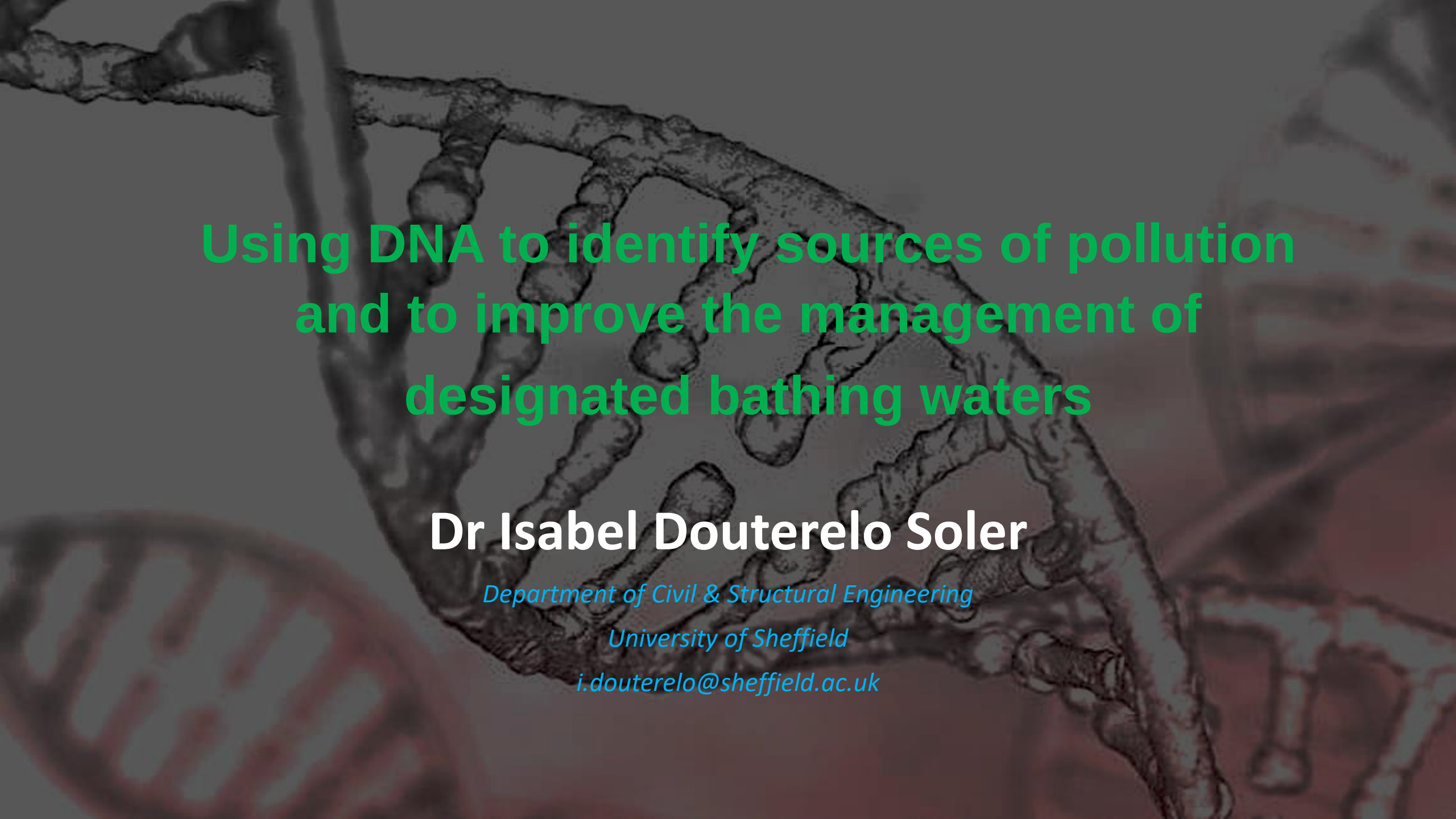

Using DNA to identify sources of pollution and to improve the management of designated bathing waters

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Rivers in England

England's rivers, including 85% of the world's precious chalk streams, are widely agreed to be a national treasure, yet only 14% are in good ecological health, and every single one fails to meet chemical standards.

https://theriverstrust.org/key-issues/state-of-our-rivers

UK Parliament

Water quality in rivers

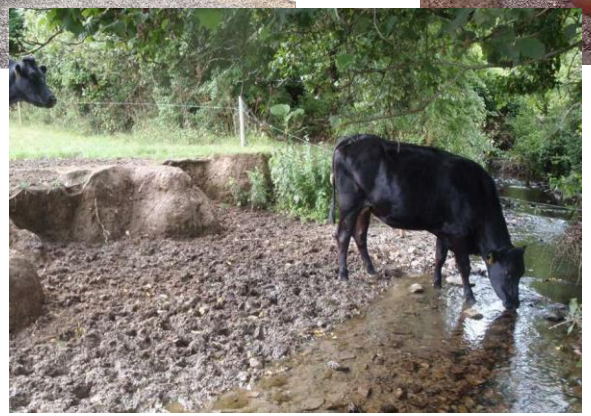
House of Commons Committee report: Only 14% of rivers in England can currently claim to have good ecological status. The Government is not on track to meet the Water Framework Directive requirement—subsequently transposed into UK law—for all rivers to reach good status by 2027. Wildlife and Countryside Link has warned that the water quality of rivers in England is the worst in Europe.

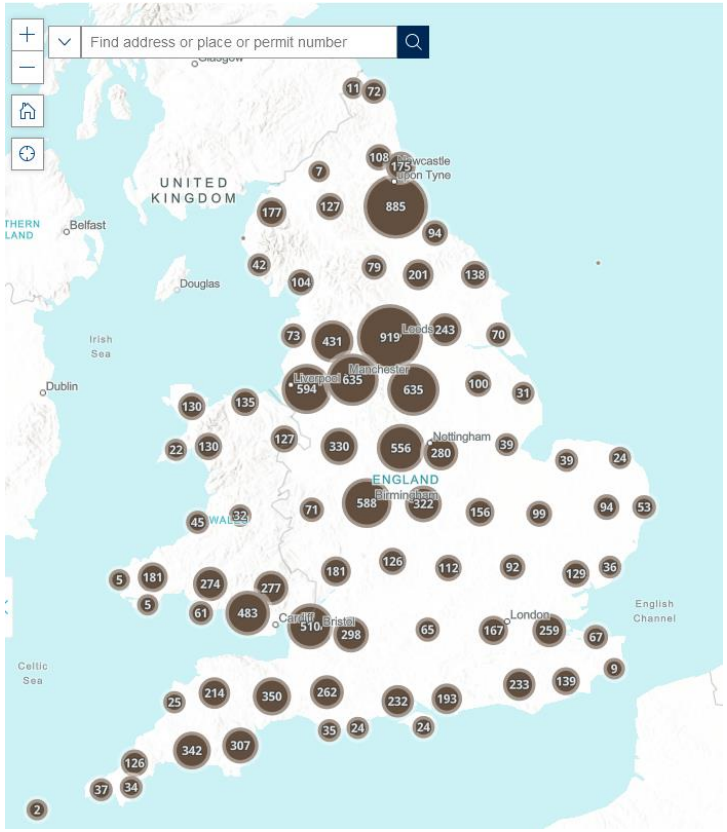
https://publications.parliament.uk/pa/cm5802/cmselect/cmenvaud/74/report.html

Where is the faecal pollution coming from?

- Wastewater infrastructure (CSOs, septic-tanks, etc.).
- Agricultural runoff (manure and livestock waste).
- Misconnections (toilets, sinks, dishwashers are incorrectly connected into drains that are intended to receive clean rainwater).

https://www.expressdrainagesurveys.co.uk/news/understanding-illegal-drain-connections





https://theriverstrust.org/key-issues/sewage-in-rivers

Citizen Scientists

ADDINGHAM CIVIC SOCIETY ENVIRONMENT GROUP

ILKLEY CLEAN RIVER GROUP

YorkshireDales Rivers Trust

Prof. Rick Battarbee
Emeritus Professor of Environmental Change at UCL
Former Director of the UCL Environmental Change Research Centre

River Wharfe@ILKLEY

Yorkshire swimming spot to get bathing water status in UK first

Campaigners in Ilkley hope River Wharfe designation will 'trigger a clean-up' of local sewage system

Ilkley River Wharfe bathing site gets 'poor' water quality rating

19 January 2022

BBC NEWS

Current Legislation

- Water quality in bathing sites & Faecal Indicator Bacteria (FIB)

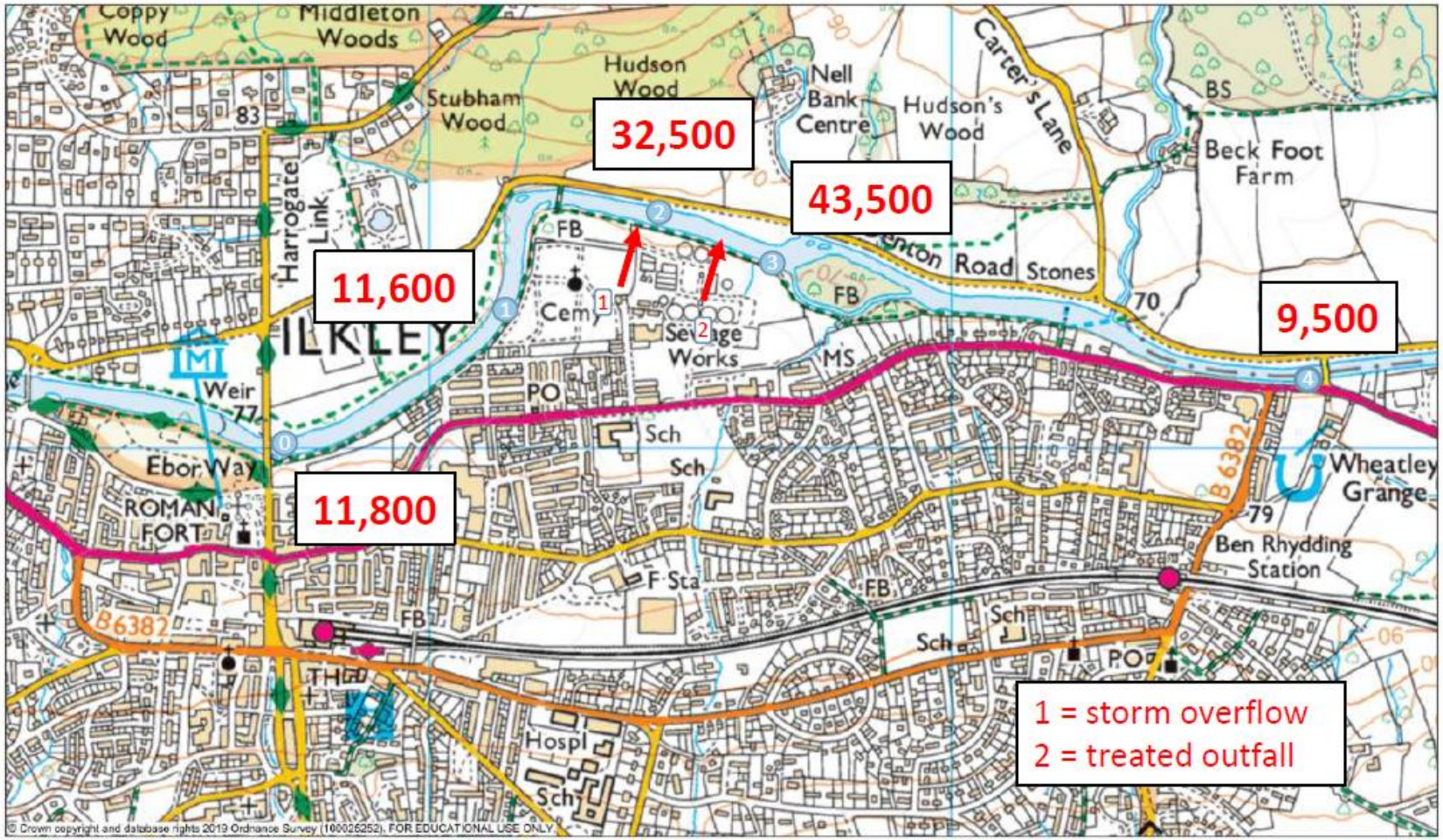
- Excellent – the highest, cleanest water quality
- Good – generally good water quality
- Sufficient – the water meets the minimum standard
- Poor – the water has not met the minimum standard

Bathing and recreational water standards, regulations, guidelines, and indicators on freshwater and marine bathing sites. INT Ent = intestinal enterococci, ENT = enterococci, GC = gene copies, STV = statistical threshold value, CCE = calibrator cell equivalents, per = percentile, GM = geometric mean, AFRI = acute febrile respiratory illness, and GI = gastroenteritis [5,6,7].

Regulation or Guideline	Indicator	Water Type	FIB Value (CFU or MPN/100 mL)	Reporting Metric	Illness Rate for Swimmers	Symptoms
[7]	Ent	Fresh/Marine	500	95 per	10% GI illness risk	AFRI, GI illness
[5]	INT Ent	Fresh	200 * (Excellent), 400 * (Good), 330 ** (Sufficient)	* 95 per, ** 90 per	AFRI: Excellent 1%, Good 2.5%, GI: Excellent 3%, Good 5%	AFRI, GI illness
[5]	INT Ent	Marine	100 * (Excellent), 200 * (Good), 185 ** (Sufficient)	* 95 per, ** 90 per	AFRI: Excellent 1%, Good 2.5%, GI: Excellent 3%, Good 5%	AFRI, GI illness
[5]	*E. coli*	Fresh	500 * (Excellent), 1000 * (Good), 900 ** (Sufficient)	* 95 per, ** 90 per	AFRI: Excellent 1%, Good 2.5%, GI: Excellent 3%, Good 5%	AFRI, GI illness
[5]	*E. coli*	Marine	250 * (Excellent), 500 * (Good), 500 ** (Sufficient)	* 95 per, ** 90 per	AFRI: Excellent 1%, Good 2.5%, GI: Excellent 3%, Good 5%	AFRI, GI illness
[6]	ENT	Fresh	30/110 STV	GM/STV	32/1000	GI illness
[6]	*E. coli*	Fresh	100/320	GM/STV	32/1000	GI illness
[6]	ENT	Marine	35/130 STV	GM/STV	36/1000	GI illness
[6]	ENT qPCR (GC)	Fresh/Marine	470 CCE/2000 CCE	GM/STV		GI illness
[6]	ENT qPCR (GC)	Fresh/Marine	1000 CCE	75 per		GI illness

Tiwari et al.,. Int J Environ Res Public Health. 2021 May 21;18(11):5513. doi: 10.3390/ijerph18115513.

Ilkley Wharfe: *E. coli* (cfu/100 ml) during high flow and a spill (13th June 2019)



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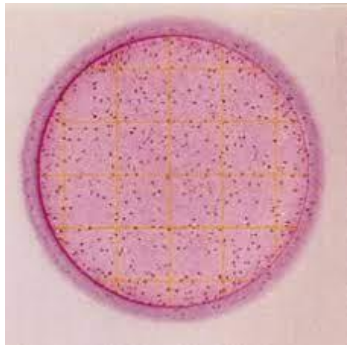
Limitations of Faecal Indicator Bacteria (FIB)

- The presence of FIB does not necessarily imply the presence of harmful pathogens.
- FIB **do not distinguish between sources** of faecal contamination. They cannot identify whether the contamination is from human, livestock, or wildlife sources.
- **Short Half-Life**: FIB have a relatively short half-life in aquatic environments, which means that their presence in water may not accurately reflect historical contamination events.



Agent	Illness	Probable Source	Transmission Pathway
Campylobacter spp.	Gastroenteritis, fever	Human and animals	Ingestion
Enteropathogenic *E. coli*	Bloody diarrhea, abdominal cramp	Human and animals	Ingestion
Helicobacter pylori	Gastritis, abdominal pain	Human and animals	Ingestion
Legionella spp.	Pneumonia, gastroenteritis	Natural	Inhalation
Leptospira spp.	Fever, headache, vomiting, jaundice	Natural and animals	Ingestion
Salmonella spp.	Gastroenteritis, fever, pain	Human and animals	Ingestion
Mycobacterium avium	Respiratory disease	Natural	Inhalation/contact
Vibrio vulnificus	Infection in pre-existed open wound	Natural	Wound infection
Shigella spp.	Bacillary dysentery, abdominal pain	Human	Ingestion
Adenovirus	Gastroenteritis, respiratory disease	Human	Ingestion, inhalation
Noroviruses	Gastroenteritis	Human	Ingestion
Rotaviruses	Gastroenteritis	Human	Ingestion
Coxsackievirus	Mild febrile illness to myocarditis	Human	Ingestion
Enteroviruses	Central nervous system, ocular and respiratory infections	Human	Ingestion
Echovirus	Diarrhea, secretions from the eyes or throat	Human	Ingestion
Hepatitis A virus	Liver disease	Human	Ingestion
Hepatitis E virus	Liver disease	Human and animals	Ingestion
Cryptosporidium	Diarrhea, abdominal pain, fever	Human and animals	Ingestion
Giardia	Diarrhea, abdominal cramp	Human and animals	Ingestion
Microsporidia	GI illness, diarrhea	Human and animals	Ingestion
Naegeria fowleri	Meningoencephalitis	Natural	Contact

Tiwari et al.,. Int J Environ Res Public Health. 2021 May 21;18(11):5513. doi: 10.3390/ijerph18115513.

Bathing Season Monitoring

River Wharfe

Sample code	Catchment Type	Description
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S3	Tributary_GW	Downstream of small STW
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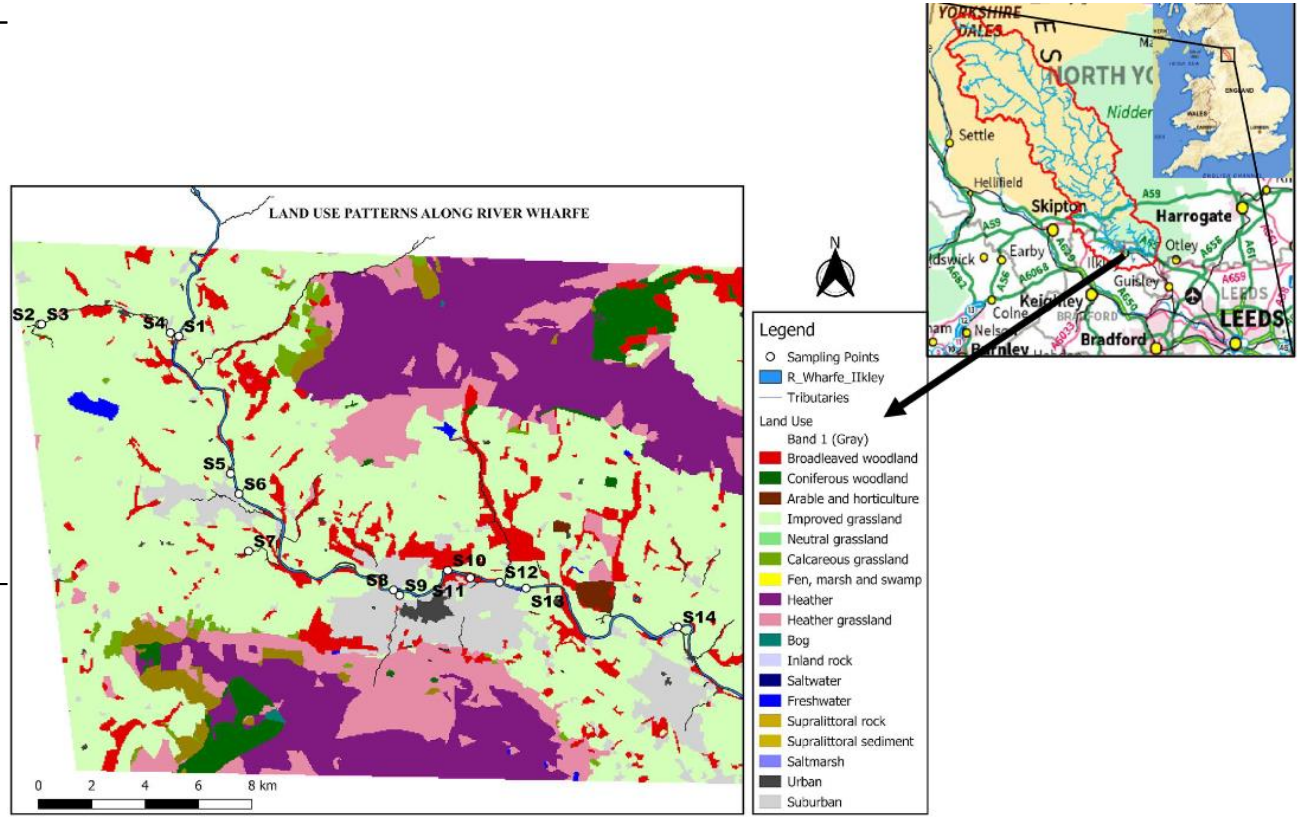
S6	Main river_U	Upstream of Sewage Pumping Station (SPS)
S7	Tributary_GW	Rural catchment, livestock and septic tanks
S8	Main river_U	Upstream of CSOs
S9	Tributary_U	Downstream of CSO
S10	Main river_U	Main bathing site, upstream of STW
S11	Main river_U	Downstream of STW
S12	Main river_U	Downstream of STW
S13	Main river_U	Downstream of STW and CSOs
S14	Main river GW/U	Downstream of STWs

GW – Grassland/Woodland, U – Urban/Suburban
STW-Sewage Treatment Works
CSO-Combined Sewer Overflow



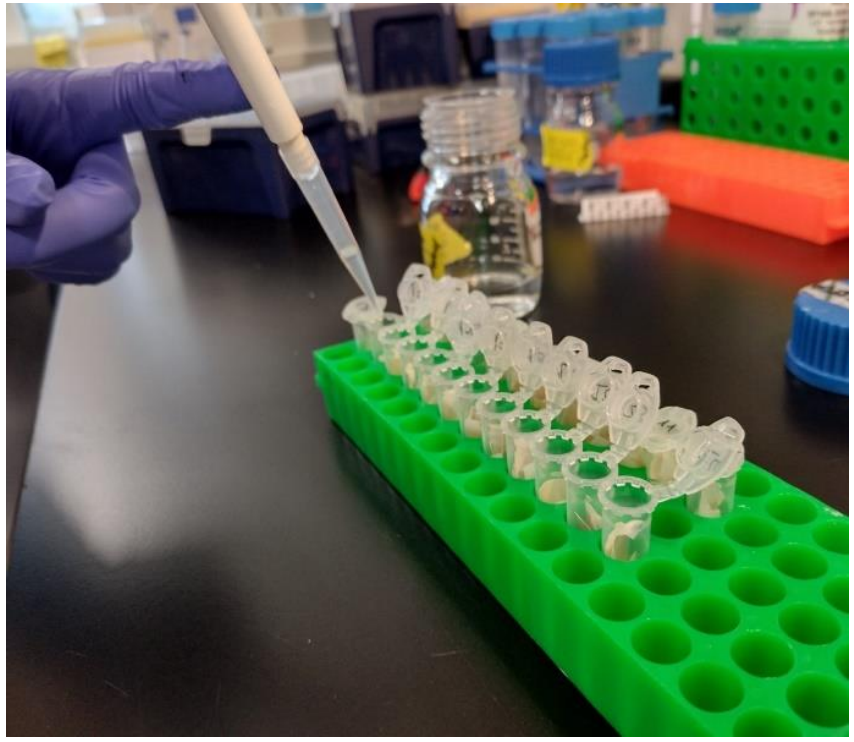
Field Work Sampling

Bathing Season 2021

- Hambleton Beck
- Lumb Beck
- Bolton Bridge
- Addingham Suspension Bridge
- Ilkley Old bridge
- Ilkley Suspension Bridge
- Beanland Island
- Denton Bridge
- Burley Weir Stepping Stones
- Spicey Beck (August)
- Wine Beck (August)
- Draughton (September)
- Stepping Stones (Denton Road)

DNA work

We can extract DNA from the microorganisms present in the samples. This involves concentrating the cells by filtering water, and breaking and opening the cells to release their DNA, which is then purified for analysis.



How can we use DNA?

DNA

Sequence the DNA to obtain bacterial diversity (16s rRNA)

qPCR to quantify specific bacterial markers to determine source of pollution

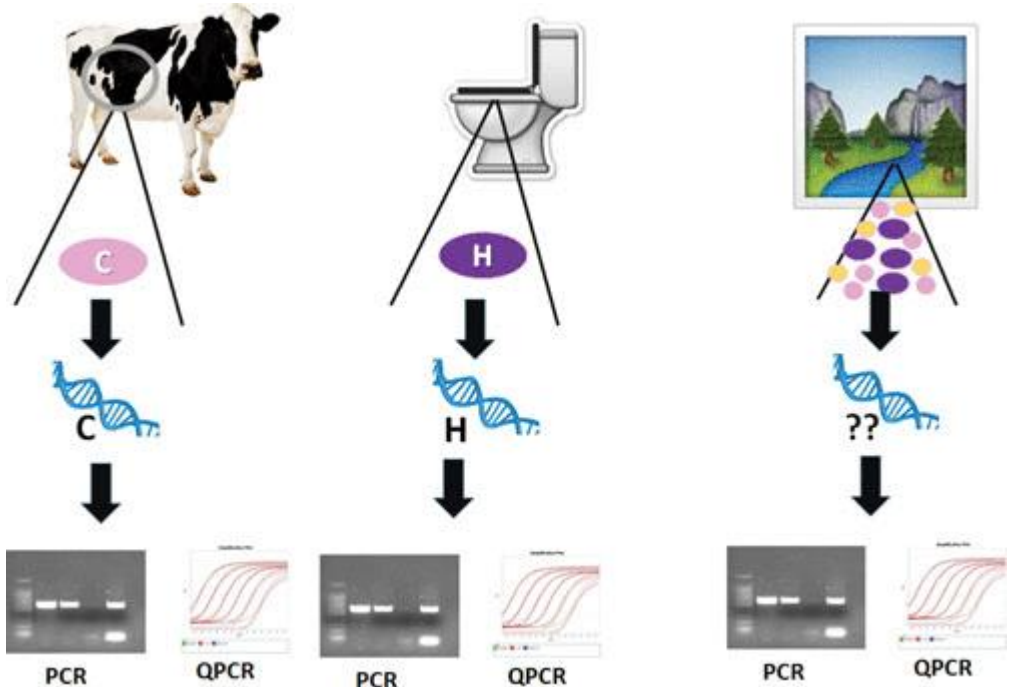
Microbial Methods

- **MST**: qPCR (quantify genes of specific markers)

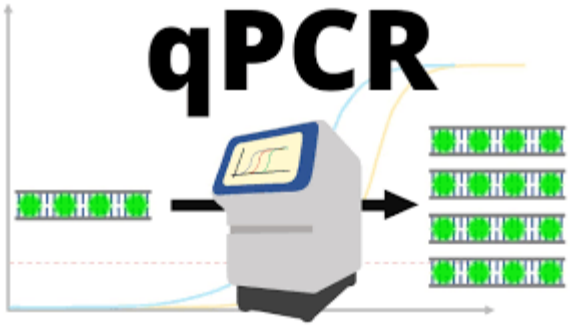
  - GenBac: general indicators of faecal pollution
  - HF183: Human
  - RumBacB2: Ruminants (cow, horse, sheep, goat and pig)

- Bacterial fingerprint using DNA and sequencing from water samples: does type of faecal pollution provide a unique fingerprint?

Microbial Source Tracking (MST)

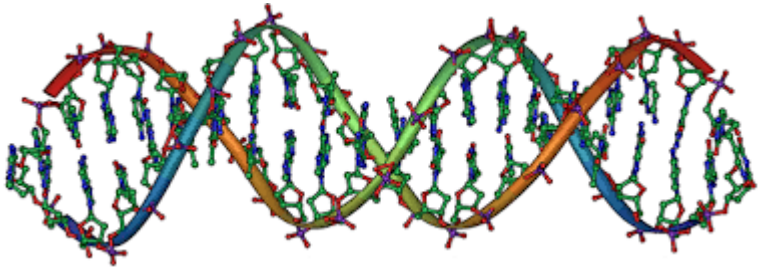


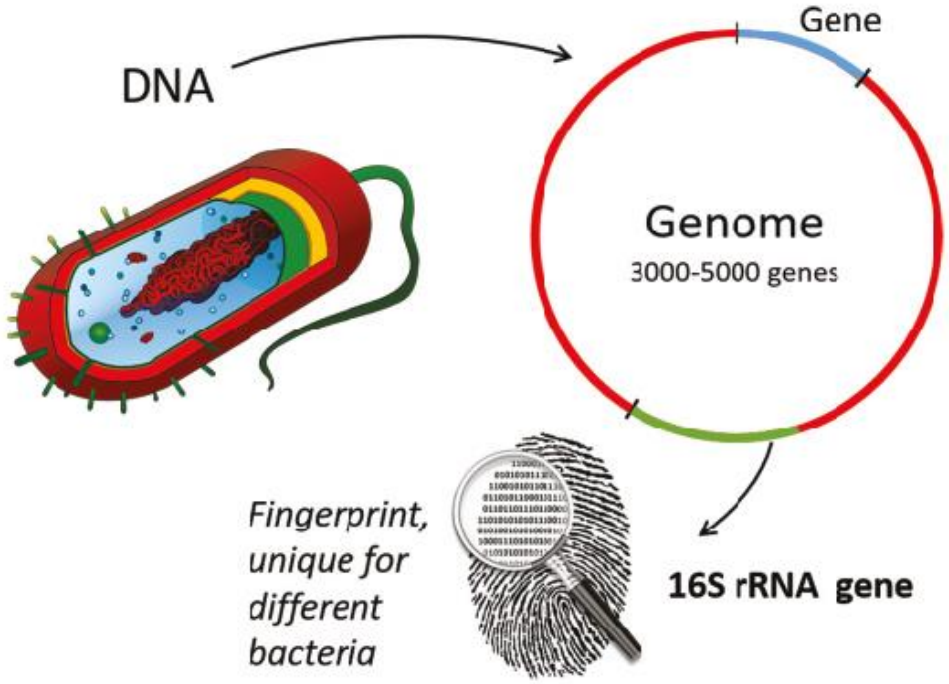
Assay	Primer/probe	Oligonucleotide sequence (5'–3')
General Bacteriodales GenBac3 (TaqMan)	GenBacF3	GGGGTTCTGAGAGGAAGGT
	GenBac4R	CCGTCATCCTTCACGCTACT
	GenBact2P	6FAM CAATATTCCTCACTGCTGCCTCCCGTA_TAMRA
Human specific Bacteriodales HF183 (TaqMan)	HF183F	ATCATGAGTTCACATGTCCG
	BthetR1	CGTAGGATTTGGACCGTGT
	BthetP1:	6FAM-CTGAGAGGAAGGTCCCCCACATTGGA_TAMRA
Ruminant specific Bacteriodales BacB2 (Taqman)	BacB2 590F	ACAGCCCGCGATTGATACTGGTAA
	Bac708Rm	CAATCGGCTTCGTGAT
	BacB2-626P	6FAM-ATGAGGTATGGAATTCGTGGTGT-BHQ1



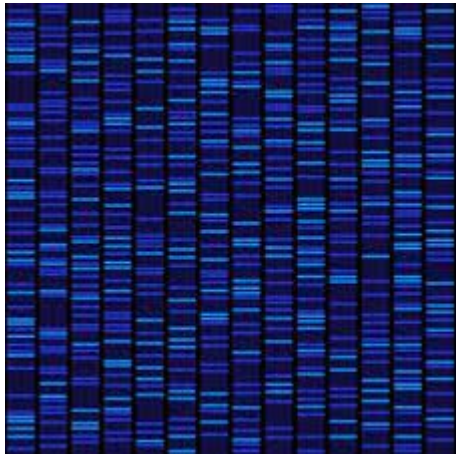
Teaf et al., 2018. https://doi.org/10.1128/microbiolspec.emf-0014-2017

Sequencing DNA





- Discover the diversity of bacteria in the water.
- Establish a fingerprint based on pollution.
- Identify potential pathogens and indicators.
- Inferred antimicrobial resistance genes.



Andersen, M.H., McIlroy, S.J., Nierychlo, M., Nielsen, P.H., Albertsen, M., 2018. Genomic insights into Candidatus Amarolinea aalborgensis gen. nov., sp. nov., associated with settleability problems in wastewater treatment plants. Systematic and Applied Microbiology. https://doi.org/10.1016/j.syapm.2018.08.001

Yorkshire Multiparametric Water Quality Sondes

- The monitors (Xylem EX02, Xylem Analytics, UK) were installed *in situ* in 7 sites along the river (upstream and downstream the bathing site) and measured levels of **dissolved oxygen, temperature, pH, conductivity** and **ammonia.**

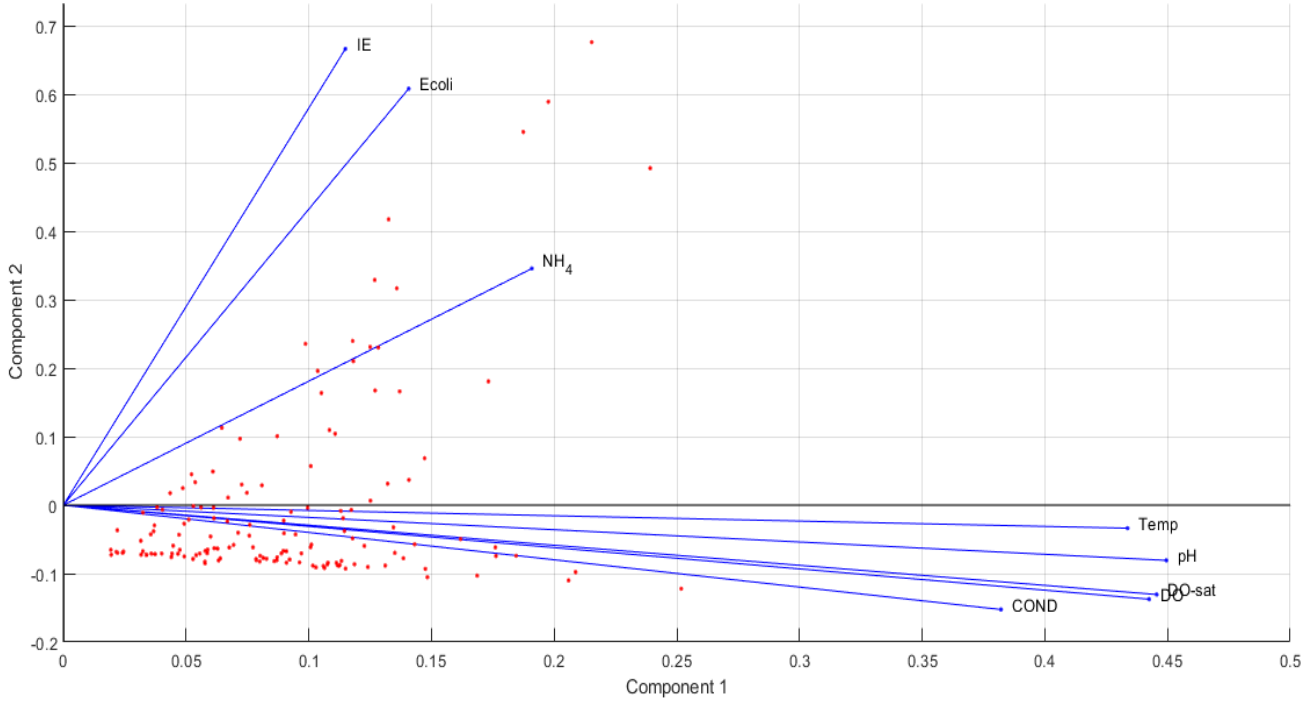
- The Environment Act 2021, requirements are yet to be finalised and will not come into force until 2025.

YorkshireWater

Karunakaran E, Battarbee R, Tait S, Brentan BM, Berney C, Grinham J, Herrero MA, Omolo R, Douterelo I. Integrating molecular microbial methods to improve faecal pollution management in rivers with designated bathing waters. Science of the Total Environment. 2024 Feb 20;912:168565.

Multiparametric Water Quality Sonde

Ammonia nitrogen in water is commonly associated with faecal pollution, it is produced by the breakdown of organic matter, including faeces. When faeces enters the environment, it can be broken down by microorganisms, which produces ammonia.



- **Sewage** contains a high concentration of faeces and other organic matter, so when it leaks into the environment, it can release a large amount of nitrogen compounds.
- **Runoff** from agricultural areas can contain manure and other livestock waste, which contains high levels of nitrogen.

$$\underset{\textit{Ammonia}}{NH_3} + H_2O \rightarrow \underset{\textit{Ammonium}}{NH_4^+} + OH^-$$

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MST (anthropogenic vs. zoogenic)

	Site	GenBac	HF183	RumBacB2
June	S1	8.7E+06 ± 1.2E+06	5.1E+04 ± 1.6E+04	B.Q.L
	S4	1.3E+07 ± 1.7E+06	7.4E+04 ± 1.4E+03	B.Q.L
	S6	6.8E+06 ± 1.8E+06	3.4E+04 ± 4.3E+03	B.Q.L
	S7	9.8E+06 ± 9.5E+05	4.6E+03 ± 1.1E+03	B.Q.L
	S8	7.1E+06 ± 2.7E+06	3.1E+04 ± 2.8E+03	B.Q.L
	S10	8.6E+06 ± 7.9E+05	5.2E+04 ± 1.0E+04	B.Q.L
	S13	2.9E+07 ± 4.4E+05	4.1E+05± 1.4E+04	B.Q.L
July	S1	1.3E+07 ± 2.3E+06	3.5E+03 ± 1.3E+03	4.2E+05 ± 8.7E+02
	S4	4.8E+07 ± 3.2E+06	2.4E+05 ± 5.5E+03	B.Q.L
	S6	4.2E+06 ± 9.6E+05	B.Q.L	B.Q.L
	S7	9.1E+06 ± 2.1E+05	2.1E+04 ± 1.8E+03	B.Q.L
	S8	7.1E+06 ± 7.1E+05	3.9E+03 ± 1.4E+03	B.Q.L
	S10	8.6E+06 ± 7.9E+05	4.0E+04 ± 2.1E+04	B.Q.L
	S13	5.4E+07 ± 7.1E+06	2.6E+05± 1.4E+04	4.7E+05 ± 2.2E+04
August	S1	1.6E+07 ± 9.9E+05	9.9E+03 ± 5.8E+03	9.5E05 ± 0
	S4	9.1E+07 ± 1.4E+07	1.9E+05 ± 1.1E+04	B.Q.L
	S6	1.7E+07 ± 1.1E+05	3.0E+04 ± 6.4E+03	9.6E+05 ± 0
	S7	4.0E+07 ± 1.4E+06	2.4E+04 ± 4.1E+02	B.Q.L
	S8	2.1E+07 ± 1.5E+06	4.3E+04 ± 7.0E+03	B.Q.L
	S10	2.9E+07 ± 1.6E+06	1.4E+05 ± 7.0E+03	9.0E+05 ± 9.9E+04
	S13	5.4E+07 ± 2.6E+06	4.3E+05± 3.4E+04	8.8E+05 ± 4.6E+04
September	S7	5.0E+06 ±1.5E+05	2.5E+04 ± 4.0E+03	B.Q.L
	S10	1.4E+07 ± 5.5E+05	2.3E+04 ± 1.4E+03	4.1 E +04 ± 0
	S12	1.3E+08 ± 2.8E+07	1.1E+06 ± 1.4E+05	B.Q.L

B.Q.L (Below Quantification Limit)

- **GenBac: Faecal pollution**
- **HF183: Human**
- **RumBacB2: Ruminants (farm animals)**

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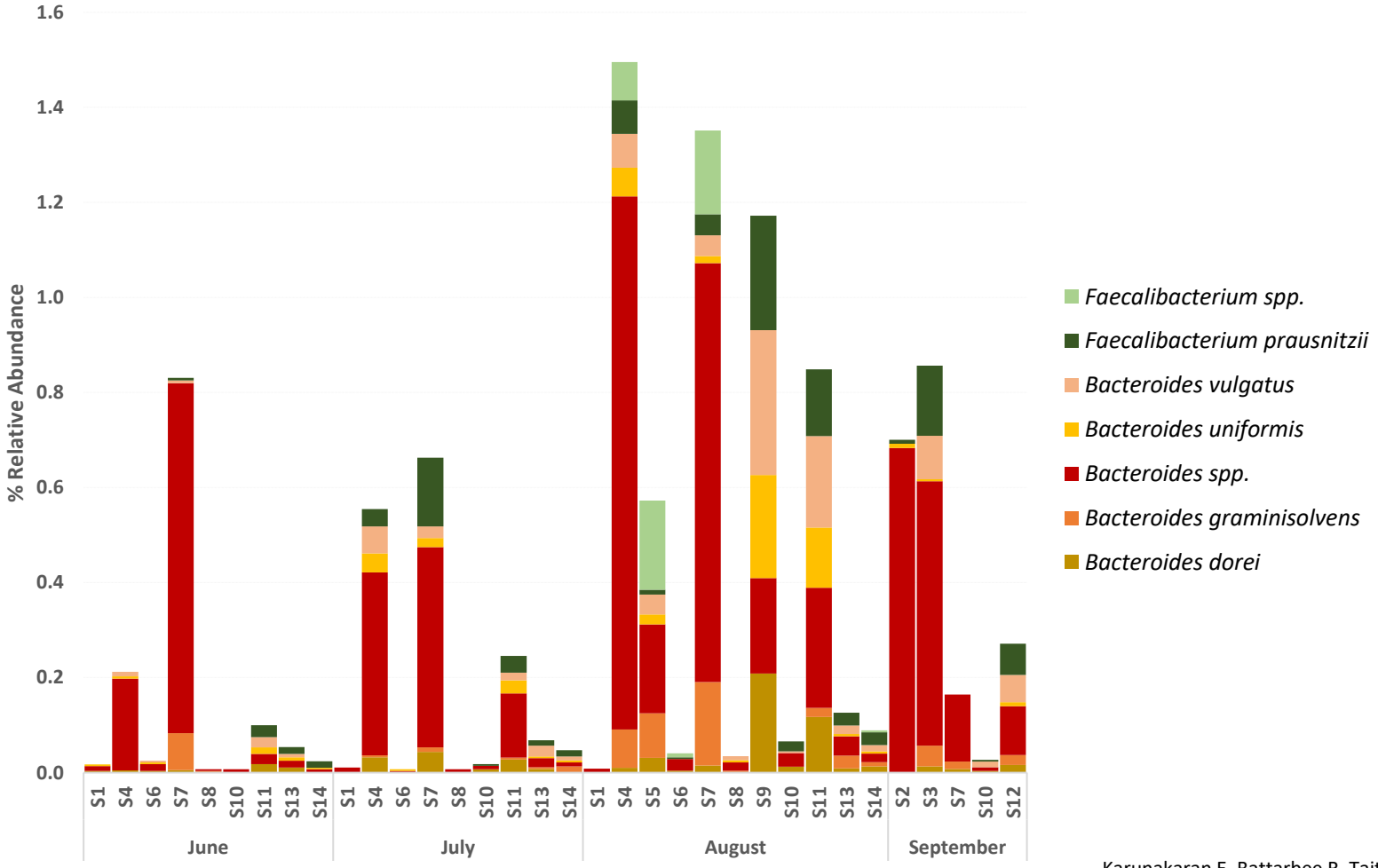
Faecal Indicator Bacteria



Sample code	Catchment Type	Description
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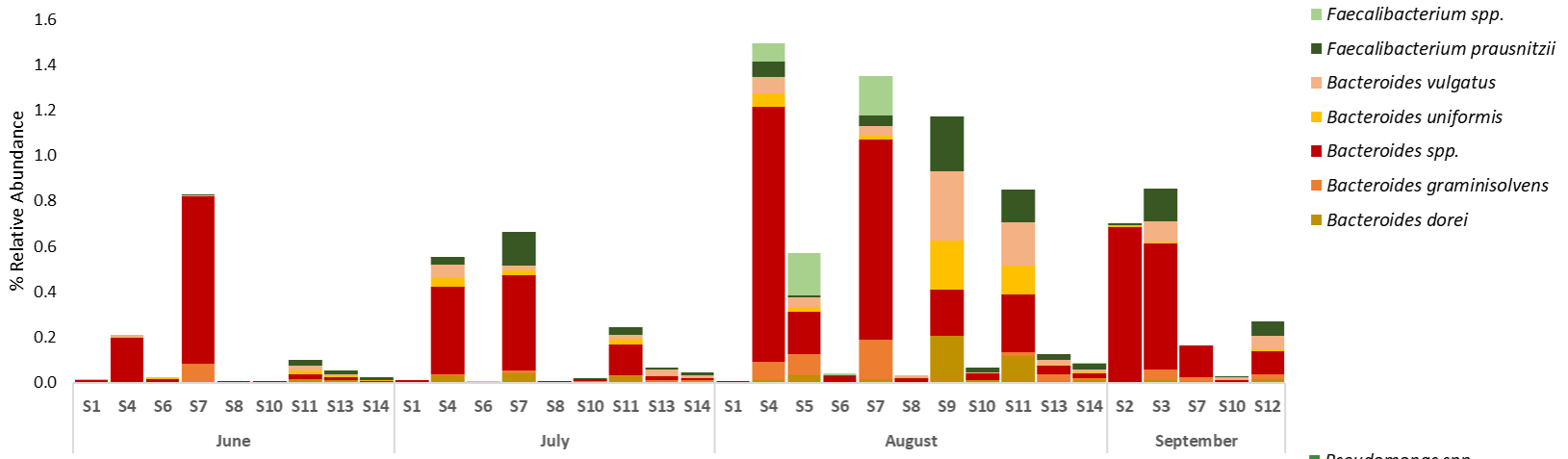
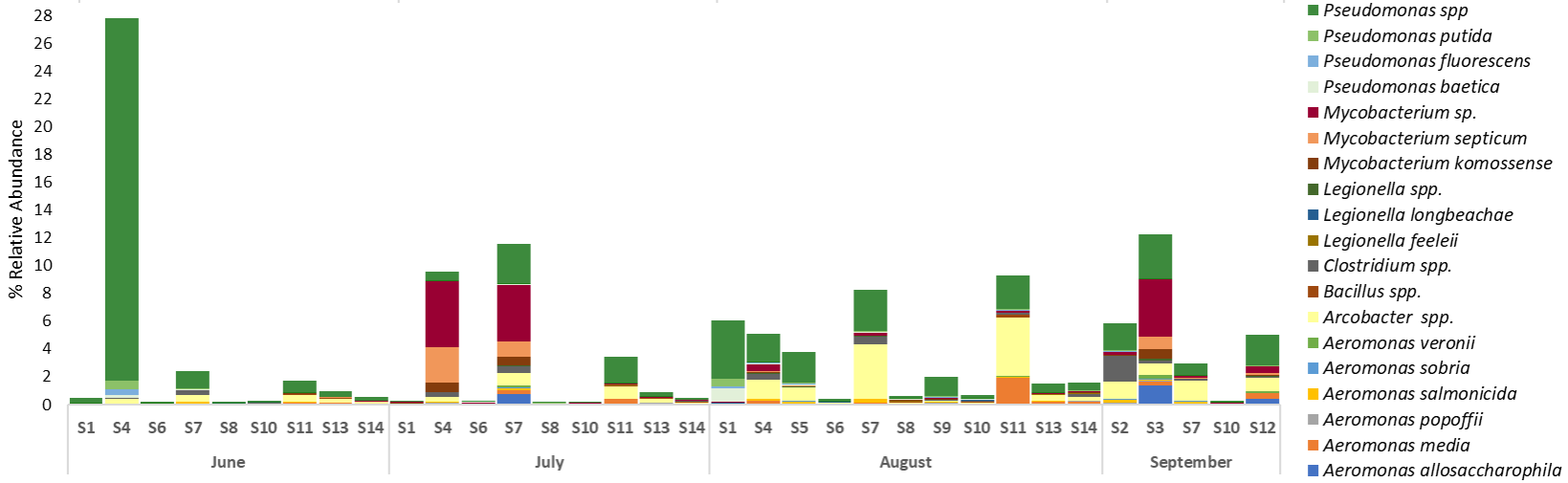
Pathogens

A)



B)



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What Yorkshire Water has done?

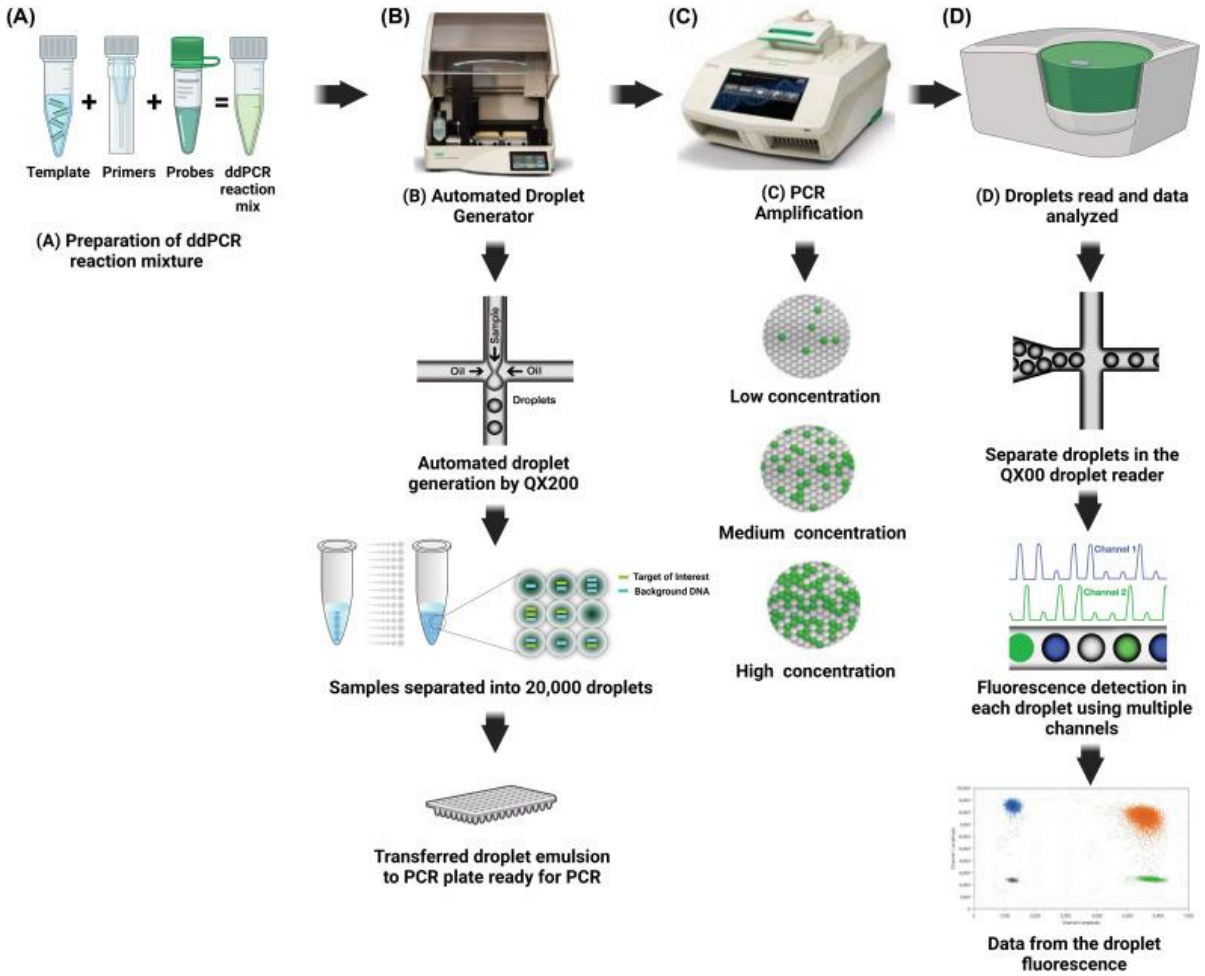
- Improvement of treatment (disinfection at small treatment plant affecting one of the becks, Draughton)
- Stormwater retention pond offered to farmers to avoid runoff.
- Upgrade the CSO, reroute the sewer network.

Yorkshire Water is committing up to £13m investment in a range of measures that aim to improve the Wharfe upstream of the bathing water. Enhanced disinfection measures will be applied to the final effluent returned to the environment at Grassington, Draughton and Beamsley treatment works, much like the measures taken on the coast, to reduce the impact on water quality.

Work will also be carried out to investigate misconnections in the catchment and a scheme to reroute the sewer network in some areas of Ilkley will be carried out to reduce discharges from storm overflows. A project is already underway to upgrade Rivadale CSO as part of this investment.

Yorkshire Water outlines £13m infrastructure investment to improve River Wharfe

Digital droplet PCR (ddPCR)



Faster and more sensitive than traditional qPCR methods

Chung, H.-K., et al. (2022). ." Microbiology Spectrum **10.**

Conclusions

1. Genetic markers work, and in the future, it will be easier, cheaper and faster to use them (policy should change!)
2. Sampling in wet/dry weather and increase frequency of sampling
3. Ammonium/Ammonia as a surrogate? for *in situ* multiparametric probes.

YorkshireWater

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YorkshireDales Rivers Trust