

ENVIRONMENTAL BIOTECHNOLOGY NETWORK

Environmental Sensors and Wastewater Surveillance WG



Environmental Sensors and Wastewater

Surveillance (ESWS WG)



Led by [Prof Zhugen Yang](#), Cranfield University and [Dr Martin Spurr](#), Newcastle University

The Environmental Sensors and Wastewater Surveillance (ESWS) WG promotes state-of-the-art sensors to enable point-of-use analysis of pollutants in water, soil, and air at sites of sample collection. Wastewater surveillance (particularly wastewater-based epidemiology), has emerged as a novel monitoring mechanism for early warning of infectious disease, including Covid-19, and is growing as a global technique to monitor public health at a community level.

The WG gathered a multidisciplinary group of researchers with interests in new environmental sensors development, including the underpinning principles, design, materials, methods, devices and applications particularly in wastewater analysis, but also water, air, soil, food, healthcare, biosecurity and beyond. This group provides an opportunity to exchange knowledge and ideas, present early findings, build collaborations and explore interdisciplinary funding opportunities. The WG also aims to enable training for early-career researchers and industry sectors.

Activity Synopsis

Timing is everything and this WG came about at a busy time for biosurveillance. A set of webinars tackled a wide range of topics.

Webinar [Biosensors and Environmental Monitoring – New Developments in Environmental Biotech](#)

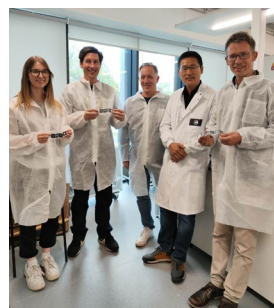
Webinar [Developing the Next Generation of Monitoring for Human and Environmental Protection](#)

Webinar [Mass Spectrometry in Wastewater-based Epidemiology \(WBE\) for the Determination of Small and Large Molecules as Biomarkers of Exposure- Needs for COVID-19 Testing with Environmental Proteomics \(EP-WBE\)](#)

Webinar [Wastewater Monitoring for Public Health: Research from the Environmental Monitoring for Health Protection](#)

Webinar [Fishing for Viruses in Sewage. A tool for understanding the molecular epidemiology of tiny human pathogens](#)

Webinar [Gout, bigger muscles and a sweet tooth – what other than Covid-19 have we been monitoring in wastewater?](#)



EBNet also supported events including a major conference ‘[Testing the Waters 6](#)’ in Oxford in 2023, a blended event at Cranfield on [Nanobiotechnology Enabled Sensing](#) of Current and Emerging Pandemic Threats, a discussion on [Nature Water](#): A Journal for All Water-related Research, and a [masterclass](#) on Advanced Sensor Technology.

Activity Synopsis ctd

Outreach activities by the WG included an article in the ADBA magazine on how [Track and Trace Tests Start to Fingerprint Environmental Pollution](#).

The WG area was popular with Early Career Researchers, with 15 presentations at EBNet's ECR events related to this topic. Two small ECR travel bursaries were awarded for a conference in London, and one larger [bursary](#) for a [presentation](#) in Houston TX, USA.

EBNet supported three very productive proof-of-concept (POC) projects in this area.

POC202214 [CRISPR/Cas-enabled paper-based sensors for rapid monitoring of antimicrobial resistance](#)

POC202113 [Fluorescent Microbiofilter Assay for Rapid Real-time Monitoring of Organic Micropollutants Biodegradation](#)

POC202101 [Faecal pollution source tracking and quantitative microbial risk assessment methods for a suitcase laboratory](#)

Follow-on funding related to POC202214 has included [MR/Y015223/1](#) Revealing new insights into MDR evolution, ecology and transmission across human, animal and environmental microbiomes and [BB/X012840/1](#) under the Anglo-Canadian Collaboration on Antimicrobial Resistance (ACCAMR).

WG Journal Papers

[CRISPR-enabled sensors for rapid monitoring of environmental contaminants](#). Wang, Y., Pan, Y., Han, W., Rossi, C.S., Hui, Q., Guo, Y., Owoseni, M.C., McAdam, E., Yong, Y.C., Wang, B. and Yang, Z., 2024. TrAC Trends in Analytical Chemistry, p.118128.

[Targeted Enrichment of Nucleic Acid Bionic Arms Enhances the Hydrolysis Activity of Nanozymes for Degradation and Real-Time Monitoring of Organophosphorus Pesticides in Water](#). Zhou, J., Xiong, D., Zhang, H., Xiao, J., Huang, R., Qiao, Z., Yang, Z. and Zhang, Z., 2025. Environmental Science & Technology.

[Environmental DNA clarifies impacts of combined sewer overflows on the bacteriology of an urban river and resulting risks to public health](#). Zan, R., Blackburn, A., Plaimart, J., Acharya, K., Walsh, C., Stirling, R., Kilsby, C.G. and Werner, D., 2023. Science of the Total Environment, 889, p.164282.

[BODIPY-labeled estrogens for fluorescence analysis of environmental microbial degradation](#). Felion, C., Lopez-Gonzalez, R., Sewell, A.L., Marquez, R. and Gauchotte-Lindsay, C., 2022. ACS omega, 7(45), pp.41284-41295.



Testing the Waters 6 Conference



Professor Zhugen Yang
Cranfield University
POC202214



CRISPR/Cas-enabled paper-based sensors for rapid monitoring of antimicrobial resistance

CONTEXT and AIM

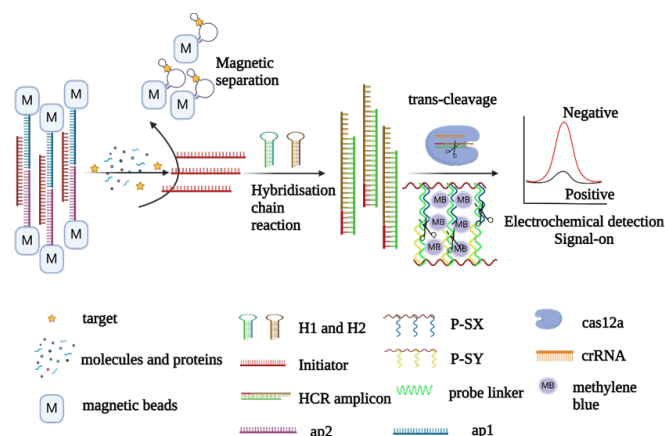
Environmental contamination with pathogenic bacteria and chemical pollutants is a global issue. However, current analytical methods for environmental samples are challenged by the complexity and heterogeneity of the matrices, as well as the ultra-low concentrations of the analytes.

Beyond its extraordinary genome editing ability, clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated systems (CRISPR/Cas) have recently initiated a new era of biosensing applications. Their high base resolution and isothermal signal amplification provide ultrasensitive, single-molecule level and highly specific sensing.

This capability can significantly improve the detection of low-level contaminants such as antibiotics (including β -lactams etc) in wastewater samples and aid in interpreting the detection with the engineering method onto devices for point-of-need monitoring (e.g. paper-microfluidic analytical devices).

The aim of this work was to develop a novel, ultra-sensitive and low-cost sensing platform to identify chemical pollutants in the environment. This in turn will leverage the CRISPR/Cas platform to offer a range of next-generation environmental sensors for rapid and on-site monitoring of chemical contaminants.

Environmental contamination is a silent crisis threatening ecosystems and human health worldwide. Traditional methods for detecting pollutants are often too slow, costly, or impractical for real-world monitoring". By harnessing the unparalleled precision of CRISPR/Cas systems alongside adaptive bio-recognition elements, we aim to redefine environmental sensing" – Zhugen Yang, Professor of Biosensing and Environmental Health



OUTCOME

A hydrogel-based HCR-CRISPR/Cas bioassay was designed for kanamycin detection. More specifically, we designed a signal amplification strategy to enhance the signal of antibiotics using an aptamer to specifically target kanamycin. The hydrogels wrapping methylene blue particles were synthesised and characterised through square wave voltammetry (SQW), which can be used as redox marker for electrochemical detection of antibiotics. We spent a long time understanding the binding between the selected aptamer and antibiotics using simulation, and we found it is important to identify the high affinity aptamer against the antibiotics.

The next tasks are to optimise the CRISPR/Cas system for signal amplification, and further evaluate the limit of detection, then use the optimised assay to detect antibiotics in real samples.

This PoC has led to two UKRI-funded international grants on AMR: UK-Canada (BB/X012840/1) and UK-China (MR/Y015223/1).





Dr Caroline Gauchotte-Lindsay
University of Glasgow
POC202113



Fluorescent microbiofilter assay for rapid real-time monitoring of organic micropollutants biodegradation

AIM

Microbial communities on biofilters form stochastically, based on the chemistry and biology in the raw water. We contend that targeted design of microbial ecology for degradation of difficult-to-treat organic micropollutants (OMPs) could be achieved by optimising said chemistry and biology using genetic algorithms. While this would require *in situ* testing of the responses of microbial communities to OMPs, no fast, inexpensive, reliable analytical methods to measure OMPs in microbial cultures are currently available.

Current gold standard methods provide precise and accurate results, but at best hours and most likely days after sampling, by which time conditions in the biological systems may have changed drastically. We aim to design and validate a new microwell plate assay to measure the biological removal of OMPs in microscale biofilters.

The plate wells will be filled with a transparent porous medium that acts as the substratum for biofilms, whose degradation of fluorescently tagged OMPs will be monitored using fluorescent excitation-emission. By the end of the project, we hope to deliver a robust, precise and sensitive assay that can be further integrated to high-throughput robotic platforms for rapid and site-specific optimisation of microbial seeds of full-scale biofiltration systems.

For video see :

https://youtu.be/SiC_MceeNAs

WHAT NEXT?

Research is continuing within the EPSRC Decentralised Water Technologies (EP/V030515/1) programme grant, in which Caroline Gauchotte-Lindsay (CGL) is a Co-I. Validation with relevant natural communities will be carried out within this grant. Once validated, she will take the project to the Converge Challenge.

RESULTS

With the EBNet PoC funding, we were able to demonstrate the feasibility of a high throughput assay for rapid selection of microbial seeds for the degradation of 17 β estradiol (E2).

Click chemistry was employed to attach a linker on a labile hydrogen of E2, and a green fluorescent BODIPY to the linker. The fluorescent tag enabled specific detection and quantification of the tagged compound in the media using a spectrofluorometer, with limits of detection much lower than standard analytical methods.

We further demonstrated in batch planktonic experiments that the fluorescent E2 could be taken up and degraded by known heterotroph E2 degraders; we were also able to accelerate this with an additional carbon source. Cell culture inserts for wells with a porous membrane at the base were shown to be a suitable medium for growth of the biofilm.

We were able to establish the conditions, necessary controls, quality control and quality assurance, and final protocol of a 24-well plate assay to select the most efficient of three degrading communities using real time fluorescence monitoring in a plate reader.



PUBLICATION:

BODIPY-labelled estrogens for fluorescence analysis of environmental microbial degradation
By: Felion, C., Lopez-Gonzalez, R., Sewell, A. L., Marquez, R. and Gauchotte-Lindsay, C. In: ACS Omega, 7(45), pp. 41284-41295.





Professor David Werner
Newcastle University
POC202101



Faecal Pollution Source Tracking & Quantitative Microbial Risk Assessment methods for a Suitcase Laboratory

“The workshop was an opportunity for me to refresh some molecular biology techniques and, most importantly, to learn and update myself on more recent techniques in the field. I also had the chance to meet other researchers from across Africa and to begin to build a network of researchers for future collaborations. Thank you for being very supportive and for investing your time and experience into building capacity on the African continent.”

George Mangse, Lecturer at Nile University of Nigeria, on molecular microbiology training with the suitcase laboratory at the Addis Ababa Water and Sewerage Authority

AIM

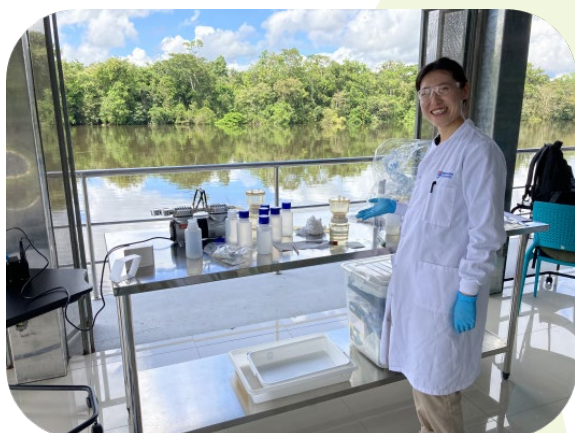
Using a suitcase laboratory that contains the MinION sequencing device of Oxford Nanopore Technologies, over 100 comprehensive water microbiome data sets have been collected from around the globe. This desk study exploits the global data for the development of multivariate data analysis tools which facilitate faecal pollution source attribution and related quantitative microbial risk assessments by suitcase laboratory users. The goal is to enable the wider uptake of an affordable suitcase laboratory for molecular water microbiology in environmental surveying and wastewater treatment biotechnology applications around the world.

RESULTS

Newcastle University researchers have developed an innovative ‘**Lab in a Suitcase**’, a portable water testing lab, that can be used to comprehensively screen water samples for faecal pollution and waterborne hazards. This suitcase laboratory brings advanced genetic testing methods within reach of water, sanitation and hygiene (WASH) researchers working in low- and middle-income countries. It is also suitable for rapid onsite water quality testing by WASH professionals around the world who run sewage treatment works, manage livestock on farms, assess bathing waters and provide humanitarian aid to communities displaced by natural or human-made disasters.

The Newcastle University researchers developed a protocol for quantitative microbial risk assessment (QMRA) which uses data generated with the suitcase laboratory to rapidly assess risks of contracting gastrointestinal diseases from water that is polluted with faecal matter.

Researchers designed and delivered a hands-on training workshop. The suitcase laboratory was used to detect genetic markers for human sewage pollution in the urban river Ouseburn. Results were obtained within 3 hours of sampling. In Ethiopia, trainees gained hands-on experience in analysing river water and wastewater samples



PUBLICATION:

Environmental DNA clarifies impacts of combined sewer overflows on the bacteriology of an urban river and resulting risks to public health. *Rixia Zan, Adrian Blackburn, Jidapa Plaimart, Kishor Acharya, Claire Walsh, Ross Stirling, Chris G. Kilsby, David Werner. Science of The Total Environment. Volume 889, 1 September 2023, 164282*





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<https://ebnet.ac.uk/resources/>.

