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@SAGB

Shellfish Association of Great Britain

The FISHMONGERS' Company's FISHERIES CHARITABLE TRUST

Department for Environment Food & Rural Affairs

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Crown Estate Scotland
Oighreachd a' Chrùin Alba

SEPAmatic

Llywodraeth Cymru
Welsh Government

DASSHH

Developing an Assurance Scheme for Shellfish & Human Health

Lewis Le Vay
Bangor University

SAGB Conference
May 2022

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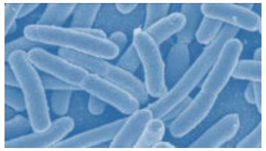
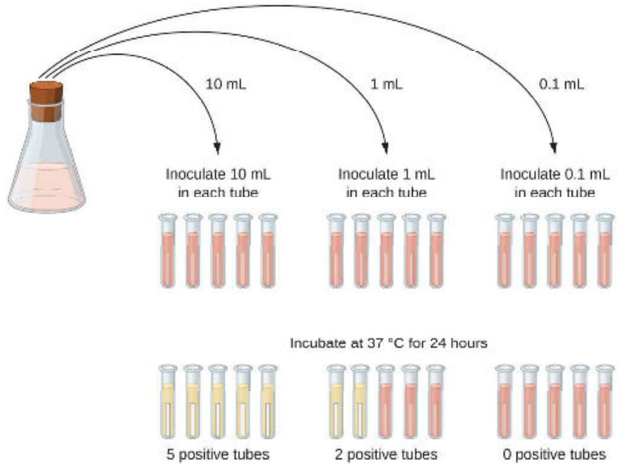
Tim & Luke Marshall
Porthilly Shellfish

The Official Control framework for shellfish hygiene (UK-retained EU legislation)

a) **Classification characterises production areas, based on *E. coli* an indicator of faecal contamination**





Most probable number method (MPN) to count *E. coli* in shellfish samples

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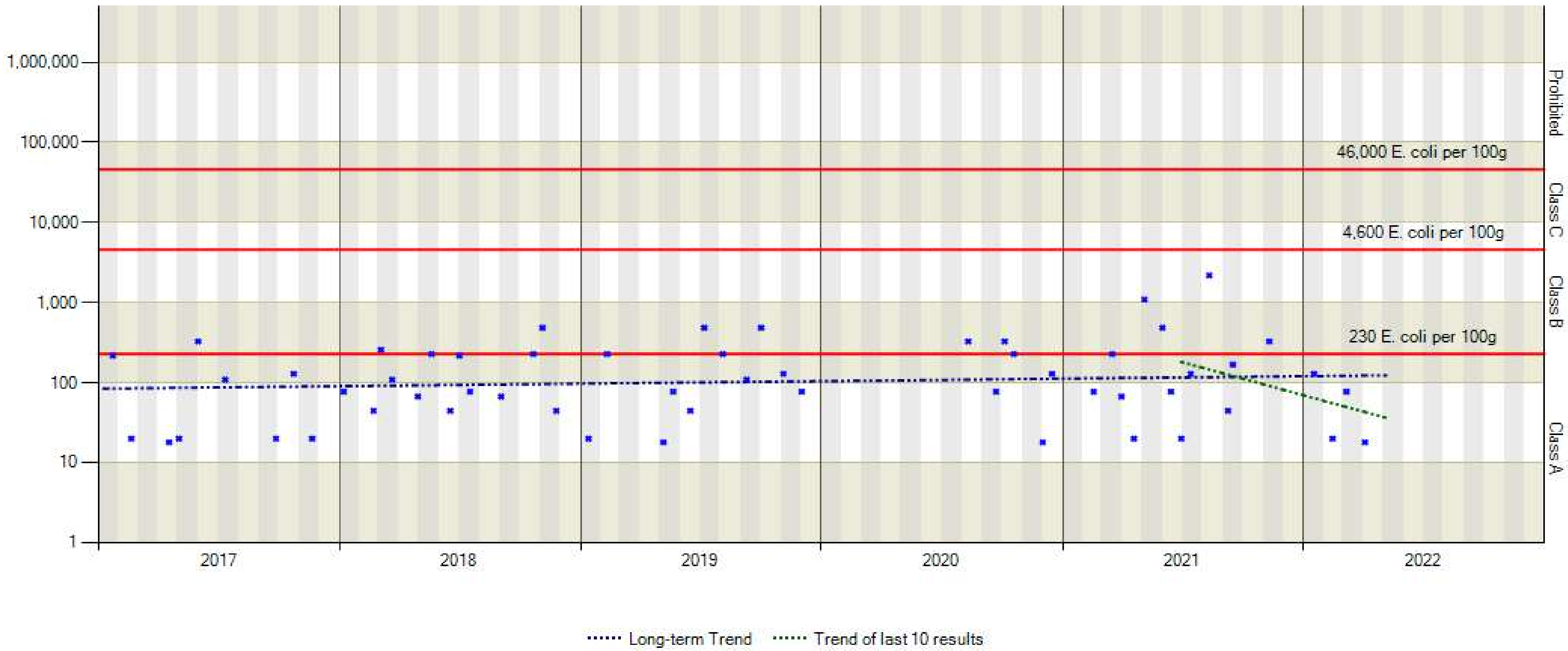
The Official Control framework for shellfish hygiene (UK-retained EU legislation, EC Regulation 854/2004)

a) **Classification characterises production areas, based on *E. coli* an indicator of faecal contamination**



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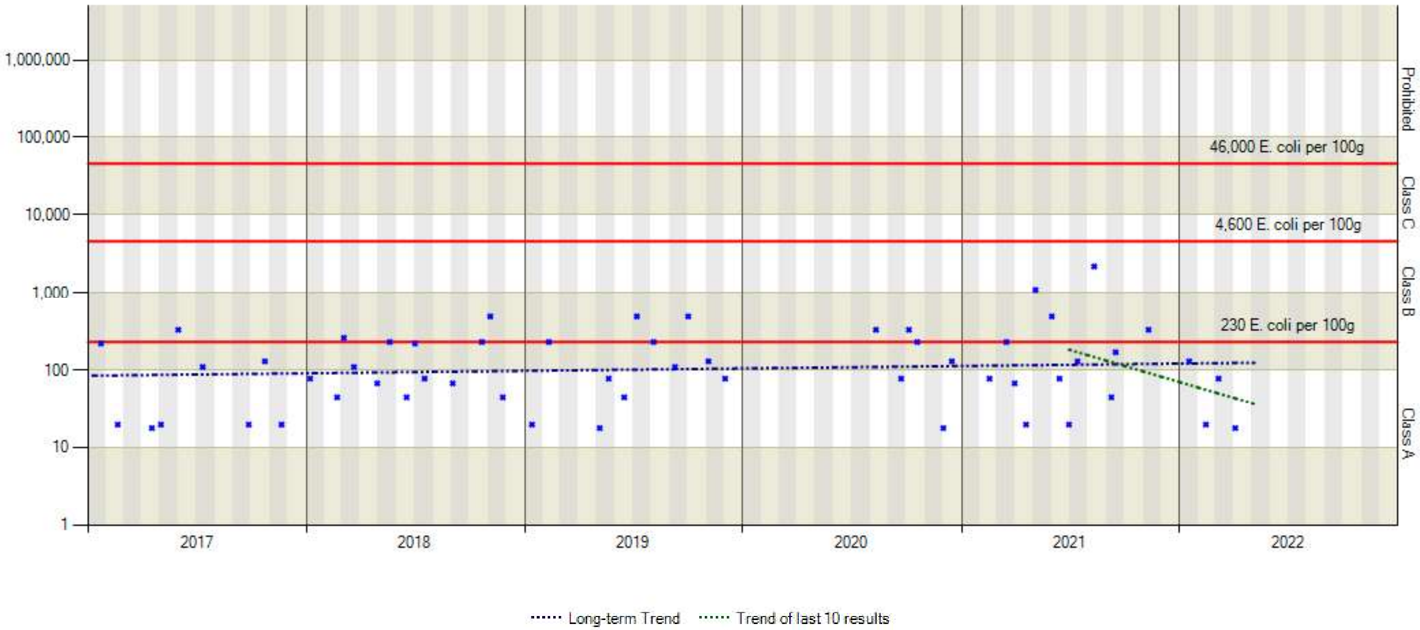
The Official Control framework for shellfish hygiene (UK-retained EU legislation, EC Regulation 854/2004)

b) **Routine monitoring reviews *E coli* levels over 3 year retrospective period**



Several days to get results

Sampling is infrequent (8-12 samples/yr)

Testing regime is not responsive enough

Producers are concerned about perceived high variability in MPN results

..this can end up with long-term area classification being applied as a responsive management tool
eg *a single result may trigger management action if area classification is affected.*

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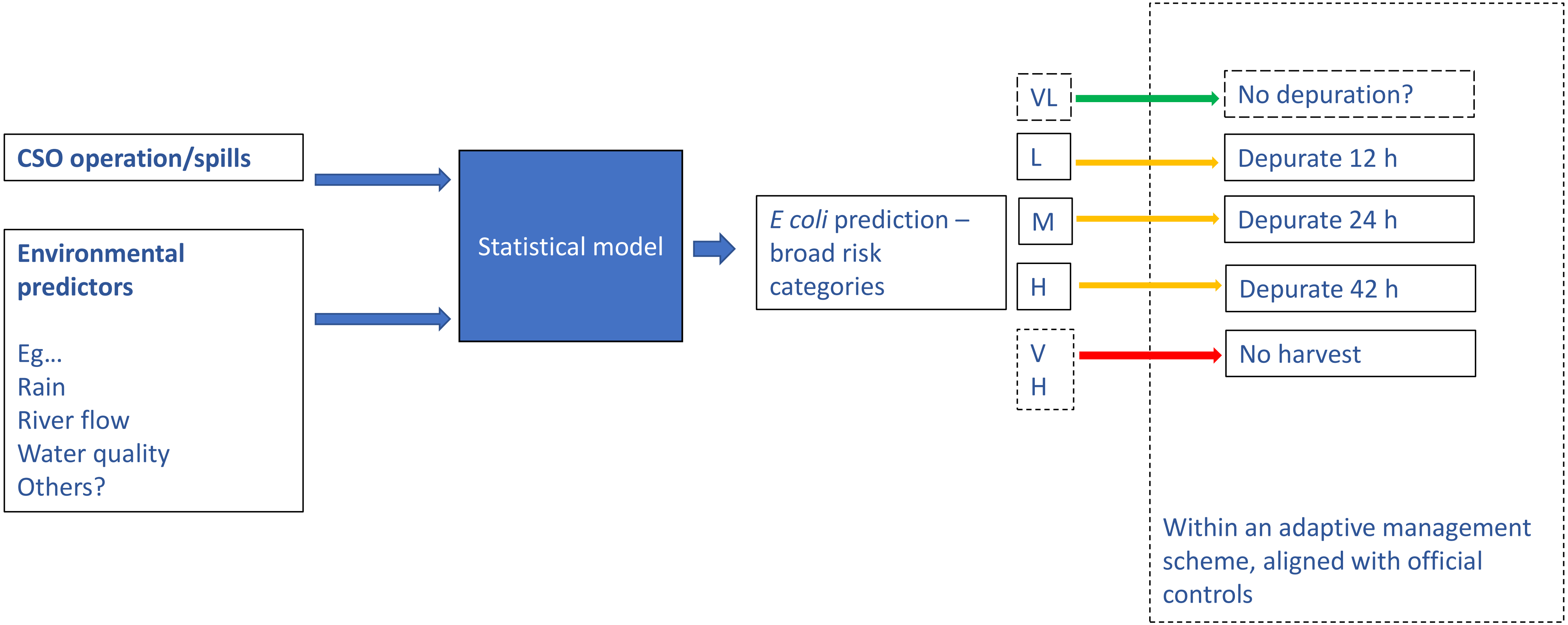
Assurance Scheme concept – towards a more adaptive approach

- Use environmental indicators to determine the optimal time to harvest, avoiding conditions where there is a high likelihood of shellfish contamination because of water quality issues.
- Reduce the regulatory burden in dealing with high *E. coli* results (downgrade/closure, investigation)
- Simpler, faster and more responsive re-opening
- Regulators gain additional information that they can consider when making decisions to manage risk.
- A tailored and proportionate approach to regulating businesses
- Greater public health assurance

ADAPTIVE MANAGEMENT
Initial concept



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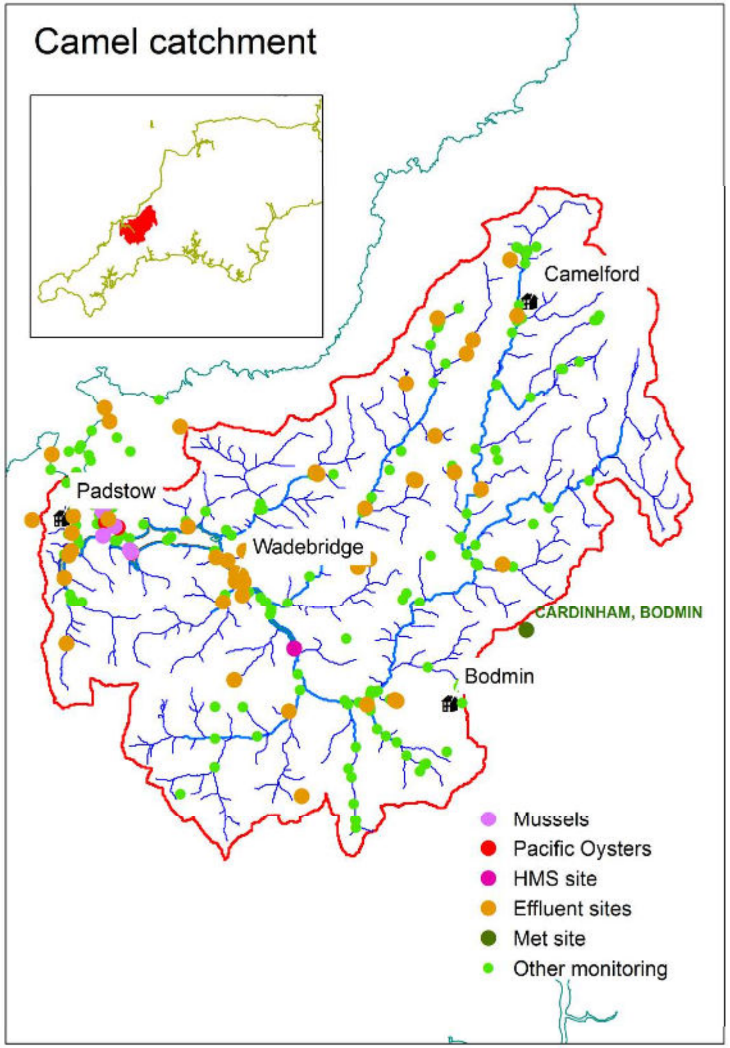
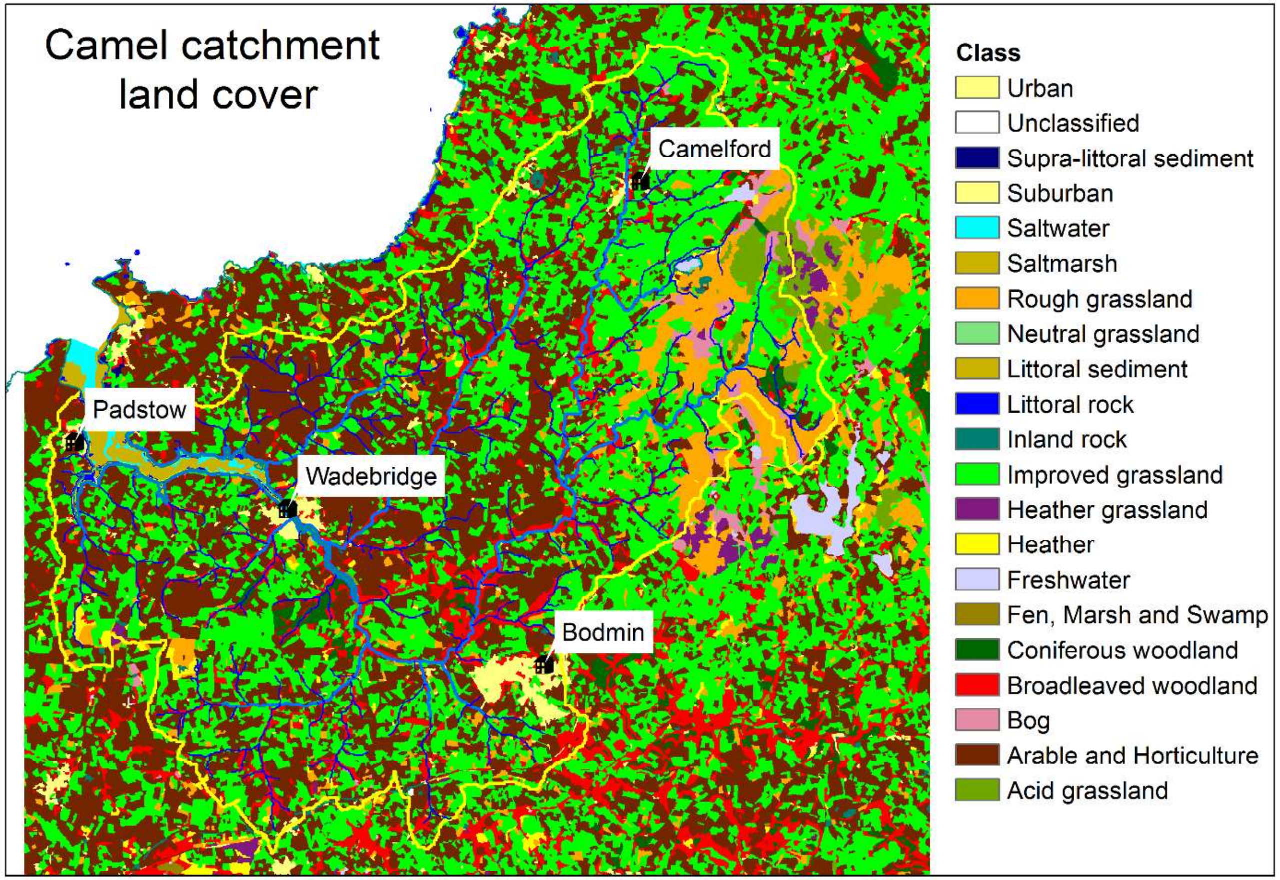
The study area; Camel catchment and estuary





Land cover map of the Camel Catchment.

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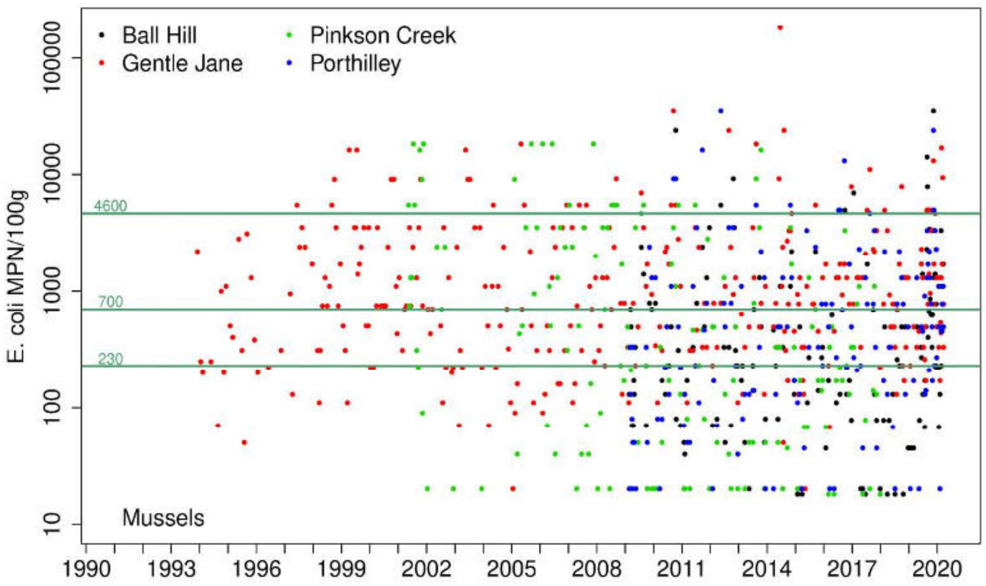
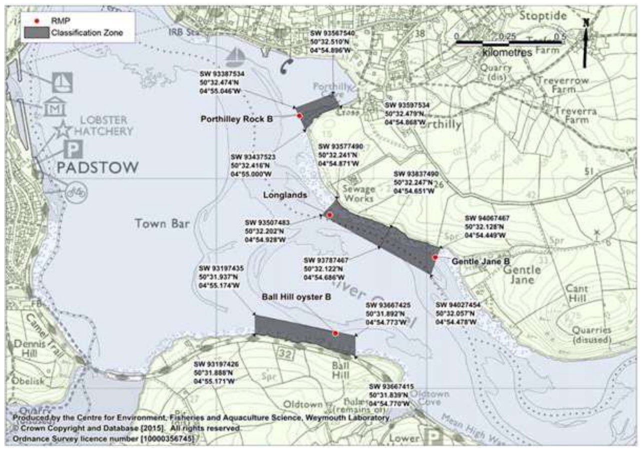
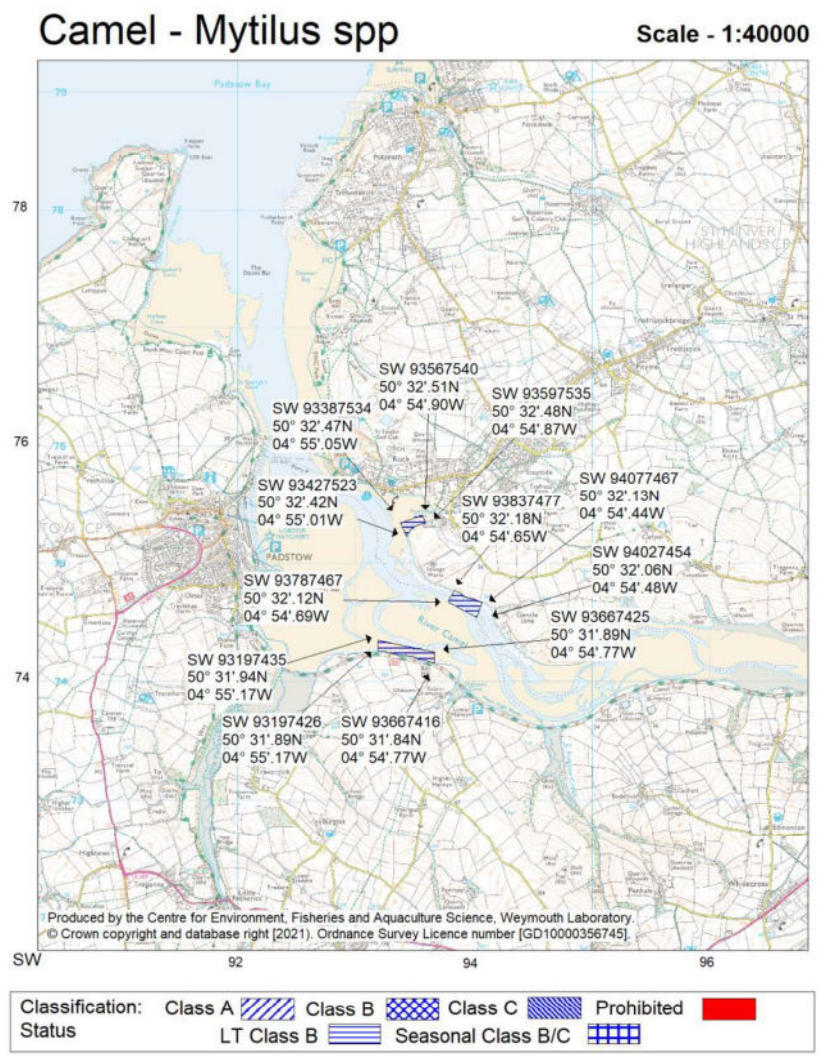
Shellfish production areas in the Camel Estuary – Porthilly Shellfish







Classification of Bivalve Mollusc Production Areas: Effective from 1 September 2021

https://www.cefas.co.uk/data-and-publications/shellfish-classification-and-microbiological-monitoring/england-and-wales/classification-zone-maps/

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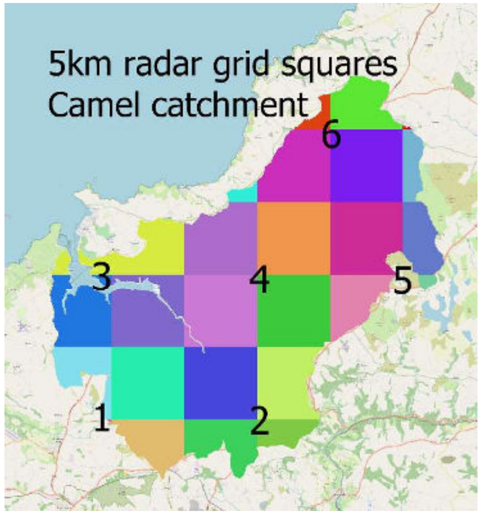
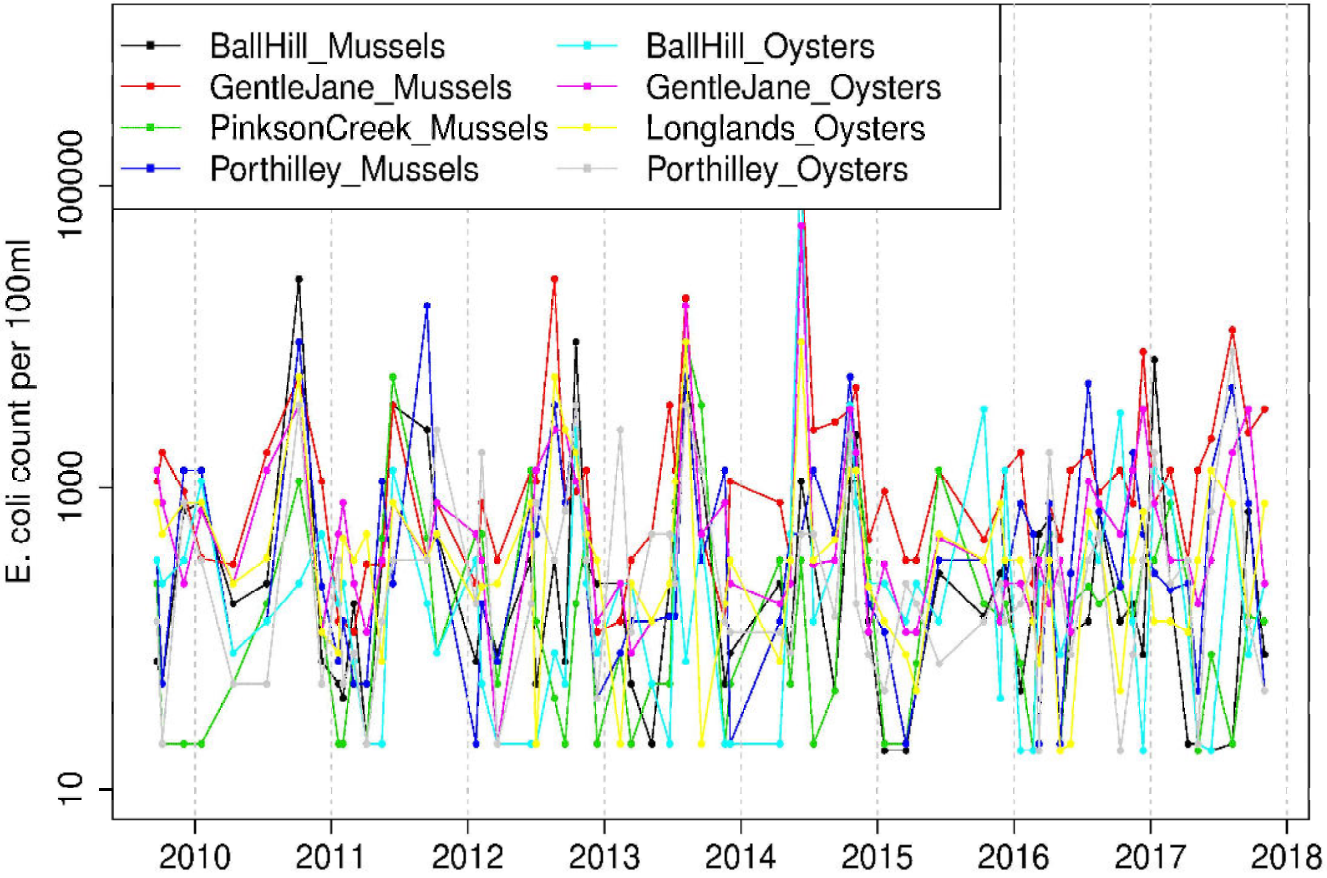
Q: How well can we predict *E. coli* in shellfish using “off the shelf” data



Data sources investigated

- Official *E. coli* monitoring data for shellfish beds (1991-2019)
- Southwest Water data on times of operation of the CSOs (2004-2019)
- Rainfall (weather station) at Cardingham (Met Office) 1991-2020
- Rain radar Met Office Nimrod (held at BADC) 2014-2020
- River flow gauges at Denby and Bodmin (EA)
- River water quality data (EA)
- Sea surface temperature (time of year)
- Tidal state

Official *E. coli* results (2010-2017) – quite inconsistent



Show a high degree of variability both within and between years.

Results from different beds don't track each other very closely over time

Neither do oysters and mussels from the same beds in some cases.

Challenging to see how a catchment-wide predictive model could work?

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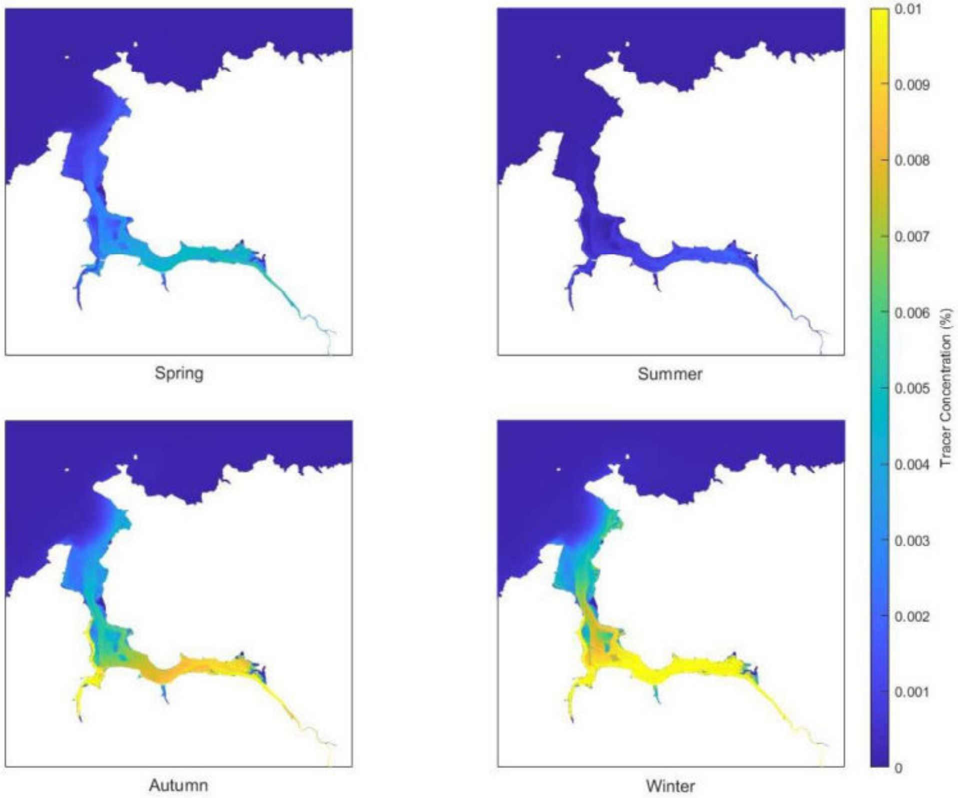
CSO operation data – not as useful as they could be?



Dispersal modelling shows CSOs influence shellfish beds – confirmed by viral indicators specific to human sources

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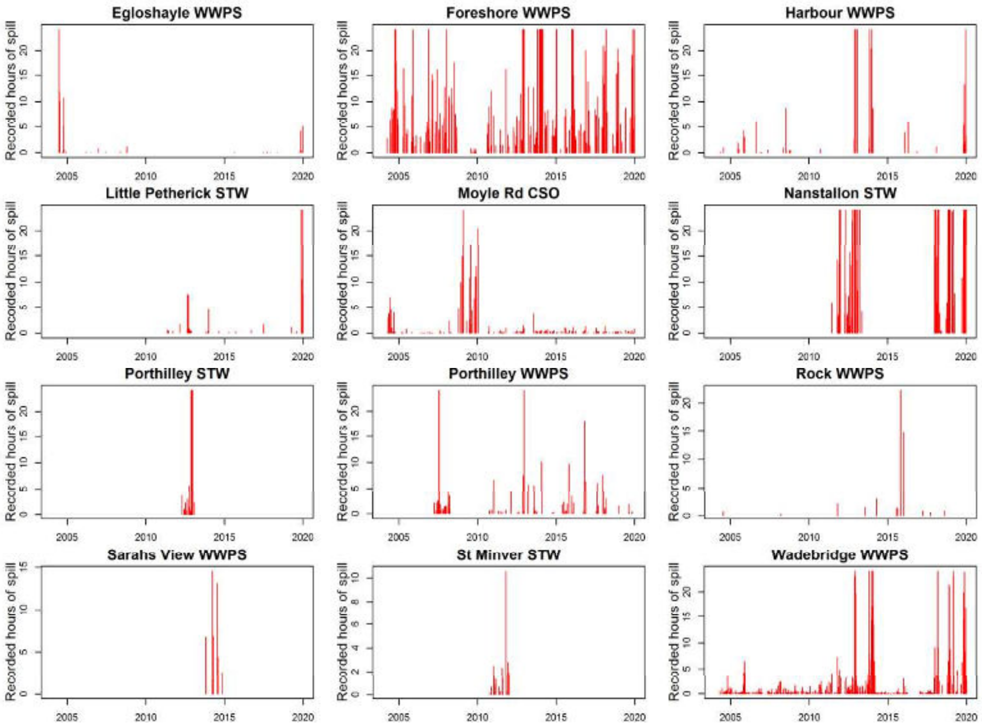


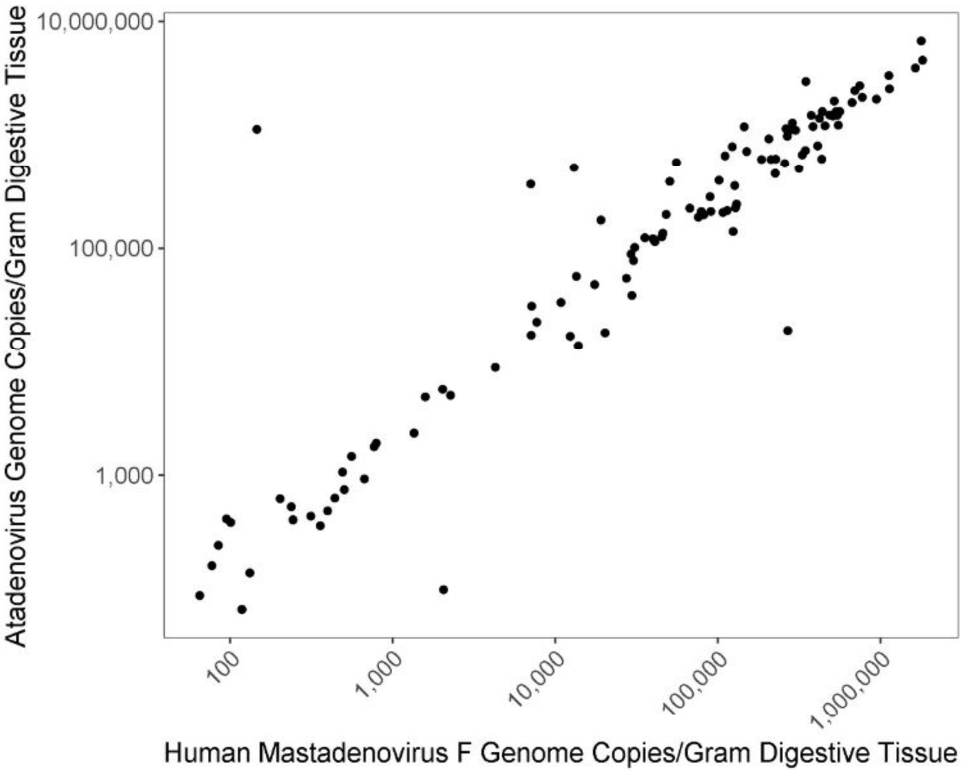
CSO operation was not a good predictor of E. coli in shellfish due to

a) gaps in the data

b) start/stop times only giving duration of operation per day.

Where there are complete records, CSO operation duration quite well related to rainfall.

CSO operation data – not as useful as they could be? **Why?**



Viral indicators showed that agricultural and human sources both contributed to contamination of shellfish

Human and animal sources are present in consistent proportions

Hence, general catchment level indicators (eg rainfall) were more effective in predicting *E. coli* in shellfish = probably because influence both CSO and catchment run-off similarly

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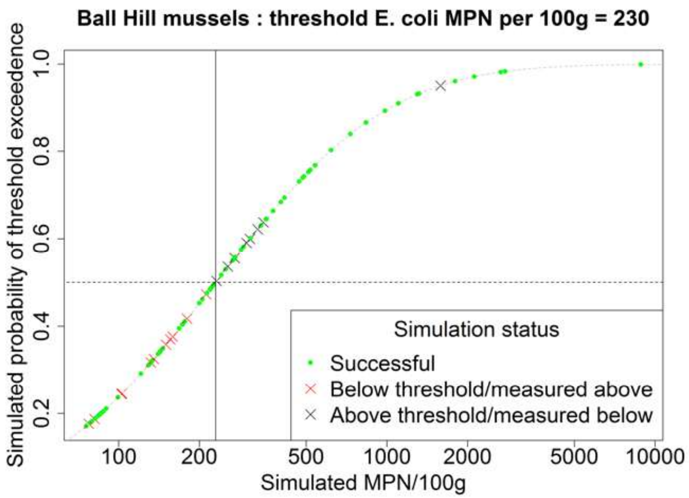

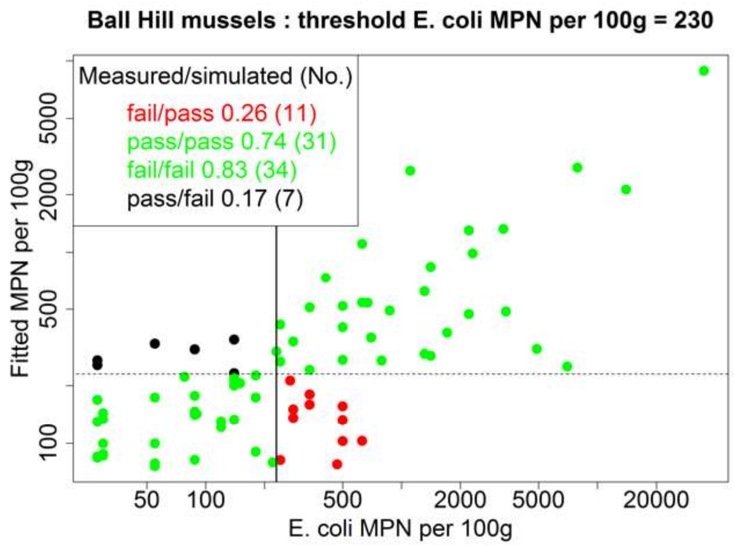

Which environmental factors contributed most to *E. coli* predictions?

- Rainfall
- River flow
- Sea surface temperature (time of year)

Can we predict *E. coli* in shellfish based on these relationships?

Not very well – only about 50% of variation is predictable overall

Reliability of predictions varies between beds – with some giving very unreliable results

Example shows best model fit predictions for one bed… still not sufficiently robust for use in management

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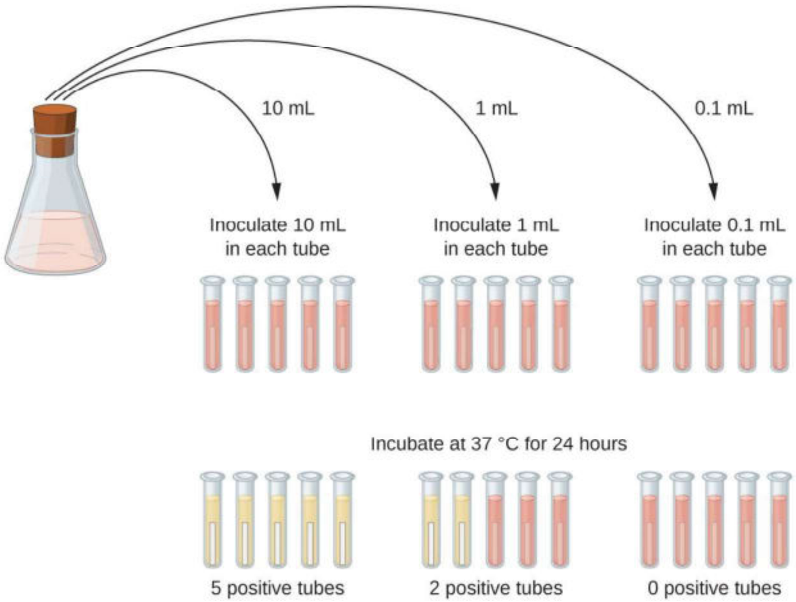
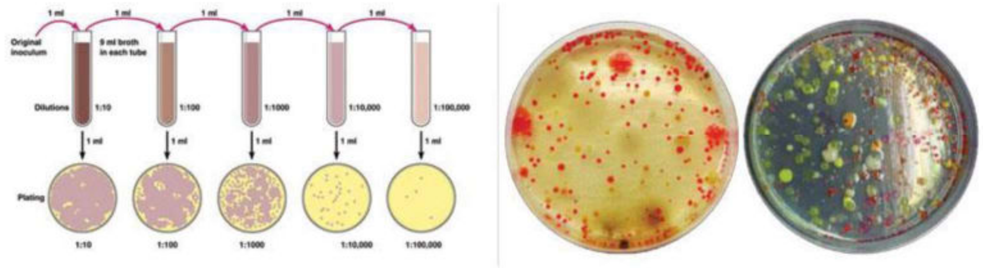
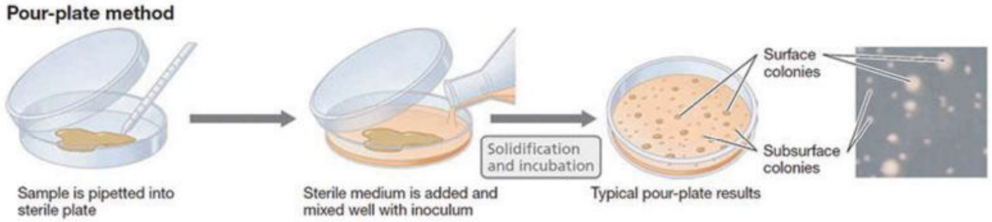
Q. Does it matter how we measure *E. coli*?



Most Probable Number (MPN) ISO 16649-3:2015





Pour Plate TBX ISO 16649-2:2001

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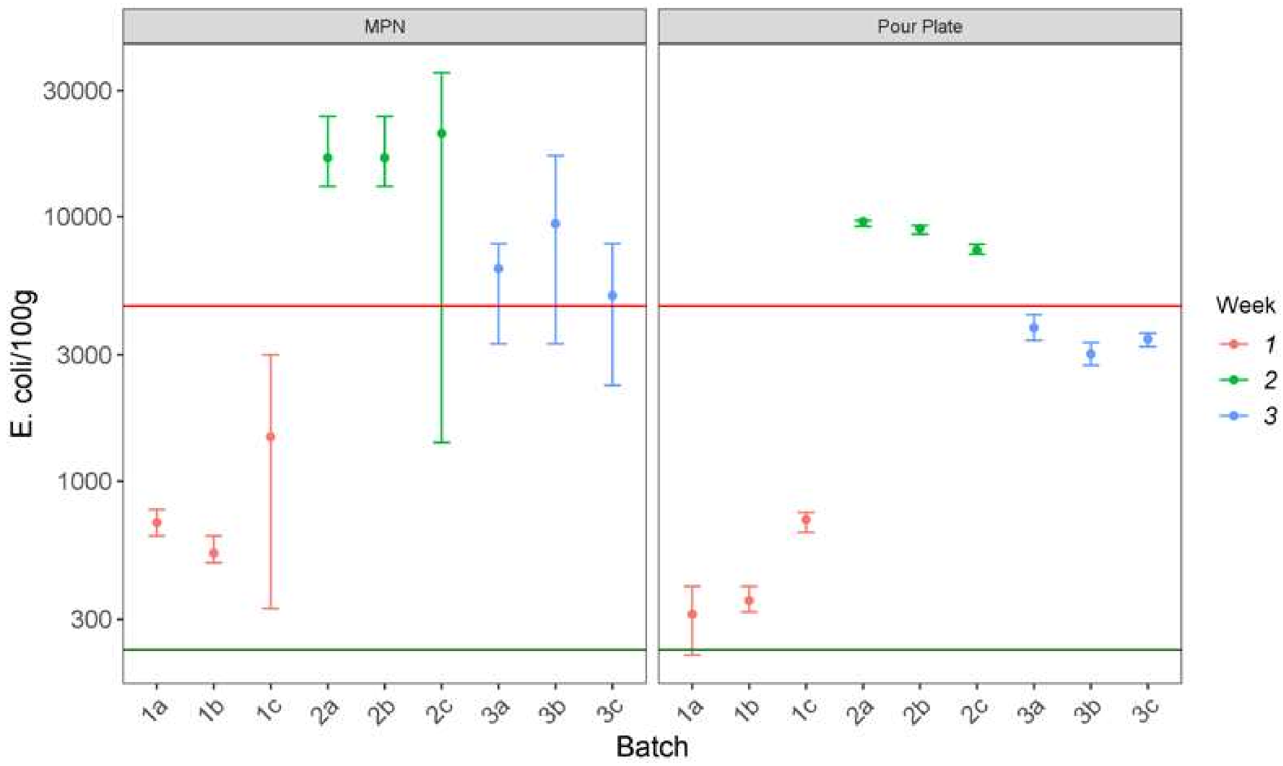
Comparing variability between MPN and Pour Plate methods



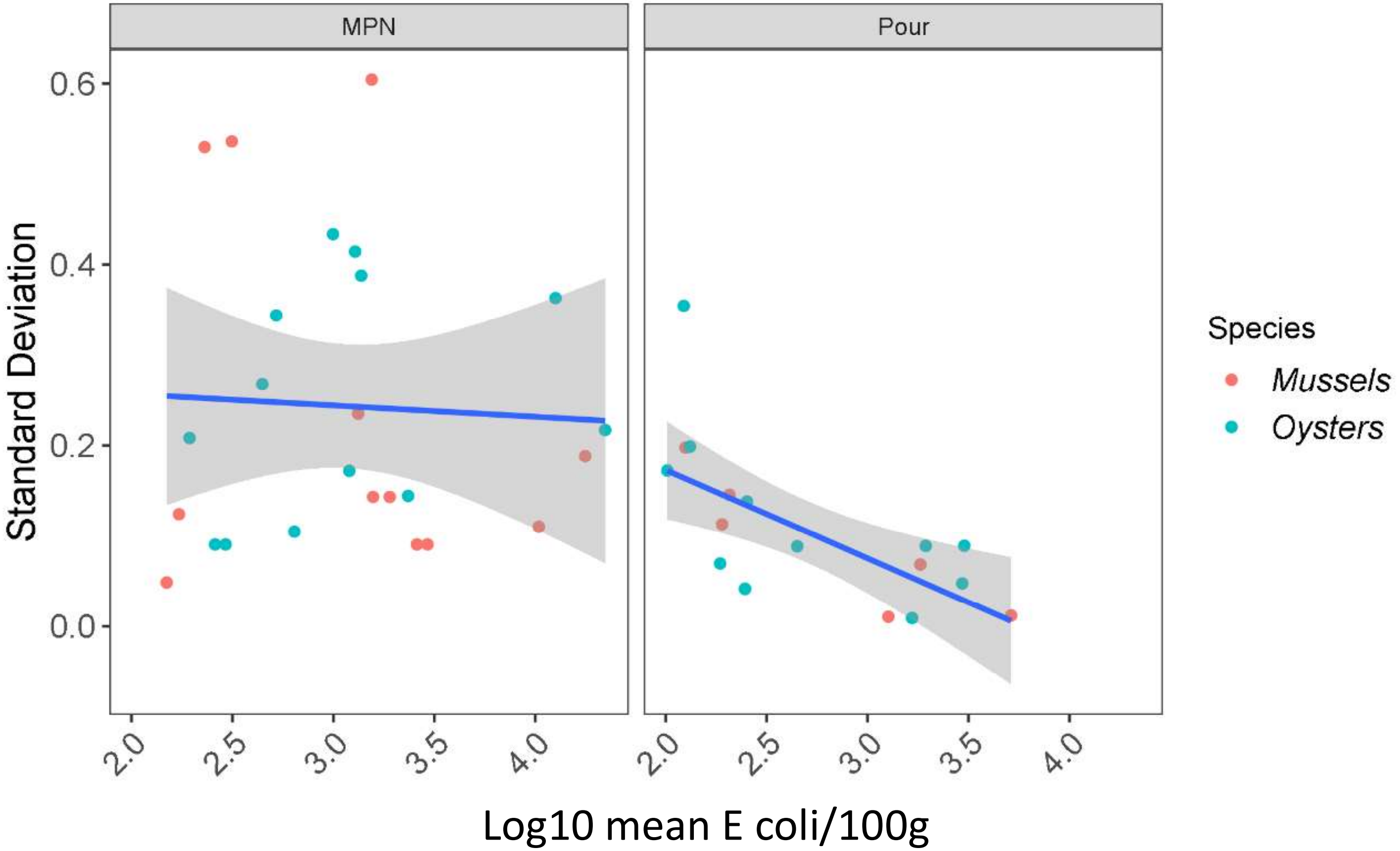
Greater variability in MPN results

Samples taken from single site (Gentle Jane)

Each sample homogenized, split and measured three times by both methods.

E. coli results for split samples of mussels using MPN and pour plate methods (Means with min/max ranges)

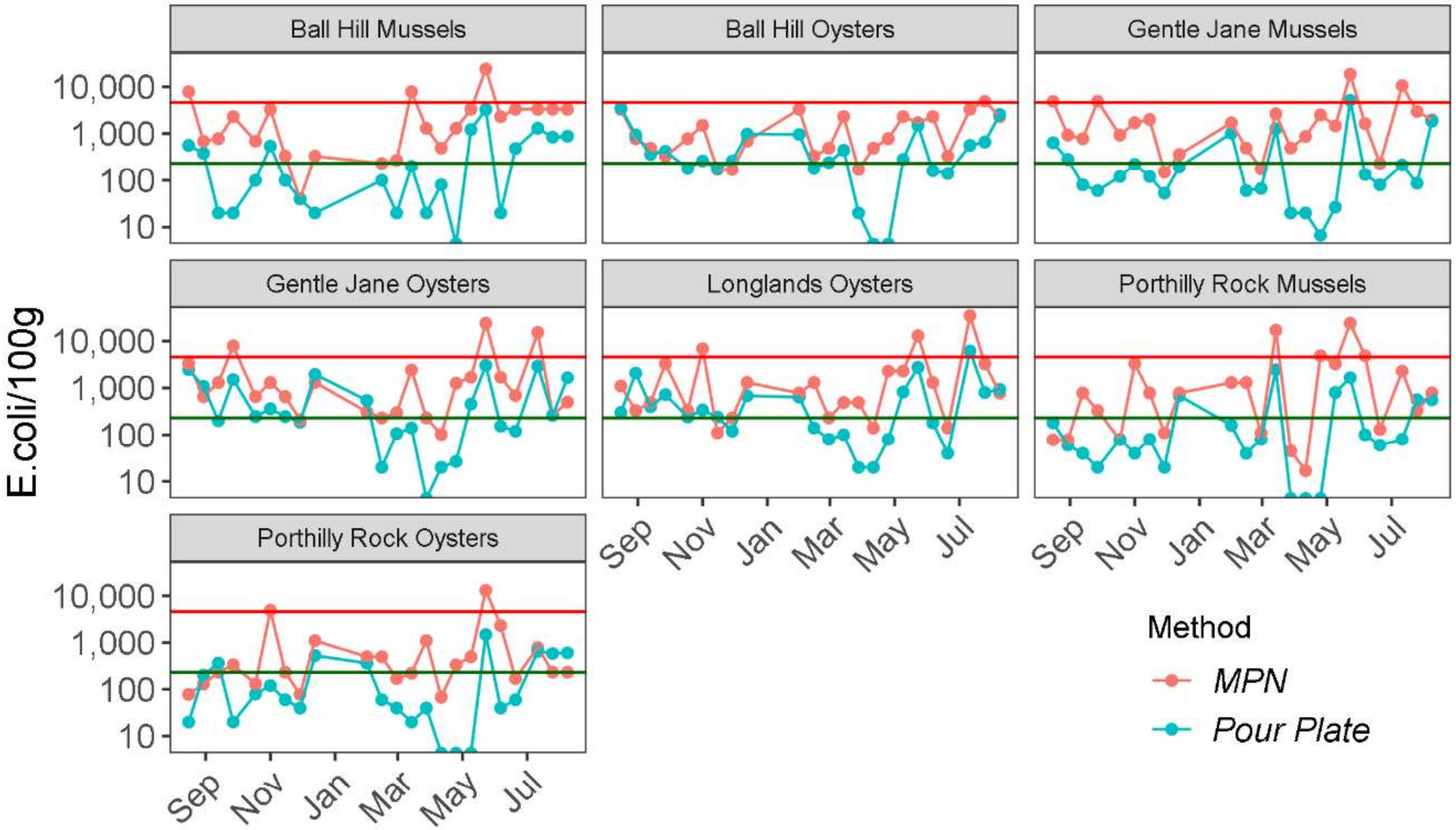
Comparing variability between MPN and Pour Plate methods



Higher variability in MPN across range

Pour plate variability lower at higher counts

How do field samples compare – 2 weekly over 12 months?



Summary of *E coli* monitoring results –August 2020 – August 2021

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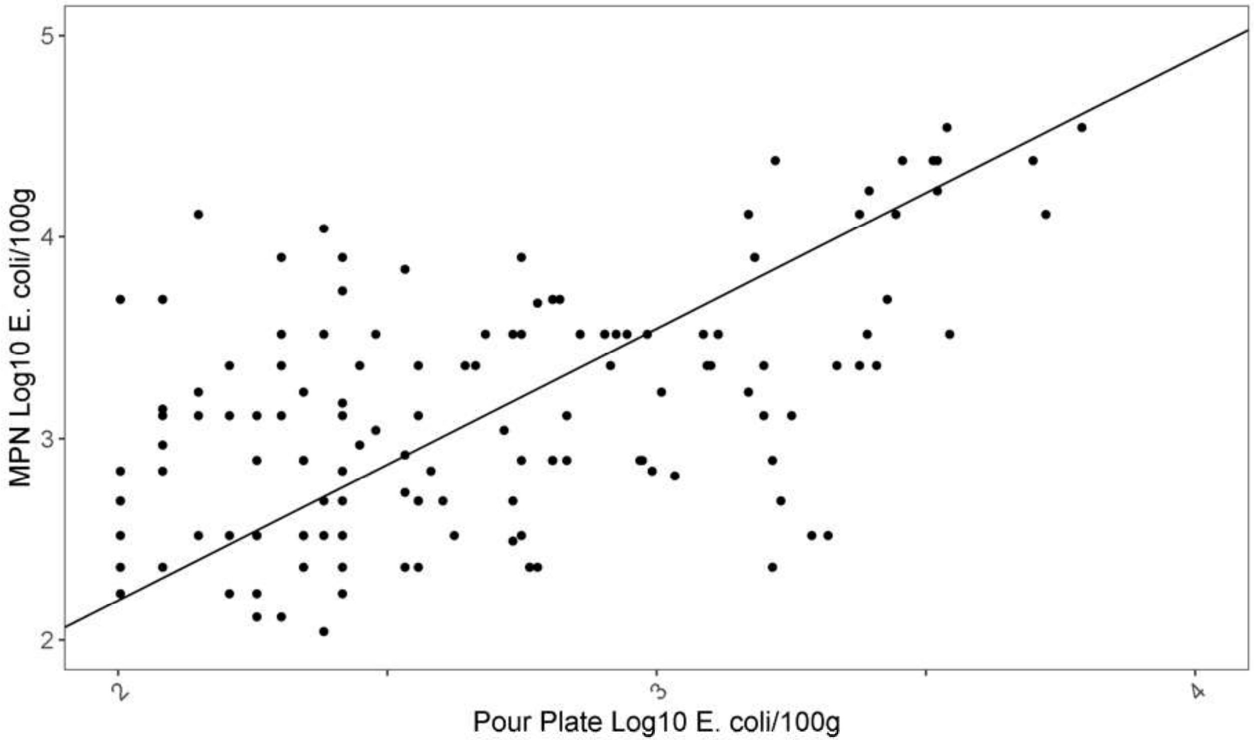

Regression plot of MPN against Pour Plate *E coli* results

Suggests the methods are broadly consistent - in line with previous cross-validation studies.

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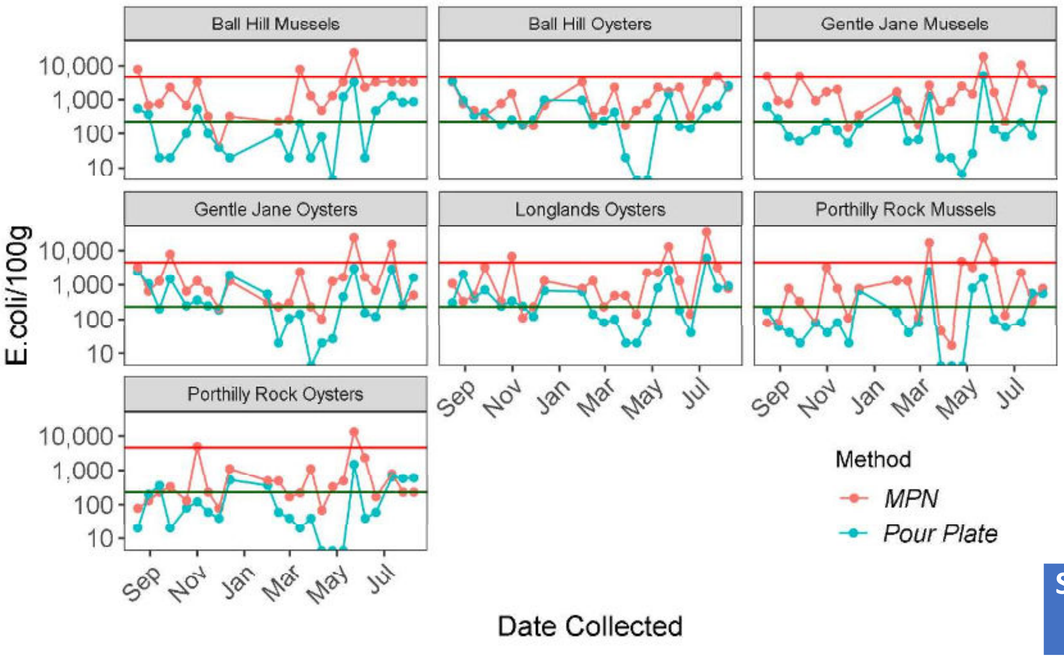

Paired t-tests & Wilcoxon's paired sample test

More appropriate statistical tests to compare results of two sets of data measured from the same samples by two methods.

Shows highly significant differences between two the methods

Site	t-test 95% CI	df	t-test p-value	Wilcoxon statistic	Wilcoxon p-value
All	0.42-0.64	215	<0.0001	16187	<0.0001
Ball Hill Mussels	0.45-1.06	23	<0.0001	276	<0.0001
Ball Hill Oysters	0.10-0.69	22	<0.001	203	0.002
Gentle Jane Mussels	0.46-0.94	45	<0.0001	738	<0.0001
Gentle Jane Oysters	0.17-0.64	50	<0.0001	985	<0.0001
Longlands Oysters	0.11-0.78	23	0.0004	226	0.001
Porthilly Rock Mussels	0.27-1.02	23	<0.001	133	<0.001
Porthilly Rock Oysters	0.05-0.64	23	0.005	147	0.008

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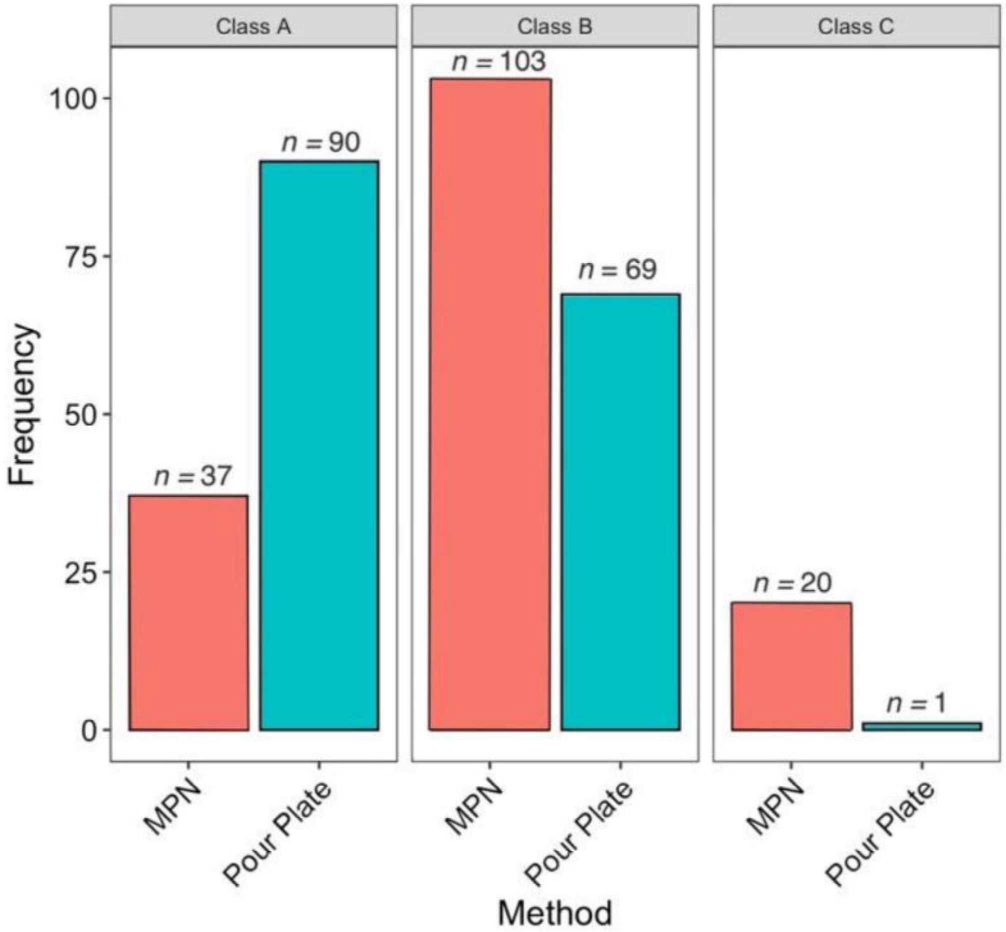
Influence on classification outcomes?



Outcome	Frequency (Percentage)
Results differ across a classification boundary (MPN higher)	74 (41.1%)
Both tests fall within same classification level	100 (55.6%)
Results differ across a classification boundary (pour plate higher)	6 (3.7%)

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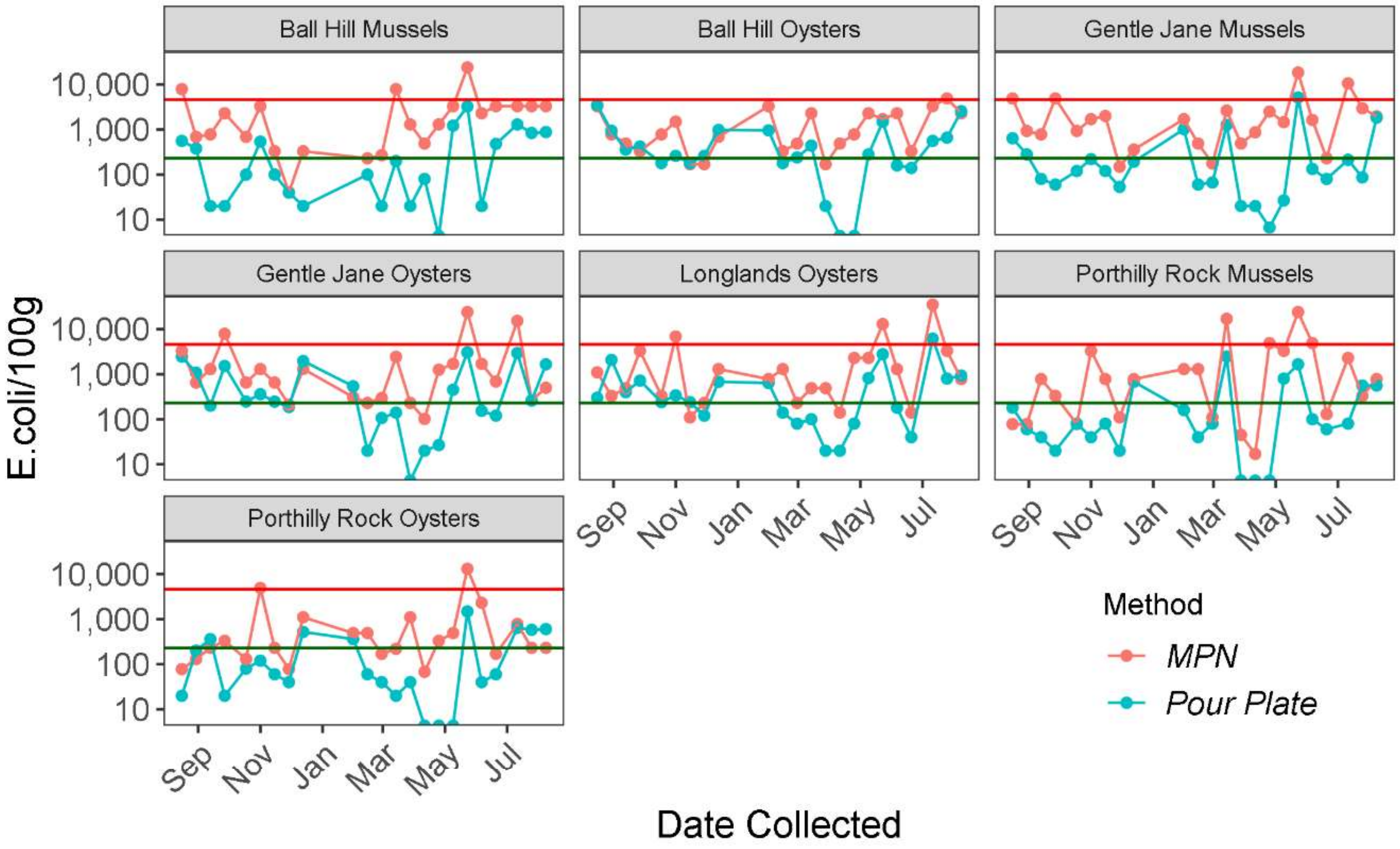
Influence on classification outcomes?



For the Camel, over the study period, use of pour plate could influence overall classification for some beds

For other sites, there may be cases where classification outcomes would be different.

Use of pour plate could reduce influence of occasional high MPN results

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Q. Revisiting predictive models with better *E. coli* data – does it help?

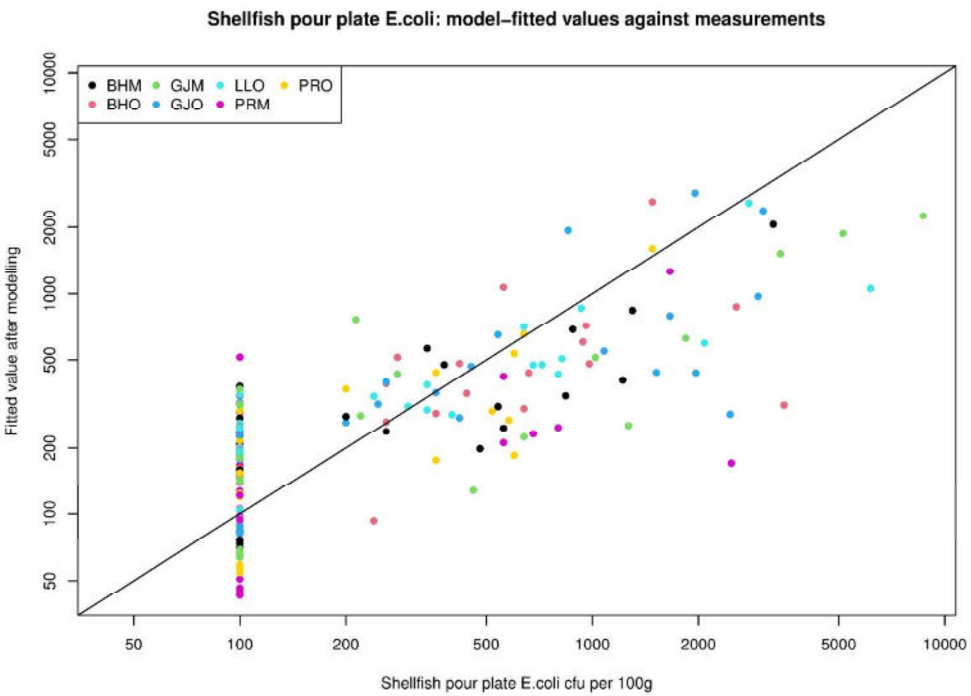
- More frequent (2 weekly) data sets for *E. coli*
- Both MPN and pour plate results

A. Yes, improved performance of predictive models using data from both methods, but substantially better using the less variable pour plate *E. coli* data

Predicting factors were:

- Sea temperature (seasonality)
- Rainfall two days previously.
- River flow (over preceding days)

All of these can be obtained in real time (or in advance), so predictive modelling possible



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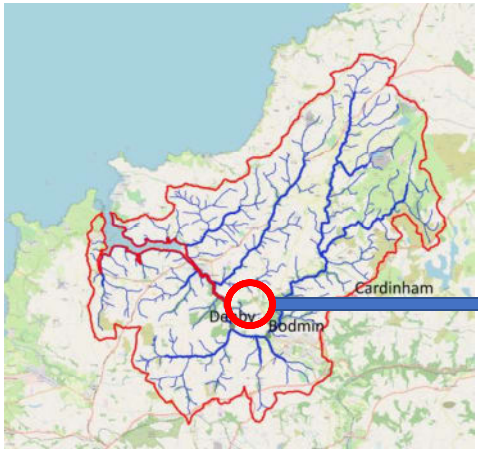
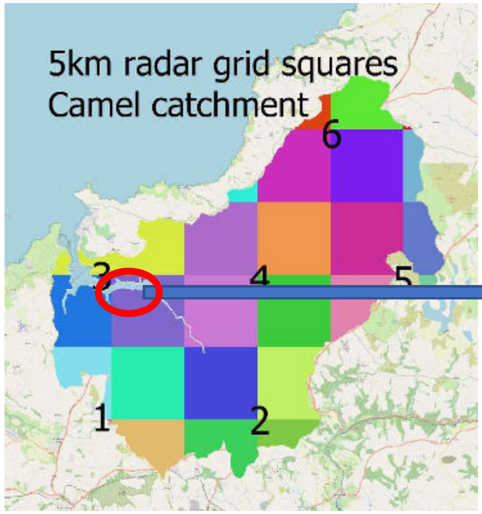
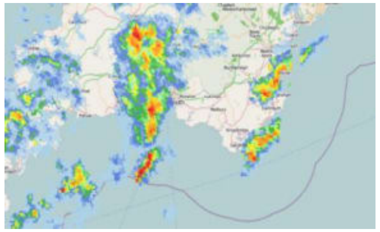


River flow



Rain radar



Model

E. coli prediction
1-2 days later

Variable	Threshold 230		Threshold 700		Threshold 4600	
	Correct <	Correct >	Correct <	Correct >	Correct <	Correct >
MPN	71%	78%	69%	71%	91%	78%
Pour plate	90%	78%	88%	70%	98%	-

Key findings & conclusions

- A real-time predictive system for *E. coli* levels in shellfish is conceptually feasible

- Relatively simple models based on available environmental data could be used to forecast risk

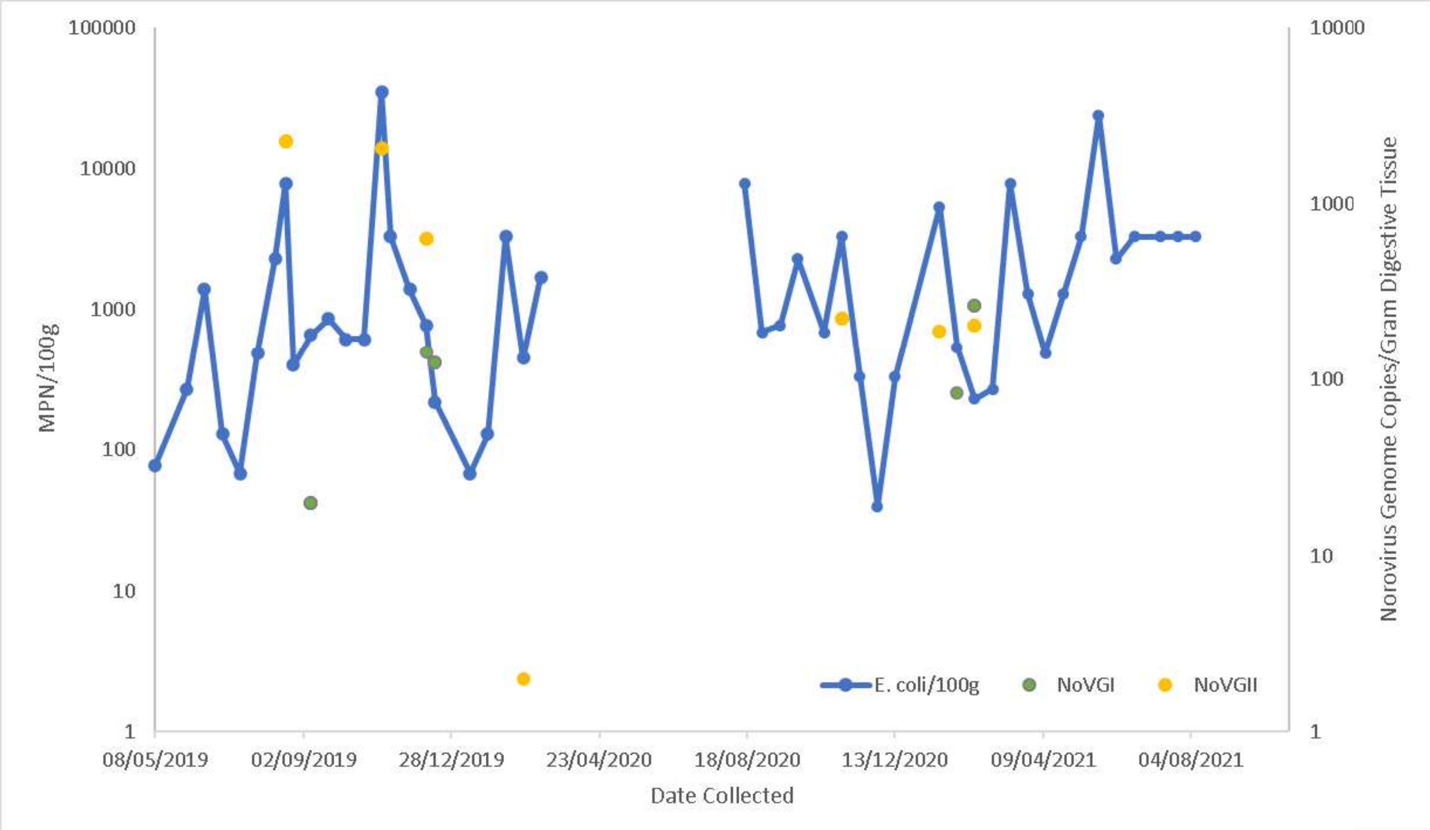
- The approved pour plate method consistently yielded less variable and lower *E. coli* results than obtained by MPN. This suggests that the MPN method may generate outlier results that explain less effective predictive models.

- Development of effective models required frequent *E.coli* sampling, using pour plate method (not just historical MPN data)

- Sewage contamination of shellfish was confirmed by hydrodynamic dispersal models and presence of human indicator and pathogenic viruses in shellfish. However, the contribution of CSO spills to *E. coli* levels in shellfish was not picked up in predictive models BUT this was due to limitations in available spill data. This may be different elsewhere and CSO data should always be evaluated.

- Agricultural run-off is clearly a contributor to *E. coli* in shellfish in the Camel estuary. CSO operation and farmland run-off respond similarly to catchment-scale environmental drivers – which may in part explain the relatively simple models developed.

- Norovirus was present seasonally and sporadically in a low proportion of samples, with no clear correlation with *E coli* numbers or environmental predictors. ***This is a key consideration in development of an Assurance Scheme…..how representative are E. coli levels of actual risk to consumers?***



Norovirus information also needed

Occurred sporadically in autumn/winter

At times > 1000 genome copies/g but often lower

No clear correlation with *E coli*

In 18-26% of occurrences, *E coli* was less than 230cfu/100g

Small data set so hard to draw conclusions

Example :Ball Hill mussels Norovirus GI and GII concentrations and MPN *E coli*

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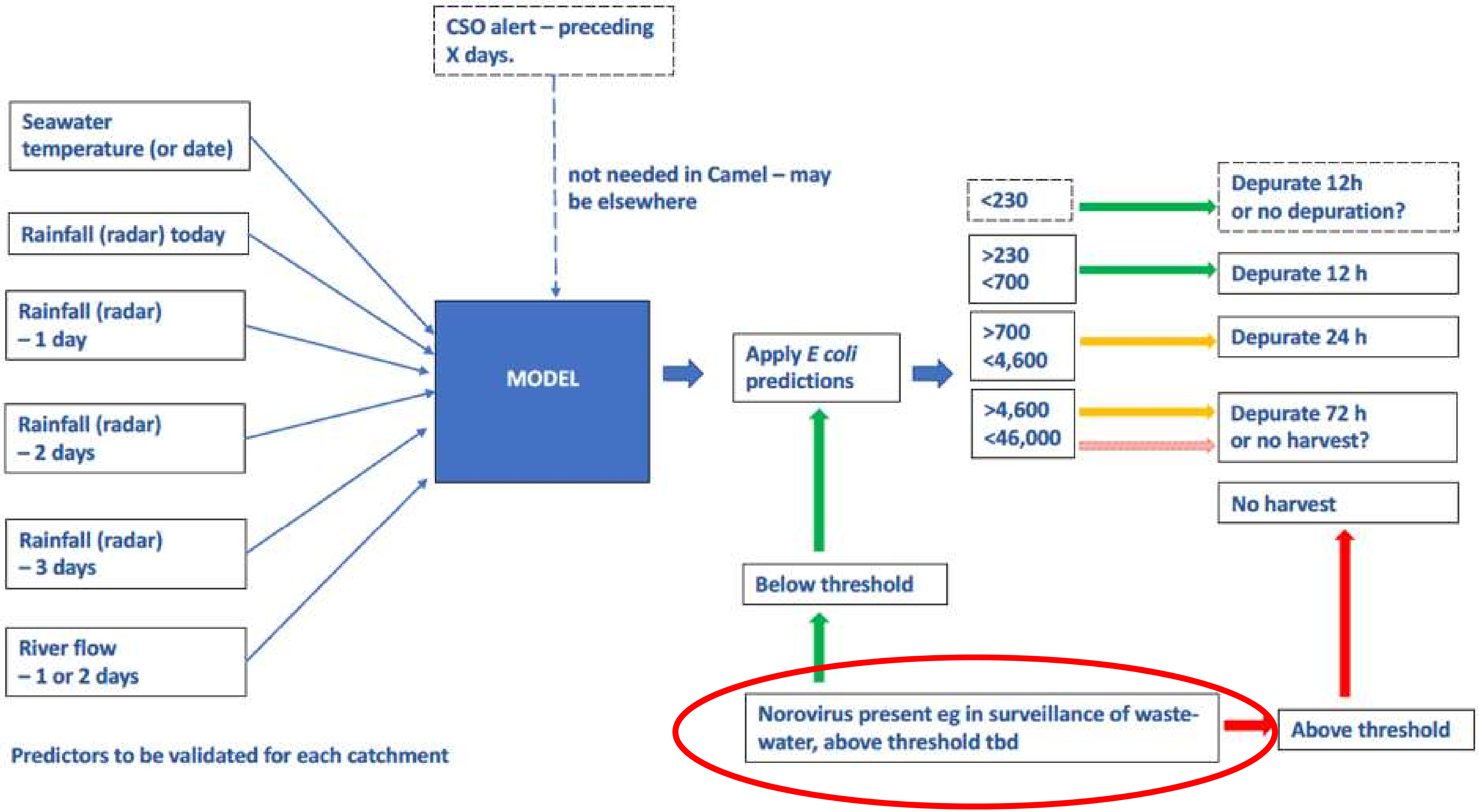

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