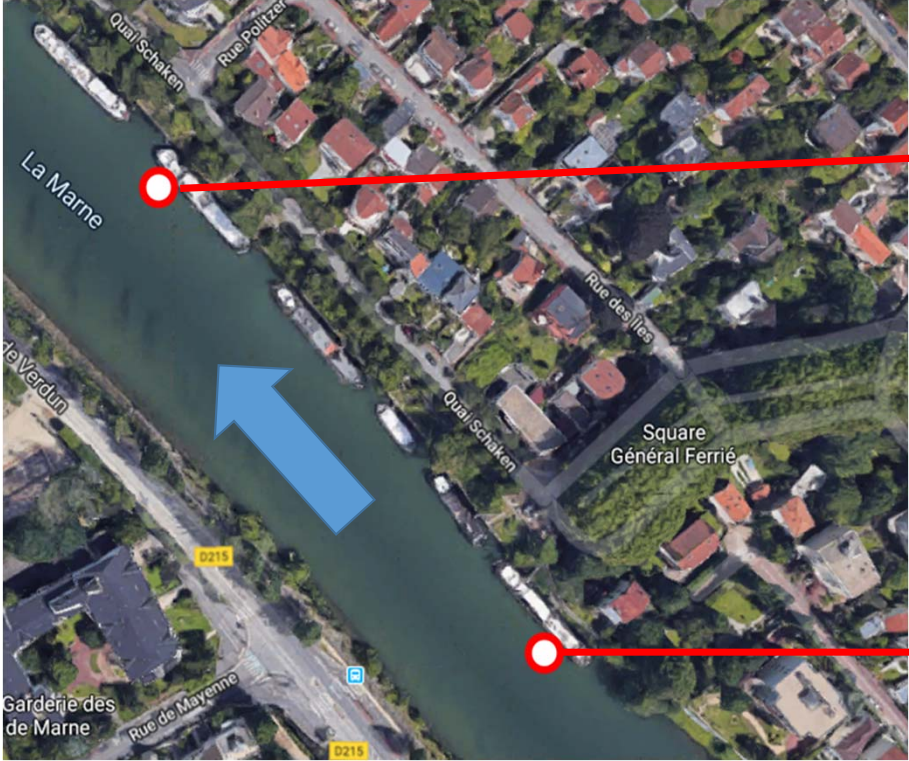
Good correlation between theory and measurement

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# Long-term installations

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Upstream installation

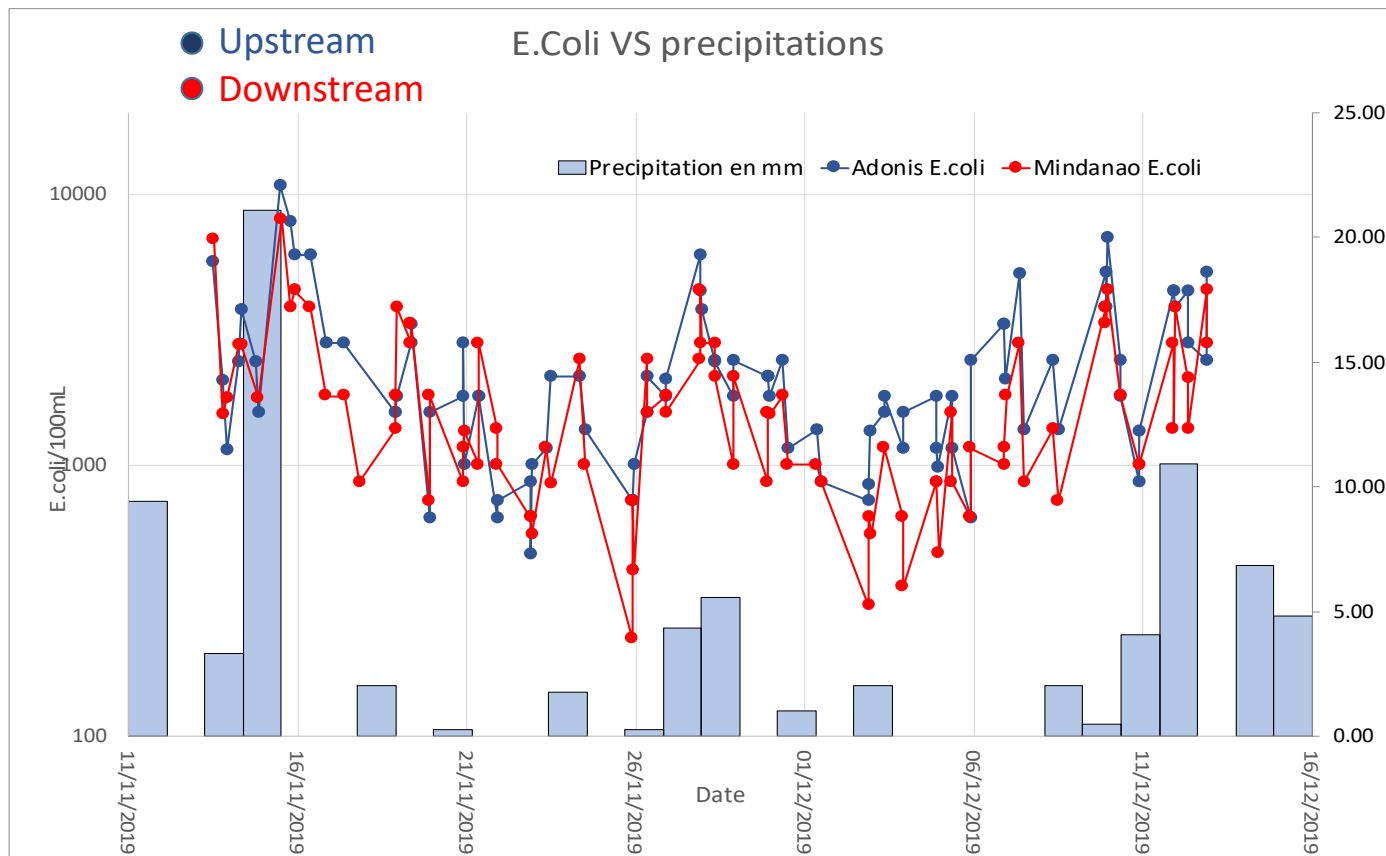


Downstream installation

# In-situ *E.coli* long-term time-series (30 days, 3 measurements / day)

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## Some interesting conclusions

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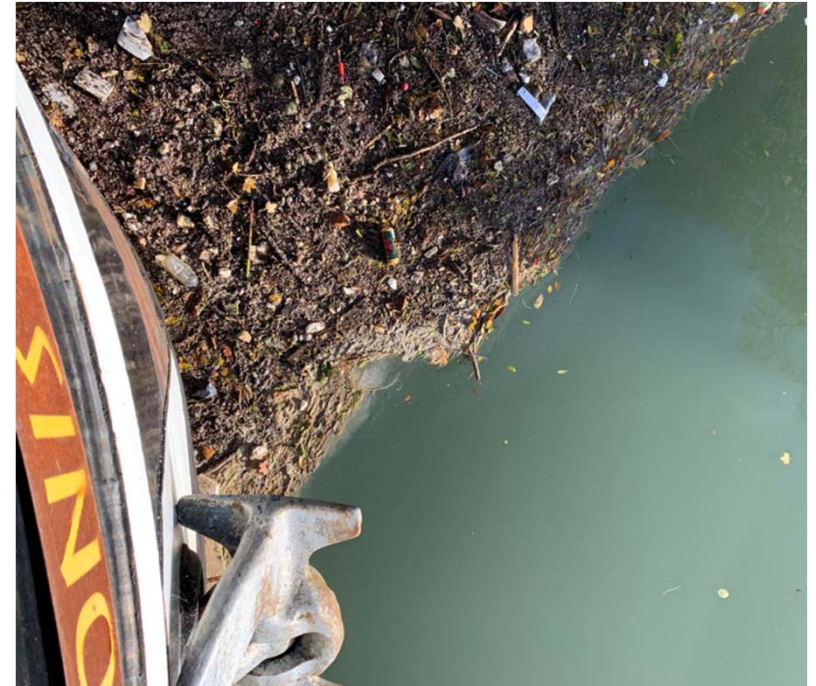
Downstream measurements consistently higher than upstream (80% of the measurement), suggesting local source of contamination upstream

High variability of greywater bacterial content

$10^{-3}$  –  $10^{-4}$  dilution factors for homogenized wastewater: 150m downstream impact non-measurable for greywater at typical volumes used

Effect of land-based outflows significantly higher than untreated boat sewage

Solid fecal matter can have large impact farther downstream: treatment and disinfection advised

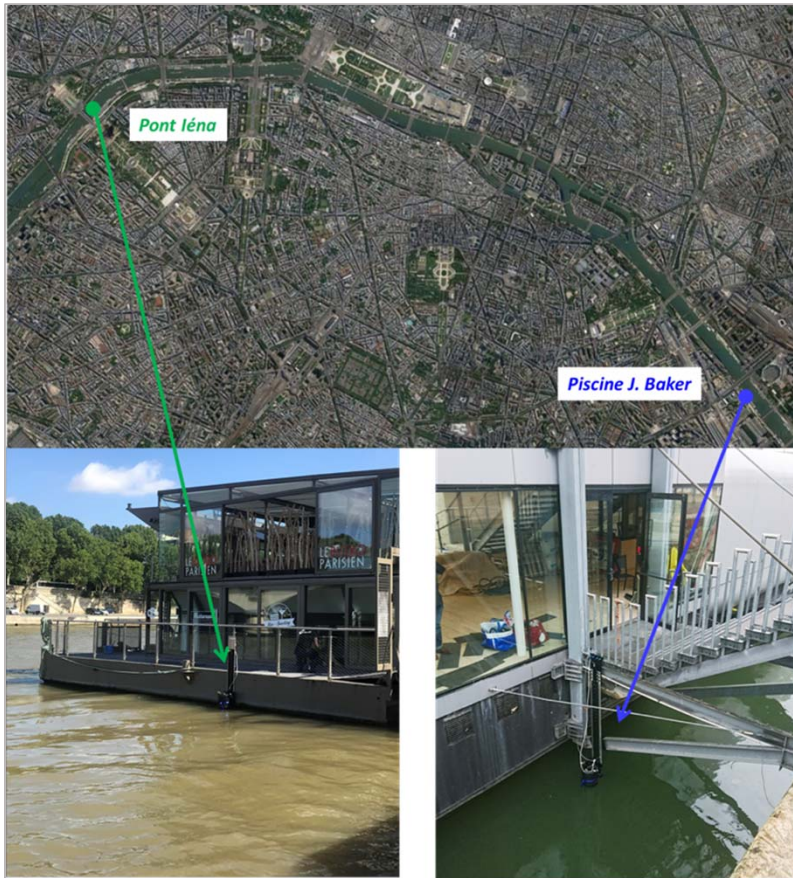


Floating debris accumulation upstream due to local river flow configuration

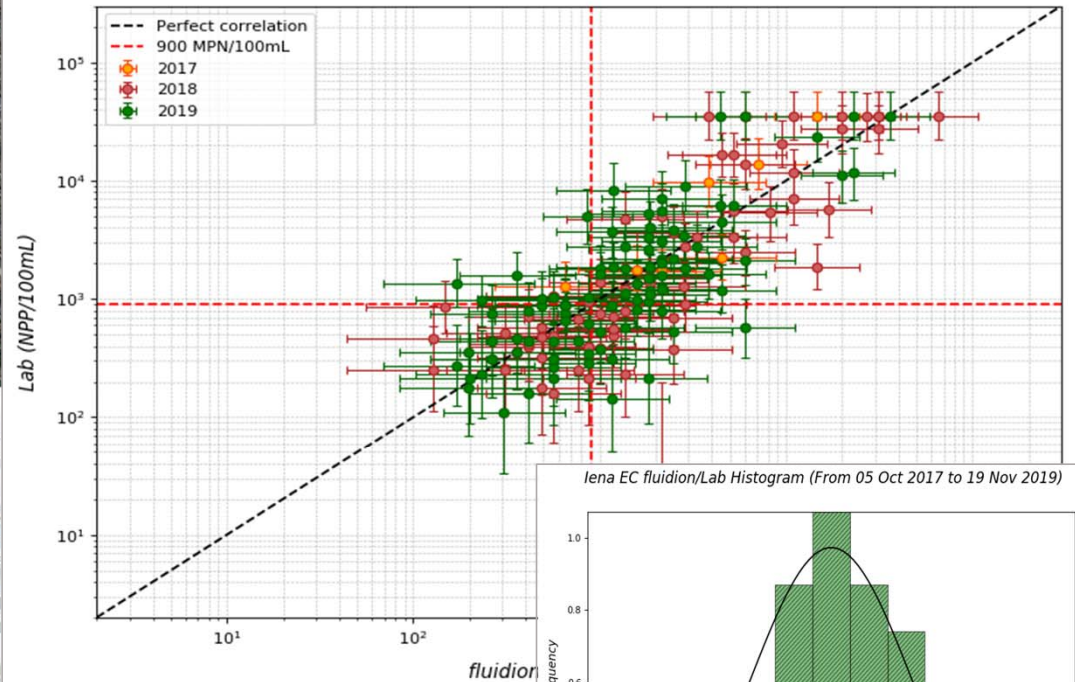
# Long-term river monitoring

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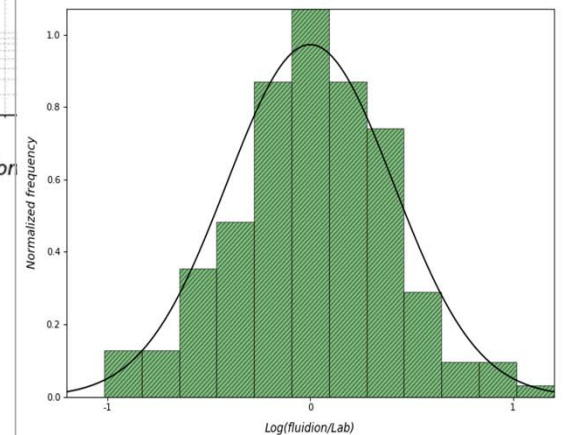
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Iéna EC fluidion-Lab correlation (From 05 Oct 2017 to 19 Nov 2019)



Iéna EC fluidion/Lab Histogram (From 05 Oct 2017 to 19 Nov 2019)

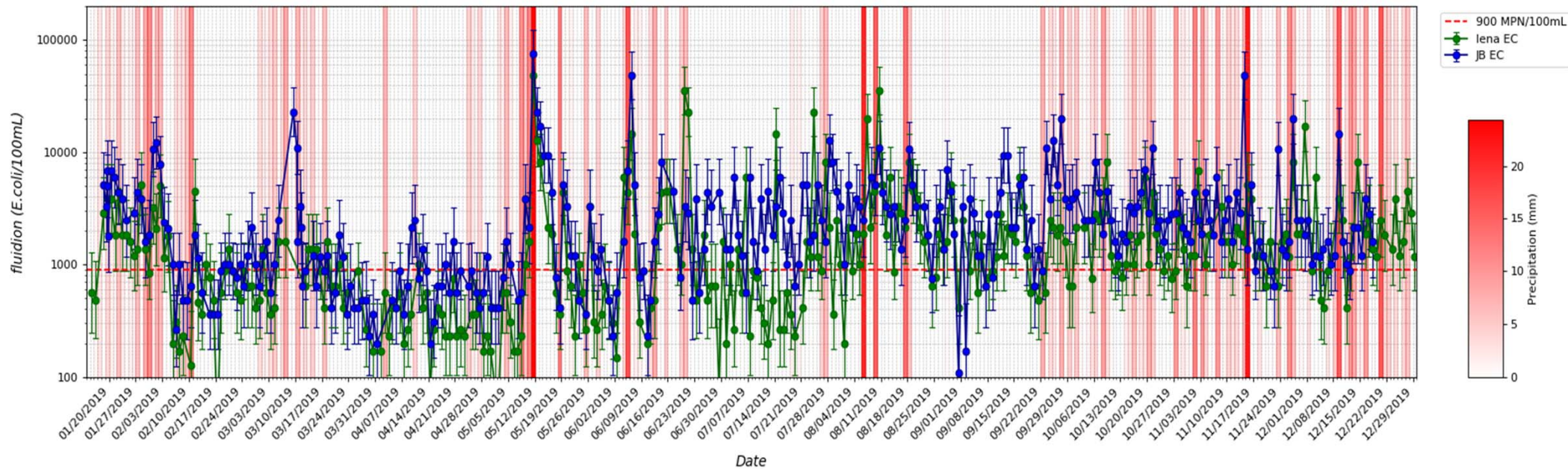


# Stormwater / CSO monitoring 2019 Paris data

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*lena and JB Time Series with Rain (From 15 Jan 2019 to 29 Dec 2019)*



# Evaluating mitigation actions

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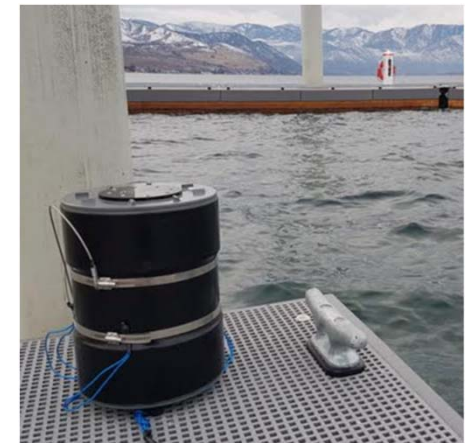
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Pump-out stations installed at Lake Chelan marinas  
Protecting a sensitive and pristine environment  
Using ALERT System to evaluate long-term effects  
Outstanding results (majority of measurements below detection limit of 4 E.coli /100mL)



Lake Chelan Research Institute



# Evaluating impact of illegal sewage dumping

Measuring bacterial contamination in Tijuana river (2017, on-going)



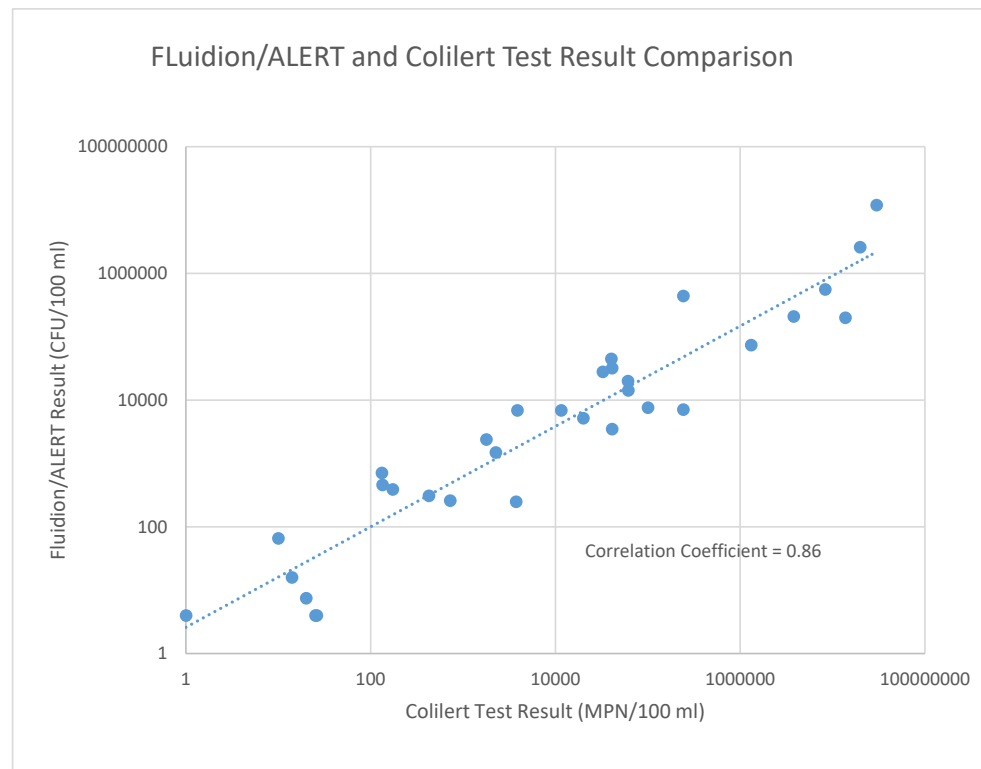
*Excellent comparison with data from EPA-approved methods over 8 LOG units.*

This study was instrumental in issuance of an investigative order by the California regional water quality control board (San Diego) for:

INVESTIGATION OF POLLUTION, CONTAMINATION, AND NUISANCE FROM TRANSBOUNDARY FLOWS IN THE TIJUANA RIVER VALLEY

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# Conclusions

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- A novel methodology for in-situ monitoring of microbiological pollution (*E.coli*)  
Identification and quantification of localized and diffuse pollution sources
- Robust impact measurement on the receiving water bodies
- Long-term time-series evaluation
- Robotic and automated technologies (drone sampling and visual monitoring, autonomous instruments)

- ❖ Impact studies in sensitive areas
- ❖ Bathing water active management
- ❖ Pollution source identification
- ❖ High-resolution environmental monitoring

# Acknowledgements

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The following organizations provided support and / or expertise that made this work possible:



Lake Chelan Research Institute



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**Thank you !**

For any questions or requests: [contact@fluidion.com](mailto:contact@fluidion.com)

