INNOVATIONS IN HOW WE MONITOR OUR WATER ENVIRONMENT

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DCU Water Institute

Overview

- Internet of things and decision support
- Current approaches
- New technologies for rapid testing
- Biosensors
- Catchment monitoring
- Innovations in sensing – eDNA
- Involving Citizens

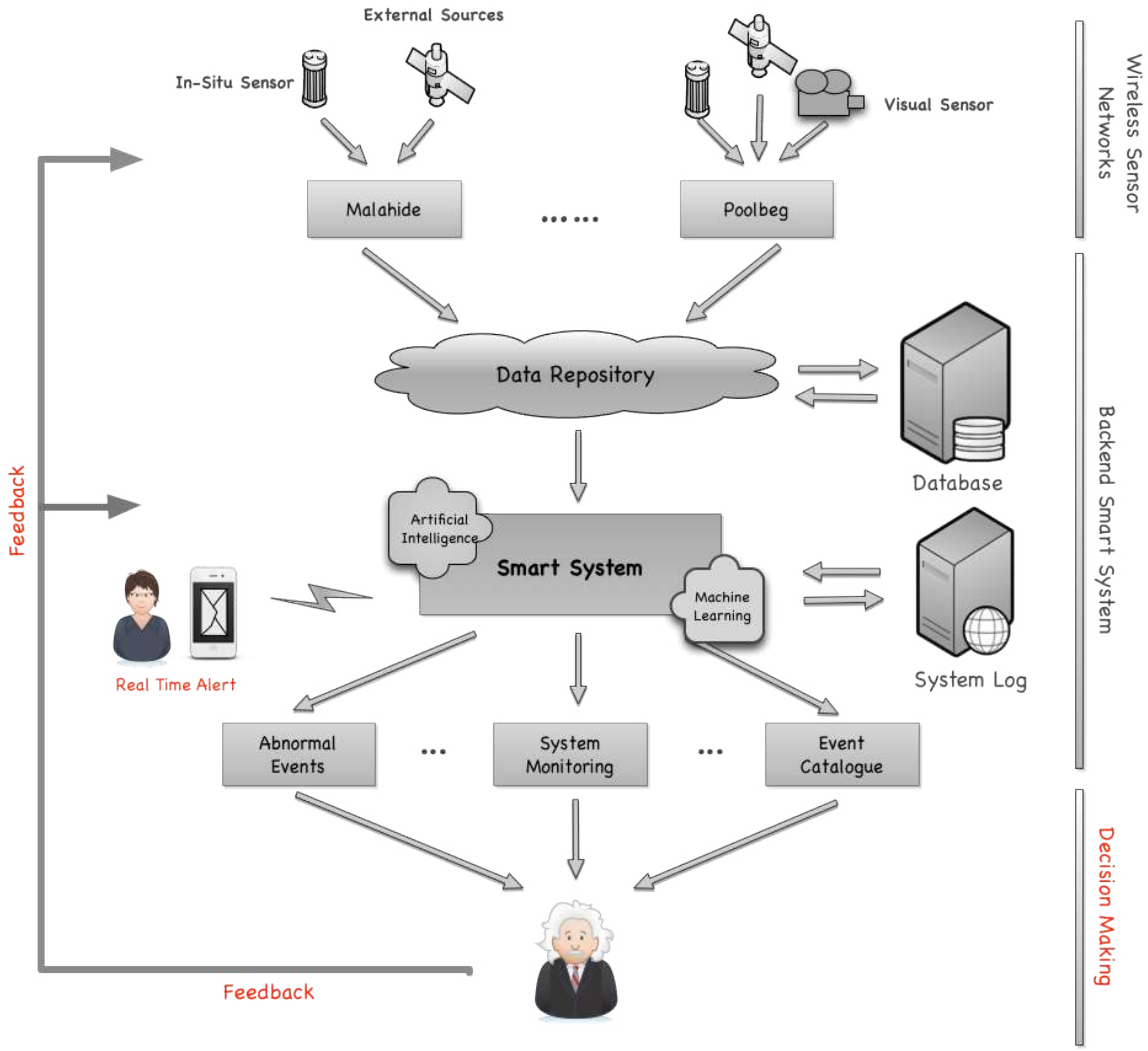
For Water

Emerging contaminants and health

Novel sensors and biosensors

Data analytics
Water IoT

Analytical methods and innovative engineering solutions

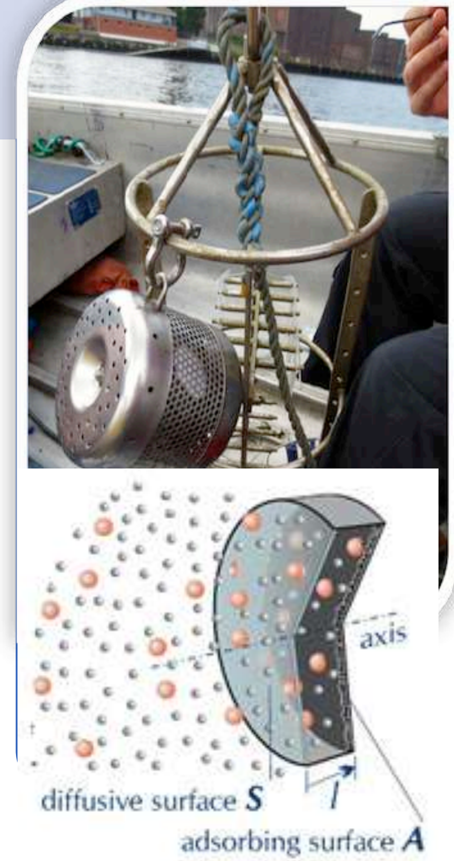


Critical to this is information

More devices collecting data

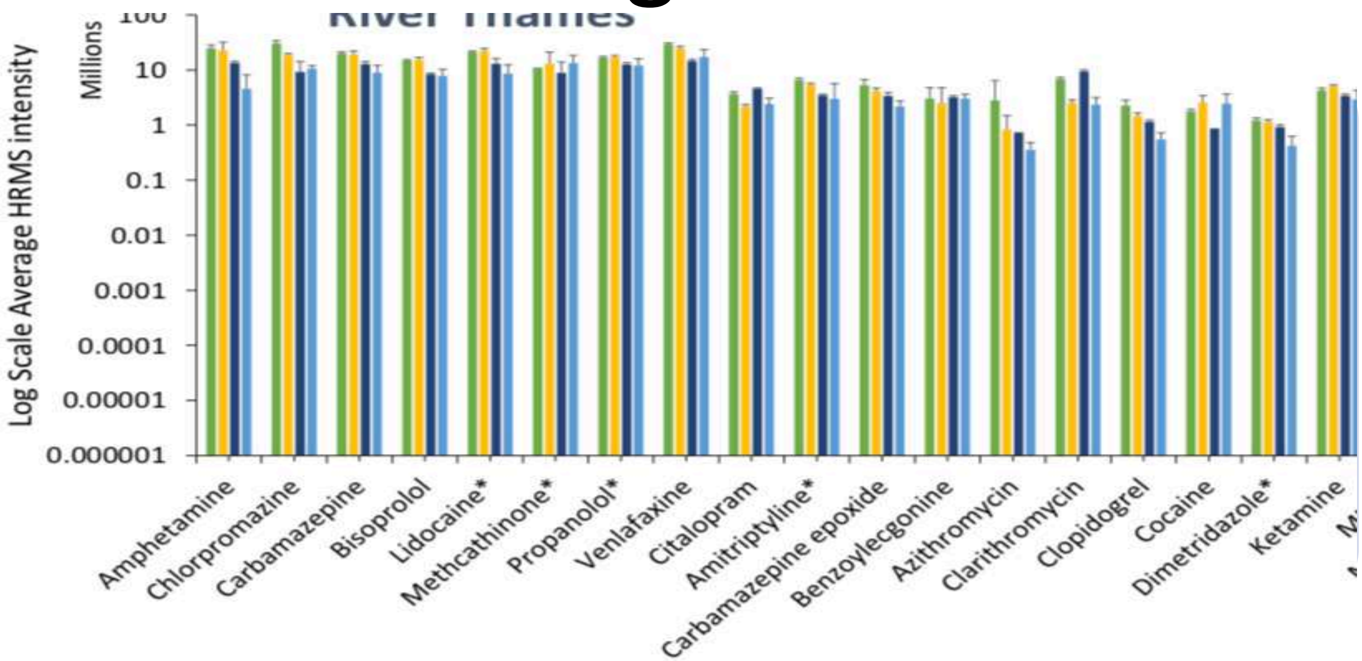
4.36m
Floating pontoon with integrated
power and telemetry
Multiparameter
sonde
2.5 m
c. 8 m
Sediment

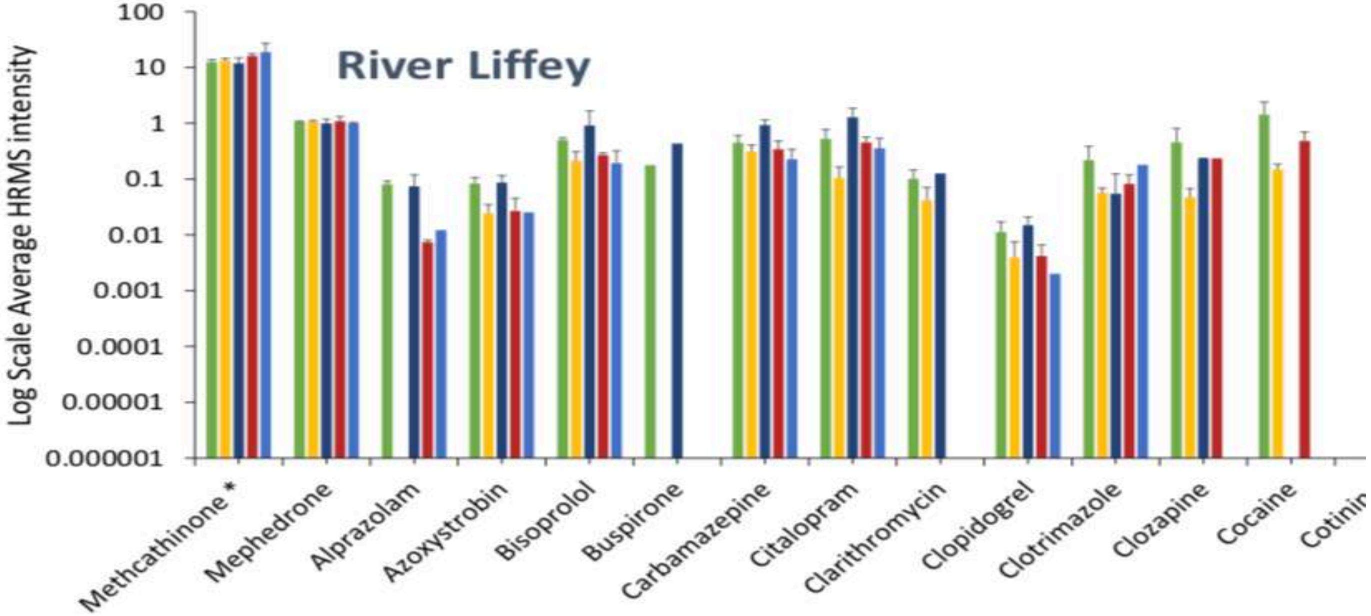
Are technology innovations meeting the needs?



Current Monitoring approach: Compliance, surveillance, investigative ➔ Levels of pollutants can vary temporally and spatially➔

Episodic events could be missed, or conclusions could be drawn on the basis of what may only be transitory high levels.

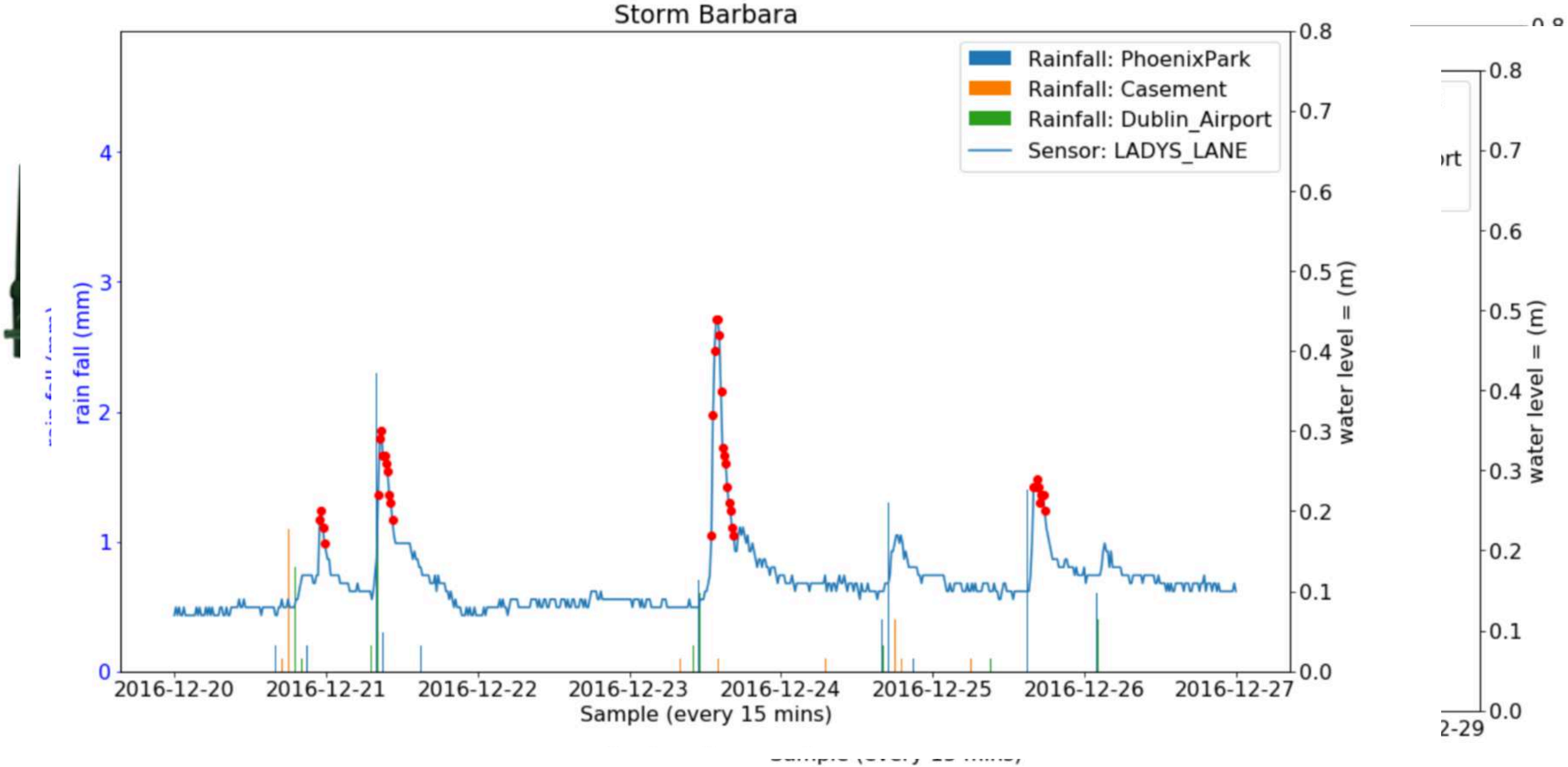




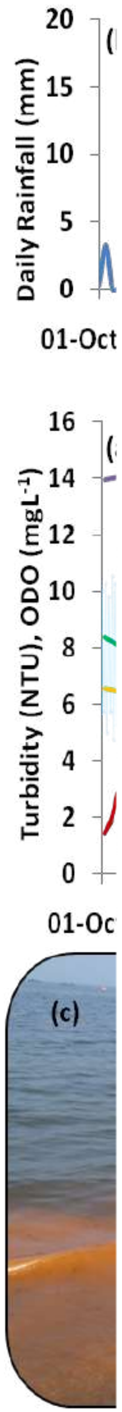
Low cost distributed sensing

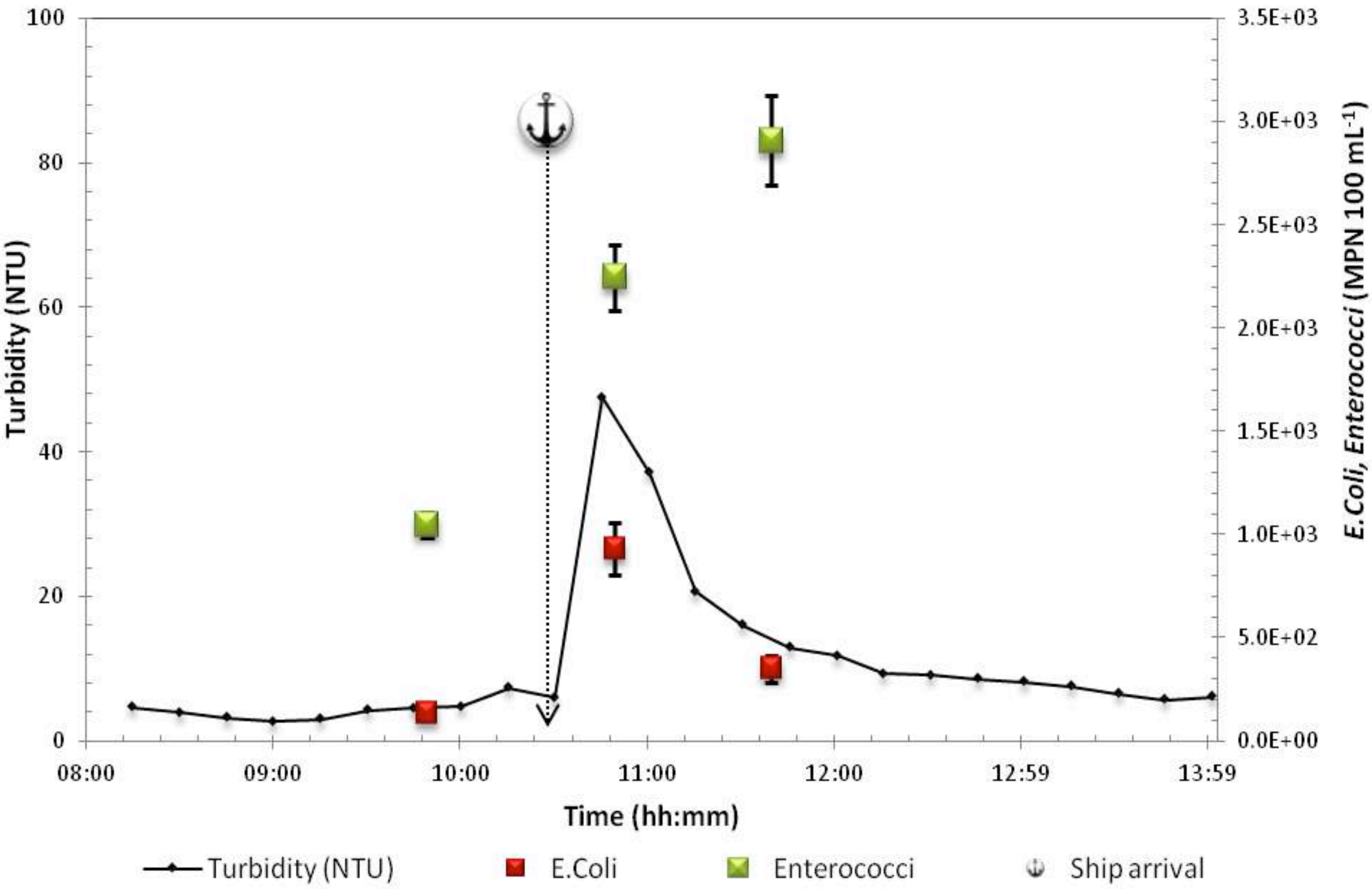
Information

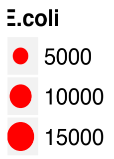
Decision support



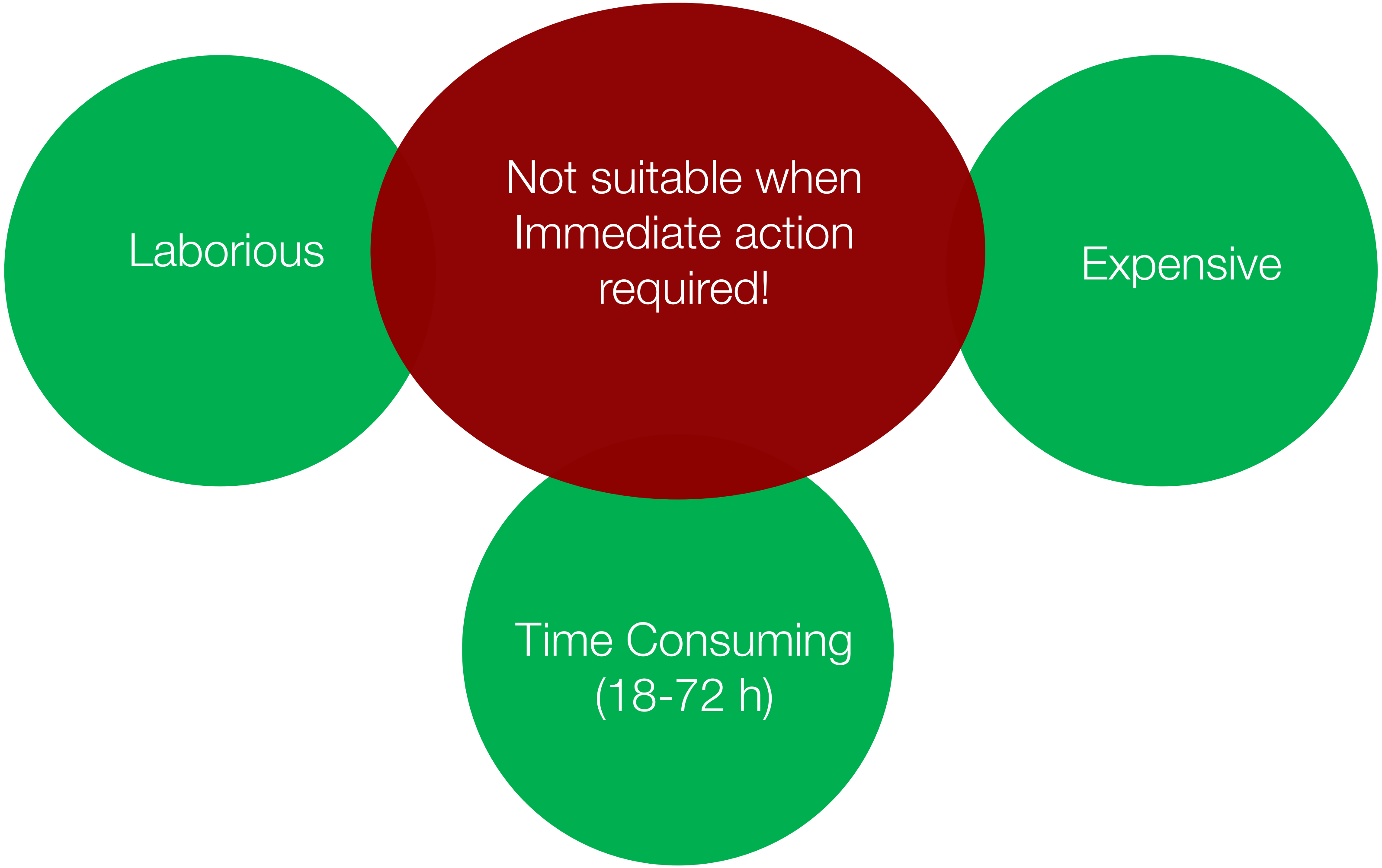
Storm Barbara
Rainfall: PhoenixPark
Rainfall: Casement
Rainfall: Dublin_Airport
Sensor: LADYS_LANE
rain fall (mm)
water level = (m)
Sample (every 15 mins)
2016-12-20
2016-12-21
2016-12-22
2016-12-23
2016-12-24
2016-12-25
2016-12-26
2016-12-27


20
15
10
5
0
Daily Rainfall (mm)
01-Oct
16
14
12
10
8
6
4
2
0
Turbidity (NTU), ODO (mgL-1)
01-Oc
(c)


100
80
60
40
20
0
Turbidity (NTU)
3.5E+03
3.0E+03
2.5E+03
2.0E+03
1.5E+03
1.0E+03
5.0E+02
0.0E+00
E.Coli, Enterococci (MPN 100 mL-1)
08:00
09:00
10:00
11:00
12:00
12:59
13:59
Time (hh:mm)
Turbidity (NTU)
E.Coli
Enterococci
Ship arrival


E.coli
5000
10000
15000

BACKGROUND-METHODS



- ➢Need for early warning technologies

Colisense assessment for rapid detection of faecal pollution in bathing areas

Sample to answer in 75 min

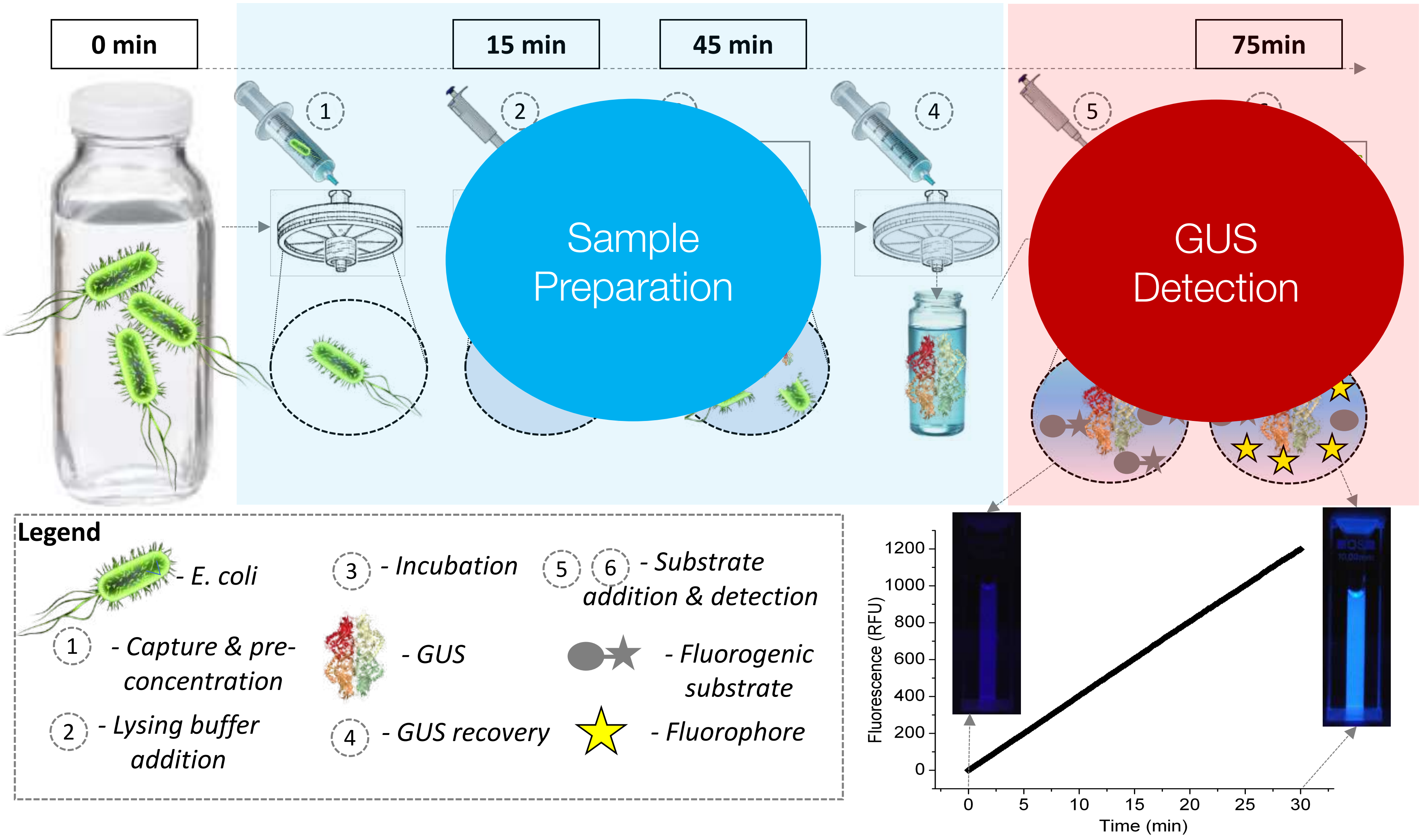
- Ciprian Briciu-Burghina
- Brendan Heery
- George Sharpson

Comhairle Contae Fhine Gall
Fingal County Council

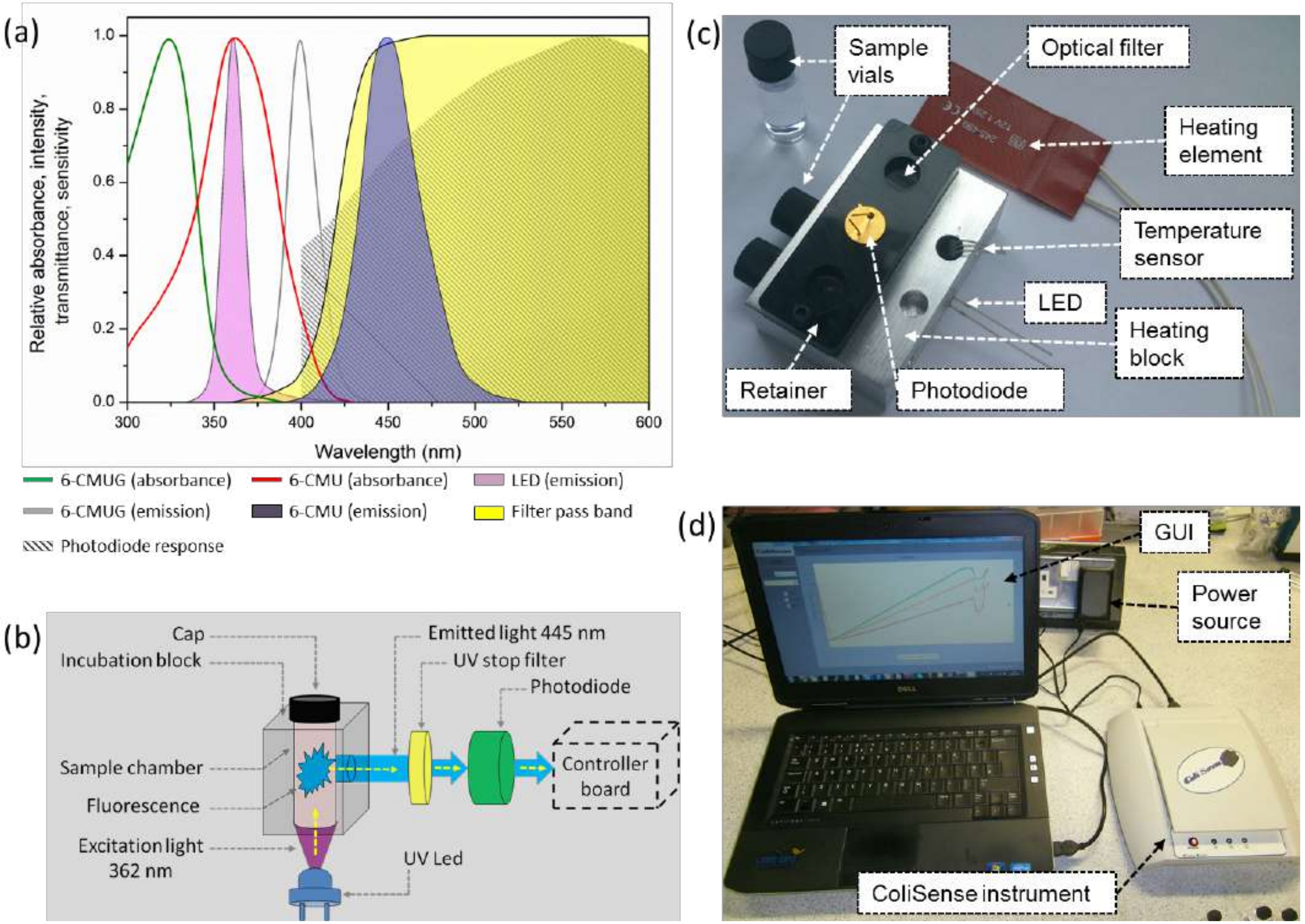
ENTERPRISE IRELAND

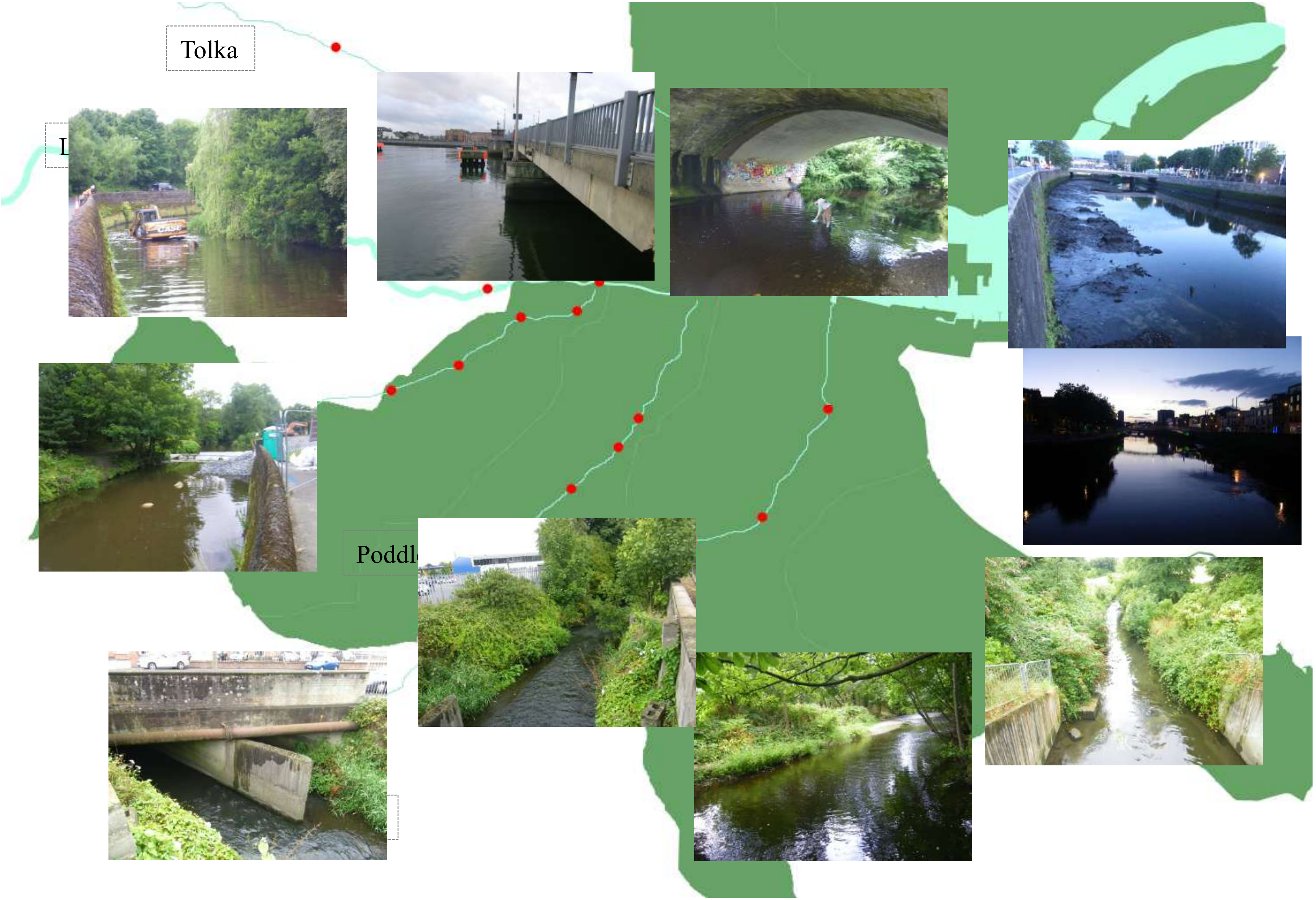
DCU | Water Institute

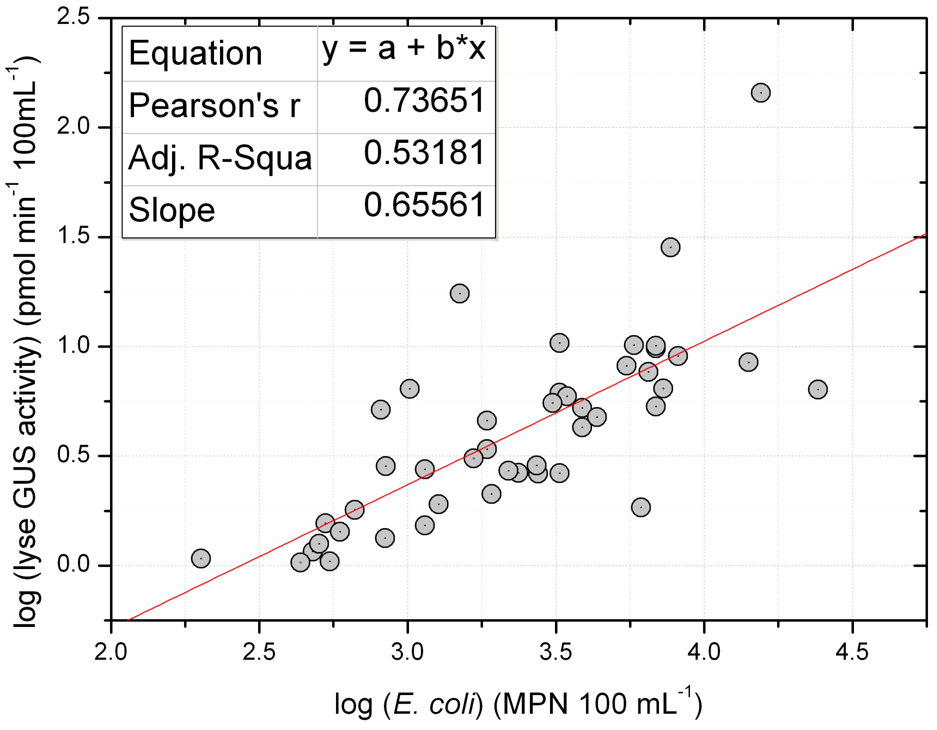
PROTOCOL DESCRIPTION

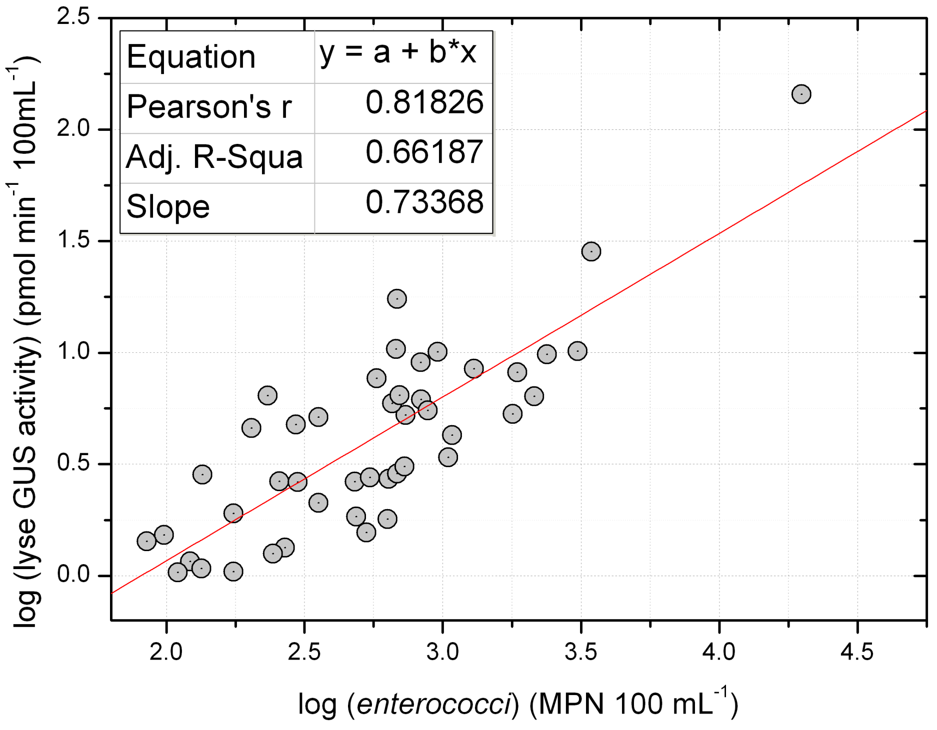


COLISENSE DESIGN




Tolka





ANALYTICAL PERFORMANCE

SAMPLE COLLECTION AND BATHING WATER SITES

Classification Standards for Bathing Waters (Single Sample Status Assessment)

Parameter	Excellent	Good	Sufficient	Poor
Intestinal Enterococci (IE)	≤100	101 - 200	201 - 250	>250
Escherichia coli (E.coli)	≤250	251 - 500	501 - 1000	>1000

Action Levels in Response to Microbiological Sample Results (HSE)*

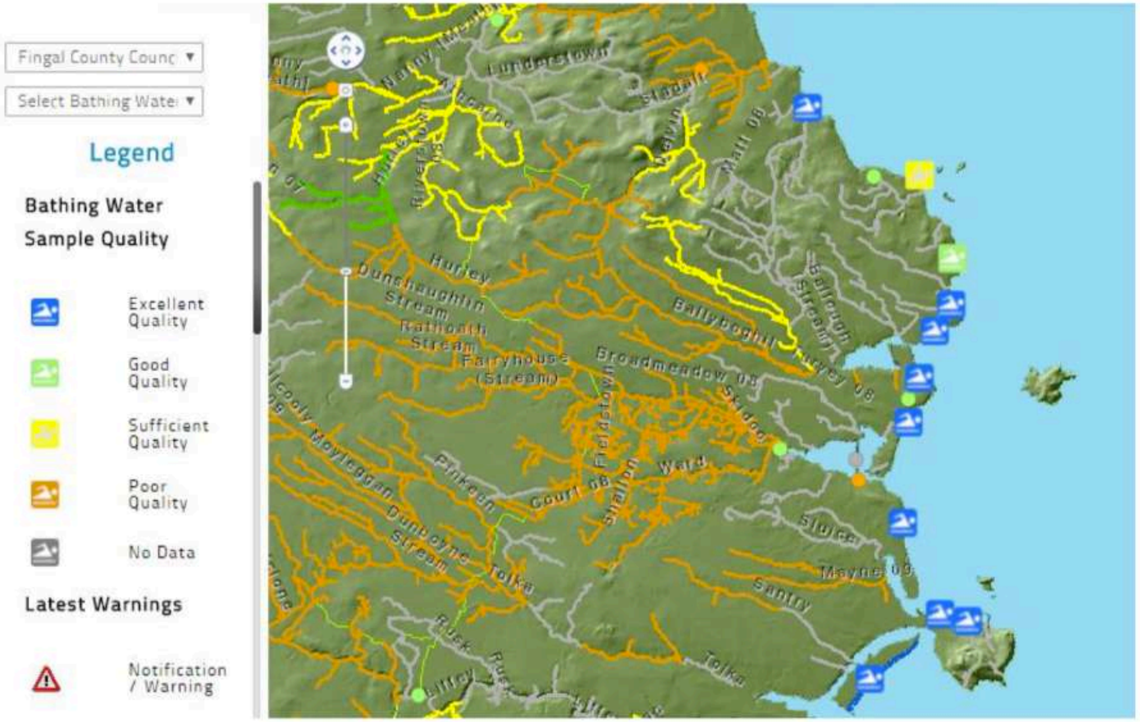
Escherichia coli (E.coli)		**Intestinal Enterococci (IE)**	**Recommended Action**
>2000 E.coli	**OR**	**>250 IE**	Issue a bathing water prohibition
≥1000 - ≤2000 E.coli	**AND**	**≥200 IE**	Issue a bathing water prohibition
≥1000 - ≤2000 E.coli	**BUT**	**<200 IE**	Issue and Advisory notice and re-sample immediately
If re-sample is still ≥1000 E.coli			Issue a bathing water prohibition
≥500 - <1000 E.coli	**AND**	**≥100 - ≤250 IE**	Monitor situation and re-sample. Decision based on evidence available/details of pollution event.

**Based on HSE risk assessment taking into account the beach profile, previous sampling history, probable source of contamination, evidence of human illness etc.*

SAMPLE COLLECTION AND BATHING WATER SITES

Samples:

- 11 designated bathing sites
- 2 added sites (Bob Davis Culvert & Balbriggan Harbour)



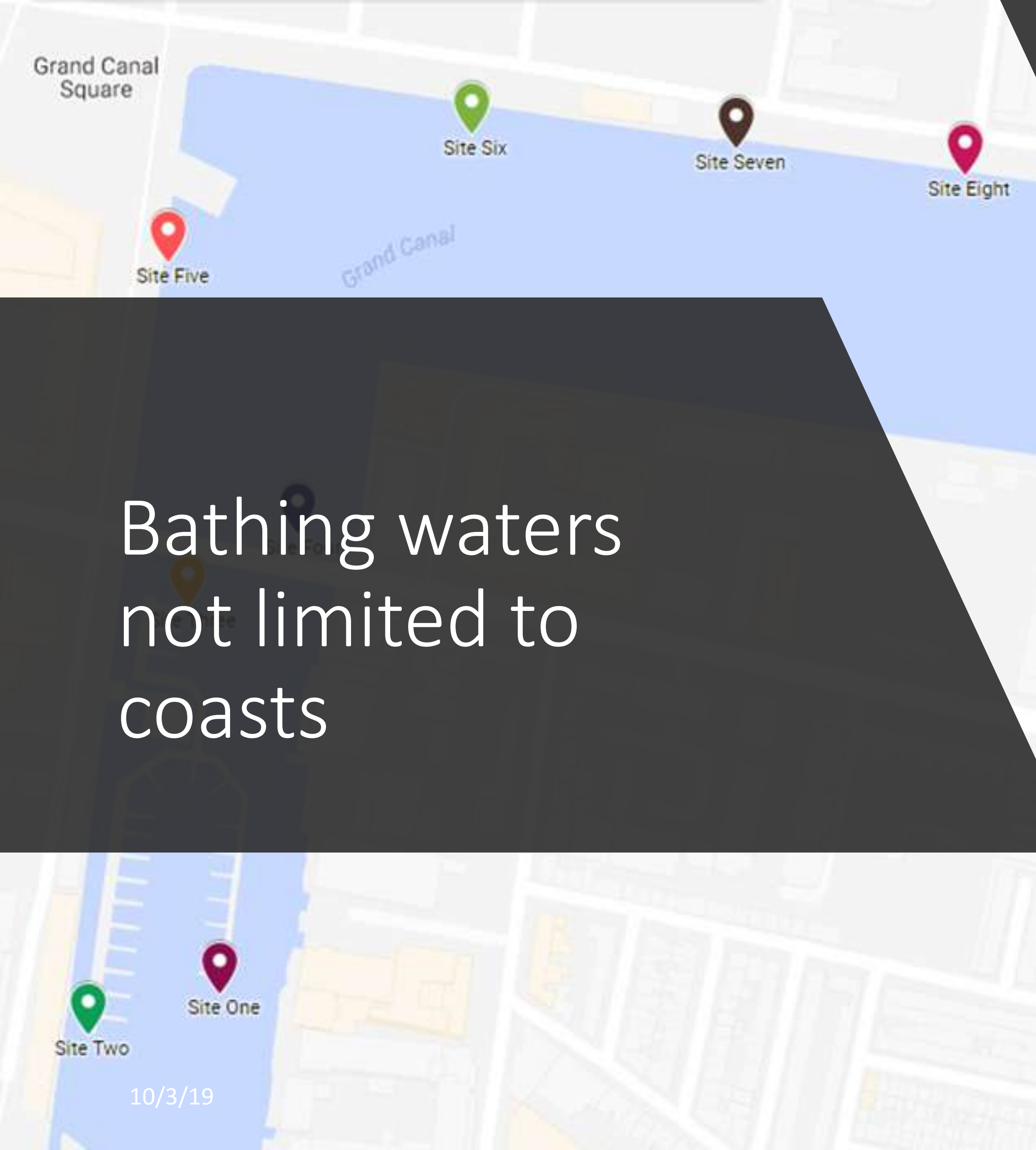
Map showing the location of the 11 Bathing sites (Fingal City Council) Source: http://splash.epa.ie/#National

SUMMARY OF DATA

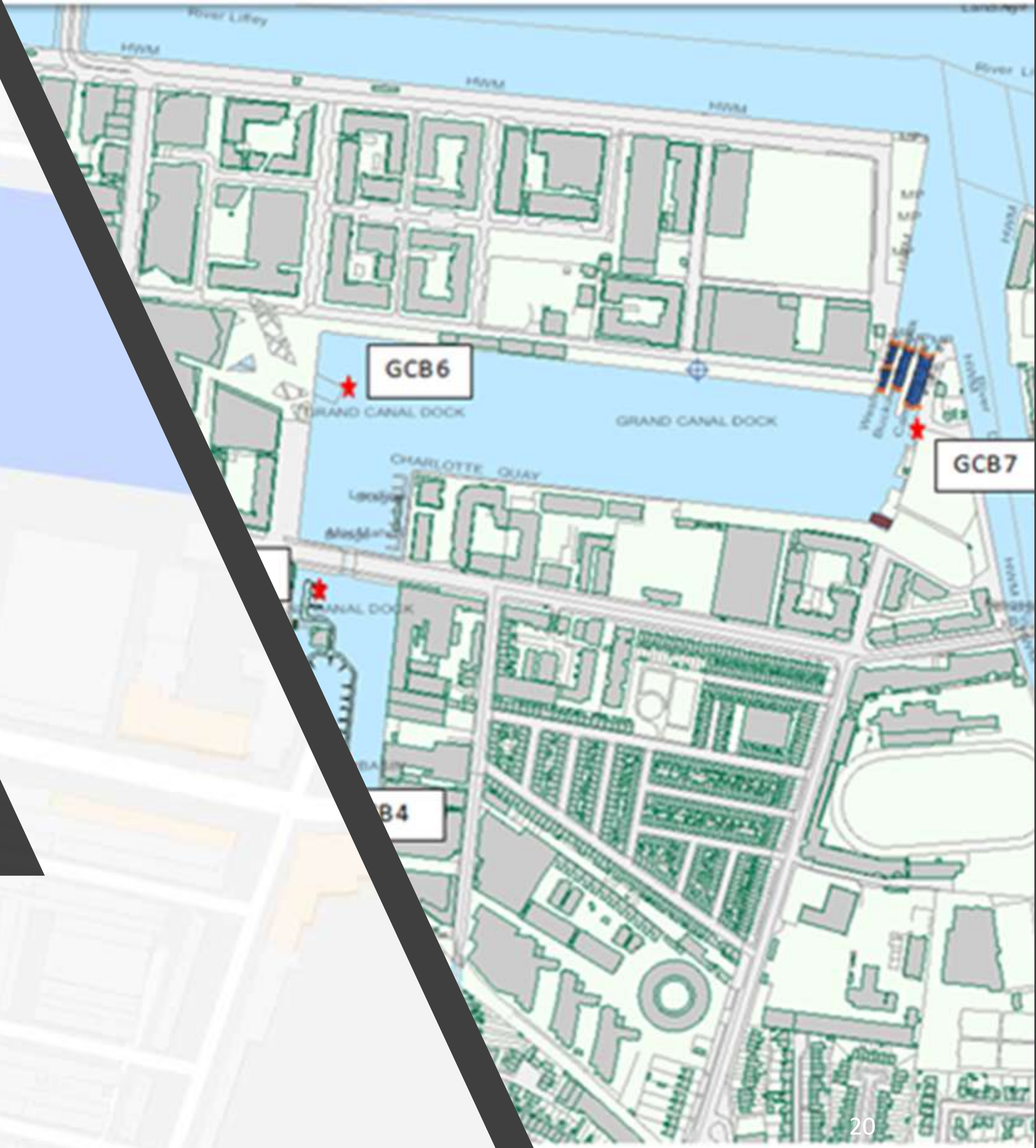
Total samples collected	N=130
Samples analysed	N=128 (2 lost)
Samples removed due to malfunction of sensor	N=0
Samples removed due to method problems	N=3
Total samples used for correlations/regressions	N=125
E. coli > 2000 MPN 100 mL^{-1}	N=5
E. coli 1000-2000 MPN 100 mL^{-1}	N=7
E. coli 500-1000 MPN 100 mL^{-1}	N=6
E. coli 0-500 MPN 100mL^{-1}	N=107

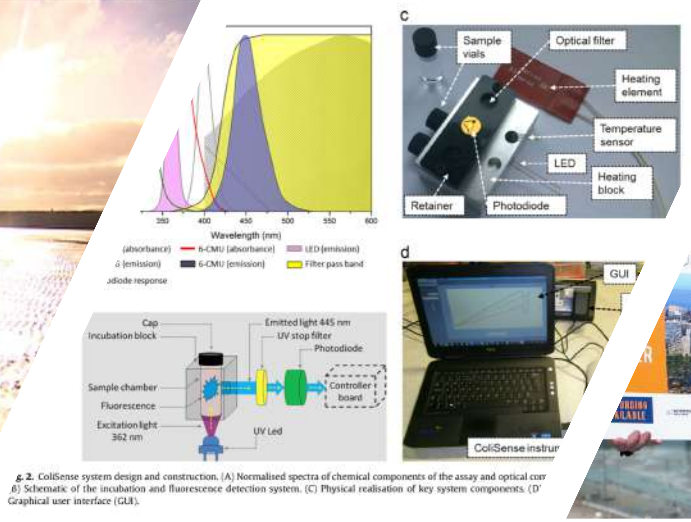
2019 data

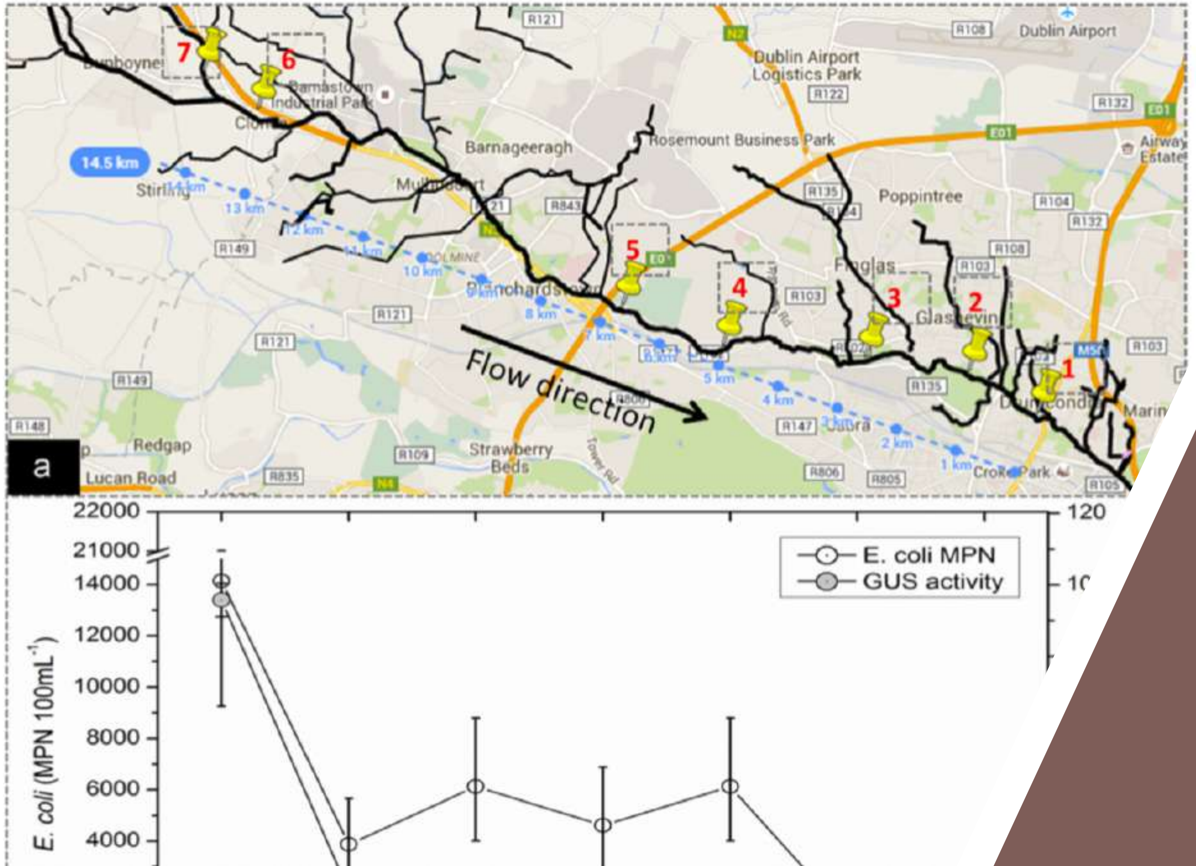
Sample ID	Sample type	Spiked with	Contamination level	ColiSense response	*E. coli* (MPN/100 mL)*	500 *E. coli* threshold prediction
1	SW	-	-	0.88	10	<500
2	SW	S	L	5.35	591	>500
3	SW	S	M	18.44	2005	>500
4	SW	S	H	133.08	19863	>500
5	SW	Sw	M	0.48	<10	<500
6	SW	Bs	M	0.72	<10	<500
7	SW	FE	L	11.19	254	>500
8	SW	FE	H	98.23	2005	>500
9	GW	-	-	0.33	<10	<500
10	GW	S	L	NA	406	NA
SW-seawater, GW-ground water, S-sewage, Sw-seaweed, Bs-beach sand, FE-farm effluent, L-low, M-moderate, H-high contamination; NA-not analysed; *-samples analysed by HSE						



Bathing waters not limited to coasts







Improved Decision Making for Bathing Waters

Are technology innovations meeting the needs?

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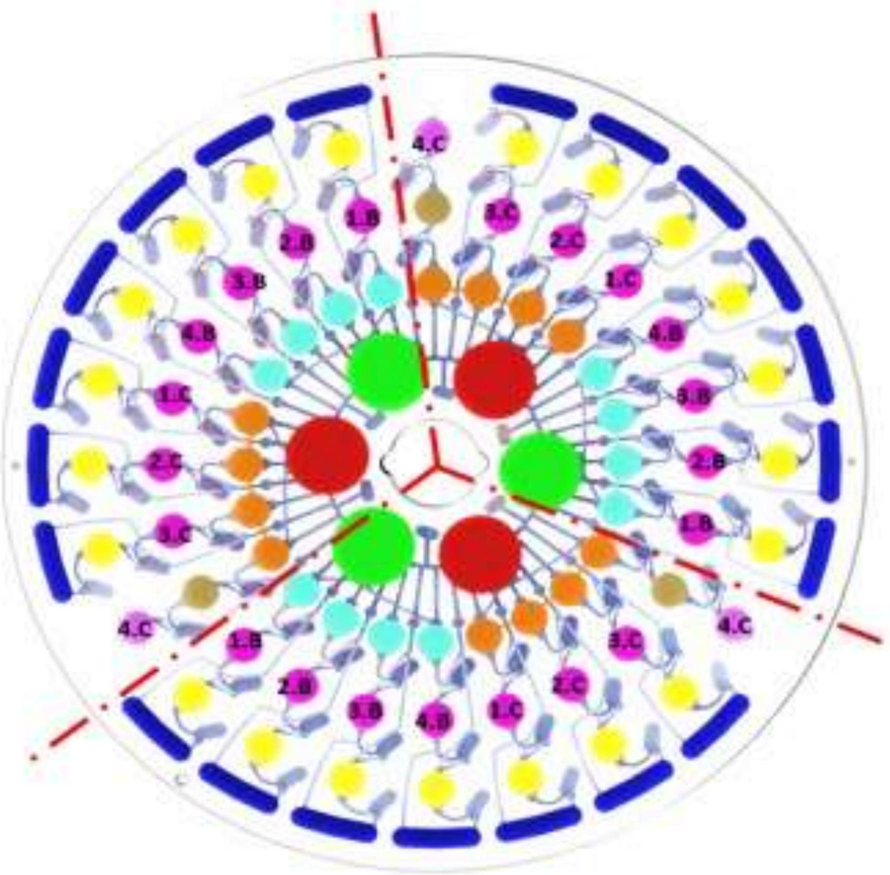
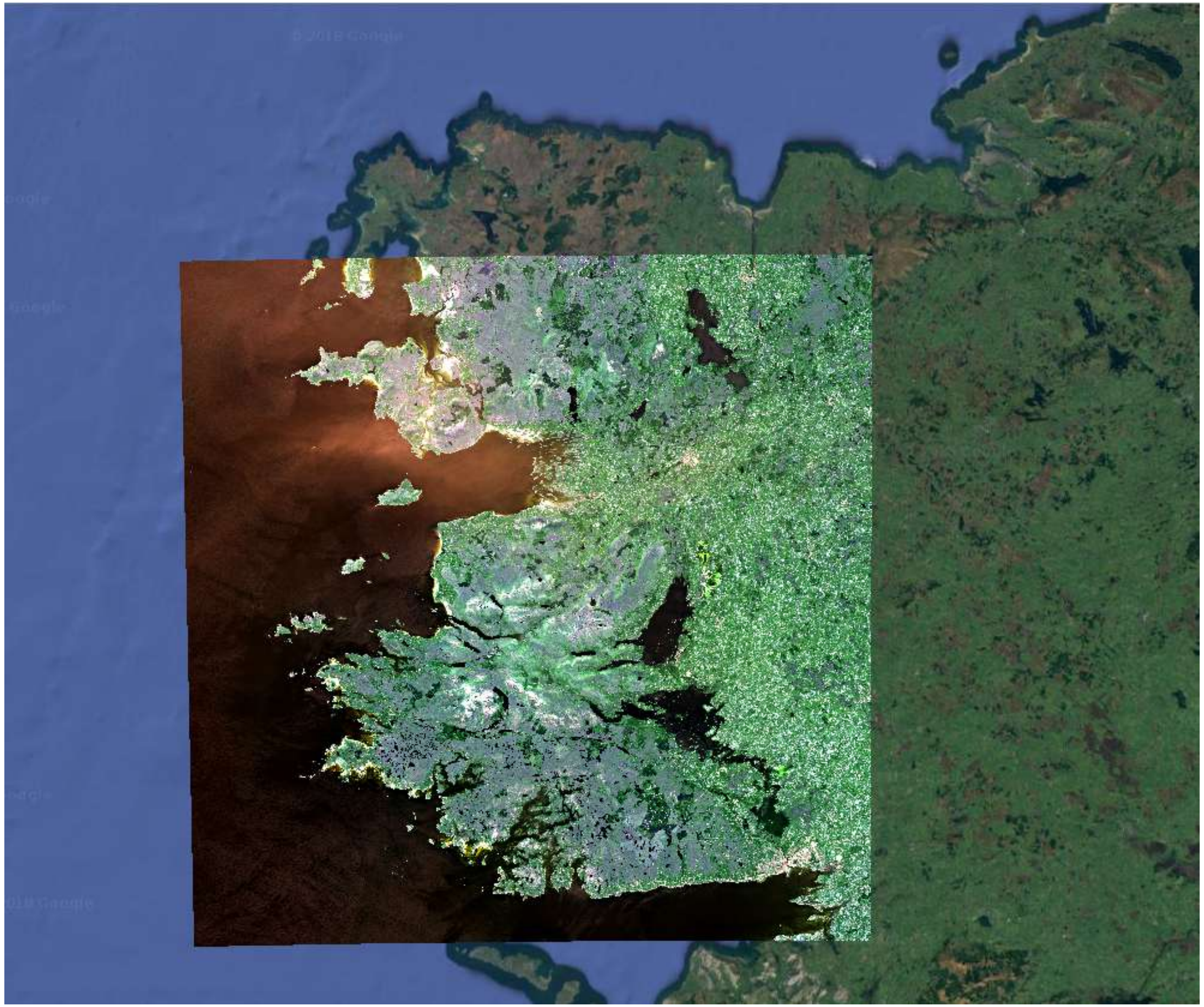
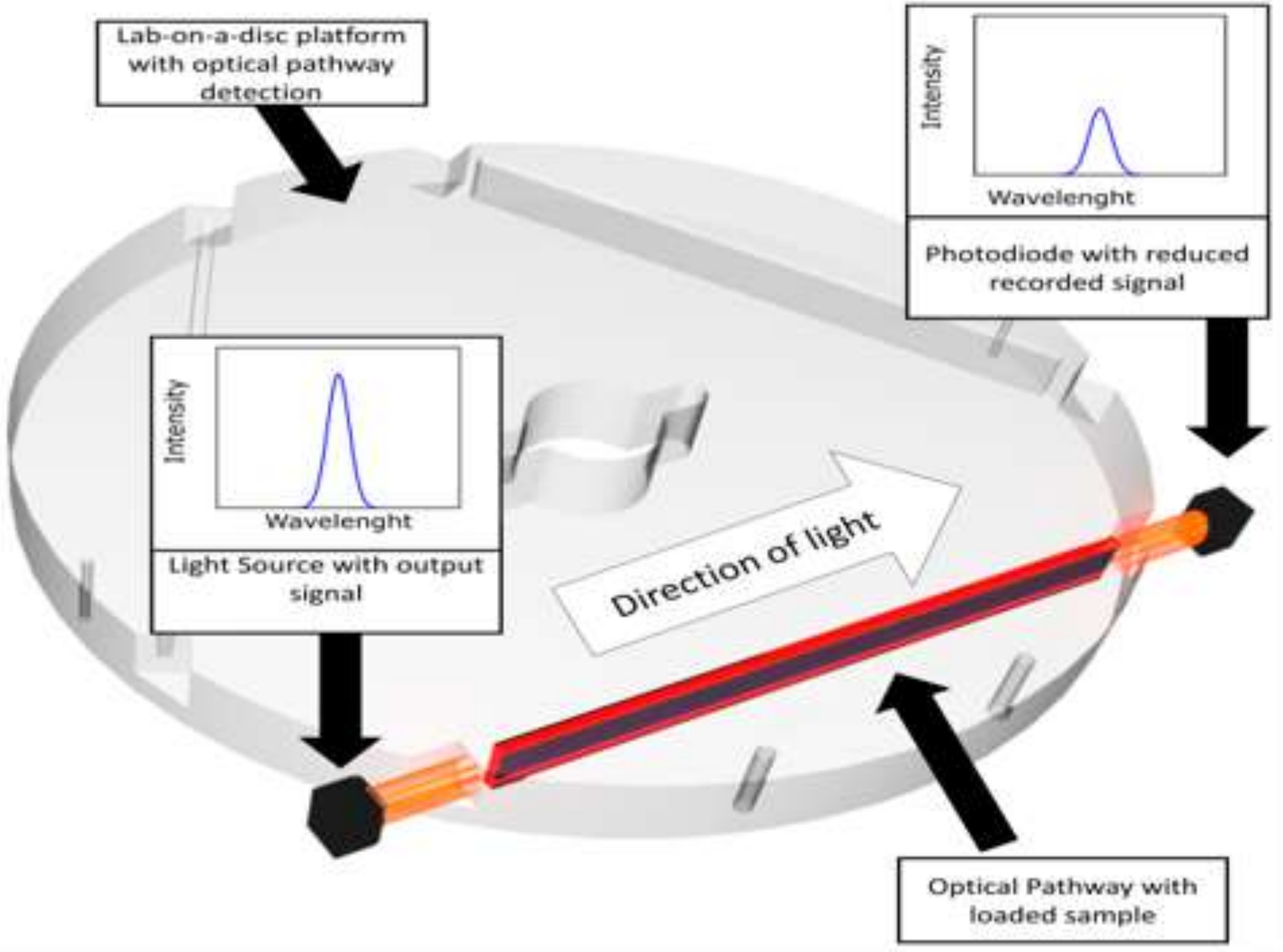


New device developments for phosphate in catchment monitoring



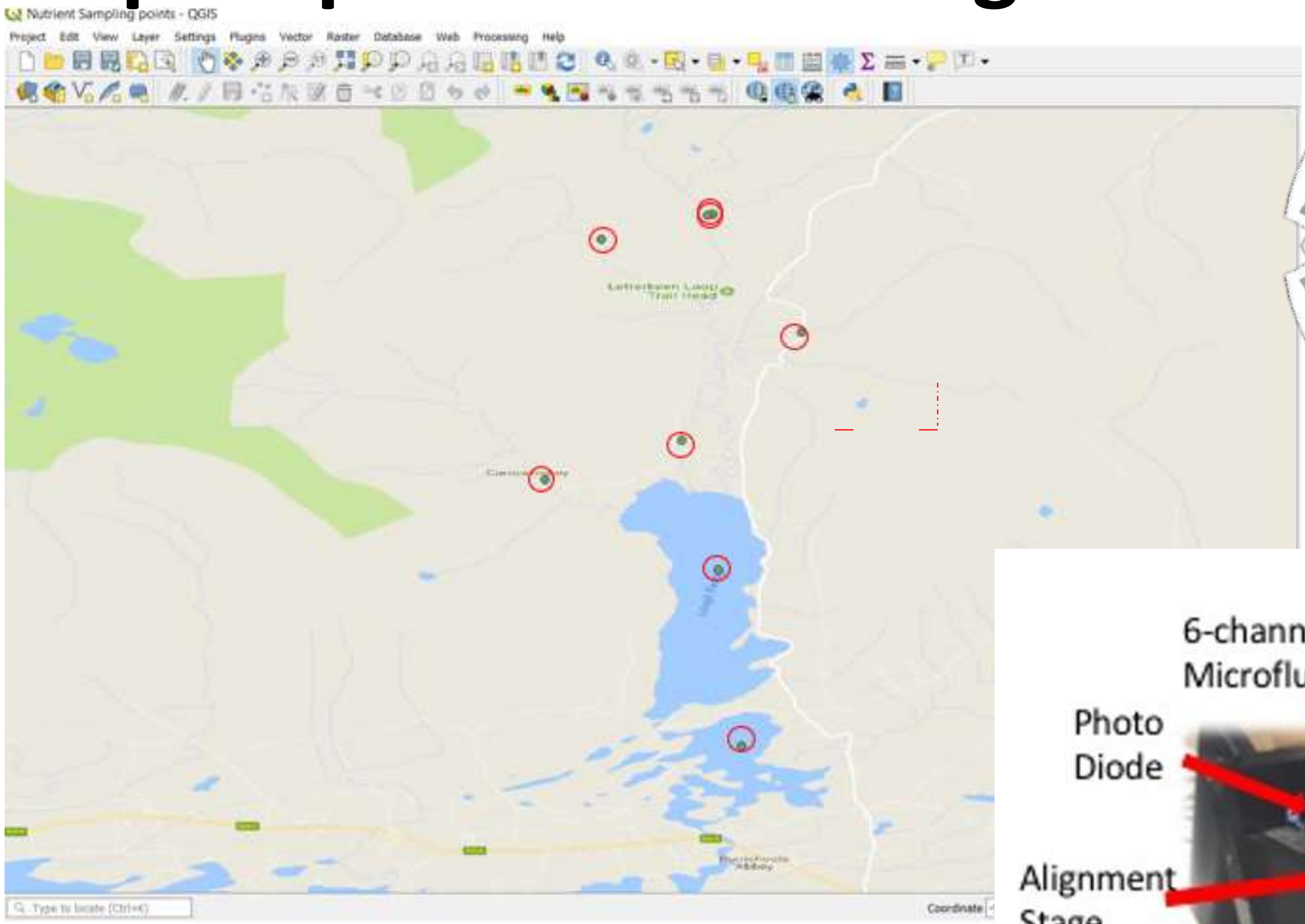
Centrifugal Microfluidics – How does it work?

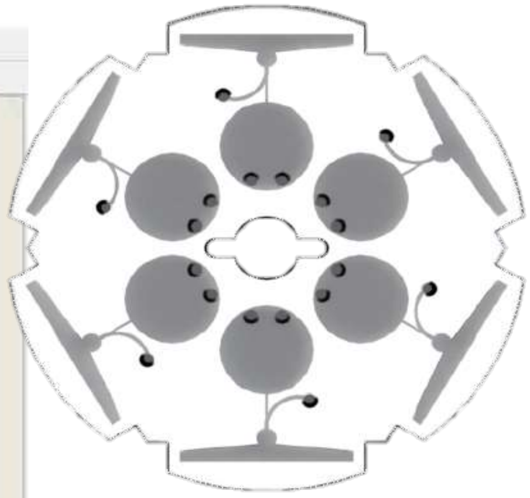
- **Lab-on-a disc for phosphate**
- **Used to integrate processes such as separating, mixing, reaction and detecting molecules**
- **Principle of microfluidics**

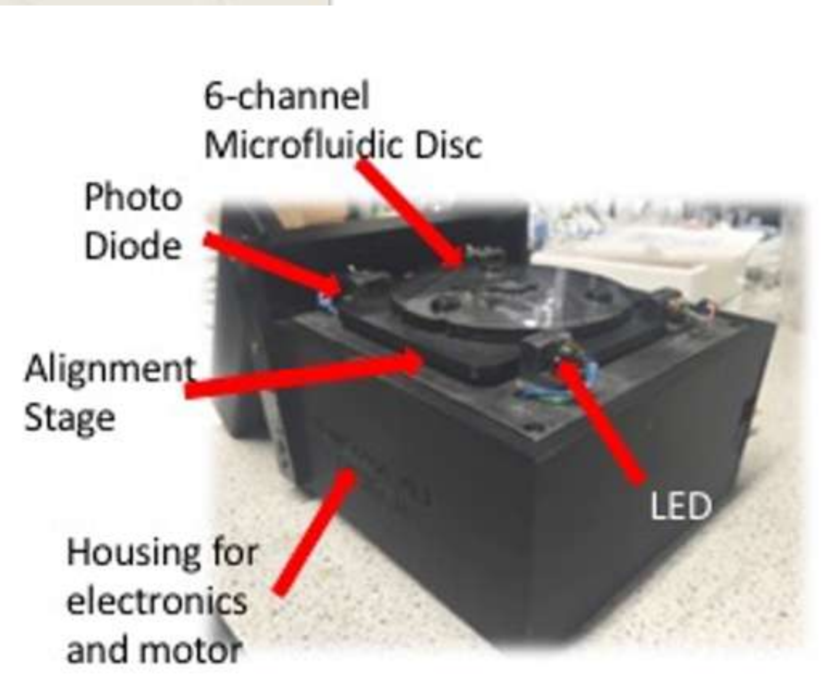


Our Vision for phosphate monitoring in a catchment

Water catchment assessments are possible where a phosphate single sensor disc can be used for multiple sites with minimal sample handling.







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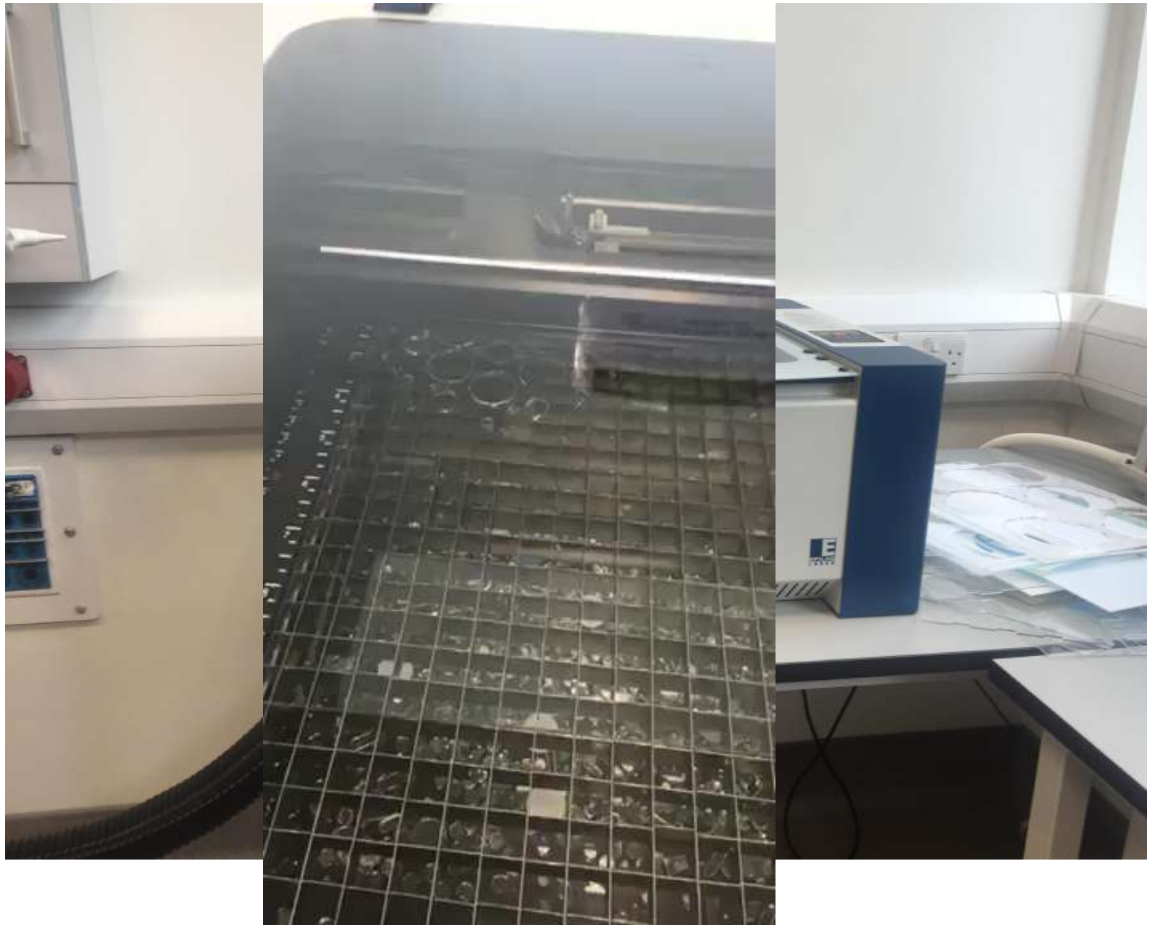
Marine Institute
Foras na Mara

DCU

Marine Institute
Foras na Mara

Manufacturing and Assembly Facilities



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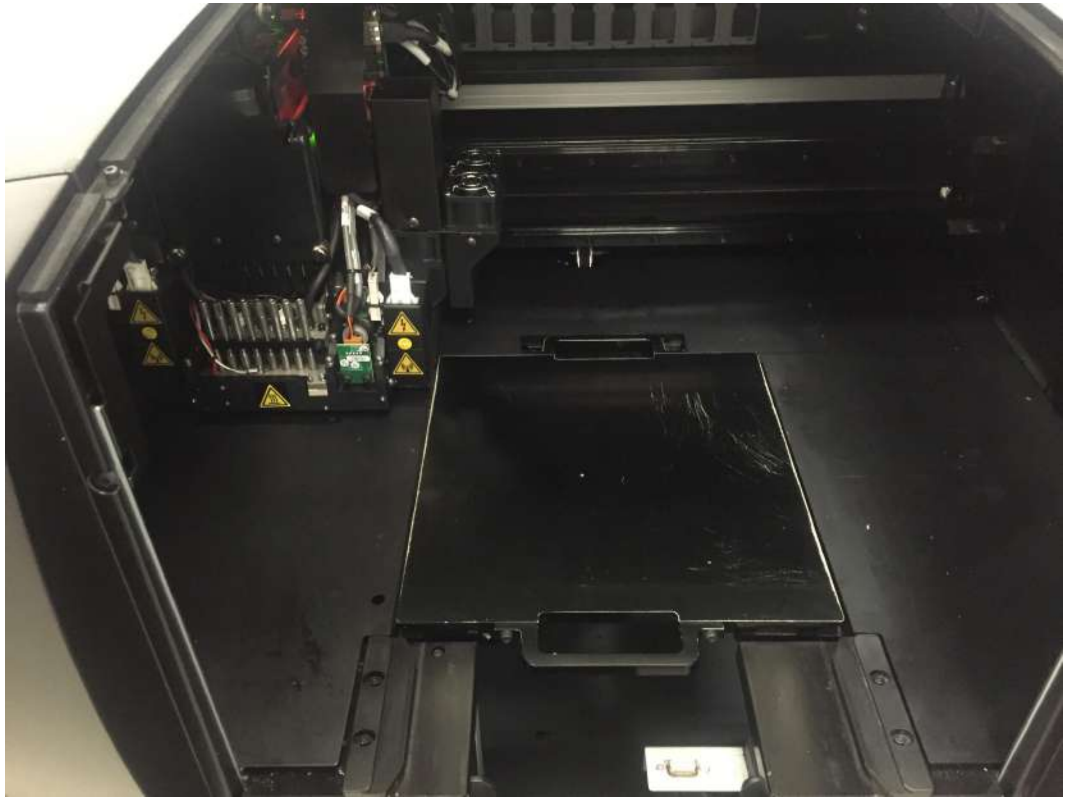
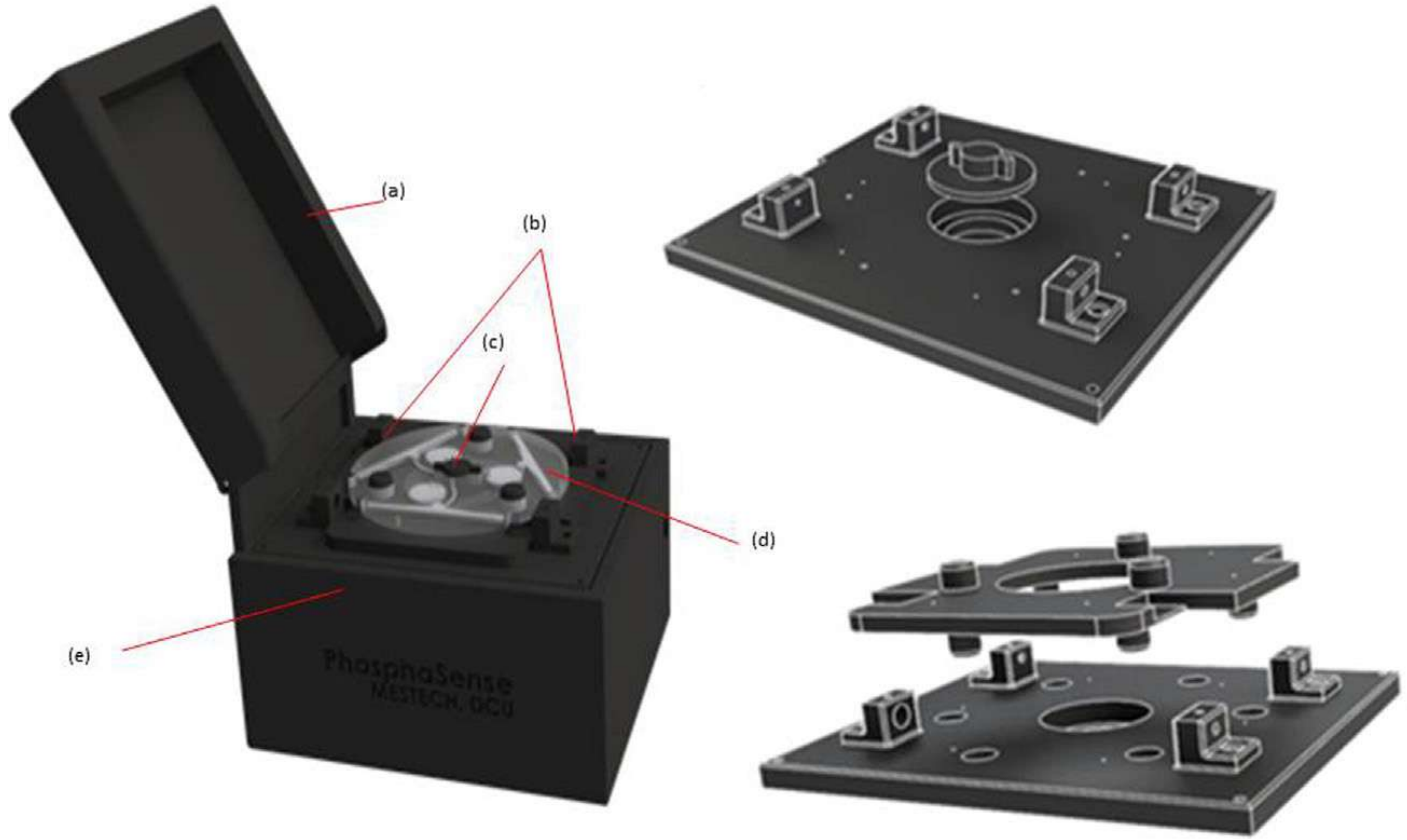
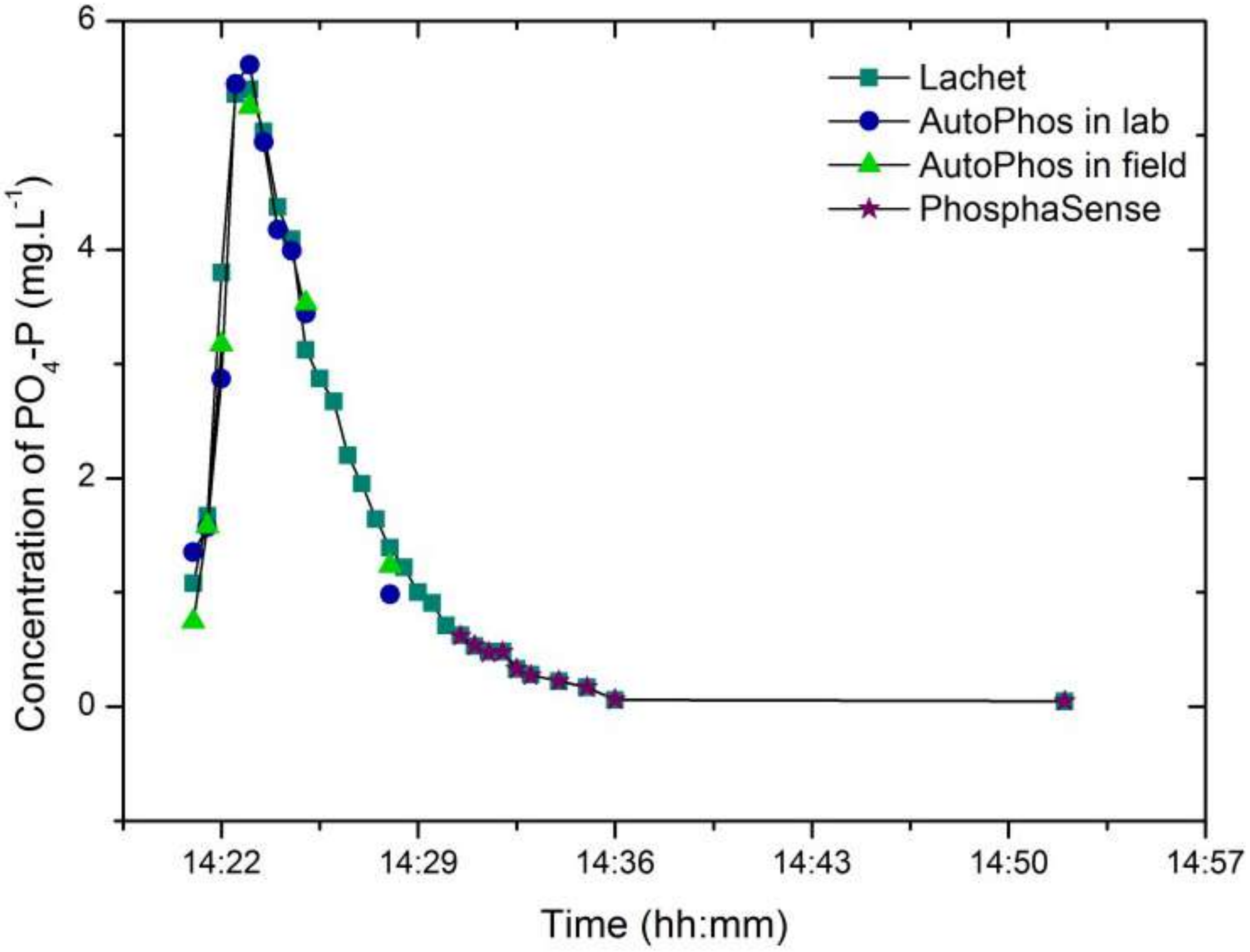
UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

3D Printing




(a)
(b)
(c)
(d)
(e)
PhosphaSense
MESTECH, DCU

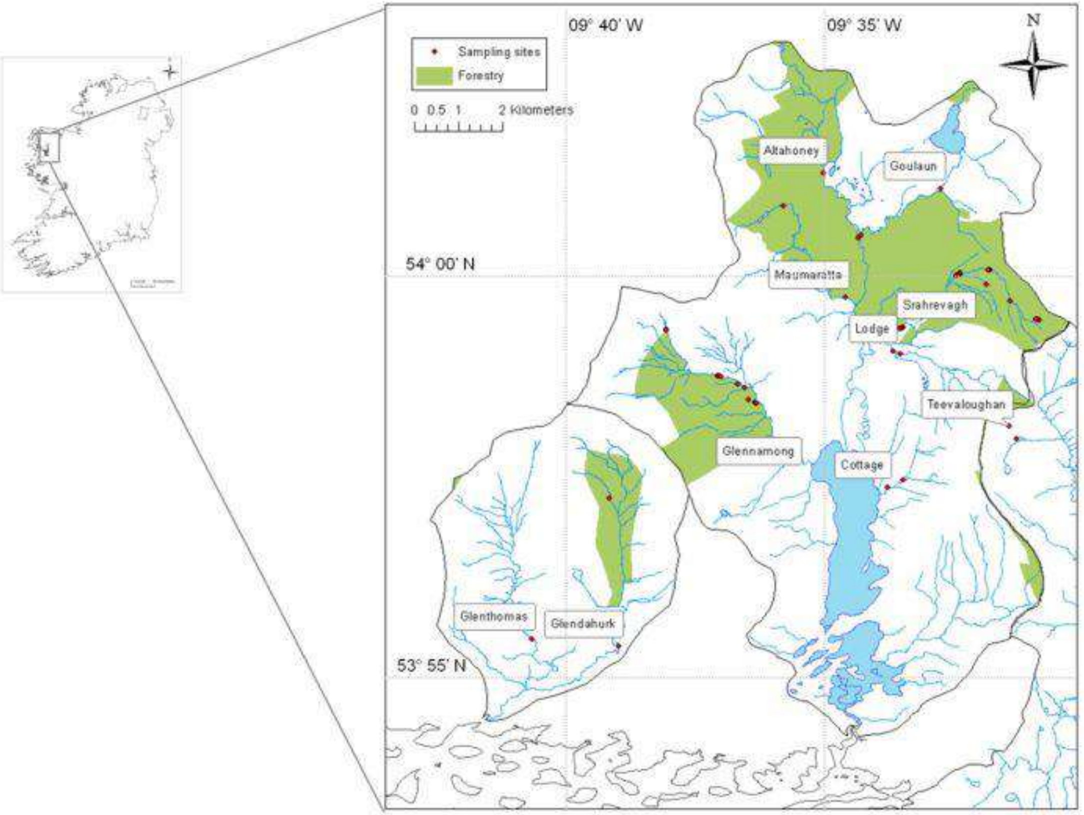


A plot of the data obtained by measuring field samples in-field and in the lab on the AutoPhos sensor, on PhosphaSense, and on the Lachet system.

Field-based assessment of the analytical performance of novel phosphate sensors for water monitoring.

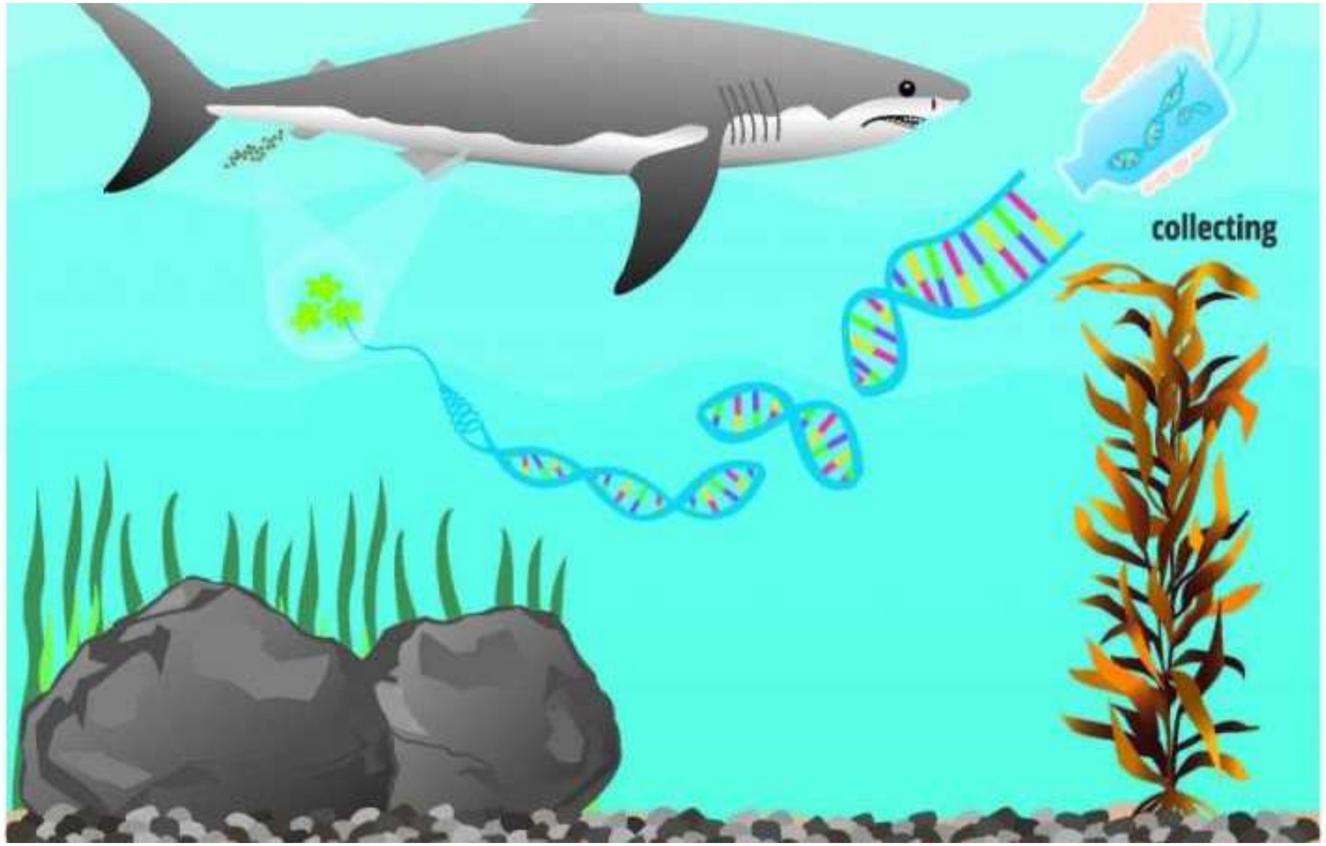
G. Duffy,[a] P. McCluskey[b], U. H. Mahl[c], N. Kent[b], Southampton people[e], Ivan Maguire[a], Margaret M[c]Caul[b], J. Tank[c], D. Diamond[b] and F. Regan[a]

UNIVERSITY OF NOTRE DAME


09° 40' W
09° 35' W
N
Sampling sites
Forestry
0 0.5 1 2 Kilometers
Altahoney
Goulaun
54° 00' N
Maumaratta
Srahrevagh
Lodge
Teevaloughan
Glennamong
Cottage
Glenthomas
Glendahurk
53° 55' N

BEYOND 2020

Translating knowledge

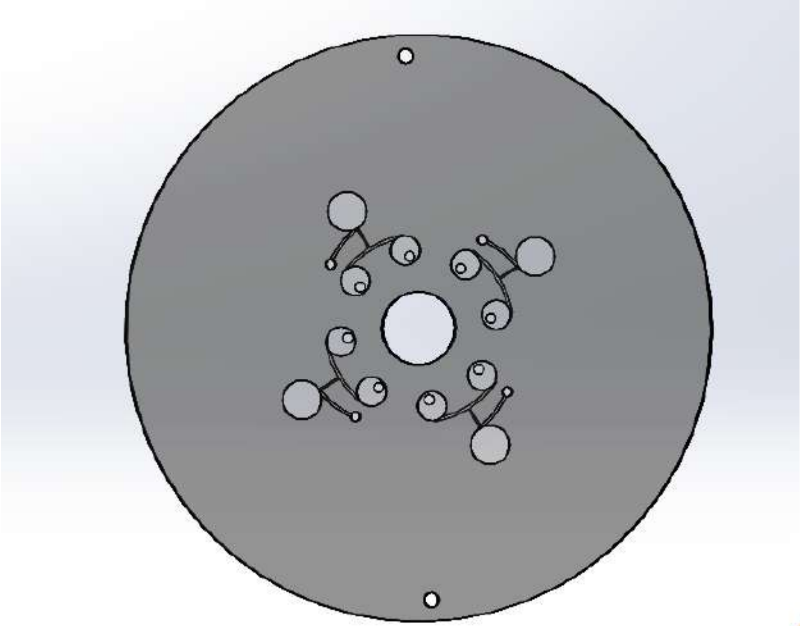


Environmental DNA (eDNA)

SalmoSense Design (Gen.1)

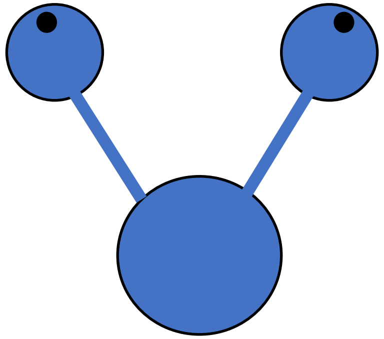
Initial Design:

Step 1: Test if amplification can be achieved on disc

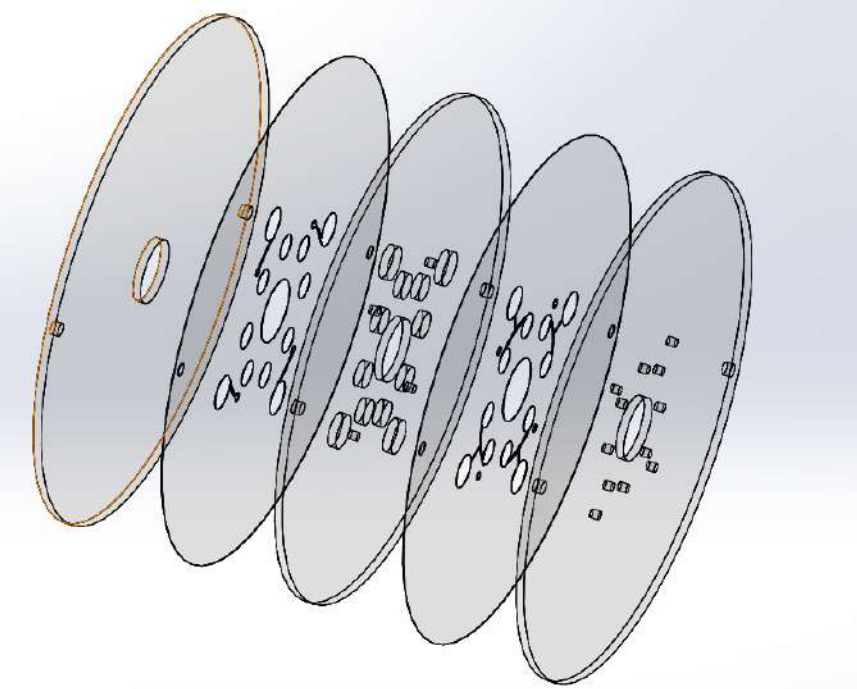


DNA

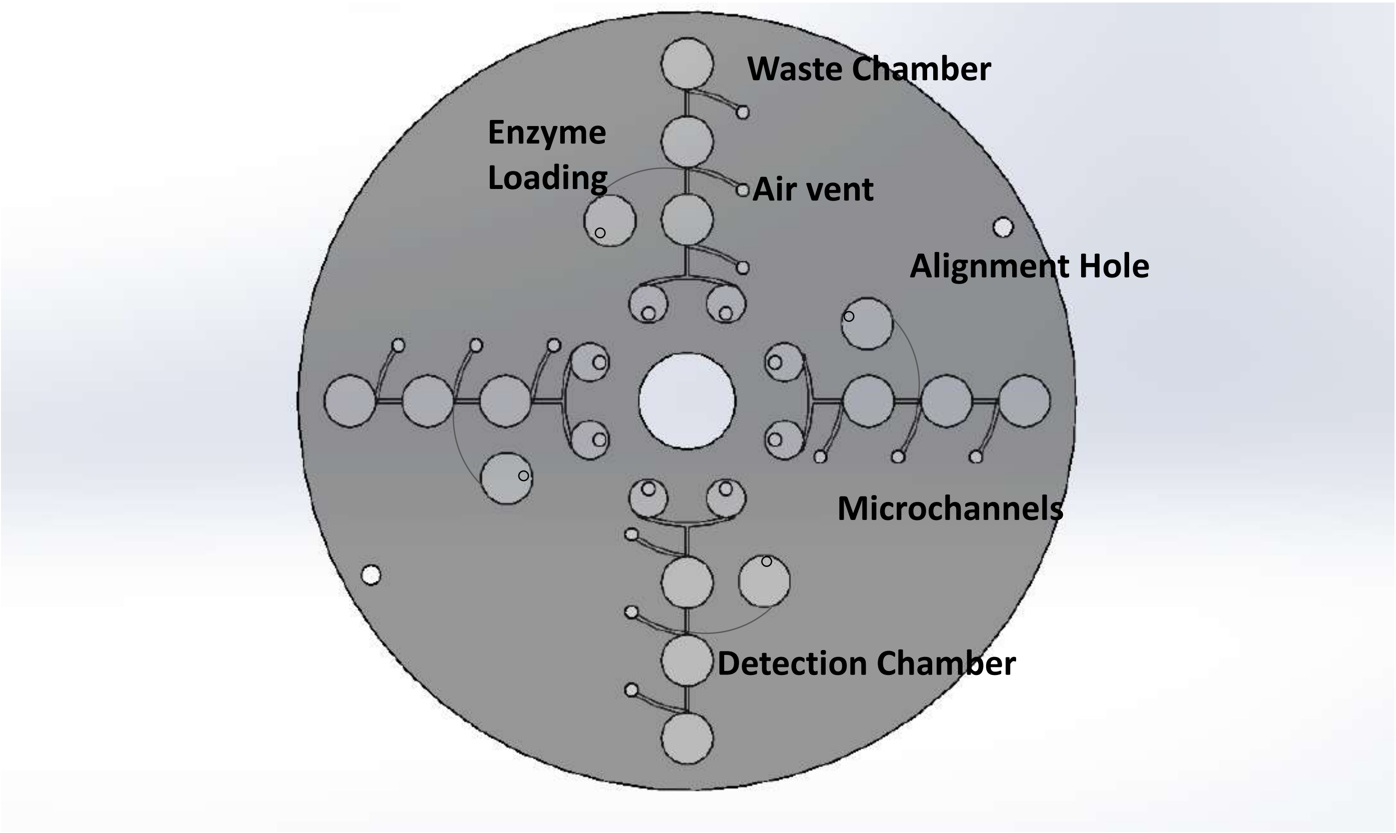
RPA Mix (buffer, primer)

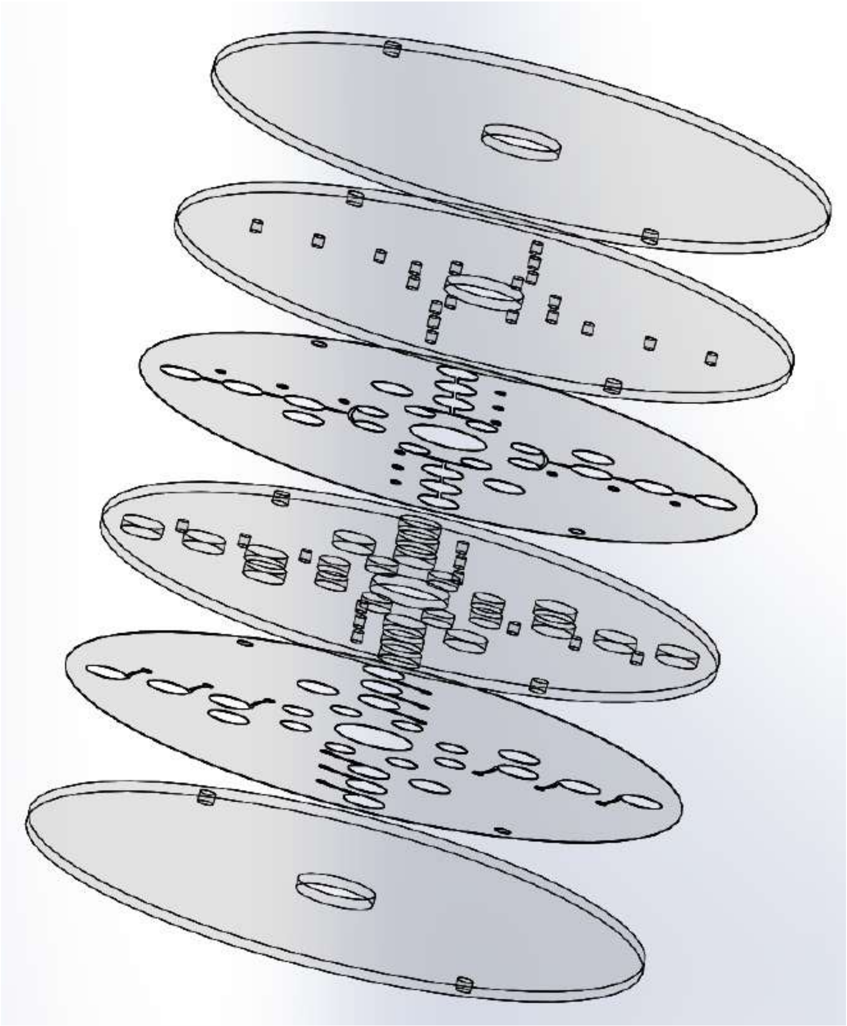


Amplification Detection chamber



SalmoSense Design (Gen 2)





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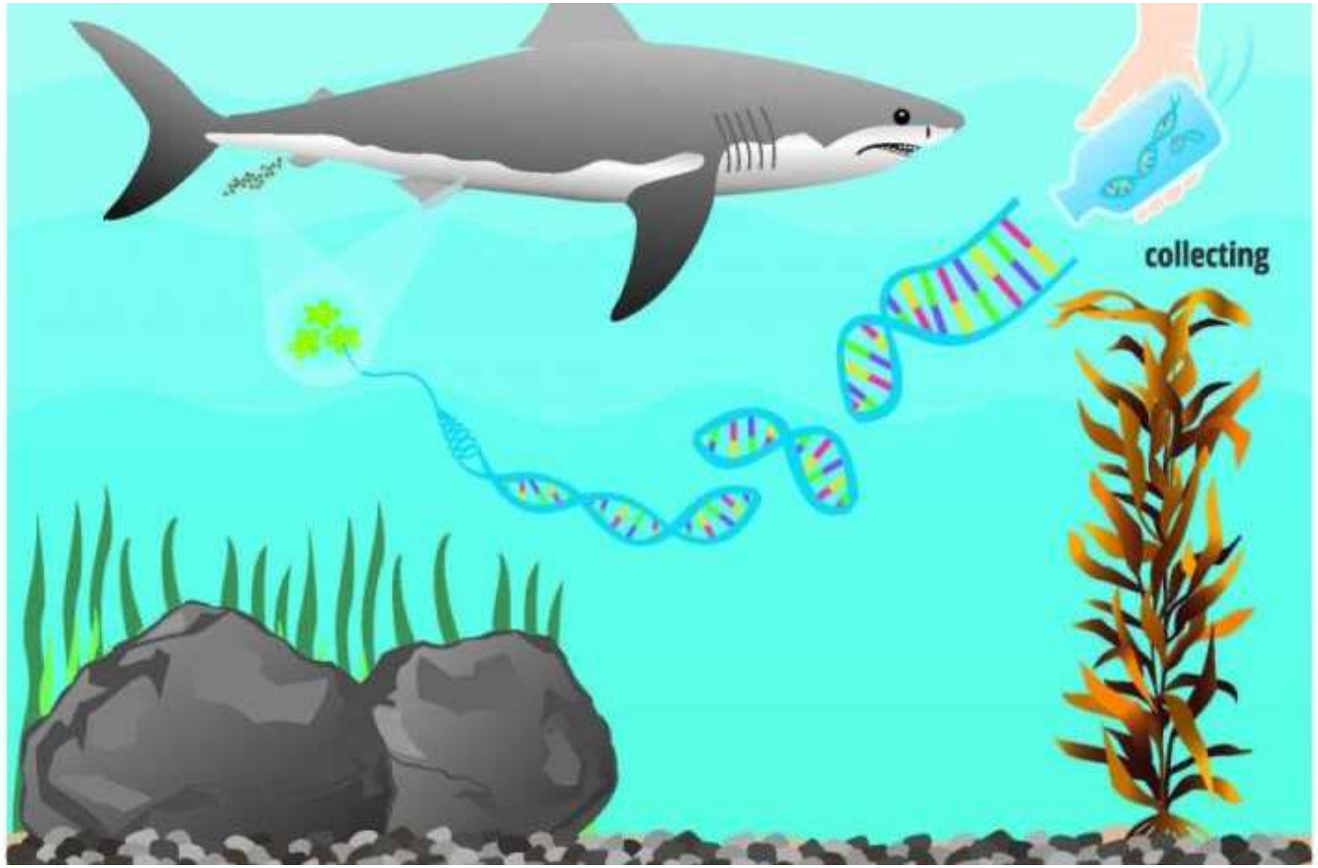
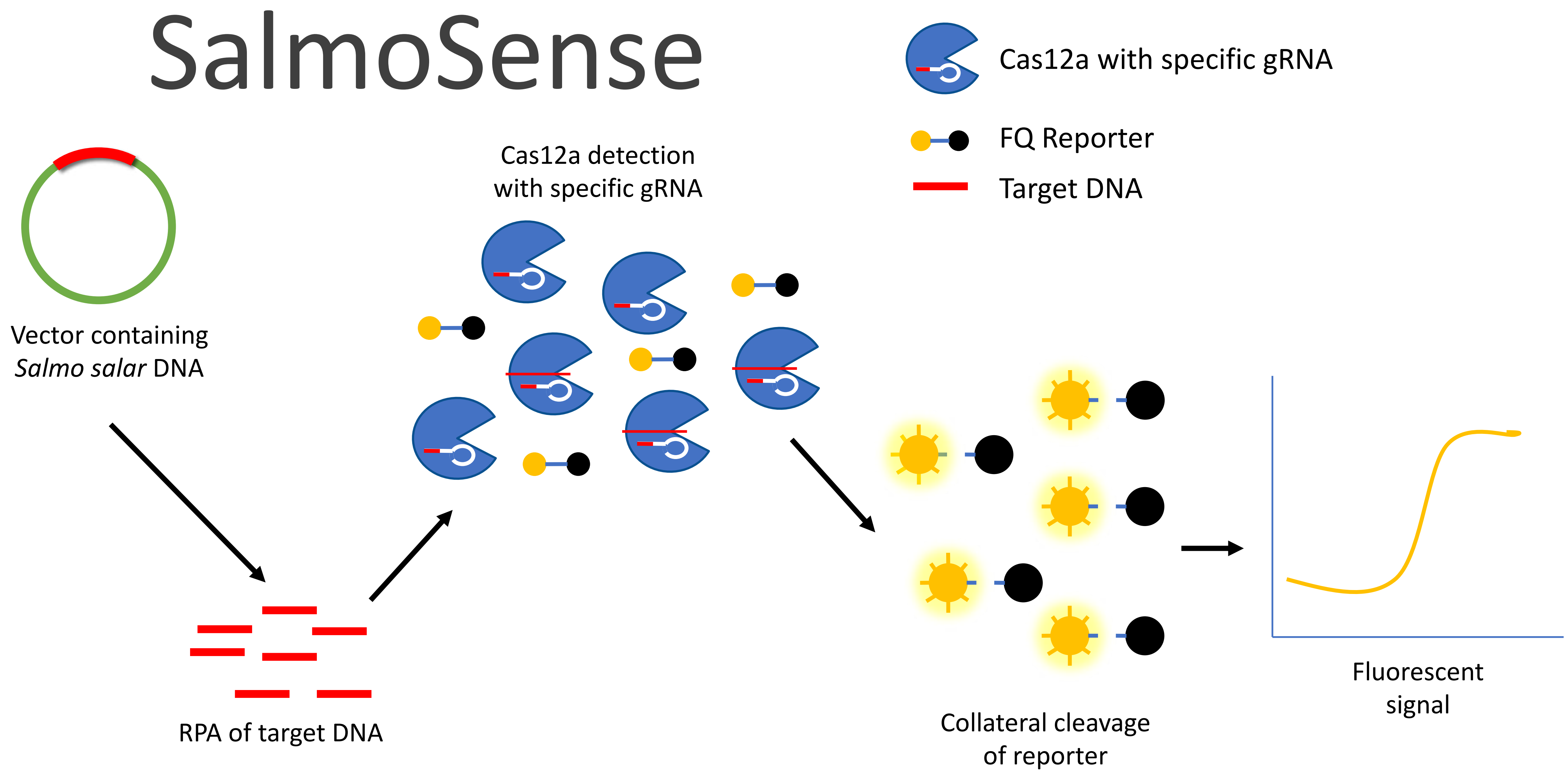
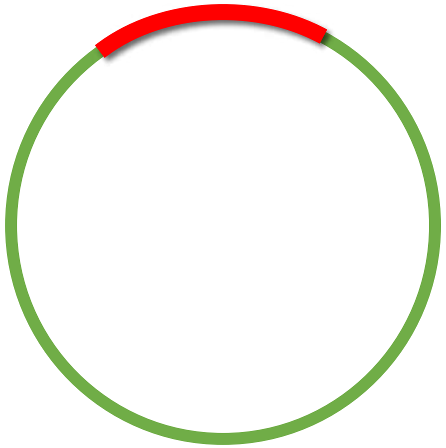


Our Vision

- Develop **a biosensor** with high **sensitivity** and **specificity** and where a single sensor disc can be used for multiple sites with minimal sample handling.
- The device should have the **minimum number of steps** required, run at **low temperatures** and ideally use a fluorescence based detection system.


SalmoSense
Cas12a with specific gRNA
FQ Reporter
Target DNA
Cas12a detection
with specific gRNA
Vector containing
Salmo salar DNA
RPA of target DNA
Collateral cleavage
of reporter
Fluorescent
signal

Increasing sample complexity

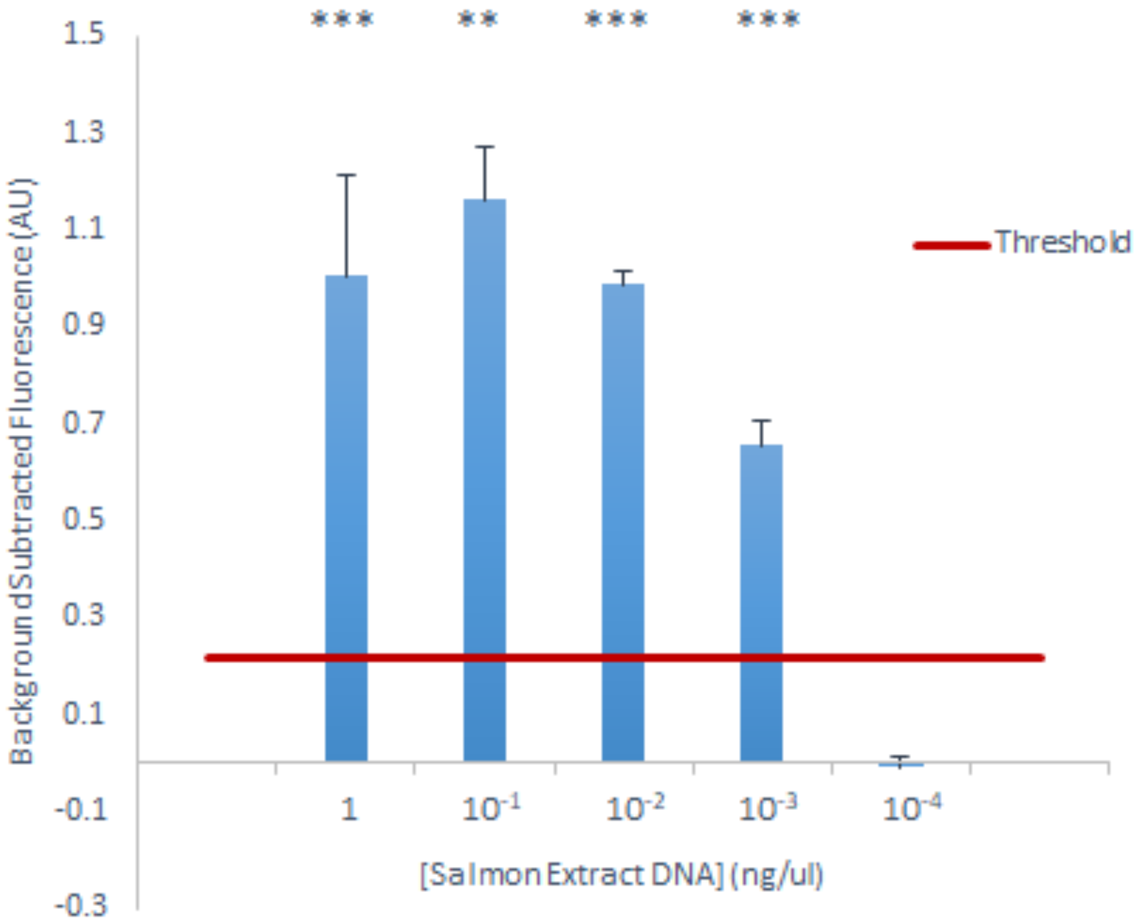


Vector with target sequence

Tissue extract with whole target genome

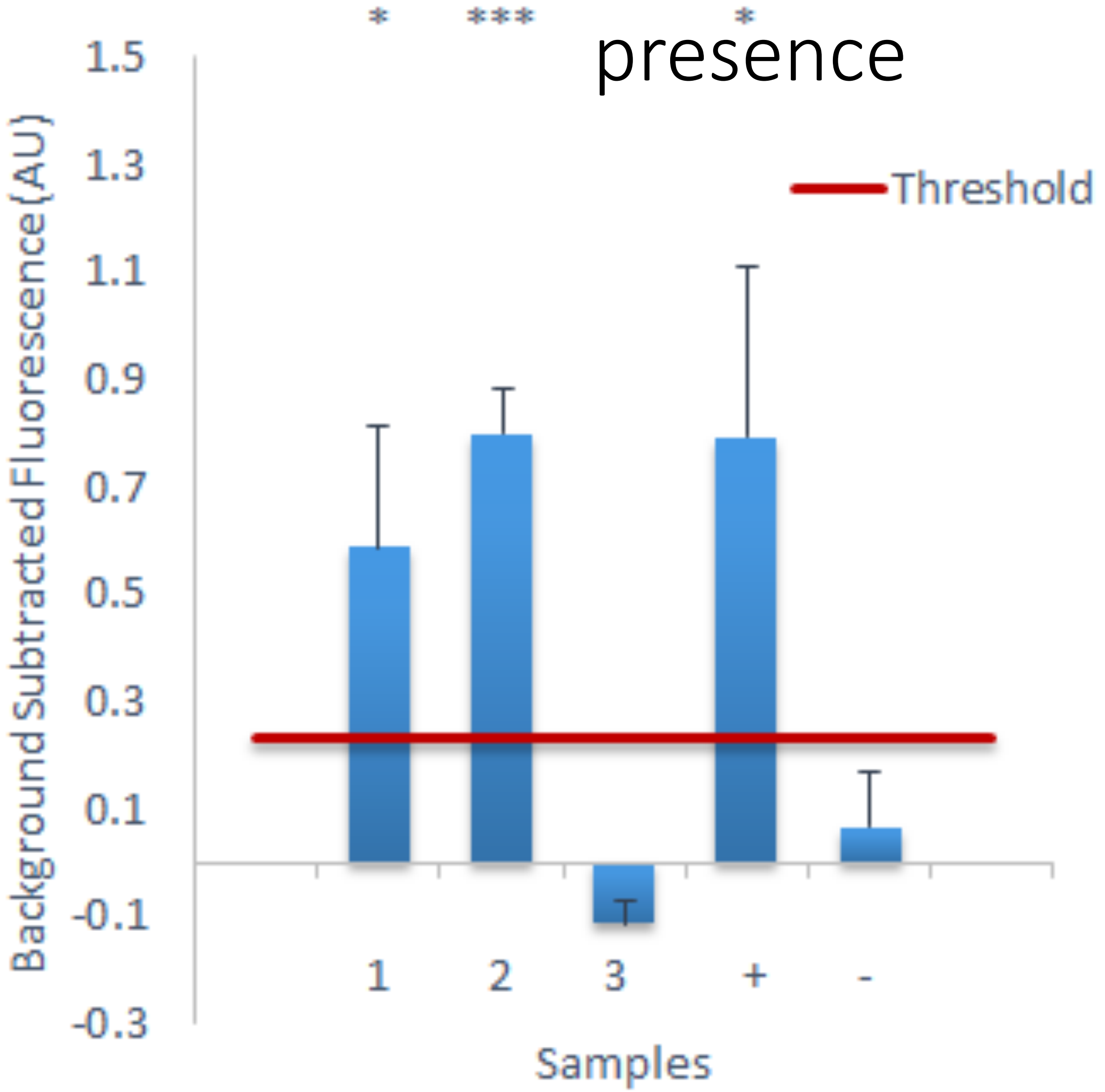
Environmental sample

Detection of *S. salar* tissue possible at 10^{-3} ng/μl

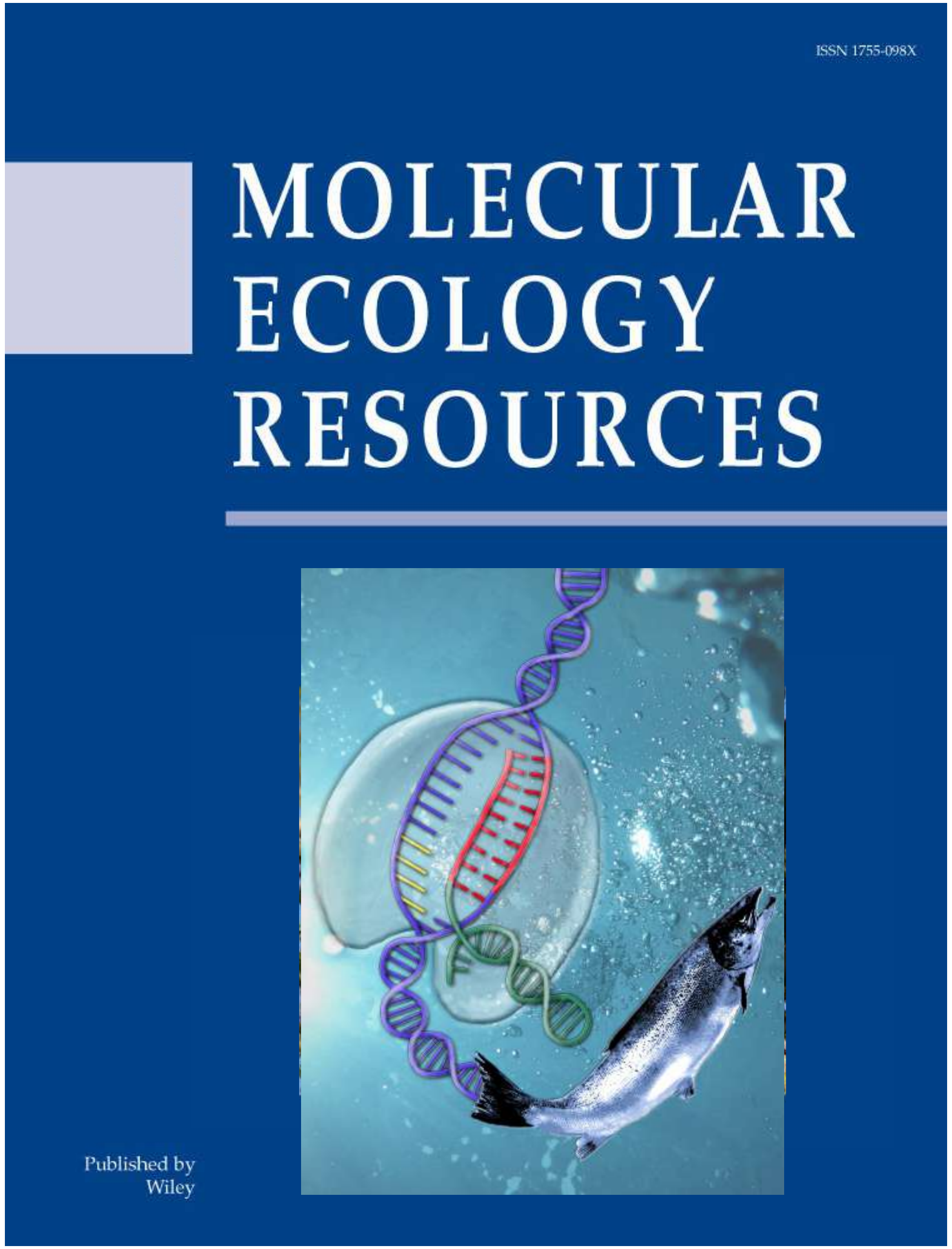


S. salar tissue extraction detected with SalmoSense

SalmoSense detects *S. salar* in eDNA sample with known presence



Environmental DNA samples (with thanks to Bernie Ball and Jens Carlsson) detected with SalmoSense



Received: 23 February 2019 | Revised: 30 May 2019 | Accepted: 31 May 2019
DOI: 10.1111/1755-0998.13045

FROM THE COVER

MOLECULAR ECOLOGY RESOURCES WILEY

The application of CRISPR-Cas for single species identification from environmental DNA

Molly-Ann Williams[1,2] | Joyce O'Grady[3,2] | Bernard Ball[4] | Jens Carlsson[4] | Elvira de Eyto[5] | Philip McGinnity[5,6] | Eleanor Jennings[7] | Fiona Regan[3,2] | Anne Parle-McDermott[1,2]

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Funding Information
Irish Marine Institute as part of the Burrishoole Ecosystem Observatory Network 2020:, Grant/Award Number: BEYOND 2020 PBA/FS/16/02

Abstract

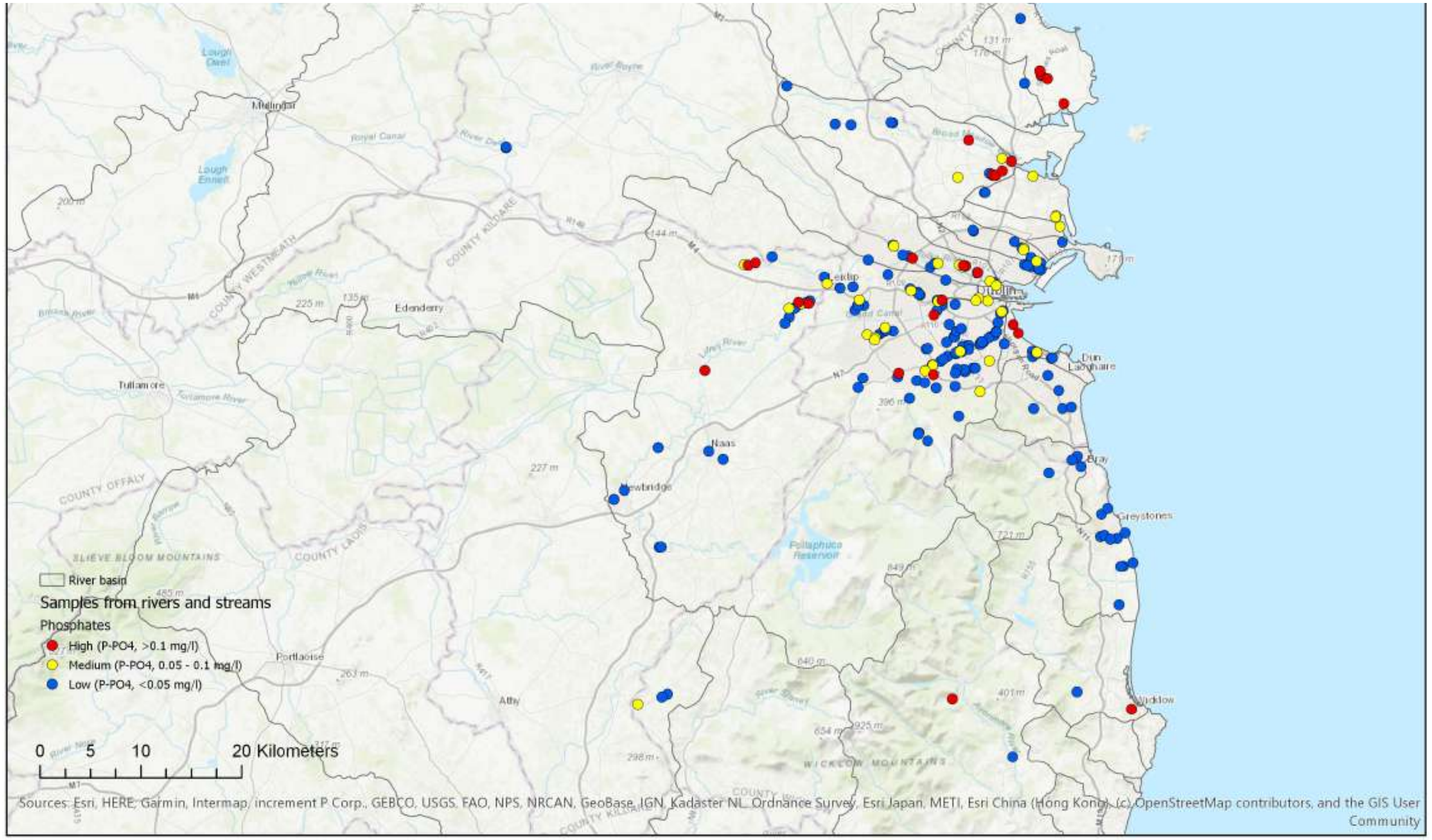
We report the first application of CRISPR-Cas technology to single species detection from environmental DNA (eDNA). Organisms shed and excrete DNA into their environment such as in skin cells and faeces, referred to as environmental DNA (eDNA). Utilising eDNA allows noninvasive monitoring with increased specificity and sensitivity. Current methods primarily employ PCR-based techniques to detect a given species from eDNA samples, posing a logistical challenge for on-site monitoring and potential adaptation to biosensor devices. We have developed an alternative method; coupling isothermal amplification to a CRISPR-Cas12a detection system. This utilises the collateral cleavage activity of Cas12a, a ribonuclease guided by a highly specific single CRISPR RNA. We used the target species *Salmo salar* as a proof-of-concept test of the specificity of the assay among closely related species and to show the assay is successful at a single temperature of 37°C with signal detection at 535 nM. The specific assay, detects at attomolar sensitivity with rapid detection rates (<2.5 hr). This approach simplifies the challenge of building a biosensor device for rapid target species detection in the field and can be easily adapted to detect any species from eDNA samples from a variety of sources enhancing the capabilities of eDNA as a tool for monitoring biodiversity.

KEYWORDS
biosensor, CRISPR-Cas, eDNA, environmental, freshwater, salmon

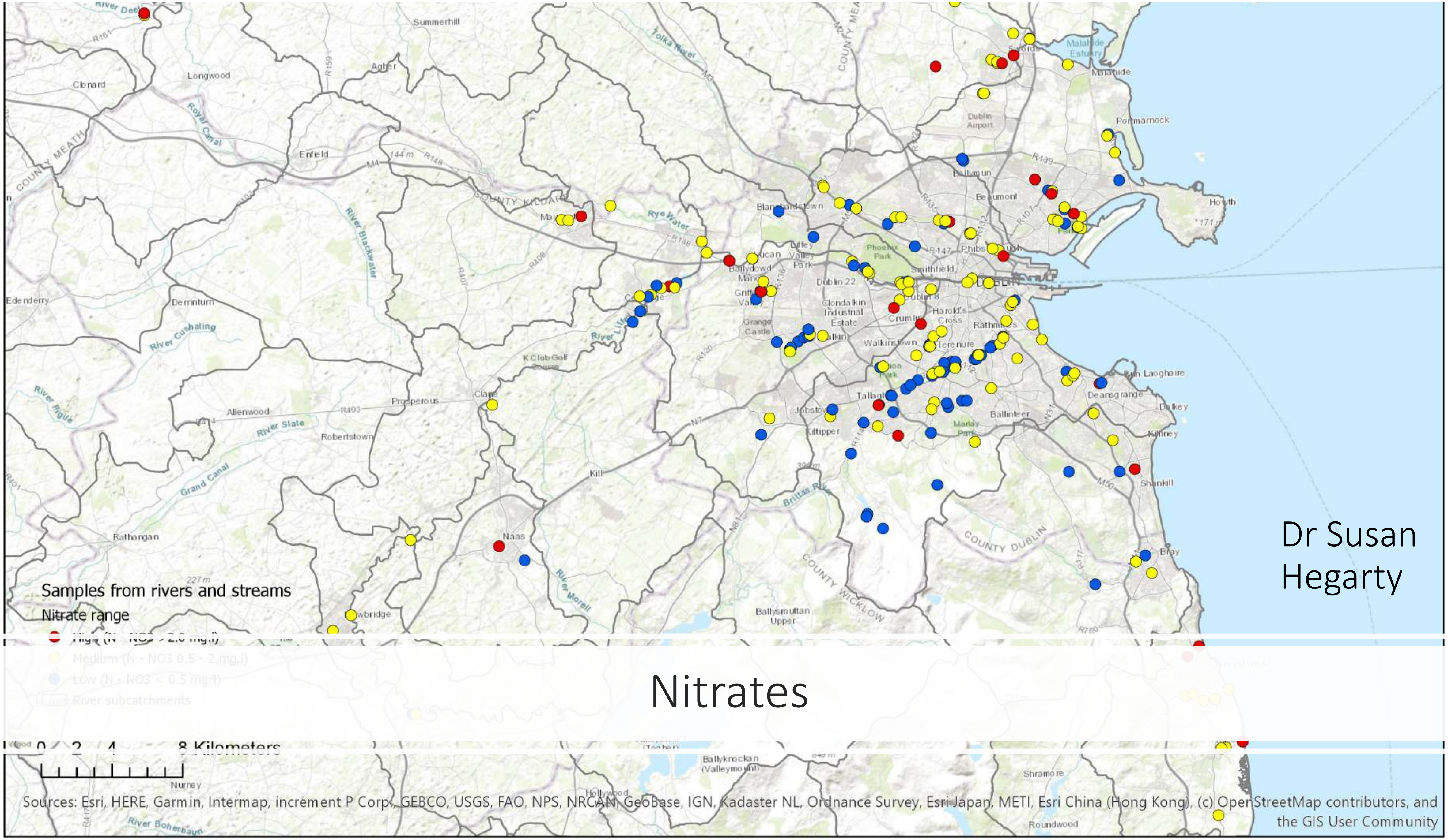
1 | INTRODUCTION

Environmental DNA (eDNA) offers a new opportunity for biologists and conservationists to monitor biodiversity and track invasive species from the organic material that they leave behind. The urgency of biodiversity monitoring is at an all-time high with the latest WWF Living Planet Index showing an overall decline of 60% in wildlife population sizes since 1970, rising to 83% for freshwater organisms (WWF, 2018). An organism can provide a rich source of eDNA in both soil and water through the cells and waste that they shed and excrete including faeces, mucus, gametes, hair and skin (Thomsen, Kielgast, Iversen, Wiuf, et al., 2012). As well as retrieving samples directly from the environment such as fresh or sea water, eDNA can also be collected from longer term deposits such as sediment and ice cores (Ficetola, Miaud, Pompanon, & Taberlet, 2008; Jerde, Mahon, Chadderton, & Lodge, 2011; Thomsen, Kielgast, Iversen, Møller, et al., 2012; Turner, Uy, & Everhart, 2015; Willerslev et al., 2007). eDNA will improve biodiversity monitoring by providing data on the variety, geographic range (Beans, 2018) and potentially the abundance of species enabling greater

Citizen science – Water Blitz

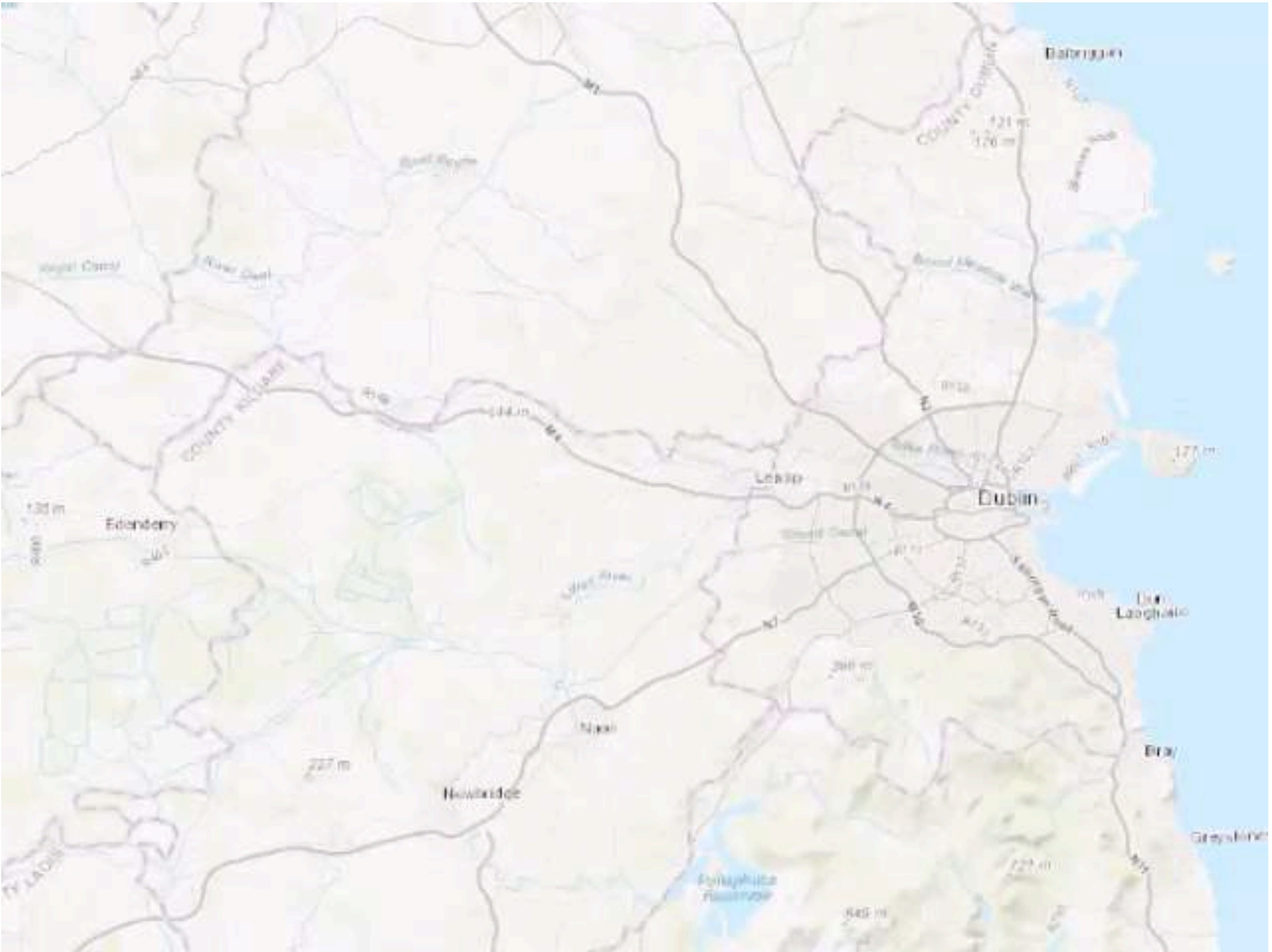


- Innovations can involve citizens
- In September 2019 we undertook the first water blitz
- >300 participants
- Nitrates, phosphates, litter, algae
- Rainfall events



Dr Susan Hegarty

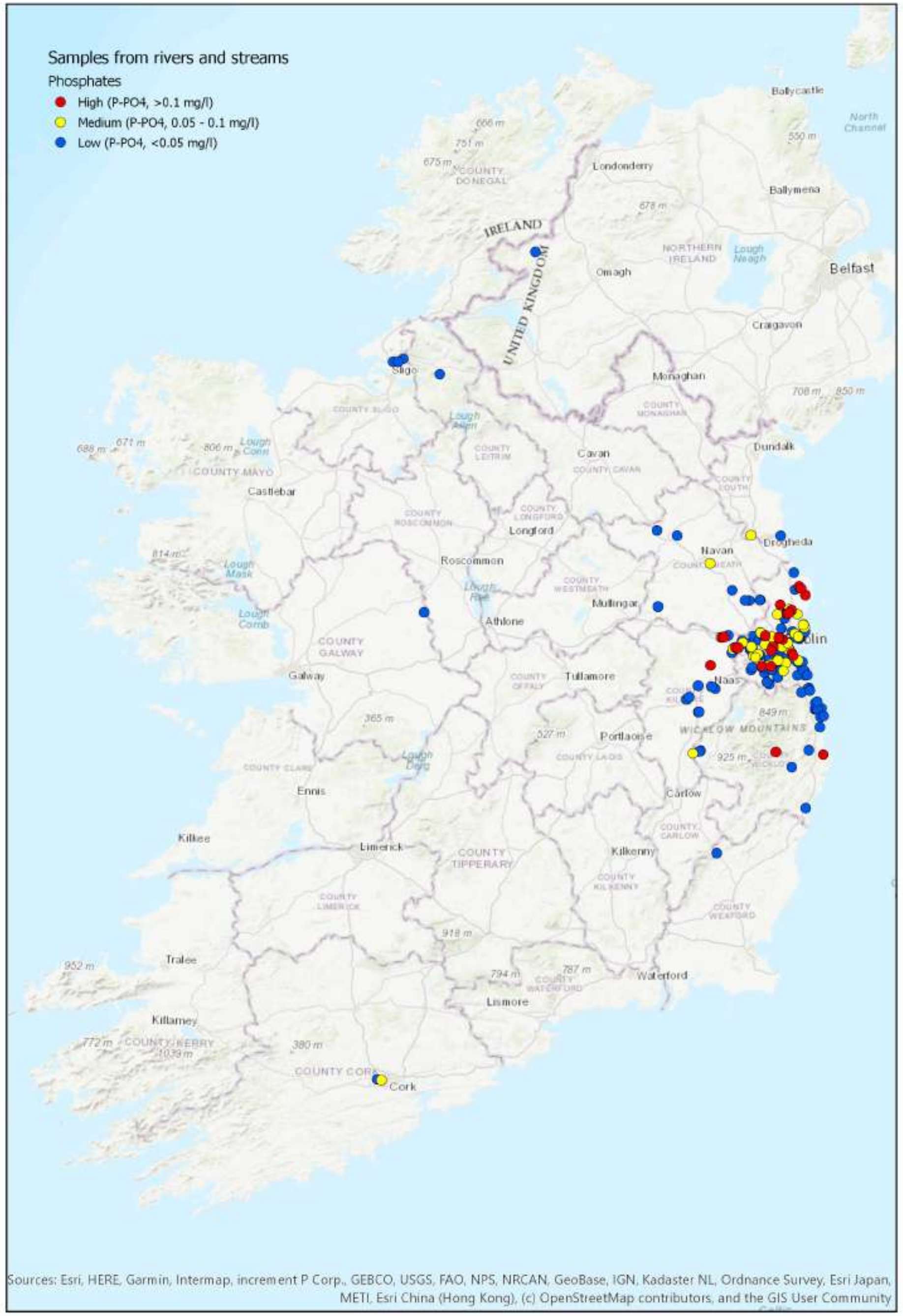
Nitrates



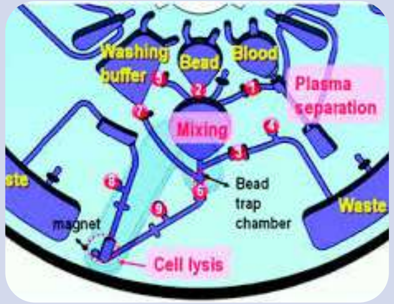
Dr Susan Hegarty

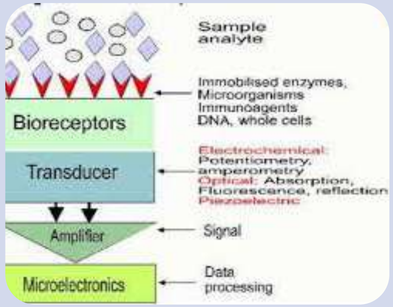
WaterBlitz

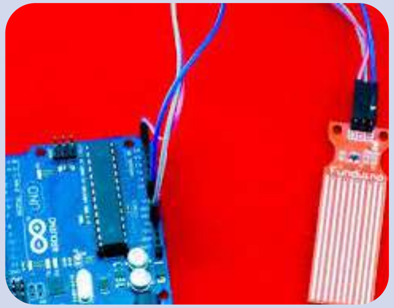
Citizens can play a really important role in monitoring



Key Messages







Need informed decision making in response to the need to manage & protect water

Be open to new ways of monitoring water for emerging contaminants of concern

Novel technology can play a role and we need to see significant investment in capacity and research

Integrate technology with data analytics – work with stakeholder to understand the problem

Better approach to management of a scarce resource





BEYOND 2020

DCU
Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

Thank you






Water JPI

Maria BOX

SEVENTH FRAMEWORK PROGRAMME

NCSR
National Centre for Sensor Research

Iascach Intíre Éireann
Inland Fisheries Ireland

Environment Agency

ASU

Marine Institute
Foras na Mara

Sea Change
Casadh na Taoide

SSTI
Strategy for Science, Technology and Innovation

epa
Environmental Protection Agency

BACKDROP