Environmental factors influencing the microbiological contamination of commercially harvested shellfish

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Abstract Filter-feeding bivalve molluscs (such as oysters, clams, mussels and cockles) can concentrate contaminants from the water column. The extent of faecal contamination in shellfish is usually estimated by determining the concentration of faecal coliforms and/or *Escherichia coli*. Three sample points in each of three geographically separate commercial shellfisheries were selected for analysis for the effect of season, spring/neap and high/low tidal cycles, rainfall and wind direction on the results of routine *E. coli* monitoring. General linear modelling was used for the analyses. The principle factors affecting the contamination of shellfisheries were season, high/low tidal cycle and rainfall. The effects varied between harvesting areas and between individual sampling points within harvesting areas. Undertaking such analyses for all harvesting areas would contribute to the management of monitoring programmes and assist in the evaluation of potentially contaminating sources, such as sewage discharges. The type of analyses undertaken on *E. coli* monitoring data would also be pertinent for the analysis of putative viral indicators, such as F+ coliphage, and could be extended to data on bacterial and viral pathogens.

Keywords *Escherichia coli*; rainfall; season; shellfish; tide

Introduction Illness associated with the consumption of bivalve molluscan shellfish has been known for hundreds of years and is still a widely recognised problem (Rippey, 1994). In the European Community, controls intended to address these problems are exerted under the Shellfish Hygiene Directive 91/492/EEC (EC, 1991) and these include classification of harvesting areas according to the degree of faecal contamination in shellfish flesh (as determined by faecal coliforms and/or *Escherichia coli*). Contamination of the estuarine/coastal environment arising from continuous sewage discharges may only produce intermittently detectable effects in shellfish monitoring programmes due to the interaction of tides, currents, wind and other variables on the discharge plume. A number of studies have looked at the effect of meteorological and environmental factors on water quality, principally with respect to bathing waters. Currents, influenced by tidal state and wind, have been determined as having a large impact (Smith *et al*., 1999). Some studies have also looked at the effect of a limited number of factors on the microbiological contamination of shellfisheries: these have shown significant effects of season, tidal state and rainfall (Brock *et al*., 1985; Wood, 1955). The present work was undertaken to investigate the significance of a range of environmental factors on the microbiological contamination of shellfish beds, as determined by *E. coli* concentration in the shellfish, and variation in such effects between and within different harvesting areas. The object was to identify whether such factors need to be taken into account when assessing the potential effects of proposed sewage discharges on commercial shellfisheries.

Materials and methods
Selection of study areas Three areas in England and Wales containing active commercial shellfisheries were selected on the basis of differing topographical and hydrographical characteristics. The quantity and
quality of microbiological and other data were also taken into account when selecting the
study areas. The shellfish present in all three areas were native oysters (*Ostrea edulis*), lay
below the low water mark and were normally harvested by dredging. Unless otherwise men-
tioned below, samples were collected by the same method. Area A consisted of a relatively
simple confined estuary containing a number of medium-sized and small sewage dis-
charges. Most of these discharges were upstream of the shellfish beds and none were known
to have been subject to improvements over the period of monitoring included in the study.
Area B consisted of a confined estuary with branching and impacted by a number of different
discharges. Only one of these was subject to significant change during the study period and
this had been assessed as not impacting on the shellfish monitoring points. Shellfish samples
for microbiological examination from this area were obtained from bagged populations kept
on the seabed. Area C consisted of part of a confined coastal location with complex tides and
currents. The main sewage discharge in the area was upgraded from crude to secondary
treatment in spring 1997 and sampling data was available both pre- and post-improvement.

**Data and data analysis**

Samples of shellfish were obtained from commercial shellfisheries on an approximately
monthly basis and *E. coli* concentrations were determined using a standard method (MAFF
*et al.*, 1992). The time series of results covered the period 1993–1999 inclusive for Areas A
and B and 1994–1999 inclusive for Area C. *E. coli* results were transformed by taking the
log10 of the original values. Tidal data for the times of sampling were obtained using
Tidecalc (Hydrographic Office, Taunton, England) using the nearest standard port for each
location. Meteorological data were obtained from the UK Meteorological Office
(Bracknell, England). Rainfall data were amalgamated to produce totals over the 2 days
before that of sampling. The predominant wind direction and speed over the 24 h prior to
sampling were determined by vector averaging of the hourly data. Data were converted into
categorical variables (factors) by assigning values to one of four groups (as shown on the x-
axes of Figures 1–4). General linear modelling and preparation of graphs were undertaken
using Minitab version 13 (Minitab Inc., State College, USA). Generalised linear modelling
was undertaken using Genstat for Windows, 5th edition (NAG Ltd, Oxford, UK).

**Results**

The main effects plots (which show mean log *E. coli* concentrations) for these factors are
shown in Figures 1–4 for areas A, B, C pre-improvements and C post-improvements respec-
tively. The associated accumulated probability values obtained from general linear modelling
are shown above each plot. Due to the unbalanced nature of the data set, with no values pres-
ent for some combinations of factors, it was not possible to undertake analysis of interactions.
Diagnostic plots showed lower variability of residuals at very low fitted values (log10 *E. coli*
≤1.6) for some of the sample points. This effect was reduced by eliminating the wind factors
or by using generalised linear modelling with a gamma distribution (data not shown).
However, the accumulated probabilities for the other factors were similar by all methods.

**Discussion**

The statistical analyses performed in this study showed that environmental factors may
have significantly affected the contamination of shellfisheries as indicated by the concen-
tration of *E. coli*. The factors that were shown to be of most significance were season, tide
(both spring/neoap and high/low cycles) and rainfall on the days prior to sampling. The
effects varied between the study areas and between sample points within individual areas.

The effect of season could be due to a number of influences acting independently or in
conjunction. These included (a) biological variation in the activity of the animals and (b)
variation in the sewage loading to discharges due to factors such as tourism or seasonal variation in rainfall patterns. A number of studies from the early 20th century identified that the \textit{E. coli} content of oysters was lower in the winter than in the summer and this effect was ascribed to a lower activity in the animals (Gorham, 1912; Smith, 1912).

The species present in Areas A, B and C was the same (native oysters) and a consistent pattern of low \textit{E. coli} concentrations in the winter was not observed. Different shellfish species may show a different response to environmental factors and therefore the different species in an area may need to be considered separately (Wood, 1955).

Increased contamination associated with heavy rainfall could arise from the operation of storm overflows in the estuary/coastal area or above the tidal limit and/or run-off from land contaminated with manure, slurry or sewage sludge. The effectiveness of sewage treatment could also be markedly less at high flow rates. Lower \textit{E. coli} concentrations at high levels of rainfall could be due to either the shellfish suspending filtering at low salinities or to the dilution of significantly impacting continuous discharges. In area C, the type of response to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{area_A_points}
\caption{Main effects plots for Area A on the east coast of England}
\end{figure}
rainfall changed with introduction of secondary treatment and this appeared to suggest dilution effects prior to the change and an increased relative impact of rainfall-related discharges afterwards.

The high/low tidal cycle altered the way that contamination from sewage discharges or other sources would have been carried over the shellfish prior to sampling. The effect of the high/low tidal cycle was only significant at some of the sampling points. This may have been due to the presence of a number of impacting sources or the fact that the concentration of E. coli in the shellfish did not relate directly to that in the surrounding water at any one time and, therefore, an averaging effect may reduce the influence of tide. The spring/neap tidal cycle would be expected to influence the flow of currents in an area but no significant effect on the shellfish results was shown in this study. Wind direction and wind speed were each only shown to be significant at one point in Area 3. It could be anticipated that an effect of wind direction and wind speed would be interdependent. However, this interaction, and that between the spring/neap and high/low tidal cycles could not be explored due to the unbalanced nature of the data.

**Figure 2** Main effects plots for Area B on the south-west coast of England
Conclusions
A number of environmental factors were shown to affect the extent of contamination detected in shellfish. Such analyses may contribute to the understanding of the nature of impacting sources when sewage improvement schemes are considered and also to the interpretation of the results from monitoring programmes. The effects were shown to be site specific and thus such analyses would need to be undertaken for each monitoring point. Much larger monitoring data sets would be required to enable analysis of the interaction of factors. The analyses performed in this study could also be applied to the analysis of putative viral indicators, such as F⁺ coliphage and could be extended to consideration of bacterial and viral pathogens.

Acknowledgements
This work was funded by the UK Food Standards Agency.
Figure 4  Main effects plots for Area C on the south coast of England: post-improvements

References


