Report No. 2: Water quality monitoring and testing in the Cam and its tributaries: bacterial indicators of faecal contamination, and phosphate and nitrate analysis

Haslingfield Sewage Treatment Works
Effluent outfall on the River Rhee, at high spate during a storm discharge

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Second Report: 1 November 2021

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Report No. 1 on Batch 1 sampling results (14th June 2021) [can be accessed here](). Please do not publish data from this report without our prior permission.

1. Summary

1.1 As part of our aspirations to safeguard and improve the River Cam, Cam Valley Forum (CVF) started the project to better understand faecal contamination in the river by evaluating counts of faecal indicator bacteria in both watercourse samples and effluent from sewage treatment works (STWs) according to seasonality, river flows, and agricultural operations. We were looking for evidence of different types of sources, but especially of point sources which might then be addressed and improved by remedial action. The faecal indicator bacteria monitored were the standard *E. coli* and intestinal enterococci (hereafter called enterococci), and total coliforms.

1.2 CVF undertook a second batch of tests on river water sampled on 24th August 2021 at 19

sites (12 sites on 14th June). The sites extended from Meldreth, on the Rhee, downstream to the Cam, through Cambridge to Clayhithe (Waterbeach) just north of Cambridge and included tributaries above Cambridge. Included were both the Haslingfield and Cambridge (Milton) STWs' effluent. Samples from both batches were taken during dry, sunny conditions during relatively low river flows.

1.3 Both STWs' discharges had much higher levels of bacteria than any in-river samples: 38,730 *E. coli* /100ml of pure effluent at Haslingfield STW, and 7380 *E. coli* /100ml at Milton. These were in '<u>treated'</u> effluent, with <u>no evidence</u> of storm overflows into the river. After taking into account the different dilution rates imposed on the effluent by the river flow, the counts of *E. coli* converted into river counts were 3495 and 2959 /100ml respectively. Thus, the estimated *river coun*t at Cambridge was 85% of Haslingfield's, on the day of sampling. The lowest river count was 55 *E. coli* /100ml in a sample from Bourn Brook.

1.4 The additional two sites on the Rhee in the Barrington to Meldreth stretch showed high levels of faecal bacteria in the river, declining over distance to Haslingfield STW. This suggests a potent source of bacteria further upstream, and most probably from one or more STWs. However, the gradual decline occurred over a considerable distance, and potent sources on the way down might possibly be adding to the bacterial load.

1.5 Total coliforms were many times more abundant than *E. coli* (also a coliform), and enterococci were least abundant. The patterns of counts over distance differed according to bacterial type. *E. coli* and enterococci behaved more similarly one to another, whereas the distribution of total coliforms was markedly different in some sections of river where counts were stable over distance whereas *E. coli*'s declined. If nearly all the bacteria originated at point sources, i.e. at the STWs, the results might suggest large differences between the types in survival rates. However, faecal bacteria can survive in the environment, albeit with declines, for several weeks, and can be released by birds and riverine animals. Runoff from contaminated soils is another source. At some sites, therefore, the clear-cut pattern of bacterial recharge of bacterial load in the river from STWs followed by declines over distance may become obscured by local 'wild' sources contributing to the counts. Although our sites are insufficient in number and too far apart to check accurately for 'wild' sources, it is unlikely that a 'wild' source will elevate the counts of *E. coli* by more than a small amount.

1.6 Cattle at Grantchester Meadows did not appear to contribute significantly to faecal bacterial counts.

1.7 Batch 2 nitrate concentrations are high, and broadly similar to those of Batch 1 despite a reduced river flow which would have concentrated the nutrient to higher levels if no other factor was operating . In contrast, phosphate levels were consistently higher than in Batch 1.

1.8 Applying the EA Water Framework Directive standards, the high phosphate levels where samples were collected on the Granta-Cam, Rhee, and Cam conferred a unofficial 'poor' status,. In August, the sampled watercourses (excluding Vicar's Brook) were shown to be eutrophic due to the high concentrations of nitrate and particularly phosphate.

1.9 High turbidity of the River Rhee continues to be a problem throughout the summer months and persists in the absence of raised flows due to rainfall.

1.10 Our bacterial data are derived from just two batches of samples and the results must be treated with caution. Batches show some inconsistencies one with the other, and some sites have been sampled only once. Further batches of samples are needed to build a more robust database.

1.11 There are two issues concerning Public Health that are not investigated in our bacterial testing beyond simple enumerations. None of our testing can provide data on the presence of carbapenemase-producing Enterobacteriaceae (CRE) -which include coliforms including some strains of *E. coli*. Their presence and abundance is a Public Health concern as strains within that group are hard to treat with antibiotics. Nor does our testing provide any information on dissemination of other antibiotic resistant bacteria and antibiotic resistance genes introduced into wastewater discharged from sewage treatment works.

1.12 This report should not be used to guide water users on the safety of any specific stretches of the river; some general guidance may become possible if further sampling shows that there are more consistent patterns in bacterial abundance. It can be stated, however, that some counts were higher than the threshold used in the standards to achieve even a 'sufficient' status according to the current EA Bathing Water Directive (2006). However, the Directive is complex practically, and we cannot apply it to our data, from only two batches, to provide guidance to river users.

1.13 In CVF's further investigations on faecal contamination, sites need to be expanded yet again, batches of samples taken in more prolonged, wetter conditions and STW effluent sampled at source both in treated form and during storm discharges. Thus, our project will run for several more months.

1.14 We welcome funding from interested parties to allow our work to continue.

2 Methods

2.1 The general procedures for Batch 2, 24th August followed the protocol used on 14th June 2021 (see Report No. 1). Sites 1–12 were sampled by Mike Foley and Bruce Huett, sites 13-19 by Stephen Tomkins and Simon Spooner.

2.2 Seven additional sampling sites were included in Batch 2. These were Meldreth (Rhee), Barrington (Rhee), Newnham Riverbank Club (Cam), upstream of Cambridge (Milton) STW (Cam), Cambridge (Milton) STW pure effluent, Baits Bite Lock (Cam), and Clayhithe (Cam).

2.3 The site map is at Figure 1, and can also be viewed online [reference[1]]. In the online map there are comments and some photos – click on the pointers. The zoom level can be changed. Click on arrow at 'base map' to change to satellite image.

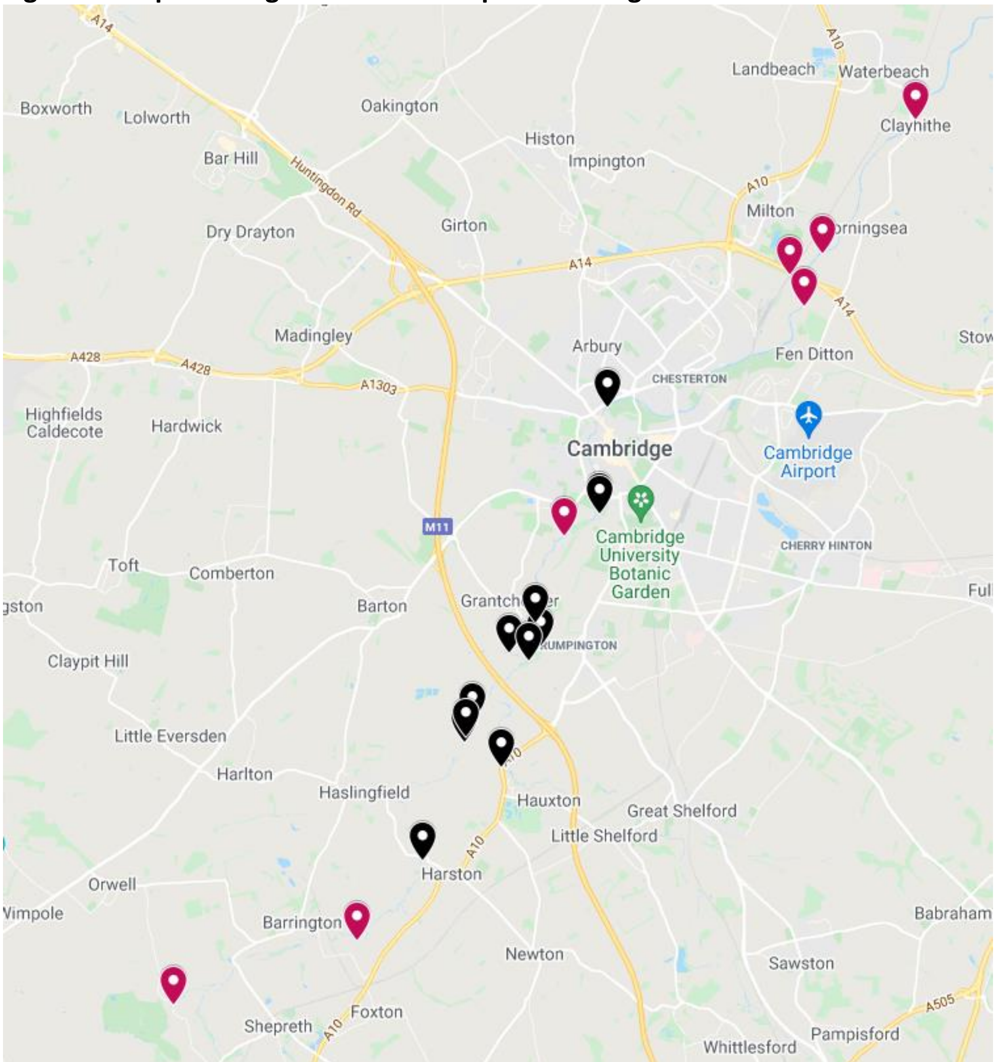
2.4 The microbial samples were tested at South East Water Scientific Services (SEWSS) for the indicator bacteria (*E. coli*, and enterococci), and total coliforms. The coliforms (*E. coli* belongs to this group) were tested by the Colilert Most Probable Number (MPN) method; and enterococci by the MGLA (Colony-Forming Units) method. Results were expressed as counts of either MPN or CFU per 100ml of sample.

2.5 At the same laboratory, the mineral samples were analysed for nitrogen (as inorganic nitrate) and soluble reactive phosphorus (SRP: phosphorus in orthophosphate). The reporting limit for nitrate is 2mg/l and for phosphate– phosphorus is 84 ppb (0.084 mg/l).

[1] Site locations Batch 2, online, interactive

The limit 0.084 was sufficient for our needs, as it is within the ‘good ’ status band according to the Water Framework Directive.

Figure 1: Map showing the 19 sites sampled 24th August 2021



2.6 Black markers show the sites selected for Batch 1, 14th June. Purple markers show the additional sites of the extended monitoring for Batch 2, 24th August.

Table 1: Location of sampling points 24th August 2021

Site code and #	Site of sampling point	Distance from STWs (km)	Lat, Long	Reason for selecting site
		(i) Haslingfield STW		
1. MORB	Rhee – Malton Lane bridge Meldreth-Orwell road.	–9.5 km	52.11588, 0.00478	Additional to Batch 1. To capture R. Mel and its STW and everything else upstream of this point.
2. BFOX	Rhee – Barrington, downstream of quarry’s railway line	–5.3 km	52.12624, 0.05285	Additional to Batch 1. Far enough downstream to allow mixing of river water with the Foxton SWT discharge.

Site code and #	Site of sampling point	Distance from STWs (km)	Lat, Long	Reason for selecting site
3. HHRB	Rhee – Harston-Haslingfield road, bridge	–3.1 km	52.13908, 0.06998	In Batch 1
4. ASTW	Rhee - Just above Haslingfield STW	–0.08 km	52.15807, 0.081	Direct comparisons just downstream and upstream of the works. Taken 80m above discharge point to be clear of works site.
5. HEFF	Haslingfield STW	± 0 km	52.19467, 0.11645	Pure effluent, taken from discharge point. Critical, to know what is present at STW source.
6. BSTW	Rhee - just below Haslingfield STW	+0.270 km	52.16148, 0.083	Direct comparison with above the works; far enough down to allow mixing.
7. GRAN	Granta	n/a	52.15412, 0.09063	Upstream of confluence with Rhee, to evaluate Granta-Cam's bacterial input. At A10 road bridge
8. ABBR	Cam – just above confluence with Bourn Brook	+1.9 km	52.17129, 0.09771	Without inputs from Bourn Brook.
9. CFAB	Bourn Brook, Cantelupe Farm bridge	n/a	52.17252, 0.09255	Upstream of confluence with Cam, to evaluate Bourn Brook's own bacterial input; coincides with EA nutrient testing site.
10. BYRO	Cam – between Byron's Pool and Brasley Bridge, Trumpington-Grantchester road	+2.35 km	52.17357, 0.10097	Below Bryon's Pool and just above private sewer effluent discharge from cottages; [Bourn Brook + 0.420 km]
11. CRIC	Cam – top of Grantchester Meadows- cricket field	+2.8 km	52.17731, 0.09964	After Mill Stream joins, and before long cattle grazing/dog walking/swimming stretch.
12. NRCL	Cam – Newnham Riverbank Club	+ 4.75km	52.19115, 0.10711	Additional to Batch 1. More precision is required to measure bacterial inputs from Meadows and closer to it than Sheep's Green; hence this site is just downstream of cattle fields.
13. VICA	Vicar's Brook,	n/a	52.19467, 0.11645	Above exit into Cam, to evaluate Vicar's bacterial input.
14. SHEE	Cam -Sheep's Green, Coe Fen footbridge	+5.5 km	52.19518, 0.11623	After confluence with Vicar's Brook, coincides with EA nutrient testing site, at popular swimming stretch.
15. JESU	Cam – Jesus Green	+7.6 km	52.21191, 0.11853	Bottom end of Middle River above Jesus Lock and just above moored narrowboats; downstream of city centre and colleges.
16. FDPF	Cam - Fen Ditton, Green End Road, paddock field	+ 12.3 km (–0.46 km to STW)	52.22801, 0.16984	To monitor from Jesus Green a large number of moored boats; various ditches; and effluent sources unknown and known.
		(ii) Cambridge STW		

Site code and #	Site of sampling point	Distance from STWs (km)	Lat, Long	Reason for selecting site
17. CEFF	Cambridge STW effluent discharge	± 0 km	52.19467, 0.11645	Pure effluent, taken from discharge well inside site. Critical, to know what is present at STW source.
18. CBBL	Cam – Baits Bite Lock	+ 0.67 km	52.2365, 0.17464	Further downstream to allow mixing of effluent; for comparison of counts just upstream and downstream of Cambridge STW.
19. CCHB	Cam - Clayhithe bridge	+ 3.7 km	52.25782, 0.19924	To monitor bacterial load over distance; just upstream of Cambridge Motor Boat Club and Cam Sailing Club

2.7 The map at Figure 2 shows the locations of the STWs. Bacterial load from those upstream of Haslingfield STW but closer to it are likely to be the sources of a large proportion of the high levels of indicator bacteria detected in our Batch 2 samples (see Figure 17). However, private sewerage systems, pipe misconnections, overwhelmed sewers, pumping station overflows, and agricultural inputs cannot be discounted at this stage in our project. Further investigations are vital to better understand the sources of the faecal contamination.

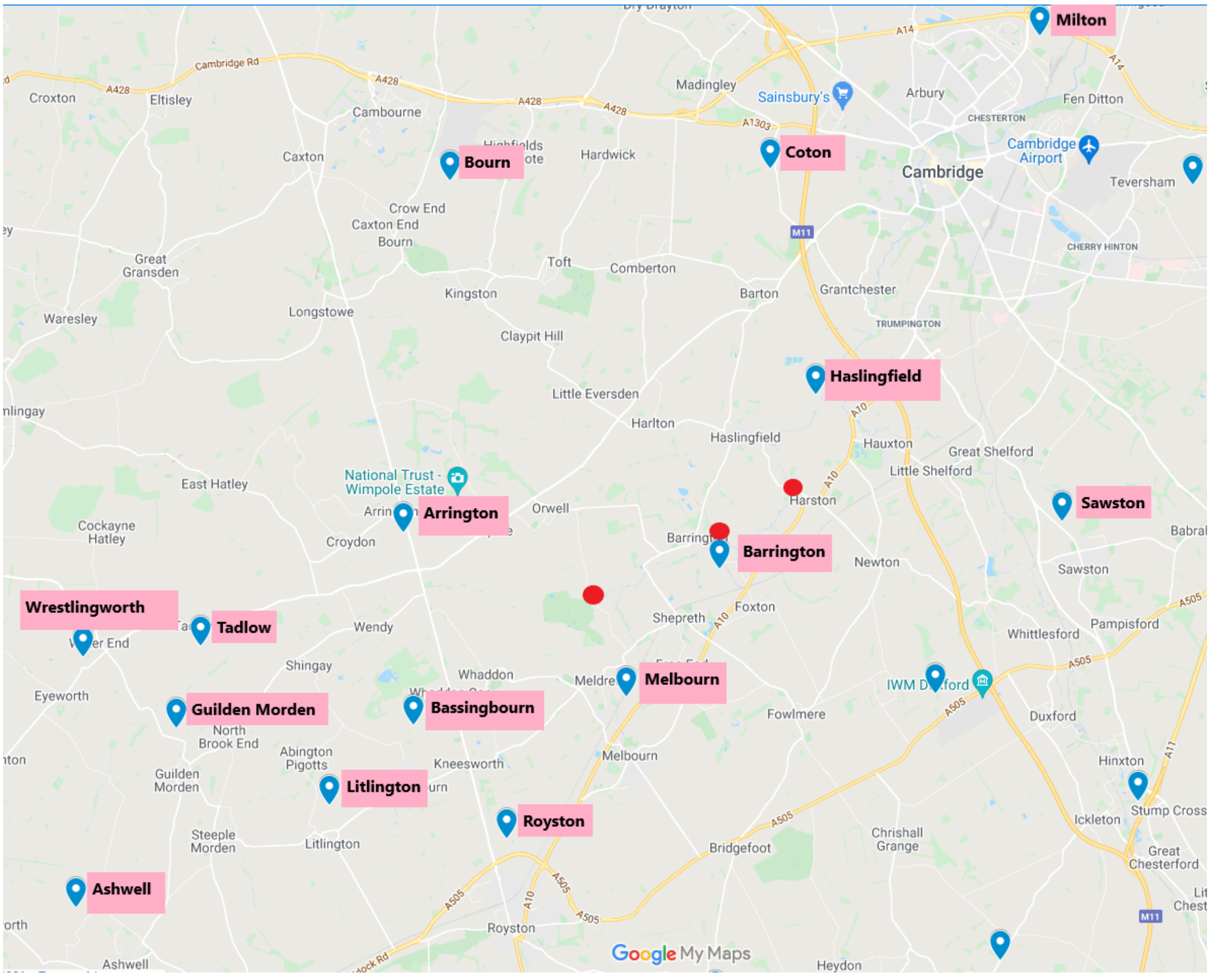
2.8 Two additional sites on the Rhee were sampled upstream of the Haslingfield–Harston road bridge (these two sites are displayed as red dots in Figure 2, the third red dot at the right hand side in the line of three dots showing the location of the uppermost sampling site in Batch 1).

2.8.1 The site east of Barrington was intended to capture <u>all sources above it</u>, include the nearby Foxton pumping station discharge point at the quarry rail line (but far enough downstream to allow thorough mixing), the Guilden Brook, Shep, Wallington Brook, and the Hoffer Brook.

2.8.2 The uppermost site at Malton Farm on the Meldreth-Orwell road intended to capture various inputs to include:

- Wimpole Home Farm stream.
- Whaddon Brook, including Royston STW.
- Mel, including Melbourn STW.
- Wellhead Spring stream (Bassingbourn Brook), including Bassingbourn STW, exiting into Mill River and eventually the Rhee.
- Chardle Ditch, including Litlington STW, exiting into Mill River.
- Guilden Morden STW
- Arrington STW
- Wrestlingworth STW
- Tadlow STW
- Ashwell STW

Figure 2: Map of sites of the three uppermost sampling points [1],[2] and [3] on the Rhee, marked as red dots, and the relevant sewage treatment works shown at blue markers. Some STWs might be considered to be too distant to contribute significantly, e.g. on the Granta, and the upper Rhee.



Base map with STW markers – credit Anne Miller

2.9 Relevant STWs that discharge directly or indirectly to the Rhee, Cam and Bourn Brook are named. Coton STW discharges directly into the Bin Brook, which exits into the Cam at St John's College.

2.10 In Batch 1, Haslingfield Sewage Treatment Works was singled out for study as it is the closest works upstream of Cambridge, and published data reveals that it experienced many storm overflows (88 events, of >1,000 hours in 2019). These overflows are of sewage which has spent time in settlement tanks, but is otherwise untreated. Anglian Water has explained that this high incidence is likely to reflect a faulty monitoring arrangement, mechanically corrected in September 2021, but this has yet to be made clear in their published record.

2.11 On 24th August air temperature was 16-21^{O}C; sunshine was intense until 1200 and reduced by 1330 by a veil of light cloud, though it was still remained very bright. Only sample [5] was taken after 1330 (taken at 1335), but if some sources were some distance upstream, UV intensity would have been higher earlier while the bacteria were still moving downstream.

2.12 Figure 3 shows the percent sunshine on 24th August 2021 with 14th June for comparison (Digital Technology Group (DTG), University of Cambridge, CB3 0FD, 6 km from Haslingfield STW). Reference[2] shows an example of their display of weather data.

2.13 This is an amateur-maintained weather station. Observations are not gathered in similar conditions to ones used by the Met Office, their sensors are not calibrated against references, and some sensors have been changed over time with ones working on different principles. However, they stay alert to faults and list them by date when noted.

Figure 3: Sunshine intensity for 24th August (sampling times 0900 – 1335)

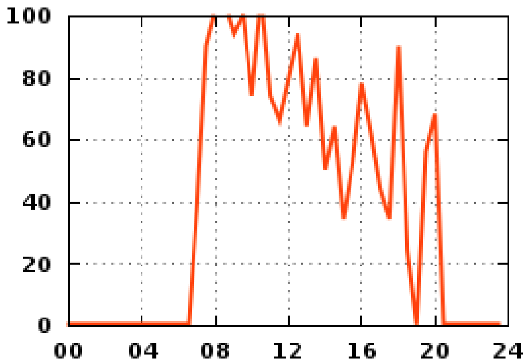
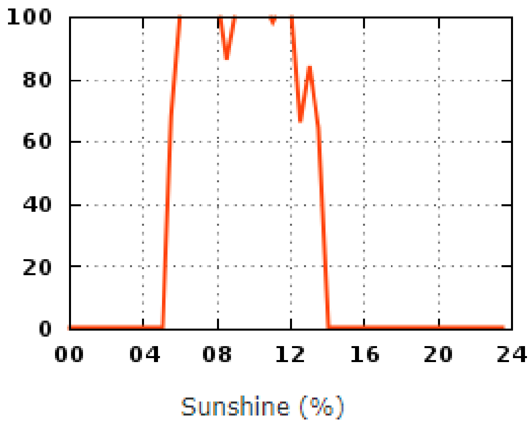


Figure 4: Sunshine intensity for 14th June for comparison (sampling times 0900 – 1335)



2.14 Ultra violet radiation (UVA, UVB) may cause some decline in indicator numbers over distance, perhaps less harmful to *E. coli* and pathogens in late August on a partially cloudy day (Batch 2) compared to mid-June on a cloudless day (Batch 1). It is also important to know if it was sunny at discharge points a few kms upstream from our sampling point, as well as judging the UV intensity at the time of sampling. Weather maps showed that the conditions recorded at DTG on 24th August were widespread.

2.15 Samples were taken after a period of dry weather, many farm ditches had very low flows or were dry, and the chances of runoff of bacteria or nutrients from farmland into ditches or directly into the river were small.

2.16 The river level at Burnt Mill, Haslingfield was about 10cm on 24th August. The level was steady and about 14 cm on 14th June, indicating a greater flow on the earlier date.

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[2] https://www.cl.cam.ac.uk/research/dtg/weather/daily-graph.cgi?2021-06-14

2.17 Observations of the Haslingfield STW effluent discharge on 24th August were similar to 14th June. The treated effluent appears clear to the eye and it flowed in an arc partly across river. Again, water turbidity was similar below and above the discharge point.

2.18 Conversion of bacterial counts and nutrient concentrations in pure effluent into river water counts

2.181 On 14th June Anglian Water data show that the effluent flow at the 'inlet' at 12.40pm (sampling time) was 37.4 l/s which is being used as the best estimate of flow. While we wait for equivalent data for 24th August, Anglian Water advise to use the same flow.

2.182 On 24th August the flow at Burnt Mill, Haslingfield was 0.374 m^3/s (checked data). The flow at Burnt Mill would have been very similar to that at the STW (1.6 km downstream).

2.183 Using actual flow data and estimates, a conversion of counts in pure effluent to counts in river water can be calculated (Table 2). <u>Note that these converted 'river water' counts are estimates.</u> The dilution figure used for Batch 2 counts for Haslingfield effluent – 11.08 – is the rate of dilution of bacteria levels in the effluent after mixing in the river water. At Cambridge STW it was 2.49.

2.184 This calculation reveals that on 24th August at Haslingfield the effluent flow made up an estimated 9% of the total river flow. At Milton it was estimated at 40%.

Table 2: Conversion of effluent count of *E. coli* to a 'river water' count using estimated flows of effluent and river

Location of STW and bacterial type	Batch 2, actual effluent count /100 ml	Batch 2 effluent count expressed as a 'river water count'/100 ml	Batch 1 effluent count/100 ml for comparison	Batch 1 'river' counts for comparison
Haslingfield STW		**(Effl count ÷ 11.08)** [1]		**(Effl count ÷ 17.9)**[3]
E. coli	38,730	3,495	38,700	2,167
Total coliforms	129,970	11730	155,300	8,696
Enterococci	3,000	271	6,200	347
Cambridge STW		**(Effl count ÷ 2.49)**[2]		
E. coli	7,380	2,959	–	–
Total coliforms	24,950	10,005	–	–
Enterococci	890	357	–	–

1. Based on 24 August: Haslingfield Burnt Mill gauging station flow: 0.374 m^3/s (EA data, checked); best estimate of Haslingfield effluent flow, 24 August: 0.0374 m^3/s. Estimated total flow passed Haslingfield STW = 0.411 m^3/s – effluent dilution factor of 11.08.

2. Based on 24 August: Just downstream of Haslingfield STW: 0.411 m^3/s; Granta (Stapleford): 0.083; Cam (Dernford): 0.372 m^3/s; Bourn Brook: unknown but low; Cambridge

   STW effluent flow: 0.583 m^3/s (five-year data 2016-2020), Anglian Water); others: small flows. Estimated total flow passed Cambridge STW = 1.449 m^3/s – effluent dilution factor of 2.49.

3. Based on 14 June: Haslingfield Burnt Mill gauging station flow 14 June: 0.631 m^3/s (EA data, checked); best estimate of effluent flow at 1240, 14 June: 0.0374 m^3/s (Anglian Water); estimated total flow passed Haslingfield STW = 0.668 m^3/s – effluent dilution factor of 17.9.

4 Results

Table 3: Bacterial counts and nutrient concentrations at the 19 sampling sites

Site	Site of sampling point	Distance from Haslingfield STW	Count of faecal indicator bacteria (coliforms inc. *Escherichia coli* – most probable number (MPN) per 100ml; enterococci – colony forming units (CFU) /per 100ml)			Nutrient concentrations		
			E. coli	Total coliforms	Enterococci	Phosphate-P) (mg/l)	Nitrate (mg/l)	Total phosphorus (mg/l)
[1]. MORB	Rhee – Malton Lane bridge Meldreth-Orwell road.	–9.5 km	3,441	9,606	1,300	0.871	62.3	
[2]. BFOX	Rhee – Barrington, downstream of quarry's railway line (discharge point)	–5.3 km	2,613	12,033	930	0.487	48.9	
[3]. HHRB	Rhee – Harston-Haslingfield road, bridge	–3.1 km	1,211	8,164	640	0.475	58.6	
[4]. ASTW	Rhee - Just above Haslingfield STW	–0.08 km	933	9,208	470	0.530	49.8	

Site	Site of sampling point	Distance from Haslingfield STW	*E. coli*	Total coliforms	Enterococci	Phosphate-P) (mg/l)	Nitrate (mg/l)	Total phosphorus (mg/l)
[5]. HEFF	Haslingfield STW pure effluent	± 0 km	38,730	129,970	3,000	1.500	73.6	1.780
[6]]. BSTW	Rhee - just below Haslingfield STW	+0.270 km	4,106	24,196	520	0.628	53.8	
7. GRAN	Granta	n/a	243	5,475	210	0.424	36.2	
8. ABBR	Cam – just above confluence with Bourn Brook	+1.9 km	683	7,701	200	0.519	45.1	
9. CFAB	Bourn Brook, Cantelupe Farm bridge	n/a	55	1,046	150	0.793	11.6	
10. BYRO	Cam – between Byron's Pool and Brasley Bridge, Trumpington-Grantchester road	+2.35 km	63	836	230	0.563	49.8	
11. CRIC	Cam – top of Grantchester Meadows-cricket field	+2.8 km	496	8,164	170	0.496	44.4	

Site	Site of sampling point	Distance from Haslingfield STW	*E. coli*	Total coliforms	Enterococci	Phosphate-P (mg/l)	Nitrate (mg/l)	Total phosphorus (mg/l)
12. NRCL	Cam – Newnham Riverbank Club	+ 4.75km	364	3,873	59	0.477	44.1	
13. VICA	Vicar's Brook,	n/a	2,046	5,475	910	<0.084	42.5	
14. SHEE	Cam -Sheep's Green, Coe Fen footbridge	+5.5 km	120	1,414	35	0.444	47.6	
15. JESU	Cam – Jesus Green	+7.6 km	365	2,420	200	0.459	44.6	
16. FDPF	Cam - Fen Ditton, Green End Road, paddock field	+ 12.3 km (–0.46 km to STW)	288	3,448	42	0.441	42.6	
		Cambridge STW						
17. CEFF	Cambridge STW pure effluent	± 0 km	7,380	24,950	890	0.127	77.9	0.260
18. CBBL	Cam – Baits Bite Lock	+ 0.67 km	1354	9,804	220	0.452	49.4	
19. CCHB	Cam - Clayhithe bridge	+ 3.7 km	754	11,199	28	0.434	49.6	

4.1 Where samples were taken on both dates, a comparison of the bacterial counts can be made to determine either increases or decreases on the second date. This is presented in Table 4. All river counts in Batch 2 should have been raised due to the reduced flow compared to Batch 1. The reduction in the river flow on the date of Batch 2 passing Haslingfield STW was 38% (including the effluent flow). Thus, several counts in Batch 2 should be higher by about 62% simply due to reduced flow. Some sites will have a slightly different flow factor influencing it, depending on its location. Ratios at several sites did not reflect this adjustment and must be different due to some other factor.

Table 4: Ratio of Batch 2 counts to Batch 1 at specific locations, where data exist for both

Shown here are the counts / 100ml for *E. coli*, total coliforms and enterococci for Batch 1 (B1) and Batch 2 (B2). The ratio B2:B1 is the Batch 2 count divided by the Batch 1 count. It shows how counts differ between the two dates.

Sites in grey are actual main river counts STWs in blue are effluent counts converted to river counts Sites in green are actual tributary counts
STWs in yellow are actual counts in pure effluent

		***E. coli* (MPN)**			**Total coliforms (MPN)**			**Enterococci (CFU)**		
		Batch 1	**Batch 2**	**Ratio B2:B1**	**Batch 1**	**Batch 2**	**Ratio B2:B1**	**Batch 1**	**Batch 2**	**Ratio B2:B1**
Actual main river counts										
1. MORB	Rhee – Meldreth-Orwell road bridge - 9.5km [1]	–	3,441	–	–	9,606	**–**	–	1,300	**–**
2. BFOX	Rhee – Barrington D/S discharge point - 5.3km [2]	–	2,613	–	–	12,033	**–**	–	930	**–**
3. HHRB	Rhee – Haslingfield-Harston road - 3.1km [3]	326	1,211	**3.7**	2420	8,164	**3**	48	640	**13**
4. ASTW	Rhee - above STW - 0.08km [4]	205	933	**4.6**	1733	9,208	**5.3**	24	470	**19.6**
6. BSTW	Rhee - below STW +0.270km [6]	1,080	4,106	**3.8**	6870	24,196	**3.5**	62	520	**8.4**
8. ABBR	Cam – above Bourn Brook + 1.9km [8]	127	683	**5.4**	2420	7,701	**3.2**	18	200	**11.1**

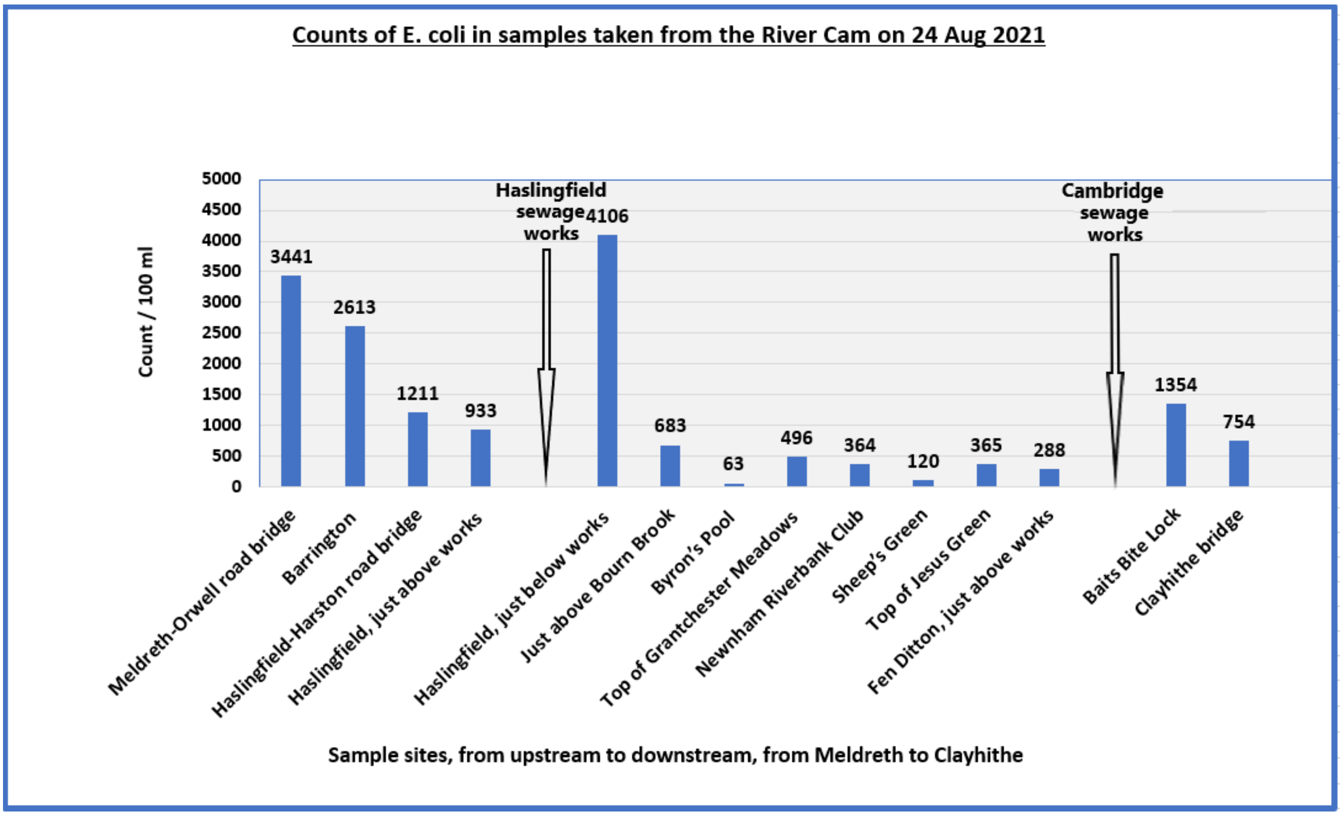
		E. coli (MPN)			Total coliforms (MPN)			Enterococci (CFU)		
		Batch 1	Batch 2	Ratio B2:B1	Batch 1	Batch 2	Ratio B2:B1	Batch 1	Batch 2	Ratio B2:B1
10. BYRO	Cam – Byron's Pool +2.35km [10]	261	63	**0.2**	2420	836	**0.3**	20	230	**11.5**
11. CRIC	Cam – top of GMs + 2.8km [11]	160	496	**3.1**	2610	8164	**3.1**	32	170	**5.3**
12. NRCL	Cam – Newnham Riverbank Club + 4.75km [12]	–	364	**–**		3,873	**–**	–	59	**–**
14. SHEE	Cam -Sheep's Green + 5.5km [14]	29	120	**4.1**	317	1,414	**4.5**	3	35	**11.7**
15. JESU	Cam – Jesus Green + 7.6km [15]	29	365	**12.6**	326	2,420	**7.4**	5	200	**40.0**
16. FDPF	Cam - Fen Ditton U/S Camb STW -0.46km [16]	–	288	**–**	–	3,448	**–**	–	42	**–**
18. CBBL	Cam – Baits Bite Lock + 0.67km [18]	–	1,354	**–**	–	9,804	**–**	–	220	**–**
19. CCHB	Cam - Clayhithe bridge + 3.7km [19]	–	754	**–**	–	11,199	**–**	–	28	**–**
Effluent counts converted to river water counts										
5. HEFF	Haslingfield STW [5]	2,167	3,495	**1.6**	8,696	11,730	**1.3**	347	271	**0.8**
17. CEFF	Cambridge STW effluent [17]	–	2,959	**–**	–	10,005	**–**	–	357	**–**
Actual tributary counts										
7. GRAN	Granta-Cam tributary [7]	167	243	**1.5**	1,986	5,475	**2.8**	6	210	**35.0**
9. CFAB	Bourn Brook tributary [9]	1	55	**55.0**	54	1,046	**19.4**	0	150	**n/a**

13. VICA	Vicar’s Brook tributary [13]	400	2,046	**5.1**	3,080	5,475	**1.8**	220	910	**4.1**
Actual effluent counts										
5. HEFF	Haslingfield STW pure effluent [5]	38,700	38,730	**1.0**	155,300	129,970	**0.8**	6,200	3,000	**0.5**
17. CEFF	Cambridge STW pure effluent [17]	–	7,380	**–**	–	24,950	**–**	–	890	**–**

4.2 Results for *E. coli*

4.21 Figure 6 shows the river counts of *E. coli* on 24th August. Neither the effluent counts nor the converted river water counts are shown here.

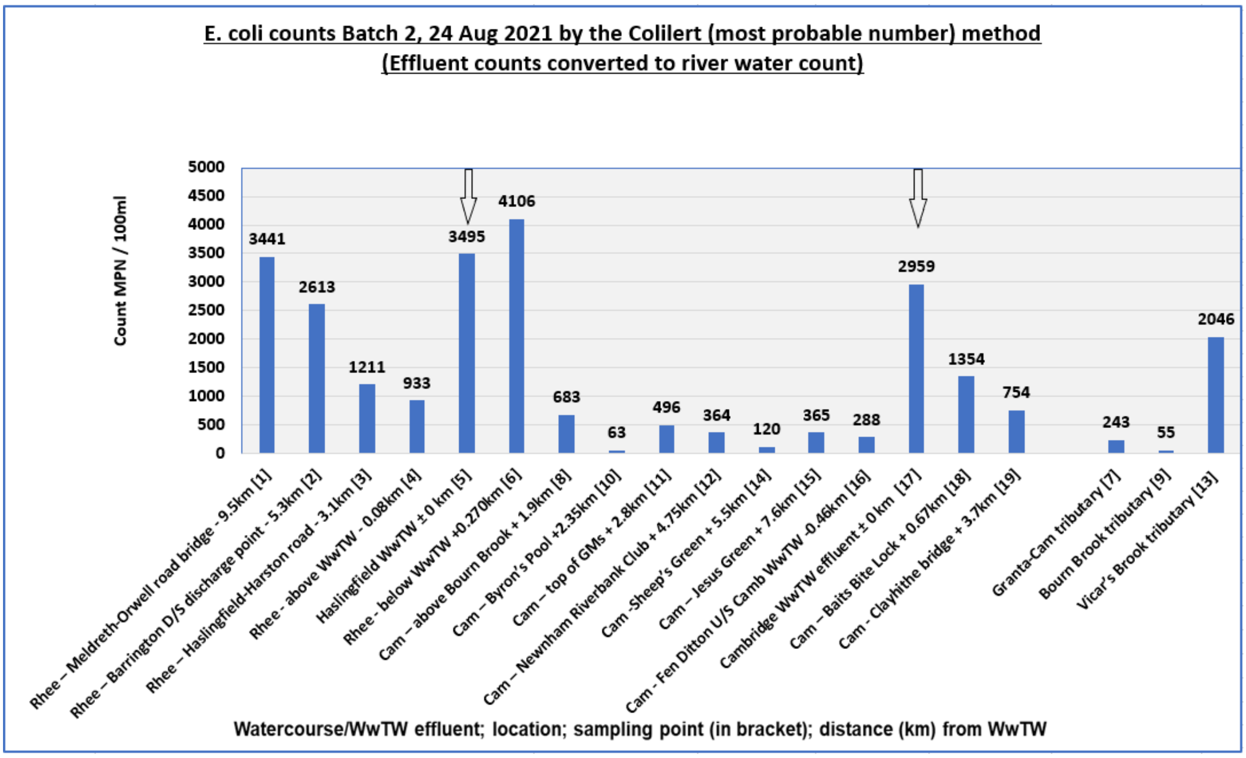
Figure 6: Actual *E. coli* Batch 2 (24th August) river counts /100ml – sites of both STWs depicted with arrows.



4.22 For Batch 2, there were two additional sampling sites above Haslingfield STW, one at Newham Riverbank Club closer to Grantchester Meadows than Sheep's Green to capture bacteria arising from the Meadows, two above Cambridge STW, the STW's pure effluent, and two below the STW.

4.23 Figure 7 shows counts at all 19 sites including the effluent counts converted to 'river water' counts.

Figure 7: Counts of *E. coli* / 100ml, Batch 2 (24th August) at all 19 sampling points (effluent counts converted to *estimated* river water counts)



4.2.4 Note: the counts of 3,495 and 2,959 MPN /100ml at Haslingfield STW and Milton STW respectively are estimated river water counts, based on calculations using the actual counts in the pure effluent and the dilutive effect of the river flow.

4.2.5 Samples in both batches were taken after period of dry weather, many farm ditches were very low or dried out, or flows were sluggish, and the chances of runoff from farmland into ditches or directly into the river were small. However, the high count (albeit in a low flow) in Vicar's Brook suggests (but is not yet confirmed) that the source is cattle on Coe Fen. However we cannot yet discount an unknown source much further upstream of the sampling site.

4.2.6 Counts in Batch 2 are relatively higher than in Batch 1

4.2.7 The inclusion of additional sites reveals clearly that STWs are important sources of bacteria that recharge the bacterial load in the river. The patterns of decline and uplift along the Cam are sufficiently clear to make this point.

4.2.8 Although we have not collected evidential data, there is a possibility that the bacterial load counted at site [1] had declined markedly over the 4.2 km stretch down to site [2], and that the high count at site [2] was mostly recharge, associated with the outfall at Barrington from Foxton STW, or some other local source.

4.2.9 There are important sources even further upstream that need further investigation. Despite declines over distance were noted in dry conditions, in wetter periods bacterial loads further upstream may become much larger and survive over longer distances.

4.2.10 There are some inconsistencies in counts at specific sites between Batch 1 and Batch 2.

4.2.11 Contributions from the three tributaries will become relatively small because of the the dilutive effect after they enter the Rhee-Cam

4.2.12 Several counts on this date did not reach the lowest EA standards of the current Directive for Bathing Waters (Figure 8) and also the directive used until 2006 which was superseded by the current directive. However, the statistical protocol of the current Directive is not being followed correctly in our samplings. The differences in counts between the two batches show that just two batches of samples are insufficient to make firm conclusions about health risks to swimmers.

Figure 8: Bathing Water Directive 2006/7/EC

Excellent	EC: ≤500 cfu/100ml ; IE: ≤200 cfu/100ml	95th percentile
Good	EC: ≤1000 cfu/100ml ; IE: ≤400 cfu/100ml	95th percentile
Sufficient	EC: ≤900 cfu/100ml ; IE: ≤330 cfu/100ml	90th percentile
Poor	means that the values are worse than the sufficient	

Key EC: Escherichia coli, IE: Intestinal enterococci, cfu: Colony Forming Units

4.2.13 One important potential source is the presence of birds, especially waterbirds of which there are several species resident, and wild mammals. From research, release of faecal bacteria from wildlife is recognised as being commonplace, and ay be contributing to a background level of contamination. They are unlikely to be the maim contributor to the higher counts found.

4.2.14 Comparing the two STWs, *E. coli* counts /100ml in the Cambridge pure effluent was only 19% of Haslingfield's. However, after taking into account the different dilution rates, the *E. coli* effluent river count at Cambridge was 85% of Haslingfield's.

4.2.14 Comparisons of counts in Batch 1 and Batch 2, samples taken 7 weeks apart, are vital to check for consistency in abundance and patterns of abundance. Figures 9 shows Batch 1 counts, annotated to focus on some key features, and Figure 10 shows the comparative counts for E.coli for the two batches.

4.2.15 The counts at the two sites above Haslingfield STW suggest some source further upstream. It is only by expanding the sites further upstream does a stronger pattern emerge. In Batch 1, there was a sharp decline only 270m from the Haslingfield STW outfall where in Batch 2 the count remained high. There is a strong suggestion that in Batch 2, *E. coli* survival rate was higher over distance.

4.2.16 A rise of 301% between Sheep's Green and Jesus Green reveals a source either in the river or entering the river after Jesus Green. No such rise was noted in Batch 1. Cattle were present on Coe Fen (although the cattle were present there on both sampling dates), but other sources such as a sewage flush, cannot be discounted. We have not collected data on the Bin Brook which exits into the river between the two sites.

Figure 9: *E. coli* counts Batch 1 (14 June) at al 12 sites for comparison (effluent counts converted to river water counts)

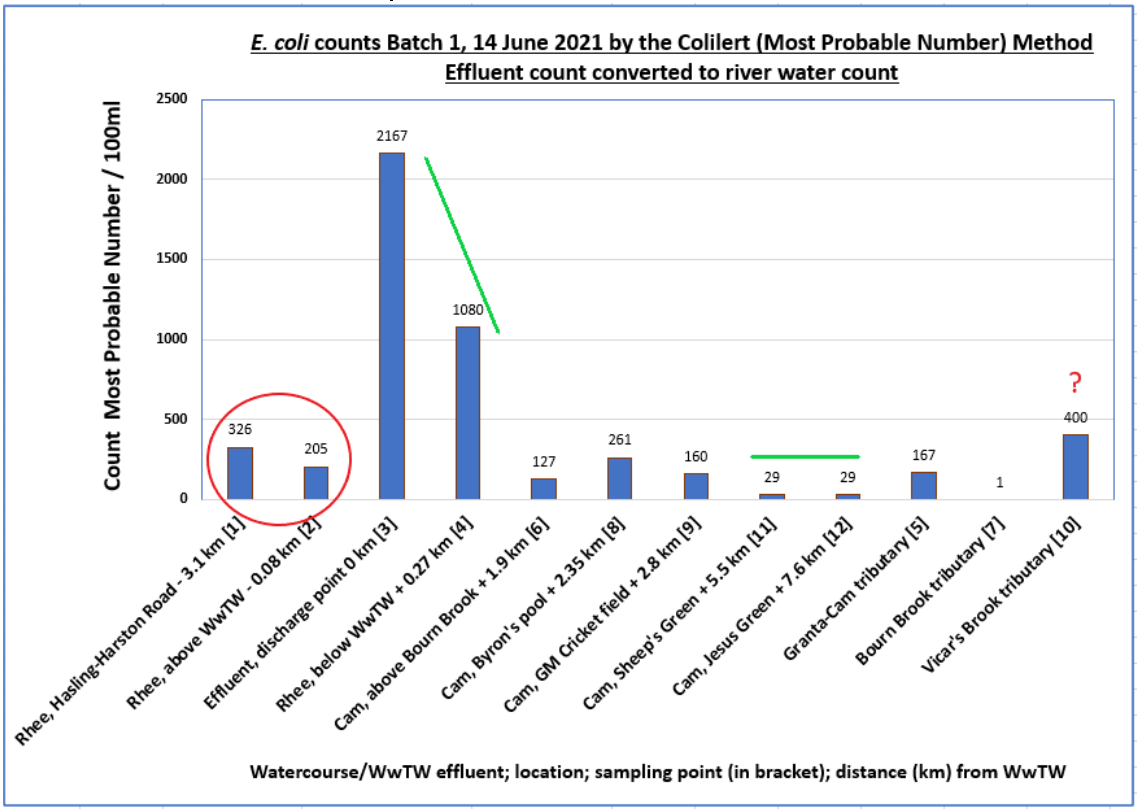
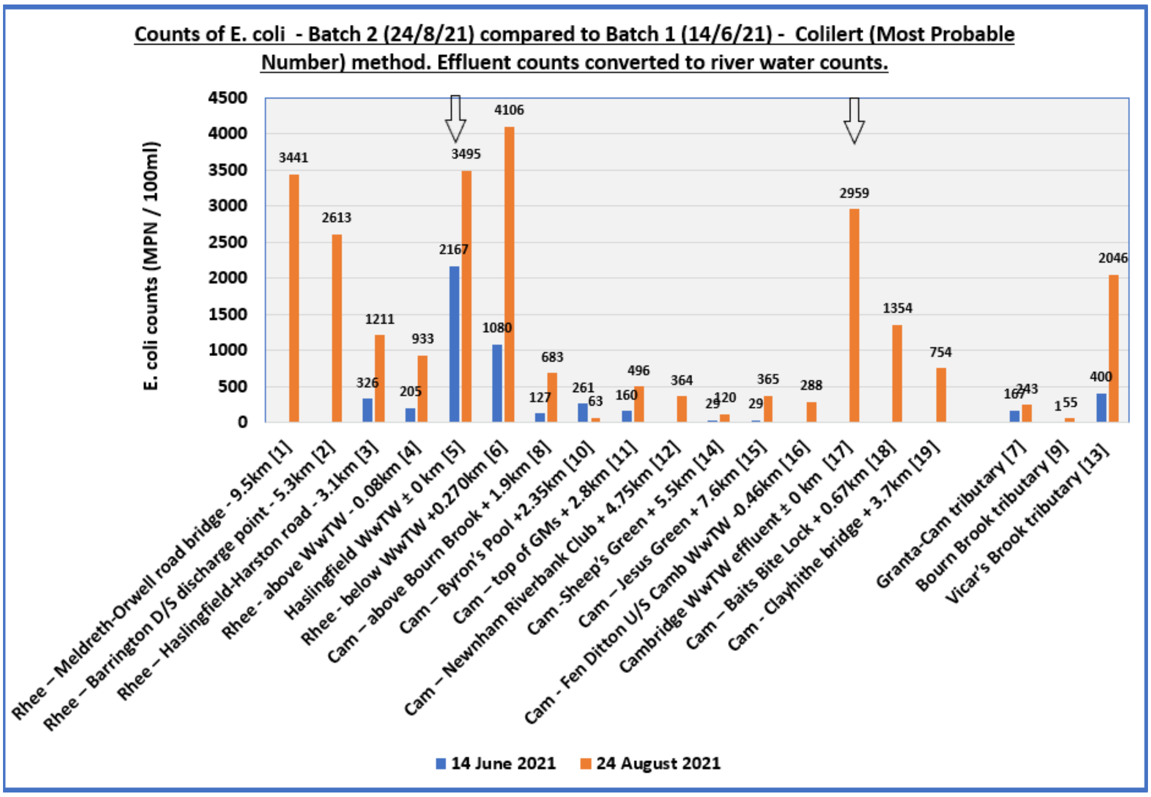


Figure 10: Comparison of *E. coli* Batch 2 counts with Batch 1 counts /100ml at all 19 sampling points (effluent counts converted to river water counts).

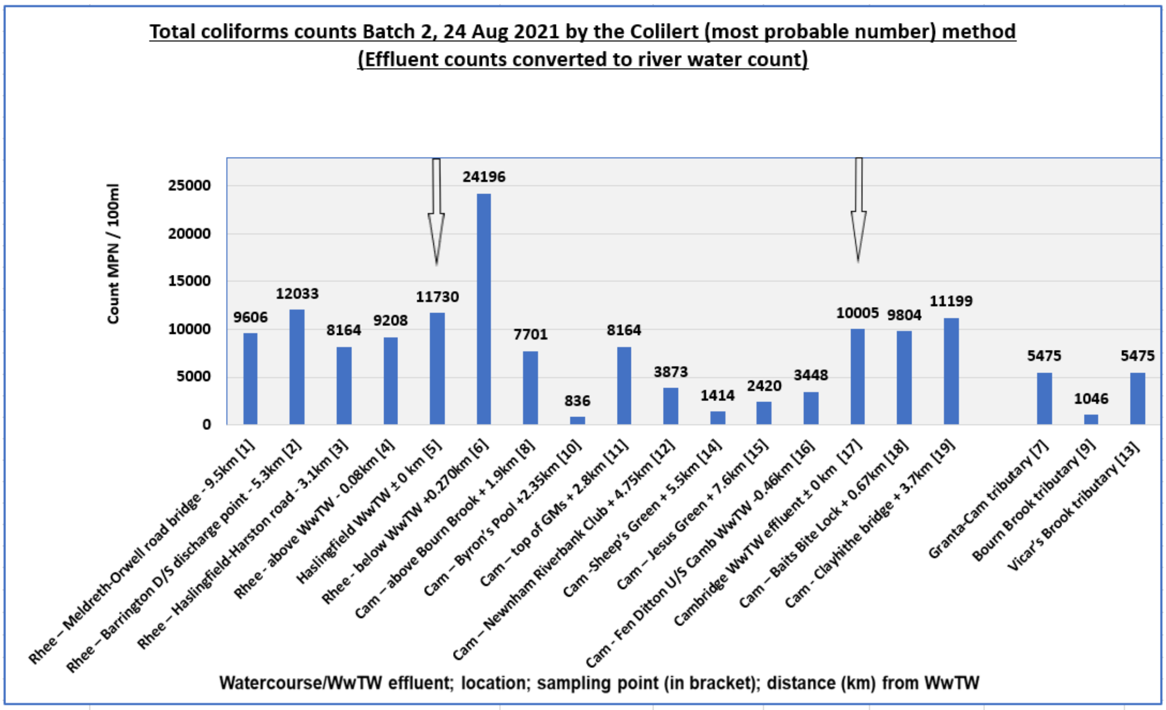


4.3 Results for total coliforms

4.3.1 Although they are not primary indicator bacteria in our project, counts of the 'total coliform' group were included in the laboratory testing. This group was originally believed to be good indicators of the presence of faecal contamination, however total coliforms can be both faecal and non-faecal in distribution and have been found to be widely distributed in nature (soils etc), and not always associated with the gastrointestinal tract of warm-blooded animals. The number of total coliform bacteria in the environment is still widely used as an indicator for contamination of potable water, but is now not used formally in the UK Bathing Water standards. Nevertheless, in the original EEC Directive on Bathing Waters (CD 76/160/EEC, December, 1975; superseded), counts of total coliforms were included as a measure. There is, therefore, merit in presenting counts of total coliforms.

4.3.2 Figures 11 and 12 chart the counts of total coliforms over distance.

Figure 11: Counts of total coliforms Batch 2 (24 August), at all 19 sampling points, effluent count converted to a river water count



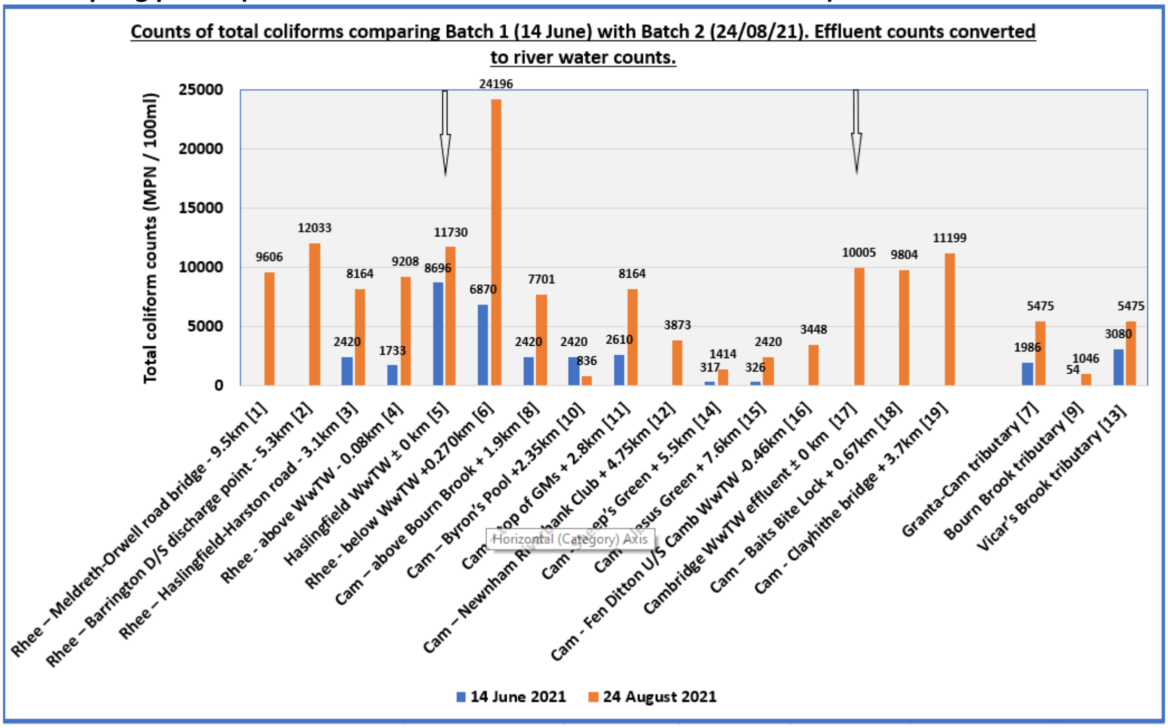
4.3.3 As *E. coli* is a coliform the counts of total coliforms should always be higher than the count of *E. coli*. Counts of total coliforms were very much higher than of *E. coli*. They were present in large numbers in the pure effluent of both STWs.

4.3.4 The count in pure effluent at Haslingfield STW was the highest count of all, and 5.2 times the Cambridge pure effluent count, seemingly demonstrating showing how efficient the Cambridge works are at destroying bacteria.

4.3.5 The count at site [5] (Haslingfield effluent count converted to a river count) was lower than the next count in sequence [6] because of dilution of the effluent. The count at [6] would appear to comprise the counts at [4] and [5] added together. There remains a possibility however, that a potent highly localised 'wild' source existed just below the STW outfall, which was not so active in Batch 1.

4.3.6 Often, counts did not usually follow the same patterns of decline over distance as *E. coli*. Counts declined steadily below Haslingfield STW but in contrast to this, they did not drop below Cambridge STW.

Figure 12: Comparison of total coliform Batch 2 counts with Batch 1 counts / 100ml at all 19 sampling points (effluent counts converted to river water counts)



4.3.7 Ratios of counts of total coliforms to *E. coli*

We are recording *E. coli* separately from other coliforms, which together make up the` 'total coliform' group. It is interesting to compare *E. coli*, a bacterium so closely associated with the intestinal tract of animals that it is used by the Envirnment Agency as an indicator species of faecal contamination, with the other coliforms, some of which may have a non-faecal origin and persist for longer periods in the environment. The ratio of the count of the coliform group to the count of E.coli is of interest. More accurately, this is the ratio of the counts of total coliforms with <u>counts of *E. coli* subtracted</u>, to the count of *E. coli*. This is included as a measure, which logically ought to provide information on the relative survival rates from point sources such as an STW. Interpretation might be clouded if there are other sources which release more total coliforms than E. coil, or *vice versa*.

4.3.8 Table 5 shows the ratios for both batches, with the ratio in the pure effluent as a baseline to compare with other sites.

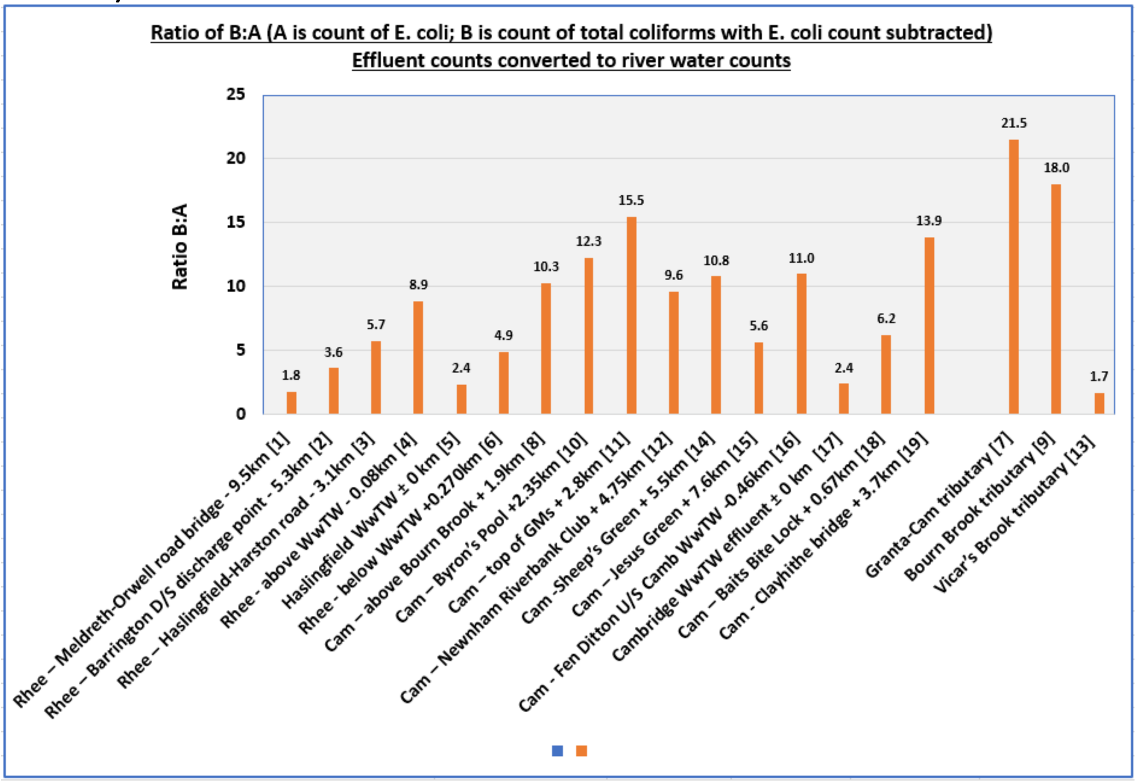
Table 5: Ratios of the counts of total coliforms to *E. coli* (after excluding *E. coli* from the total coliform count by subtracting counts of *E. coli* from the counts of total coliforms). Ratios in pure effluent at the two STWs are in bold.

Sampling site		Ratio of B:A (B is count of total coliforms with *E. coli* count subtracted; A is count of *E. coli*)	
		Batch 1, 14 June 2021	Batch 2, 24 August 2021
5. HEFF	**Haslingfield STW ± 0 km [5]**	**3.0**	**2.4**
17. CEFF	**Cambridge STW effluent ± 0 km [17]**	**–**	**2.4**
1. MORB	Rhee – Meldreth-Orwell road bridge - 9.5km [1]	–	1.8
2. BFOX	Rhee – Barrington D/S discharge point - 5.3km [2]	–	3.6
3. HHRB	Rhee – Haslingfield-Harston road - 3.1km [3]	6.4	5.7
4. ASTW	Rhee - above STW - 0.08km [4]	7.5	8.9
6. BSTW	Rhee - below STW +0.270km [6]	5.4	4.9
8. ABBR	Cam – above Bourn Brook + 1.9km [8]	18.1	10.3
10. BYRO	Cam – Byron's Pool +2.35km [10]	8.3	12.3
11. CRIC	Cam – top of GMs + 2.8km [11]	15.3	15.5
12. NRCL	Cam – Newnham Riverbank Club + 4.75km [12]	–	9.6
14. SHEE	Cam -Sheep's Green + 5.5km [14]	9.9	10.8
15. JESU	Cam – Jesus Green + 7.6km [15]	10.2	5.6

16. FDPF	Cam - Fen Ditton U/S Camb STW - 0.46km [16]	–	11.0
18. CBBL	Cam – Baits Bite Lock + 0.67km [18]	–	6.2
19. CCHB	Cam - Clayhithe bridge + 3.7km [19]	–	13.9
7. GRAN	Granta-Cam tributary [7]	10.9	21.5
9. CFAB	Bourn Brook tributary [9]	53.0	18.0
13. VICA	Vicar’s Brook tributary [13]	6.7	1.7

4.3.9 Figure 13 shows the same data in a graphical format.

Figure 13: Batch 2 ratios of the counts of total coliforms to *E. coli* (after excluding *E. coli* from the total coliform count by subtracting counts of *E. coli* from the counts of total coliforms). Effluent counts converted to river counts.



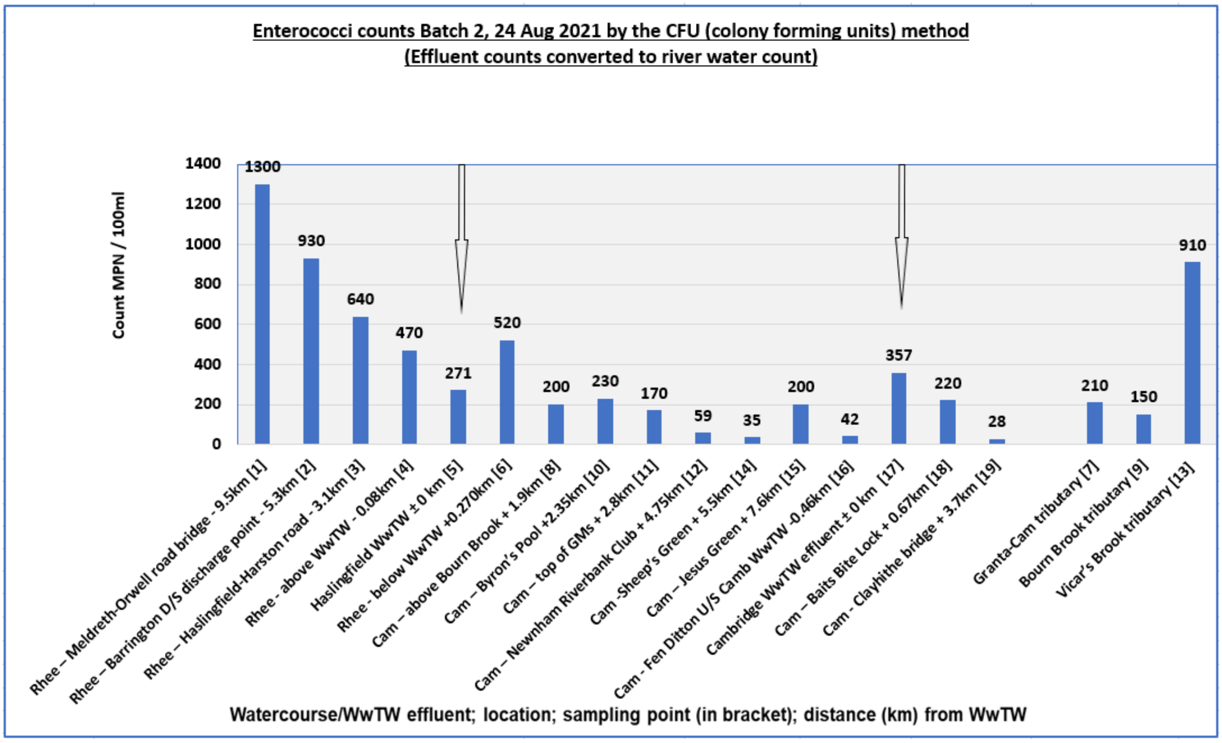
4.3.10 By comparing the ratios at all locations for Batch 2, a pattern becomes clear. Distances vary between sites, there is a clear increase in the ratio down to but not including the Haslingfield STW, a clear increase from the STW to the top of Grantchester Meadows, and a clear increase from the Cambridge STW to Clayhithe.

4.3.11 Interestingly, the ratio is the same at both STWs in Batch 2, after sewage treatment.

4.4 Results for intestinal enterococci

4.4.1 Figure 14 shows the baisc counts at all 19 sites, the effluent counts having been converted into river water counts.

Figure 14: Counts of enterococci of Batch 2 (24 August) / 100ml at all 19 sampling points (effluent counts converted to river water counts)



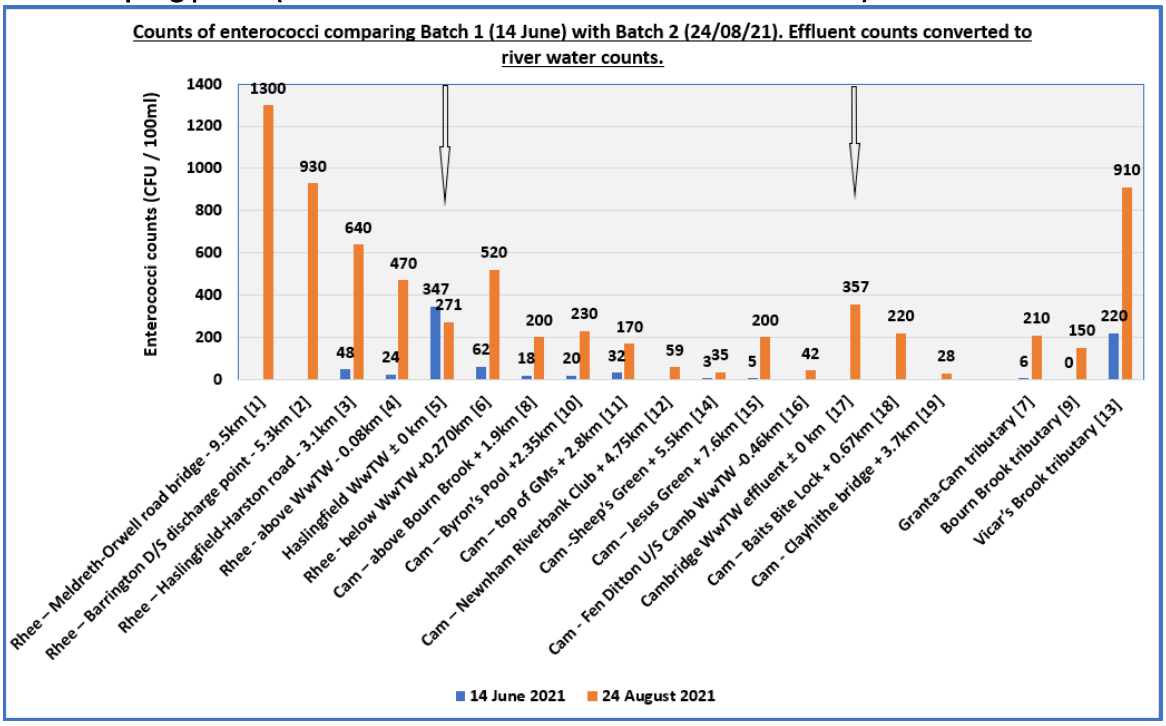
4.4.2 Enterococci counts were much higher than in Batch 1. A comparison of Batch 1 with Batch 2 is presented as Figure 15.

4.4.3 Counts follow a pattern of decline and rise broadly similar to counts of *E. coli*.

4.4.4 Counts in pure effluent in Batch 2 at Haslingfield were only 48% of Batch 1 counts. This contrasts with 100.1% for *E. coli* and 84% for total coliforms. The count in pure effluent at Milton was 29.6% of that at Haslingfield, whereas when these values were converted to river water counts, the count was now 132%, reflecting the differences in dilution.

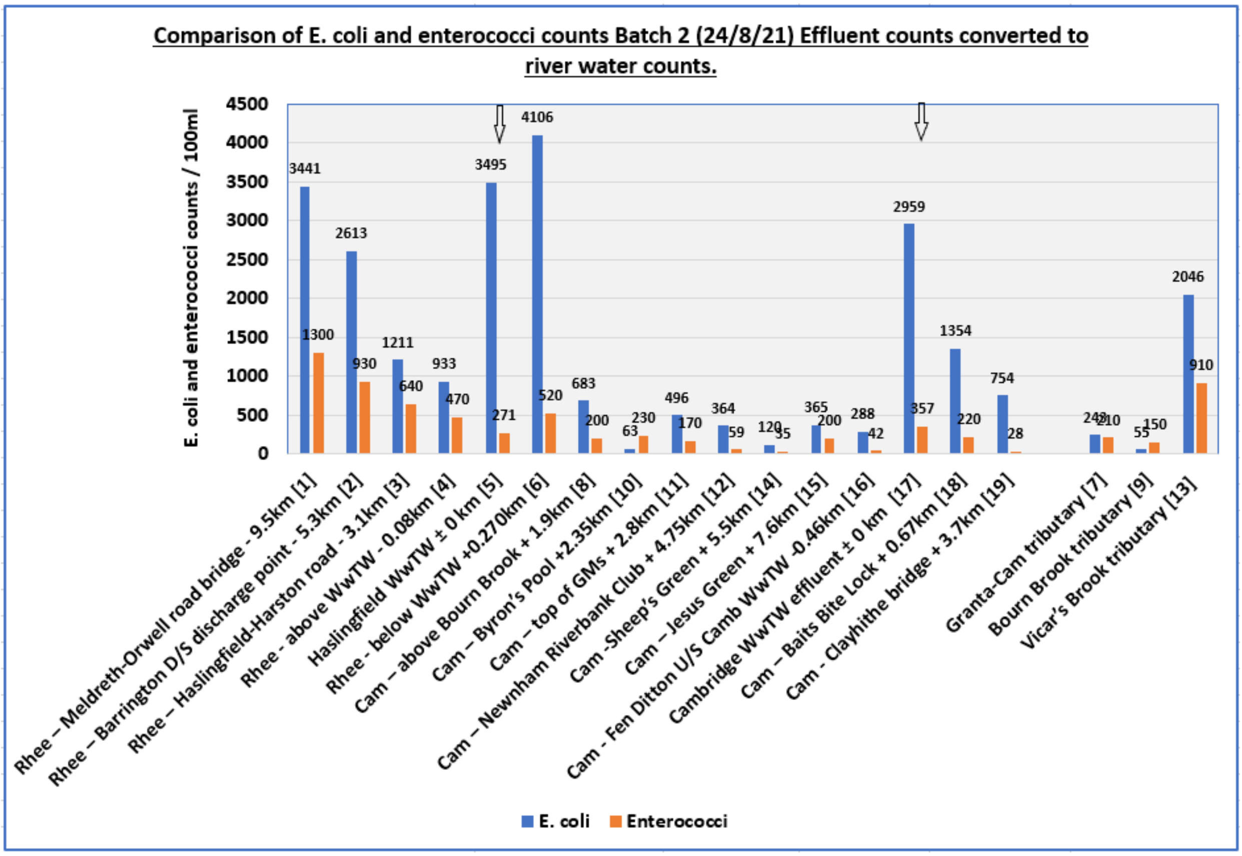
4.4.5 Several counts <u>on this date</u> did not reach the lowest EA standards of the current Directive for Bathing Waters (Figure 8) and also the directive used until 2006 which was superseded by the current directive.

Figure 15: Comparison of enterococci counts of Batch 2 with counts of Batch 1 / 100ml at all 19 sampling points (effluent counts converted to river water counts)



4.4.6 Figure 16 shows the relative values of *E. coli* and enterococci in Batch 2.

Figure16: Relationship between counts of *E. coli* and counts of enterococci – Batch 2 (24 August)



4.4.7 The ratio of counts of E.coli to counts of enterococci in pure effluent were:

- Haslingfield Batch 1 .... 16:1
- Haslingfield Batch 2 .... 13:1

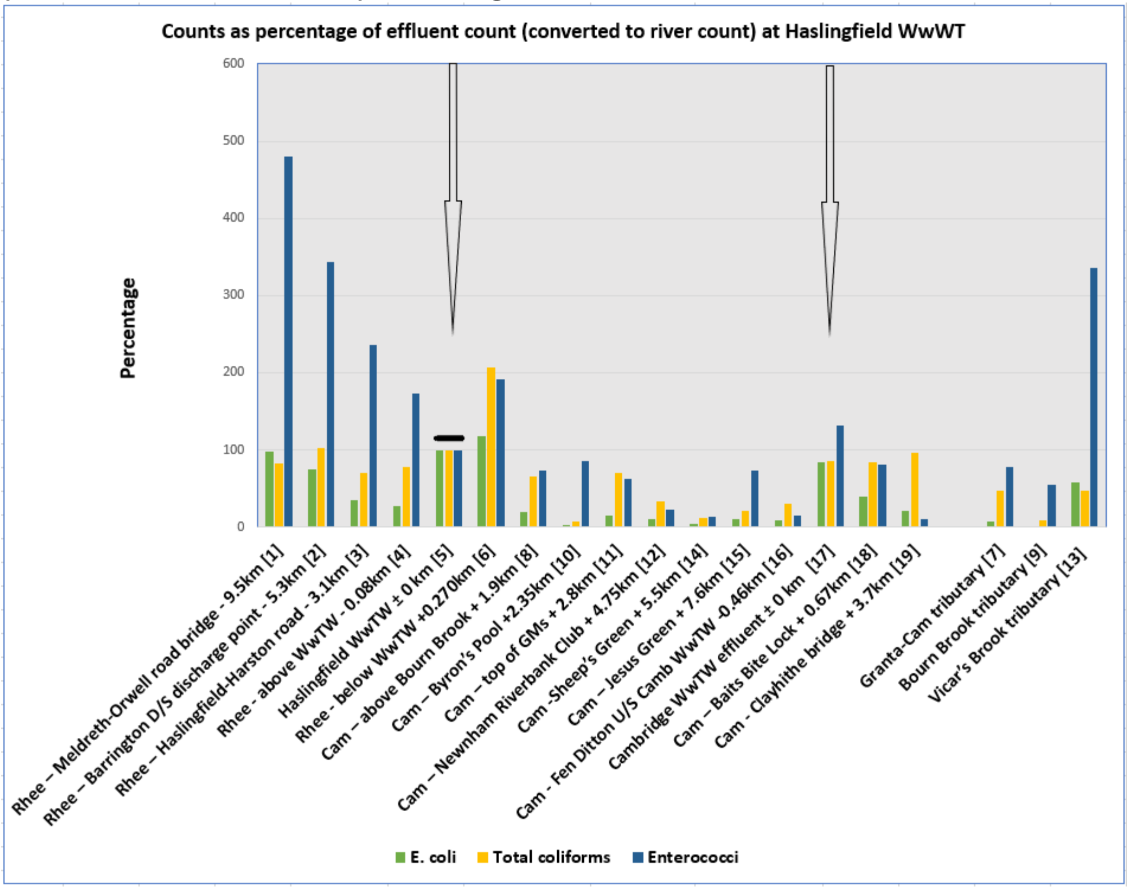
- Cambridge (Milton) Batch 2 only 8:1

4.4.7.1 At Haslingfield the ratio was broadly similar between the two batches whereas at Milton the ratio was approximately half of those. The reason for this is not clear; perhaps the treatment process at Milton affects enterococci less so than coliforms.

4.4.7.2 The ratios varied more considerably at some other sites, in river samples. At site [6] just before Haslingfield STW it has dropped to 8:1. At Vicar's Brook it is only 2.2:1. At Byron's Pool it is 0.27:1. The differences in ratios may have some intrinsic value to better understand how the two different types of bacteria behave in the river environment after release from a faecal source.

4.4.7.3. A pictorial view of the relative differences in levels of *E. coli*, enterococci and total coliforms over the 19 sites in Batch 2 was constructed (Figure 17), by setting the counts of these groups each to be 100 in the Haslingfield STW effluent converted to a river count. Presented in this format, all three bacterial types can be charted together despite their having vastly differing ranges of counts.

Figure 17: Counts of all three bacterial types as a percentage of the effluent count (converted to a river count) at Haslingfield STW. Effluent river counts = 100%



4.4.7.4 This shows clearly any difference between sites for between bacterial types. For instance, the enterococci value at Byron's Pool [10] is close to the effluent river value whereas at the same site *E. coli* is very low compared to the effluent. Another example is the lack of marked differences in values of total coliforms from sites [1] to [4] over 9.5 km, whereas at these same sites *E. coli* and enterococci seems to behave very differently (but similarly to each other), albeit with the values of *E. coli* counts all below 100% of the effluent, and of enterococci in a much higher range.

5. Phosphate and Nitrate results – Batch 2

5.1 The limited monitoring is not sufficient to provide a robust phosphate status for stretches of river within our sampling area, nor should we attempt to do this. When phosphate status based on the results of CVF's testing is mentioned within this report, it is done in an informal manner and should not be confused with official classifications (reference[3]).

5.2 Laboratory analyses of phosphate and nitrate are reported as concentrations in mg/l of soluble reactive phosphorus (i.e. phosphorus in orthophosphate), and nitrate (not nitrogen in nitrate). These terms are used throughout.

5.3 In the Water Frame Directive (Table 6), rivers are classified for phosphate status according to the bands of phosphate-phosphorus.

Table 6: Water Frame Directive standards for phosphate-phosphorus in lowland (<80m AOD – above Ordnance Datum), high-alkalinity rivers)

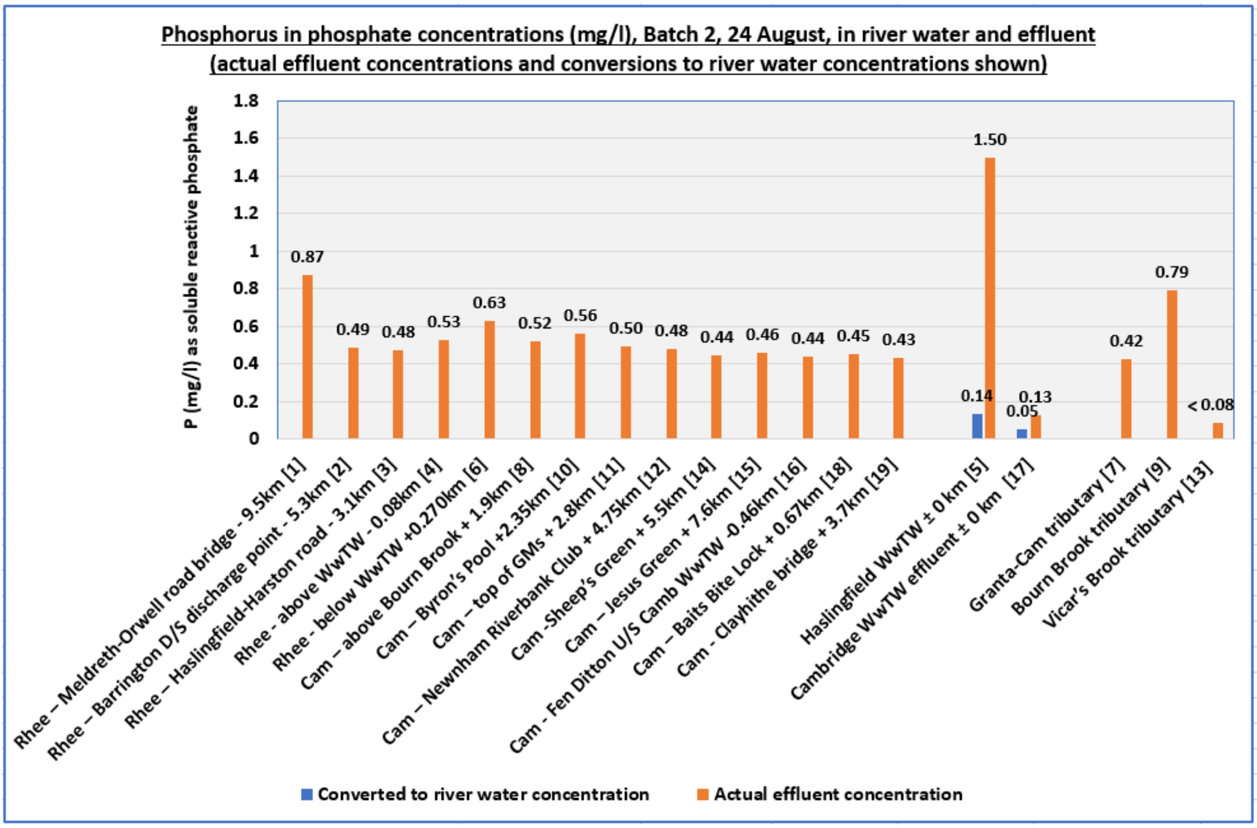
	Status				
	High	**Good**	**Moderate**	**Poor**	**Bad**
Bands, P (ppm)	0.00 - 0.05	0.051- 0.089	0.090 - 0.211	0.212- 1.089	> 1.089

5.4 **Phosphate results**

5.4.1 In Batch 2, samples from all 19 sites were analysed for phosphate, and in addition total phosphorus was analysed in the two samples of pure effluent. Figure 18 shows the results, sites being grouped as main river sites, effluents, and tributaries.

[3] Draft river basin management plan: maps (arcgis.com)

Figure 18: Phosphorus (mg/l) as soluble phosphate at all sites – Batch 2 (24 August)



5.4.2 The phosphate–P concentration in Haslingfield STW's pure effluent was the highest, at 1.5 mg/l, nearly three times the concentration in Batch 1, and markedly higher than in the Cambridge STW effluent. The time of sampling in the day, and conditions on the two sampling dates should not create such differences. At Haslingfield, the concentration of total phosphorus in the effluent was 1.78 mg/l, and in the Cambridge STW effluent was 0.26 mg/l, also markedly different.

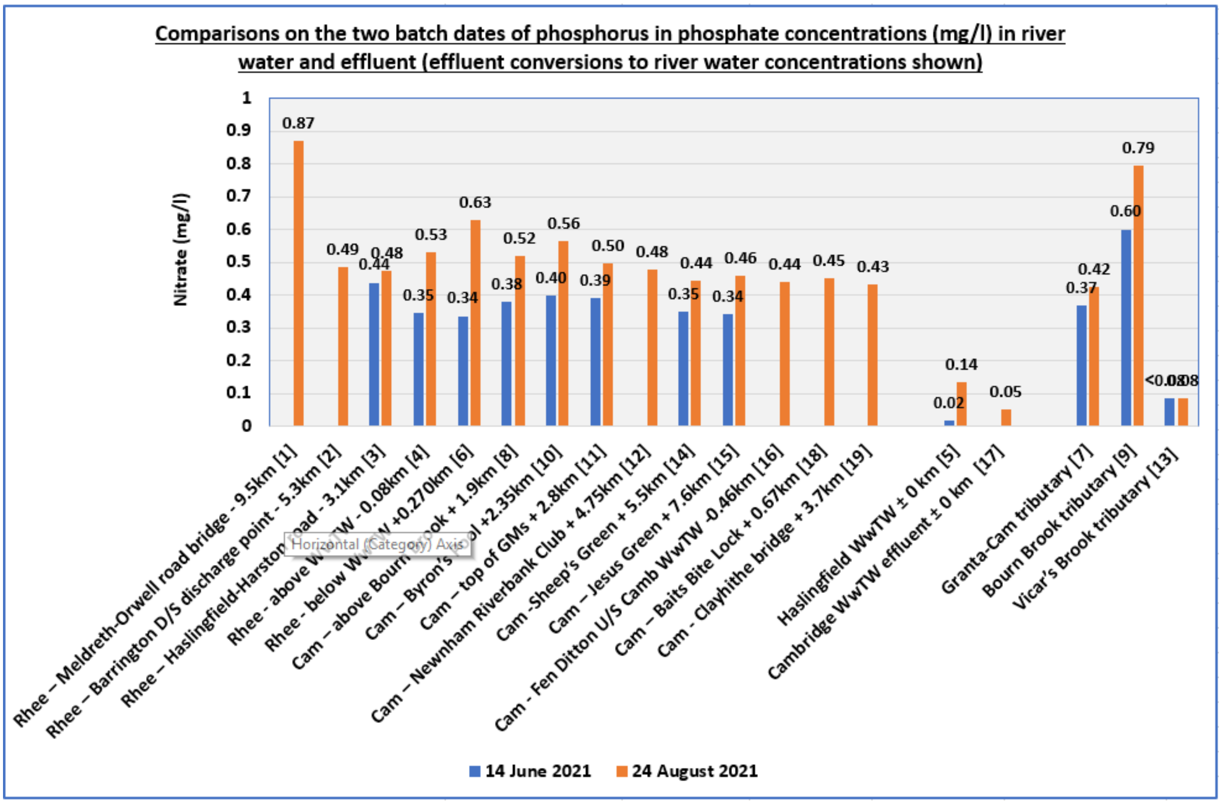
5.4.3 Concentrations in effluent converted into river water values were considerably lower than those samples taken directly from river water. At Haslingfield STW, the diluted effluent concentration was 0.14 P mg/l (the blue bar in the chart above, at site [5]).

5.4.4 In the run from Barrington downstream, to Clayhithe, the chart shows relative constancy of phosphate in the river, the highest value being 0.63 mg/l, measured at the site 270 m downstream of the Haslingfield STW outfall. The figures show that Haslingfield effluent contributes sufficient phosphate to raise the river concentrations by a measurable amount – from 0.53 P mg/l at site [4] immediately above the works. In contrast the contribution from Cambridge STW is much smaller.

5.4.5 Two sites stand apart from the general broadly similar and higher concentrations – site [1] near Meldreth, and Bourn Brook. The concentration in Vicar's Brook, a chalk stream, was below the limit of detection (<0.084 mg/l).

5.4.6 Comparisons of the batches of river samples show a general increase in concentrations between the two dates (Figure 19). At the 10 river sites where comparisons can be made, the mean increase was 35%.

Figure 19: Phosphate-P concentrations Batch 2 (24 August) compared with Batch 1 (14 June), at all sites.



*

5.4.7 In Batch 1 at Haslingfield STW, the higher flow and the lower concentration of phosphate in the effluent led to a river water concentration of only 0.02 mg/l P. However, these additions to the river would be continuous at all STWs upstream of Cambridge. As soluble phosphate is likely to travel long distances despite a part being utilised by plant life or becoming bound up, many STWs are implicated. Runoff of soil from fields, ditches, slurries etc into rivers will also be contributing varying amounts of phosphorus.

5.4.8 As part of a small monitoring project using a Hanna HI-713 hand held phosphate checker, Mike Foley recently started testing effluent at some STWs and the watercourses into which they discharge. Bassingbourn STW's effluent was analysed on 27th October, and the results (Figure 20) show a clear-cut increase in phosphate concentration in a branch of the Wellhead Spring chalk stream (also known as Bassingbourn Brook). The phosphate status of the stream has been reduced from 'high' to 'poor'.

5.4.9 Litlington STW's effluent was analysed on 7th October, and apparently had high levels. In future work, confirmatory laboratory analyses should be undertaken where the concentrations seem to be very high.

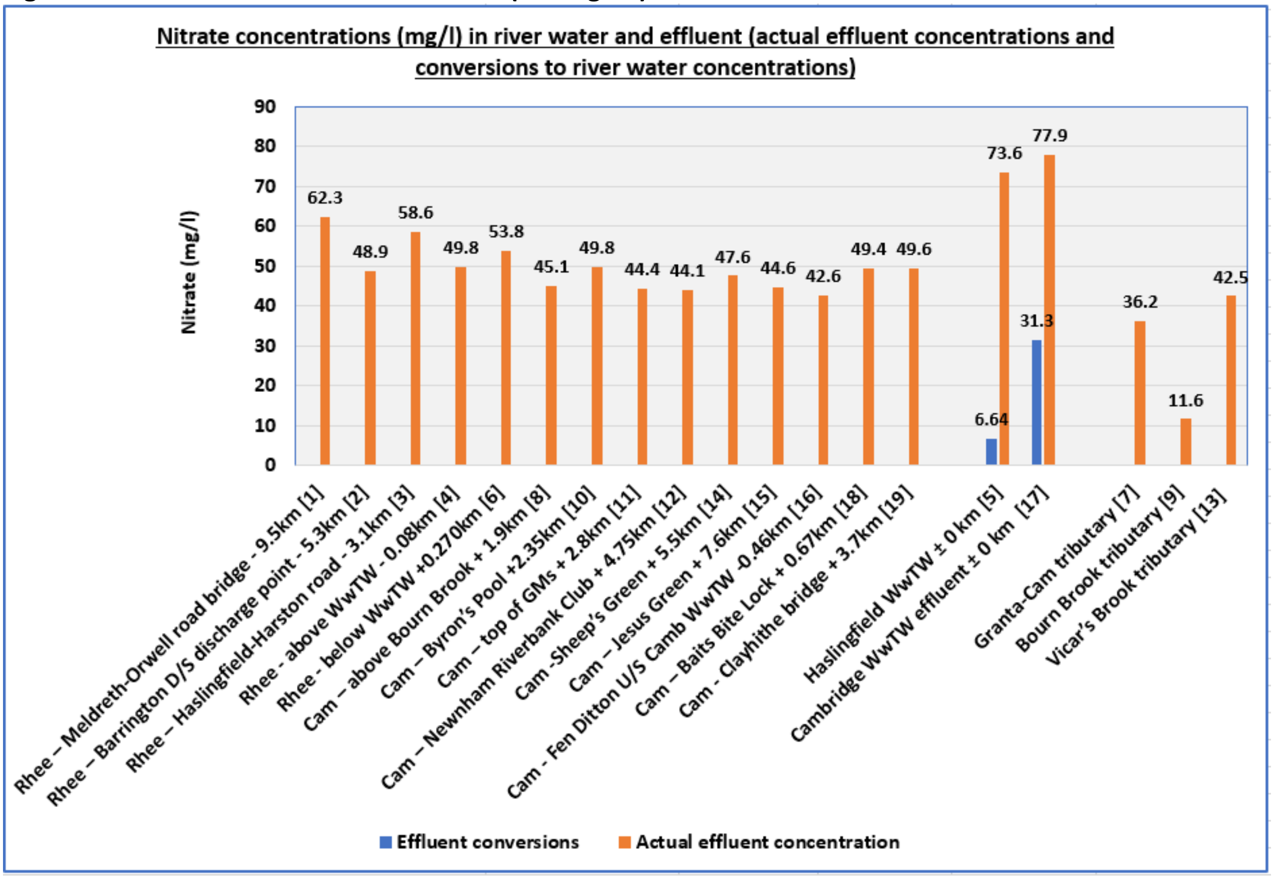
Figure 20 Phosphate-phosphorus concentrations in the effluent from Bassingbourn STW, and in the Wellhead Spring chalk stream in single samples taken on 27th October 2021 and analysed using a Hanna HI-713.

5.5 **Nitrate results**

5.5.1 Nitrate analyses from all Batch 2 sites revealed high concentrations, both effluents having the highest by some margin. The third highest value was at site [1], which also had the highest phosphate value. The lowest value was found in the sample from Bourn Brook.

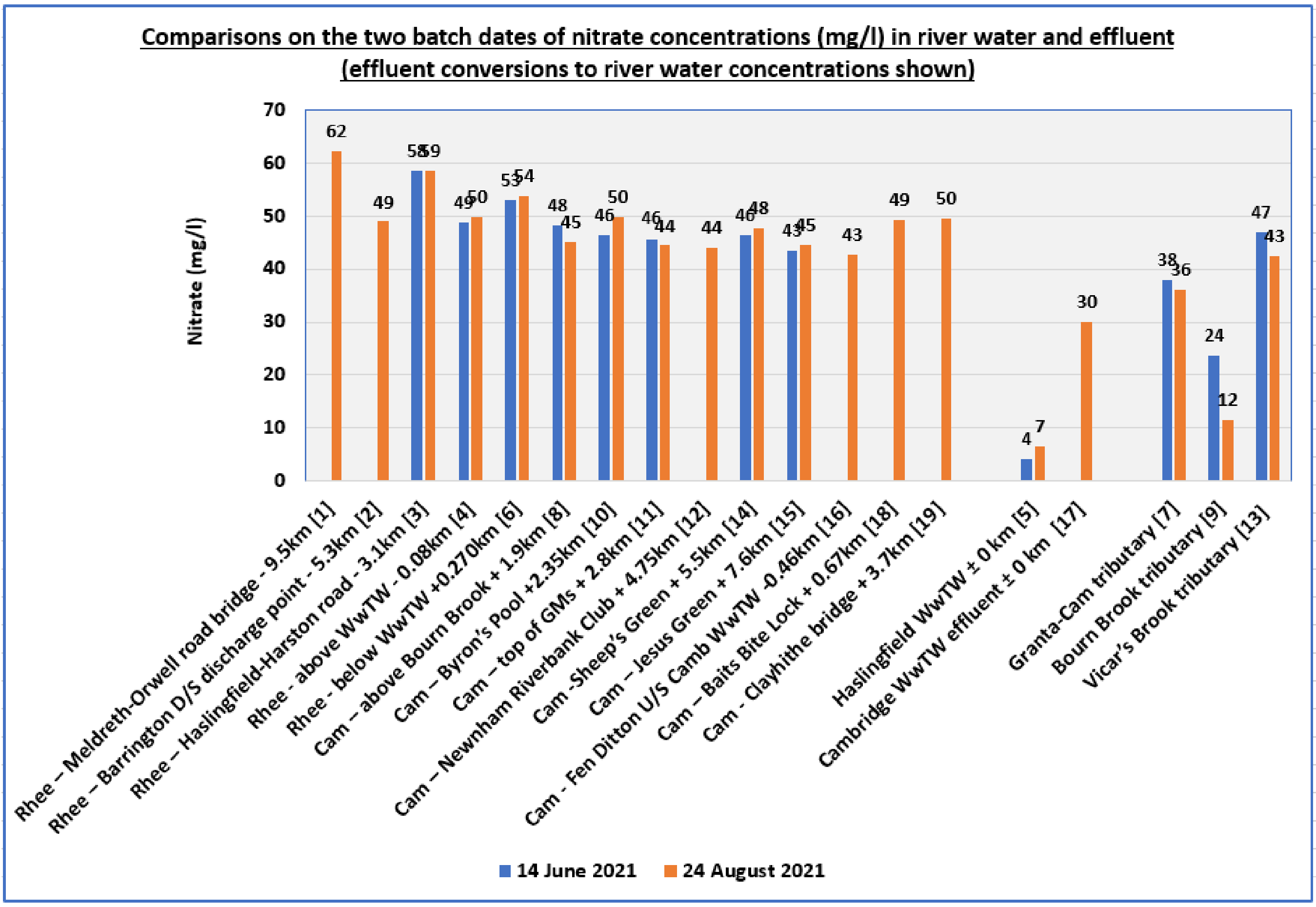
5.5.2 As previously reported, much of the aquifer at Ashwell, which supplies the Rhee at source, is known to have high levels of nitrate. The somewhat lower level in the Granta-Cam reflects the often lower, but variable, nitrate concentrations measured by the Environment Agency in samples at sites on the Granta such as Linton.

Figure 21: Nitrate concentrations Batch 2 (24 August) at all sites



5.5.3 In Figure 22, nitrate concentrations for both batches are compared at each site. In contrast to phosphate, values were not consistently higher, several sites having similar values with no overall trend towards either higher or lower values in Batch 2. The Bourn Brook value was noticeably lower at the second sampling date, and the lowest of all river samples.

Figure 22 Nitrate concentrations Batch 2 (24 August) compared with Batch 1 (14 June), at all sites.



6. Water turbidity

6.1 Observations continue to be made on turbidity levels of the Rhee at scattered points in the upper Rhee, having firmly established it can be highly turbid at Cambridge. It was found to be turbid at least as far upstream as Northfield Road bridge near Guilden Morden. The Rhee has yet to be monitored between this point and Ashwell End, where the water was clear in early September and mid October and the bed has exposed gravel. Undoubtedly there is more suspended soil in the river water after heavy rain which colours the water, yet the Rhee is turbid during prolonged periods of dry weather.

6.2 We recognise that depth of water, light reflection, bed composition, benthic plants and other factors can affect the appearance of the water, and perceptions can be distorted. We think that these have been considered, and that the Rhee has a turbidity problem.

Clear water Ashwell End (52.049234, -0.168970). 7th September 2021

Rhee – markedly turbid water – Potton Road bridge, Guilden Morden 52.093912, -0.146384. 7th September 2021

Rhee – Northfield Road bridge, upstream from the previous location, 52.079844, -0.160220

6.3 The Rhee continues to be murky at many points for much of the time, e.g., near Haslingfield – 24 August 2021 (sampling site [4]).

7 Further monitoring

7.1 Vicar's Brook needed more samples to be taken, some further upstream from Coe Fen and beyond Trumpington Road. If necessary, samples can be taken at the balancing ponds associated with Hobson's Park, and outfalls from Addenbrookes Hospital. We are liaising with Hobson's Conduit Trust.

7.2 For the next batch of samples, sites must need to be further expanded in number to include stretches even further upstream.

7.3 Samples need to be taken at all relevant STWs so that counts in pure effluent can be added to the database.

7.4 Having sampled twice during benign and dry conditions, another batch of sampling needs to be undertaken both when autumnal / early winter weather creates generally wetter conditions, when ditches are filling and soils are approaching saturation. It is vital that another batch of samples is taken during or shortly after a period of heavy rainfall when Combined Sewer Overflows / storm discharges are in operation.

7.5 DelAgua dipslides can quantify abundance of total coliforms in samples to different orders of magnitude may become part of our testing procedure. Using this method, although not as accurate as laboratory testing, we will be able to find sites with high counts, and if necessary these sites can be further investigated.

7.6 We probably have sufficient data on nitrate concentrations in river water. Phosphate monitoring needs to continue.

8. Amendments (8 September 2021) to Report No. 1 after issue on 24 August.

5.2.1 The vertical axis of the *E. coli* chart was originally titled $\log_{10}$ scale of *E. coli* counts. This has been changed to counts on a non-log scale.

5.2.2 The amendment in this paragraph is emboldened: The calculated count of *E. coli* in the river after effluent was mixed with river water forms an important part of the overall picture. Counts at eight sites along the river can be compared between each other but valuable information is gained by having a river water count from the STW's discharge. The count of *E. coli* immediately above the STW at point [2] was 9.4% of the effluent 'river count' at the STW and was 15% higher further upstream at point [1]. **Both counts above the STW were higher than at point [6] which is 1.9km below the STW, suggesting that there is either a moderate source close to and above point [1], or a much more potent source further upstream.** Potential sources of *E. coli* further upstream need to be investigated.

5.4.3 Figure 12: *E. coli* count was 2167 MPN / 100ml, not 1733. The count was first calculated using a lower estimated effluent flow before Anglian Water provided a more accurate figure.