A comparative study of the elimination rates of *E. coli* and F+ bacteriophage in native oysters following a sewage spill event in Langstone Harbour, United Kingdom

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Introduction

The main agents of illness in Europe are viral and are principally Hepatitis A Virus (HAV) and Norovirus with the latter predominating in Northern Europe (Lees 2000). In the European Union, assessment of the risk of contamination of bivalve shellfish harvesting areas by sewage-derived pathogens is undertaken by classification on the basis of monitoring for *Escherichia coli* in samples of shellfish. At the time that this study was undertaken, the requirements were derived from the 'Shellfish Hygiene Directive' 91/492 EEC and were implemented in England and Wales by the Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998. The classification categories are given in Table 1.

Classification category	*E. coli* / 100g Flesh	Comment
A	Less than 230	Suitable for consumption without processing.
B	Less than 4600 in 90% of samples	Depuration or relaying in a category A area or cooking by an approved method.
C	Less than 46000	Relaying (minimum of 2 months) in category A or B area needed or cooking by an approved method.
Prohibited	Above 46000	Cannot be placed on the market.

Table 1. Criteria for the classification of bivalve molluscan shellfish harvesting areas (Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998).

It is recognized that there are certain limitations in the use of *E. coli* as an indicator, particularly in reflecting the viral content of shellfish on a single sample basis (Lee 2000). Brion *et al* (2005) have shown that F+ bacteriophage can be a better indicator of the possible presence of Norovirus in shellfish.

The study described here was carried out following a sewage spill in a natural harbour (Langstone Harbour) on the south coast of England following a period of heavy rainfall. The area contains a flat oyster (*Ostrea edulis*) fishery harvested by dredging.

The sewage discharge arrangements and routine shellfish monitoring point locations in Langstone Harbour are shown in Figure 1a. Figure 1b illustrates the density of population in the adjacent area and thus the faecal loading potential of the Harbour from the local sewage discharges. The short sea outfall (SSO) at the mouth of the Harbour discharges 6mm screened storm sewage effluent when the sewage treatment works is operating at full capacity and the storm tanks are full. Given the SSO is located in the mouth of the harbour, the actual impact of each episode will depend on the flow of the tide.

Materials and methods

The samples were collected and transported to the Cefas Weymouth Laboratory in accordance with a standard sampling protocol (Cefas 2006).

Microbiological testing Shellfish flesh testing for *E. coli* was carried out using a standard 5 tube by 3 dilution Most Probable Number (MPN) method used for official control testing of shellfish in the UK (Donovan et al 1998).

Testing for F+ bacteriophage in shellfish was carried out according to ISO 10705-1 with the addition of a sample preparation step specific for shellfish (Cefas 2003).

Other data Rainfall data was obtained from the UK Environment Agency from a raingauge situated in the catchment for the shellfishery.

Results

The first notification of a problem was received on 28th November 2003 following high results from routine classification monitoring undertaken by the Local Enforcement Authority (LEA) of the class B oysters in Langstone Harbour on 24th November (results shown in Table 2). It was arranged with the LEA that they should undertake some investigatory sampling for *E. coli* and F+ bacteriophage to enable some assessment to be made of the relative viral loading. No Norovirus analysis was carried out due to resource constraints and so no direct linkage with pathogen content is possible in this case.

Site	*E. coli*/100g
North end main channel (A)	54000
North End Broom Channel (B)	17000
Broom Channel Salterns (C)	17000
Sinah Lake (D)	24000

Table 2. Routine shellfish monitoring results of 24 November 2003.

Subsequent investigations by the Environment Agency showed that sustained heavy rainfall throughout November had caused an exceptional number of discharges from the Eastney short sea outfall (SSO) at the mouth of Langstone Harbour (see Table 3).

Date	Duration of spill	Rainfall (mm)
22 November	2 hrs 35 mins	14.7
23 November	11 hrs 35 mins	7.5
26 November	8 hrs 20 mins	19
30 November	4 hrs 40 mins	13.8

Table 3. SSO spill and rainfall data.

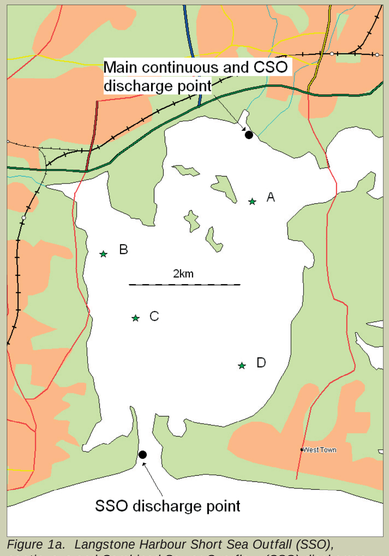

Figure 1a. Langstone Harbour Short Sea Outfall (SSO), continuous and Combined Sewer Overflow (CSO) discharges and monitoring point locations A, B, C, and D

a) Oyster Ostrea edulis
b) Oysters Ostrea edulis

Figure 1b. Langstone Harbour Short Sea Outfall (SSO) aerial image illustrating density of population (and thus faecal loading) in the area.

Bed name	Date	F+ bacteriophage	WEMS *E.coli*
N End Main channel (A)	03/12/2003	50000	13000
Broom Channel (B)	03/12/2003	72000	4300
Salterns (C)	03/12/2003	48000	5000
Sinah (D)	03/12/2003	102000	1300
N End Main channel	10/12/2003	20000	750
Broom Channel	10/12/2003	38000	500
Salterns	10/12/2003	28000	310
Sinah	10/12/2003	29000	220
N End Main channel	15/12/2003	7200	500
Broom Channel	15/12/2003	7800	750
Salterns	15/12/2003	2100	220
Sinah	15/12/2003	6200	1400

Table 4. E. coli and F+ bacteriophage shellfish results of investigative samples taken on 3, 10 and 15 December

The spatial and temporal variation characteristics of the two indicators differ markedly (see figures 2 & 3 and Table 4). For example, *E. coli* results would suggest that point A was the most contaminated initially, whereas the F+ bacteriophage results would suggest point D was the most contaminated (note, result for point D fell below the 4600 *E.coli*/100g class B threshold).

Enforcement action

Following receipt of the high results and after obtaining details of the emergency discharges, a Temporary Prohibition Order (TPO) was issued for the whole Harbour on the 28th November, prohibiting the collection of any live shellfish from that area.

The TPO was lifted and the beds reopened on 17th December following results at all points falling below the 4600 *E. coli*/100g class B threshold accompanied by a distinct decline in the levels of F+ bacteriophage.

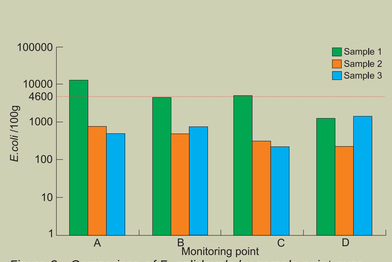

Figure 2. Comparison of E. coli *levels by sample point over the three samples (sample 1, 2 and 3) taken on 3, 10 and 15 December 2003 - statutory class B threshold of 4600* E. coli/ *100g marked as red line.*

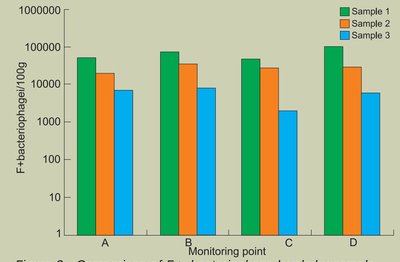

Figure 3. Comparison of F+ bacteriophage levels by sample point over the three samples on 3, 10 and 15 December 2003.

Discussion and conclusion

Norovirus is the commonest bivalve-associated viral pathogen, however, the testing methodology for Norovirus in shellfish is still being developed. F+ bacteriophage testing on the other hand is much simpler and cheaper. Studies carried out here at Cefas Weymouth looking at outbreak samples and samples after purification (Dore *et al* 1998) have shown that F+ bacteriophage is a better indicator of possible Norovirus presence than *E. coli* in these situations. A sewage spill and the time following such an event when cleaner water conditions should return could be likened to a purification tank scenario.

It is assumed that there would have been an increased risk of Norovirus presence where the F+ bacteriophage content of the shellfish was at elevated levels.

E. coli levels were back within the class B range at 2 of the 4 sites after only 4 days following the cessation of spills (on 30th November) whilst F+ bacteriophage levels were still elevated (although decreasing) after 16 days at all sites compared with what would be considered typical for class B sites in England and Wales.

Based on the results of this study and the findings from other workers demonstrating the value of F+ bacteriophage as an indicator of Norovirus presence in shellfish, we would suggest that the use of F+ bacteriophage would be a more useful indicator than *E. coli* in deciding when shellfisheries should be reopened following a sewage spill.

If F+ bacteriophage is to be used in this way it would be advisable to carry out monitoring to determine 'normal' background levels on a site-specific basis for each area.

Acknowledgements
The authors would like to thank Portsmouth Port Health Authority for their help in providing samples for this study.

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