FACTORS INFLUENCING FAECAL CONTAMINATION OF SHELLFISHERIES AS DETERMINED BY STATISTICAL ANALYSIS OF FAECAL INDICATOR CONCENTRATIONS

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Introduction

Bivalve molluscs being filter feeders may concentrate contaminants found in water, including pathogenic bacteria and viruses. The public health risks associated with the consumption of contaminated shellfish are well documented (Rippey, 1994)[1]. In the European Union controls to protect public health from such risks are exerted under the Shellfish Hygiene Directive 91/492/EEC (European Communities, 1991)[2]. These controls include the classification of shellfish harvesting areas according to the degree of faecal contamination found in shellfish flesh, as indicated by faecal coliforms and/or *Eschericha coli*. The classification determines the extent of any post harvesting processing that may be required, in extreme cases harvesting may be prohibited. Some of the hydrographical and environmental factors that influence the contamination of shellfisheries are examined. Additionally, comparisons are made between the concentrations of Male-specific RNA bacteriophage* (FRNA bacteriophage) and *E. coli** from paired samples taken from a limited number of sites. FRNA bacteriophage has been put forward, putatively, as a replacement faecal indicator for *E. coli*. The results are discussed in relation to how such contamination may be reduced.

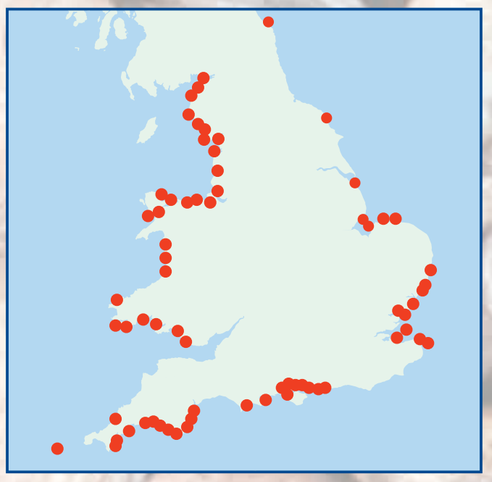

Figure 1. Shellfisheries included in study

Data

Under the Shellfish Hygiene Directive, 90 commercial shellfish harvesting areas have been sampled using more than 350 monitoring points during the period 1992 to the present. The Centre for Environment, Fisheries & Aquaculture Science (CEFAS) maintains a database of microbiological data from these shellfisheries. Information held on the database includes counts of *E. coli*, and environmental data recorded at the time of sampling, which was supplemented by data from the Met. Office. A separate database of paired *E. coli* and FRNA bacteriophage determined in shellfish flesh from a subset of the classified areas, was also available.

Site selection

A subset of data from the classified microbiological database was extracted using shellfish species+ and a minimum of 20 samples per species per monitoring point as criteria; resulting in 79 shellfisheries with 156 associated monitoring points being selected (see Figure 1). Figure 2 illustrates the use of trestles in shellfish cultivation, a method used in some of the shellfisheries included in this study. Four sites with paired FRNA bacteriophage-*E. coli* samples were selected (sites 1 - 4). Consideration of varying tidal effects, average annual rainfall and other environmental influences together with geographical factors, resulted in the selection of three sites (A, B and C) where the influence of these factors could be analysed.

Figure 2. Shellfish farming operation

Selected shellfisheries

Descriptive statistics for each of the 159 points from the selected shellfish harvesting areas were calculated, using Excel[3] and Minitab[4]. Mean $\log_{10}$ *E. coli* counts were calculated for each point (the antilogarithm of this figure is equivalent to the geometric mean). Figure 3[5] illustrates the spread of mean $\log_{10}$ *E. coli* levels found at these points, which range from 1.43 logs to 3.90 logs (equivalent to geometric means of c. 30 and c. 7,900 respectively).

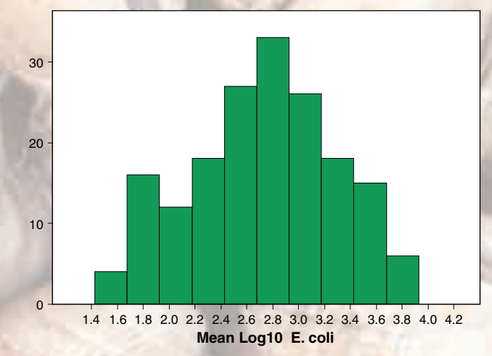

Figure 3. Distribution of mean $\log_{10}$ E. coli counts of monitoring points from the selected sites

Male-specific RNA bacteriophage - E. coli: comparison of paired samples

Mann-Whitney tests were used to test the equality of the medians of each set of paired samples (the data were found to be non-normal). Tests were conducted using a 95% confidence level and adjusted for ties. Each set of paired samples from Sites 2, 3 and 4 were found to have significantly different medians, this was not true of the paired samples from Site 1. Figure 4[5] illustrates the differences in concentrations of FRNA bacteriophage and *E. coli* from Site 3. The plots divide the frequency of the $\log_{10}$ counts into four equal parts. The "T" bars represent the maximum and minimum values. The top of the box indicates the start of the top quarter of results, the base of the box the end of the bottom quarter and the line through the box the median value. Although, the spread of *E. coli* counts was greater than that observed for FRNA bacteriophage, the median value for the latter was almost a logarithm higher. At site 1 both the median value and the distribution of each indicator were almost identical (results not shown).

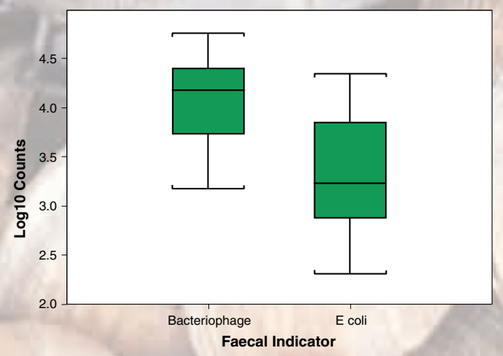

Figure 4. Comparison of paired FRNA bacteriophage and E. coli samples from Site 3

Foot notes

* Figures given for shellfish contamination are expressed in the following units: for *E. coli* counts per 100g of shellfish flesh and for FRNA bacteriophage the number of plaque forming units (pfu) per 100g of shellfish flesh.

\+ Species included in the study were, mussels, clams, native oysters and Pacific oysters.

References

1 Rippey, S R (1994), Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Rev.*, **7**, 419-425.

2 European Communities (1991). Council Directive of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve molluscs (91/492/EEC). Official Journal of the European Communties L 268, 24.9.91 : 1-14

3 Statistical analysis conducted using Excel, Microsoft Corporation, Redmond, WA, USA.

4 Statistical analysis conducted using Minitab, Minitab Inc., State College, PA, USA.

5 Graphs were produced using S-Plus, MathSoft Inc., Seattle, WA, USA.

Influence of environmental and other factors

Environmental and microbiological data were extracted and collated for Sites A, B and C. The assumption was made that there was no association between levels of *E. coli* contamination and three environmental parameters (total rainfall for the seven days preceding the date of sampling; water temperature at time of sampling and seasonal effects). This assumption was tested using Spearman's rank correlation test[4]; the results are detailed in Table 1. The pattern of association between the chosen parameters and recorded *E. coli* varied across the sites. The marked seasonal differences between sites A and C is illustrated in Figure 5[5]. Such differences were not observed at site B. However, the positive association between 7-day rainfall totals and associated *E. coli* levels for this site increases with distance from the estuary mouth. This marked variation in association between this parameter and *E. coli* levels was not observed at the other sites. At site A, where all the monitoring points are located near the centre of a large shallow harbour, the variation in association between 7-day rainfall totals and *E. coli* counts was less than 0.05. At site C, no significant association was observed, this site is located in relatively deep water that is subject to strong tidal streams. The association between *E. coli* concentration and water temperature also showed marked differences between sites. No significant association was found at site B. At the other sites significant associations were detected, however their magnitude differed and the association was positive in one instance and negative in the other.

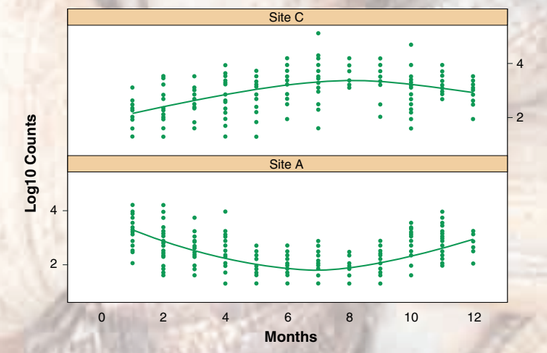

Figure 5. Comparison of shellfish E. coli concentrations between Sites A and C on a monthly basis

Table 1. Association of environmental factors with E. coli *contamination of shellfish sampled from Sites A, B and C++*

Parameters	Study Sites Site A	Site B	Site C
Total rainfall for the 7 days prior to sampling	0.42**	0.39**	-0.04
Water temperature at time of sampling	-0.53**	0.09	0.33**
Season	-0.28**	0.17**	0.26**

++ Data from the monitoring points at each site were aggregated ** p<0.01

Discussion

All three data sets showed marked variations. Levels of contamination in the points from the selected shellfisheries differed by c. 2.5 logarithms, indicating significant inter-site variation. Site-specific differences were also observed for the paired FRNA bacteriophage - *E. coli* samples, sites 2, 3 and 4 showed differences in both levels and variance of contamination between the two indicators, a result not found at site 1. This suggests that the relationship between these indicators may be determined, in part, by inter-site differences.

The association between levels of contamination and environmental factors also varied significantly between shellfisheries. The association between rainfall and contamination levels may be due to spill events from over loaded sewerage systems and/or run-off. The positive association between rainfall and *E. coli* counts observed at sites A and B suggest that such effects operate at these sites. The lack of any association between rainfall and *E. coli* levels at site C may be due to the fact that the nearest river/estuary mouth to this site is c. 4 kilometres to the west and to the absence of any sewer overflows in its vicinity.

The marked seasonal differences between sites A and C, a maximum difference of c. 1.5 logarithms, during the summer months is significant. This is approximately equal to the logarithmic reduction in *E. coli* concentrations that result from secondary sewage treatment.

Despite the relationship between the three chosen environmental parameters (both rainfall and water temperatures vary seasonally), the results reported here indicate that the effect of each variable differs from shellfishery to shellfishery.

Water quality models and other techniques and methods currently used to estimate contamination levels in coastal and estuarine waters take little or no account of seasonal and other factors. The effect of rainfall in many water quality models is often omitted. The results presented here suggest that environmental and other factors can have a significant effect on observed levels of contamination and ought to be taken into account when planning and implementing measures to reduce contamination.

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