Analysis of bacterial measurement in the Char catchment from 4th June to 1st October 2024

Four sites were selected for monitoring on the Char and a fifth one on the Monkton Wyld stream. The sample sites are shown in Figure 1. There are environmental discharge permits along the rivers including north of our sampling points. Samples were taken at approximately 14-day intervals. The discharge points of Wessex Water are only between STW and the lagoon. The first sample was collected on the 4th of June and the last one considered in this report on the 1st of October 2024. Samples were collected using an agreed protocol in duplicate. One duplicate was sent to Wessex Water (WW) for analysis and the other set retained for analysis by Char catchment volunteers with the bacterial incubation and counting carried out by John Kenward. The resultant abundance of bacteria *Escherichia coli* and Enterococci species detected are used internationally as the standard indicators of the faecal pollution of bathing waters.

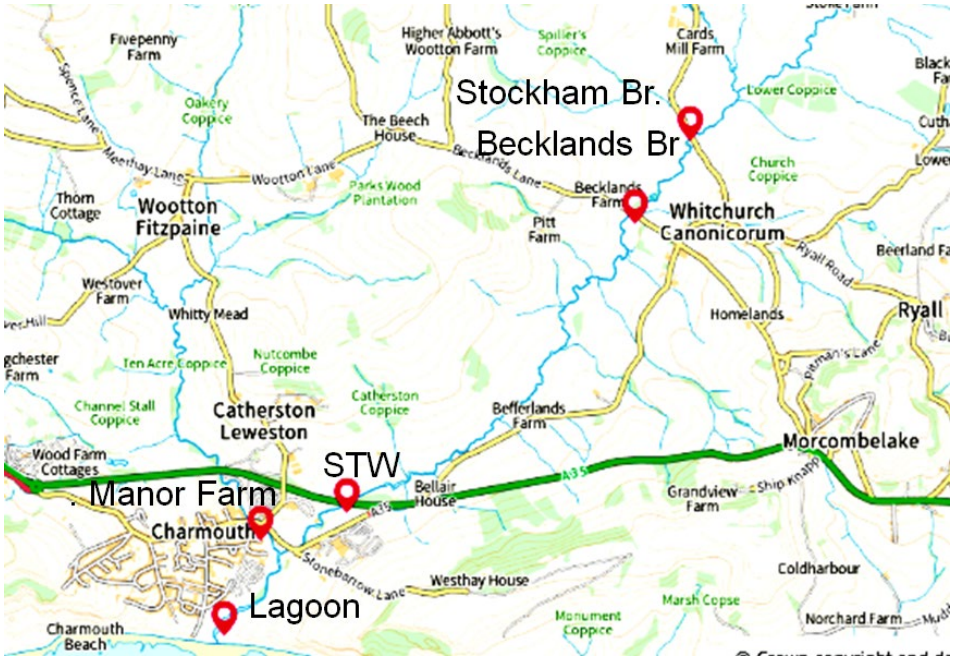

Figure 1: Sampling sites for bacterial monitoring

The upper limits for each category of water quality based on the abundance of the two bacterial groups are given in Table 1. The assessment requires many samples to take account of variation in bacterial densities between occasions.

Classification	Thresholds (percentile)	
Excellent	*E. coli* ≤500 cfu/100ml **and**	Enterococci ≤200 cfu/100ml (95th percentile)
Good	*E. coli* ≤1000 cfu/100ml **and**	Enterococci: ≤400 cfu/100ml (95th percentile)
Sufficient	*E. coli* ≤900 cfu/100ml **and**	Enterococci ≤330 cfu/100ml (90th percentile)
Poor	indicates that the values are worse than the sufficient	

Bathing Water Quality (data.gov.uk)

Table 1: Water Quality classifications for inland bathing waters

The upper thresholds that must not be exceeded for the *E. coli* and Enterococci values are given in Table 1. These values are based on the average (μ) and the spread of values assuming there are a similar number above and below the average. This spread is defined by the standard deviation (σ) of the data set. It defines a spread of 67%.

Both *Excellent* and *Good* categories use a 1.65 multiplier for σ to widen the spread to cover up to 95% of the data set. The two different upper thresholds allow different combinations of the average and the spread with *Excellent* setting a more stringent upper threshold than *Good*. The *Sufficient* category narrows the spread to

90% using a lower multiplier (1.282) for σ. It accommodates an even wider range of the average and spread combination. It sets a slightly lower threshold than *Good* which limits the extent of its laxer requirements.

All bathing waters that fail all three thresholds for both bacteria are classified as *Poor*. The regulations require all such bathing waters to carry a notice advising against swimming. The Environment Agency then investigates the cause of the *Poor* status for designated bathing water sites only.

The standard analysis is based on logarithmic transformation of the data. This is a more appropriate basis for assessing bacterial populations than arithmetic values. The approach allows for 15% of readings over 4 years to be discounted. This is to dismiss the effects of temporarily high values. This 15% omission has been applied in the analysis (Figure 2). It establishes that the water quality is in the *Poor* category and so unsafe for bathing based on measurements by both WW and CROWD.

Calculation	*E.coli cfu/100ml*		Enterococci cfu/100ml	
	WW	CROWD	WW	CROWD
number of measurements	42	32	41	24
number to dismiss (highest 15% of values)	6	5	6	3
log mean (μ)	3.11	2.57	2.30	2.36
log StDev (σ)	0.40	0.78	0.58	0.77
arithmetic mean	1284	369	200	230
upper 95 percentile = (μ + 1.65 σ)	3.78	3.86	3.26	3.63
arithmetic upper 95 percentile = antilog (μ + 1.65 σ)	5,979	7,230	1,814	4,313
upper 90-percentile = (μ + 1.282σ)	3.63	3.57	3.05	3.35
arithmetic upper 90-percentile = antilog (μ + 1.282σ)	4243	3723	1109	2244
Category (inland bathing water)	Poor			

Table 2: Water Quality classification for the Char catchment

This consensus of WW and CROWD on a *Poor* category for water quality is encouraging for CROWD's approach. More detailed analysis indicates that CROWD normally underestimated values provided by WW. The data in Figure 2 have regression lines fitted with a slope parallel to that when the two values are the same (green line) but with a reduction in elevation indicating the extent of overall under reading by CROWD relative to WW of 40-45% (red numbers).

Further work is required to determine the cause of the normally underestimated values by CROWD. This could be due to the different methodologies used by the two laboratories. Further work aims to reduce the discrepancy. CROWD values could be adjusted to match those of WW using a c2.5x multiplication factor. This would only be of possible interest if future CROWD assays indicate the water quality is better than the *Poor* category. This conclusion might arise from CROWD's underestimation of likely WW values.

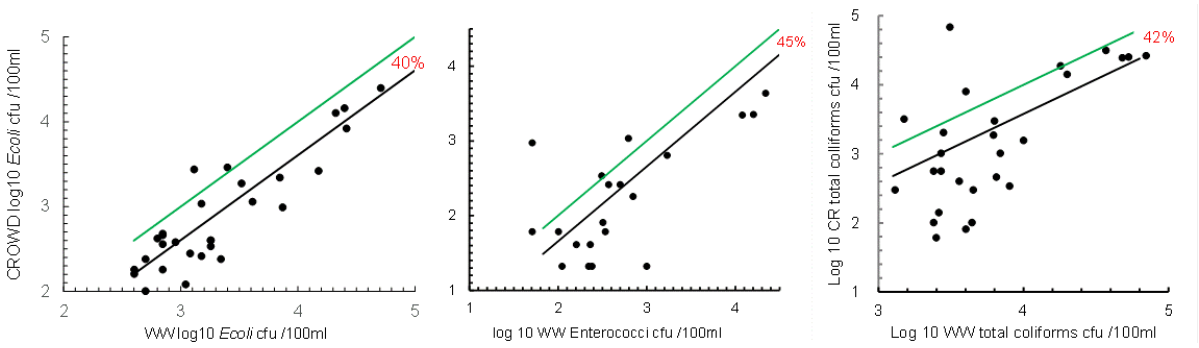

Figure 2: The relationship between values obtained by WW and measured by CROWD for *E. coli*, intestinal enterococci and total coliform bacteria. The green line indicates the relationship if WW and CROWD values are identical. The number in red is the average CROWD percentage relative to that of WW.

The maximum depth of the Char at Whitchurch Canonicorum (Hydrology Data Explorer - Whitchurch Canonicorum) had a significant effect on the numbers of both *E coli* and enterococci found the sampling days at the four sample sites on that river (Figure 3). Sub-groups assigned different letters differ. Any average can belong to more than one subgroup. There was a greater than tenfold difference between the numbers of both bacteria when the depth was 0.21 metres relative to the next highest average. The river was occasionally much deeper than on the days of measurement.

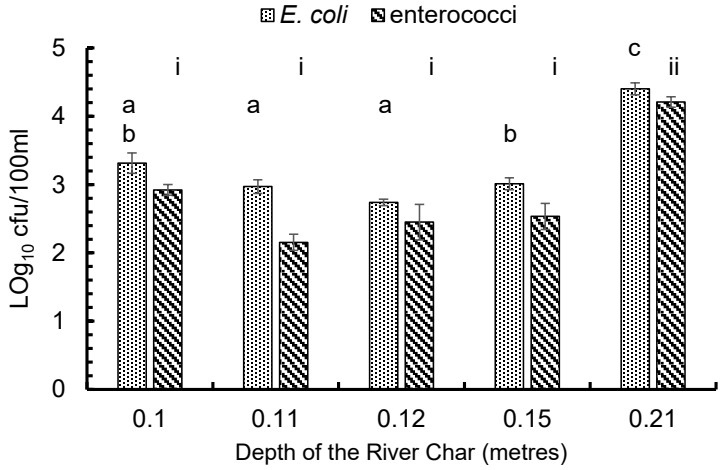

Figure 3: The density of colony forming units of *E. coli* and enterococci at four sites measured by Wessex Water on the River Char and its depth on the sampling dates at Whitchurch Canonicorum. Averages for depths that are statistically significantly different do not share the same letter (P<0.05. SNK Oneway ANOVA).

Another aspect of the analysis is to determine if the bacterial counts vary with location. This was carried out using the percentage of counts at each site relative to the average for that site on each occasion. This eliminates the variation due to differences in bacterial densities among sample occasions. The analysis establishes that there are no statistically significant differences between the sites for the density of total coliform bacteria (Figure 4). In contrast, there are such differences (P< 0.05; SNK, Oneway ANOVA) for both *E coli* (Figure 5) and enterococci (Figure 6). Sub-groups assigned different letters differ. Any average can belong to more than one subgroup. The same approach is taken in Figure 5. The site STW provides the lowest percentage average score for *E coli*. Breckland bridge and the lagoon have the highest average *E coli* densities. Stockham and Becklands bridges have the highest average densities for enterococci. Manor Farm has the most variable data for enterococci and belongs to all three sub-groups.

A second approach to looking at the relationships between the sites is to determine the extent to which they provide the same bacterial profiles using an approach known as Cluster Analysis. This indicates that Breckland bridge is the most divergent of the five sites (Figure 7).

Further investigation is required to determine the origins of the *E coli*. This is likely to require DNA-based analysis of the biotypes involved. There is a need for caution in interpreting likely origins of the detected bacteria until that work is completed, They could be from sewage outlets, farm livestock, fields to which added manure has not fully degraded or arise from wildlife. Similar analysis for enterococcus may not be so informative as some of its species also persist outside of the guts of humans, livestock, or wildlife.

The data in this report does not consider any impact of storm overflows from WW assets. Sampling would have to coincide with such events to analyse for this. Such outflows would only impact on water quality downstream of them and so only the lagoon of the five sites studied in this work could be affected.

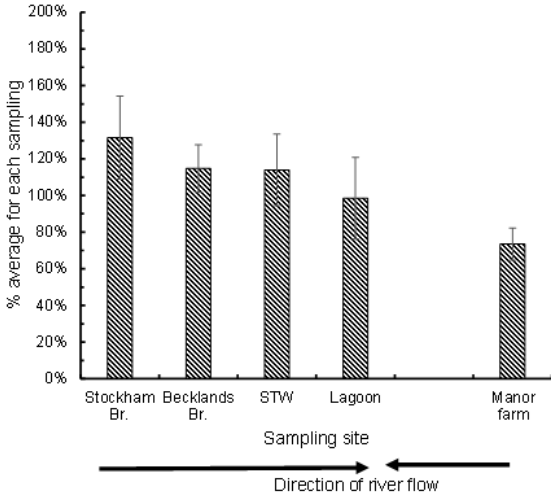

Figure 4: The percentage of total coliform bacteria cfu/100ml recorded by WW and CROWD based on the average for all sites on each sampling occasion.

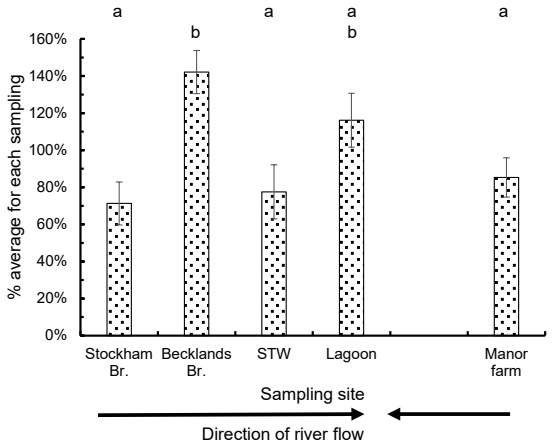

Figure 5: The percentage of *E. coli* cfu/100ml recorded by WW and CROWD based on the average for all sites on each sampling occasion.

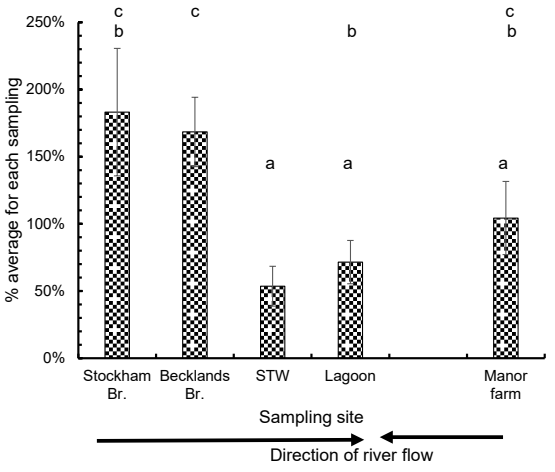

Figure 6: The percentage of enterococci cfu/100ml recorded by WW and CROWD based on the average for all sites on each sampling occasion.

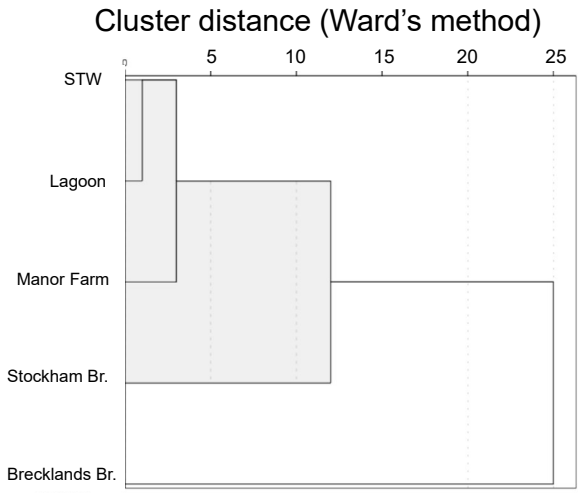

Figure 7: The relationship between sites indicating similarity of sites for all bacteria results (Cluster analysis).

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