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A view on the most promising molecular tools for the UK regulatory community

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Help deliver government environmental priorities

Protect and improve the environment, promote sustainable development.

Protect human health, by regulating activities that can cause pollution and by monitoring the quality of air, land and water.

Identify the best environmental options and solutions, after considering the different impacts on water, land, air

The impact of a changing climate, the needs of a fast growing population, the need to secure future supplies of energy and food, the impact of waste, all during an economic downturn

Environment Agency

SEPA Scottish Environment Protection Agency

NIEA Northern Ireland Environment Agency www.ni-environment.gov.uk

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Pragmatic considerations for the UK regulatory community:

- (molecular ecology) research paper phrases:
  indicate, suggest, probably, further work is needed

- regulatory bodies would prefer more certainty
  you must act (£££s)
  you must stop (£££s)
  if we do this, we will see… ✓

- quantification of actions
  improve water quality by *X*%

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Why would regulators use molecular biological techniques?

1. Faster
2. Access information unavailable by other routes
3. “Nice to do” as well as “Have to do”

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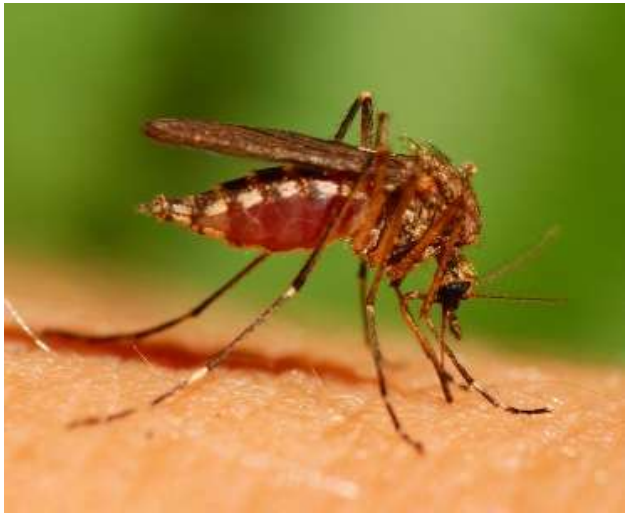
Why would regulators use molecular biological techniques?

1. Faster:

    - rapid measurement that is useful (e.g. comparable to regulatory method)
    - detection of viruses



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Why would regulators use molecular biological techniques?

2. Access information unavailable by other routes

e.g. microbial source tracking, norovirus, diversity indices, identification

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Why would regulators use molecular biological techniques?

3. “Nice to do” as well as “Have to do”

- "background" data

- spread (control?) of invasive species

- quantification of the effects of pollution

- mystery problems

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Useful data relies on a chain of events:

Sampling, processing, molecular assay, data analysis, interpretation

In a robust system, the “molecular assay” is often the easy bit...

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Fantastic new approaches; NGS, emulsion/bead PCR, huge database

Compare "molecular" with "culture" - 100 ml sea water, *E. coli*, sampled 9 am Monday.

Sample, filter, incubate (e.g. TBX), count, report = Tuesday, 2pm
Sample, concentrate, lyse, extract/purify, aliquot, qPCR, data, report Monday at 5pm, Tuesday at 9am

When would this make a difference to the regulator?

Spin 1 – costs, staff, labs, culture works, 100ml processed.
Spin 2 – so much more *could* be done – stored, uncultured species, etc.

What is the specific regulatory question?

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Fantastic new approaches; arrays, gene expression, bioinformatics

Not all necessarily ready (yet) for routine use?
Data need interpretation.

Depending on question, may not necessarily need data immediately, e.g. NGS sequencing costs per run, sample DNA could be stored and combined

Lab vs. field - look to the military?
Quality control from the back of a van?
Immunoassays? Direct (non-amplified) detection?
Isothermal amplification?

Identification of useful sequences vs. utility of those seqs in regulation

Routine monitoring of sequences then uses most appropriate approach…

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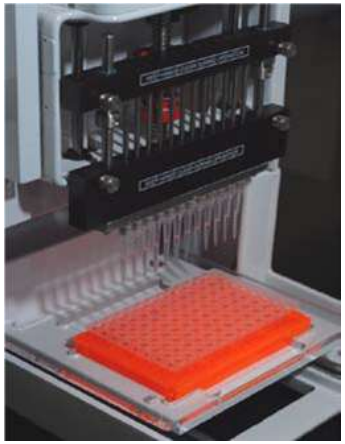
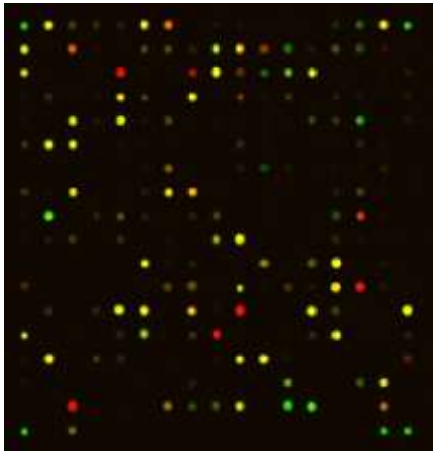
Useful data relies on a chain of events:

Upstream of all of this:

Assay development

Sampling, processing, molecular assay, data analysis, interpretation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Useful data relies on a chain of events:

Sampling, **processing**, molecular assay,data analysis, **interpretation**

BIAS - in capture, cleaning, amplification, hybridisation

NUMBERS - e.g. Bacteroidales 10^8, mtDNA 10^2 per 100 ml

DETECTION LIMITS - how many target molecules can you reliably quantify in a qPCR well? 20? 10? 5? <1? in 1-5ul, representing cubic metres of water?

SEQUENCE LENGTH - qPCR, 80-150 bases?

SPECIFICITY - amplification - primers/probe; capture - capture probe/detection probe

e.g. nucleic acid extraction - reliable, robust, routine, matrix range, environmental conditions, viral RNA , mRNA , rRNA, DNA – all are different

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Example: sources of bacteria within UK waters

Bacteroidales: human, ruminant

Mitochondrial DNA: avian (& human), dogs, sheep, cows, pigs

All use TaqMan qPCR analysis; with UKAS accreditation

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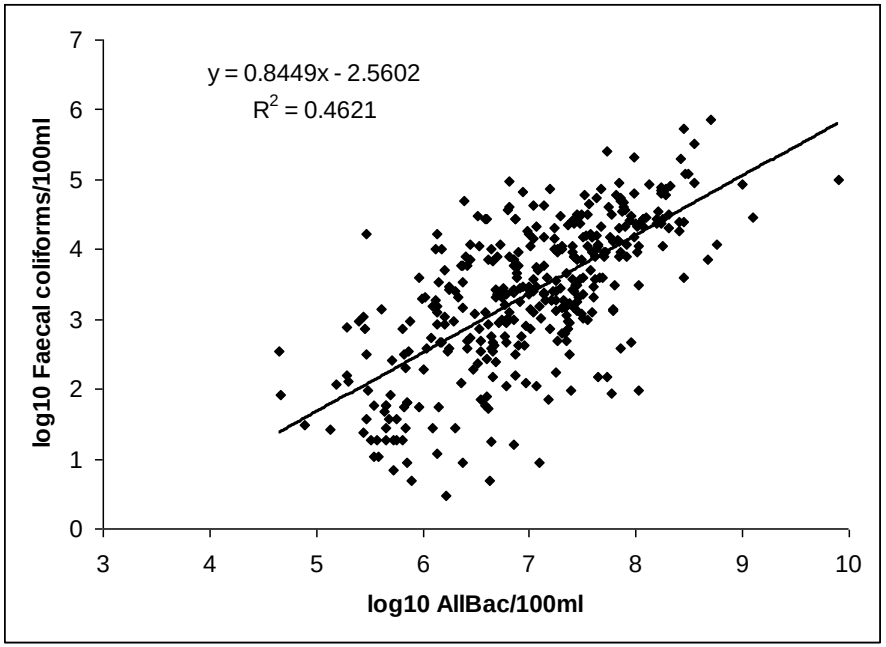
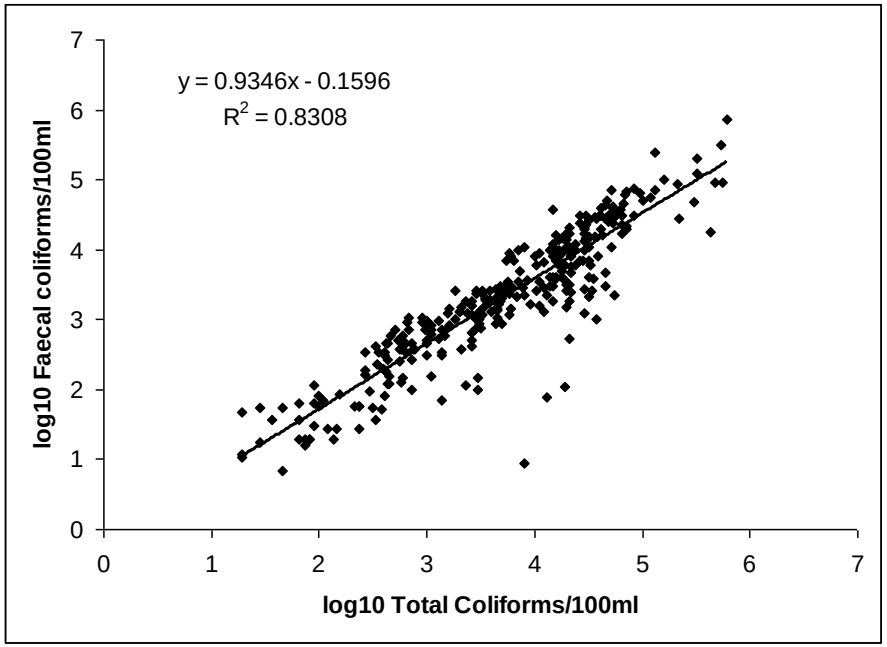
Using Bacteroidales to predict FIOs





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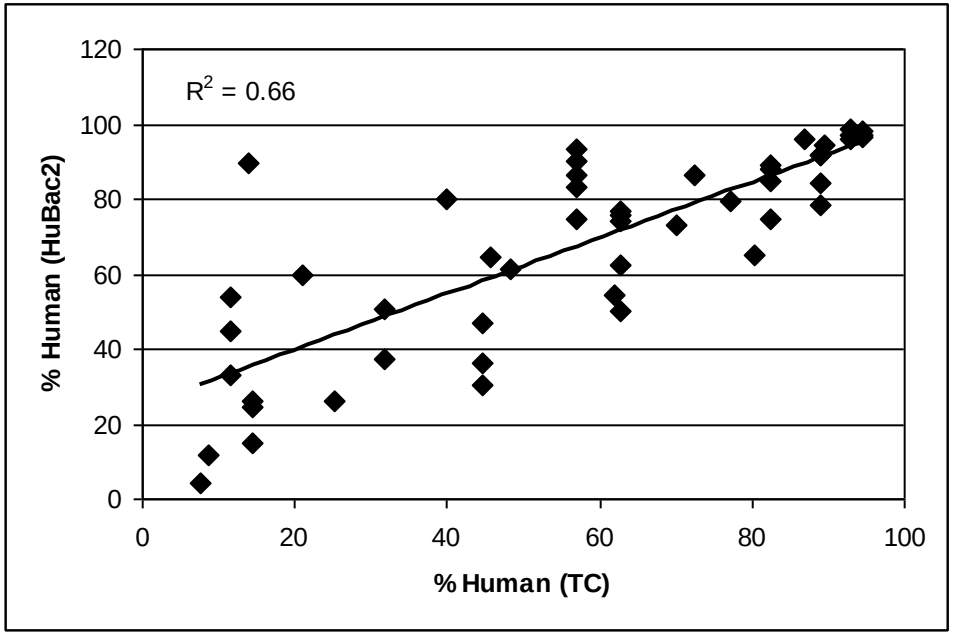
Artificial mixtures: Cattle/human

Overall plot (freshwater and seawater)

n=49 from duplicate runs

Similar from sheep/human (R-sq = 0.75)



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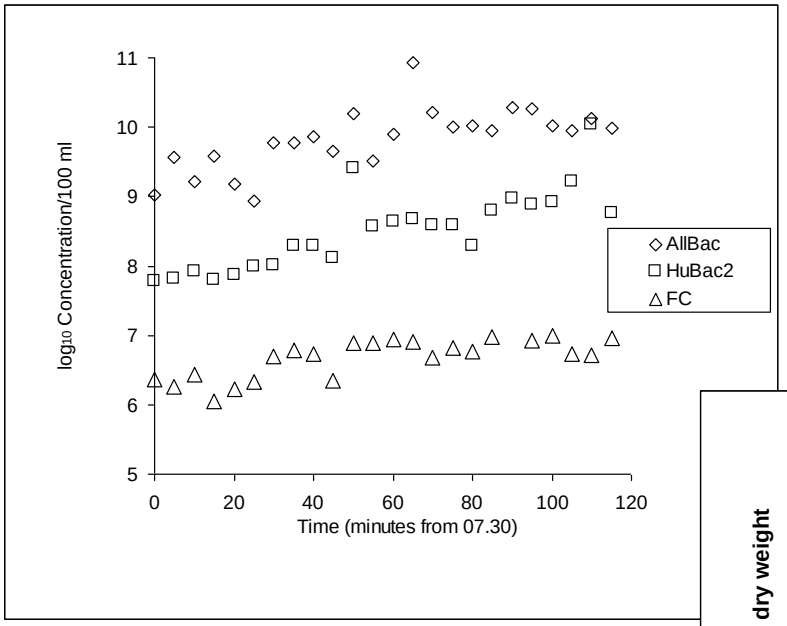
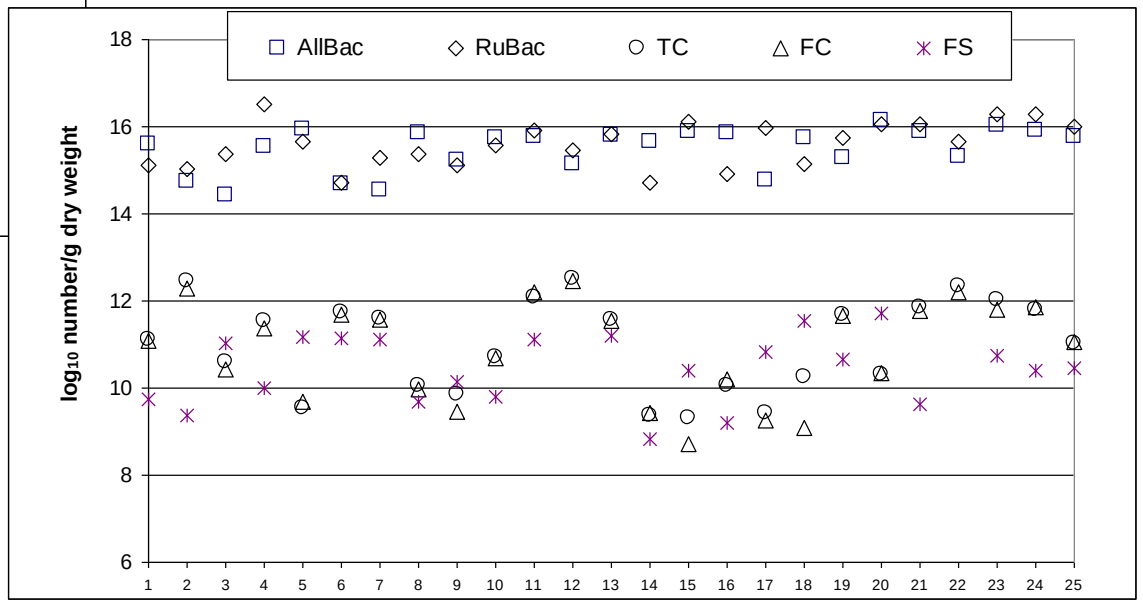


Estimating the variability in the real world…



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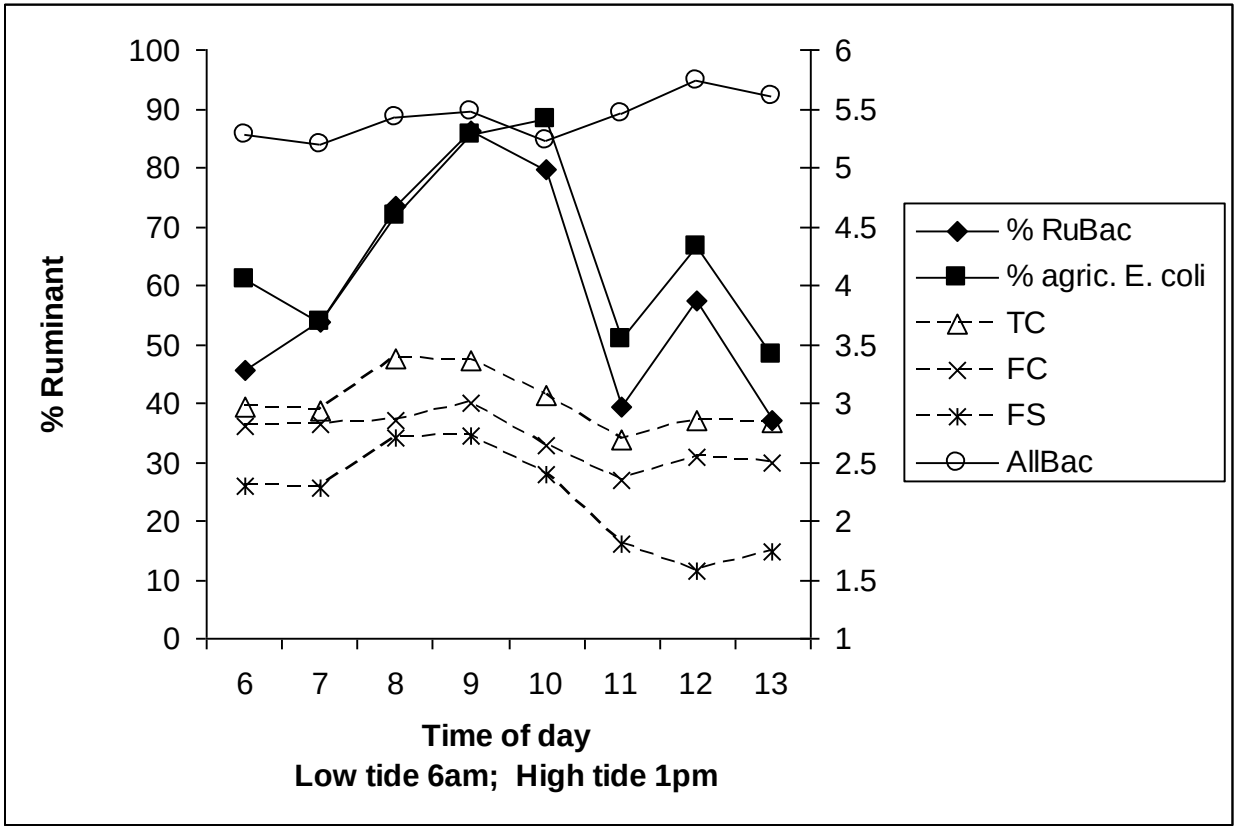
SAMPLING

Bacterial sources at Sidmouth

Similar data collected from the river Exe, Axe/Seaton, and Sidmouth

The environment is complex!

Explaining the dynamics of these systems requires focused and intensive sampling effort



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Routine MST data. What does it tell you? – case study.

~40 bathing water samples collected and stored.

Eight samples were judged polluted.

Agricultural sources dominated seven out of eight.

We are NOT able to state (67+99+87…..)/7 = 79% ruminant sources.

We ARE able to state that faecal bacteria associated with ruminant animals dominated in these samples.

THIS REFLECTS OUR CURRENT GUIDANCE ON USING MST DATA.

%Human	%Ruminant
33	67
1	99
13	87
4	96
80	20
18	82
6	94
16	84

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How far can we extrapolate?

☑ Presence/absence

☑ Ranking of sources (multiple samples)

☒ Accurate quantification of FIO sources

Data recommended as supporting evidence

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“one or two key questions to be considered in moving from culture to molecular-based tools”

Narrowed it down…

Regulatory staff education
- DNA isn't difficult
- traditional techniques aren't always right!

Academic staff education
- DNA doesn’t always give an answer

Historical data sets have value
Culture may always have a place!
Can be combined with a molecular assay

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Explanatory data vs. regulatory data
- sampling, sampling, sampling, sampling - VARIABILITY

Local knowledge, add David Kay, obtain the “typical” picture
Sampling/analysis - gives a snapshot of the sampled environment; what was happening right then

Modelling - feed sample (molecular) data into modelling?

The need to develop more rapid, sensitive, and accurate methods for detecting specific NA sequences in complex, heterogeneous mixtures is still evident from the research literature.

Regulatory community may appreciate a clear answer to the question “What is the useful information that can be gained by profiling microbial communities and quantifying changes in soil, sediment, and water organisms?”

Obvious “lab assay questions” can be dealt with
ability to discriminate sequences
quantitative precision and accuracy in measuring abundances of specific sequences
ability to measure low concentrations of sequences
ease and speed of preparation and processing of samples
degree of multiplexing (i.e., the number of sequences that can be detected in a single run)
cost (perception vs. actual, but also if it solves a problem…?)

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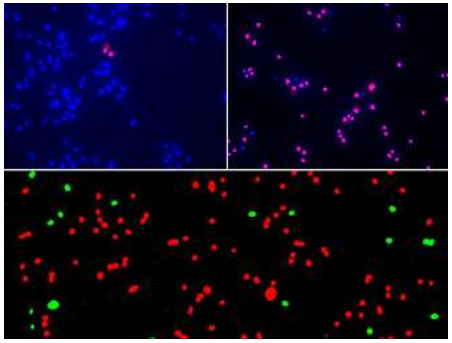
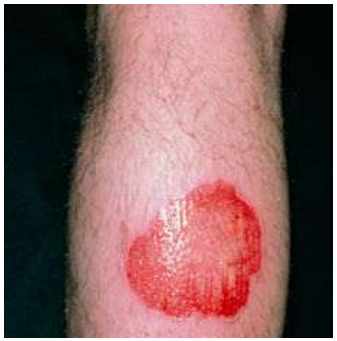
Protect human health, by regulating activities that can cause pollution and by monitoring the quality of air, land and water.

Live vs. dead infective vs. non-infective





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defra
Department for Environment Food and Rural Affairs

dwi
DRINKING WATER INSPECTORATE

SEPA
Scottish Environment Protection Agency

The Scottish Government

UKWIR
UK WATER INDUSTRY RESEARCH LIMITED

NATURAL ENVIRONMENT RESEARCH COUNCIL

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