THE IMPACT ON SHELLFISHERIES OF RUNOFF FROM LAND RECEIVING ORGANIC WASTE AND PROCEDURES TO DISTINGUISH ORGANIC WASTES OF ANIMAL AND HUMAN ORIGIN IN BIVALVE MOLLUSCAN SHELLFISH

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Introduction

- It is widely accepted that Norwalk-like virus (NLV) and hepatitis A (HAV) from sewage contaminated bivalve molluscan shellfish (BMS) account for a large proportion of shellfish associated outbreaks in the community. The accumulation in shellfish tissues of these human origin enteric viral pathogens is perceived as constituting a greater human health risk than consumption of BMS exposed to animal faeces. Whilst reliable epidemiological evidence to support this hypothesis is not well documented, it is widely accepted that shellfish associated zoonoses present less frequently.
- Under Council Directive 91/492/EEC classification of shellfisheries is based on enumeration of *Escherichia coli* / faecal coliforms per 100g shellfish flesh. This is used as an indication of faecal contamination status of shellfish and prescribes the level of processing required before consumption.
- The practice of applying farmyard manures, dirty water and other organic wastes to land is widespread providing both a means of disposal and allowing valuable nutrient recycling. These wastes all contain micro-organisms including potentially high numbers of *E.coli* / faecal coliforms.
- Attempts to link degradation of a fishery with possible sources of contamination are not always successful. Specific data are often available on point source inputs from sewage outlets and associated contamination incidents but comparable data on run-off from agricultural land receiving organic wastes have not been included in impact assessments.
- The classification of shellfisheries, whilst giving an indication of the risk of the presence of pathogens, does not enable human and animal sources of pollution to be distinguished. Consequently, shellfisheries may on occasion receive a poor classification in the absence of sewage contamination. Differentiation of human sewage contamination and agricultural run-off would allow management strategies and investment policies to be targeted to ameliorate the affects of the differing types of estuarine pollutants.

Objectives

- The overall scientific objectives of this combined desk and laboratory based research programme are to:
- To establish if a linkage can be found through comparing predicted run-off pollution events with known incidents of shellfishery contamination.
- To predict the *E. coli* loading in a river at the tidal limit following a hydrological event.
- To develop, evaluate and validate microbiological procedures for distinguishing between sewage contamination and agricultural run-off impacting upon shellfisheries.
- To provide advice to FSA, DEFRA and water regulators on future policy affecting shellfishery production.

Catchment/estuary selection

There are 43 estuarine commercial shellfisheries throughout England and Wales of which 22 were selected for risk analysis of the impact of human (sewage) and animal (run-off) wastes. Of these 2 were selected for intensive evaluation. One site was representative of a catchment receiving mainly agricultural inputs, whilst the other was typical of an estuary impacted upon by a mixture of human and animal contamination.

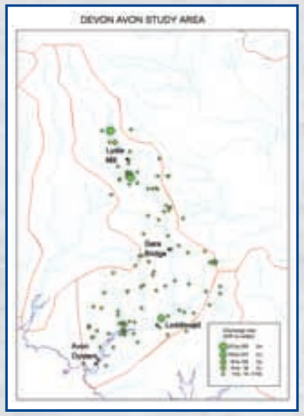
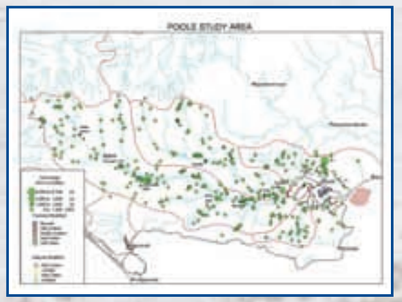


Figure 1: Map of rural sampling catchment showing sampling locations (★) and discharges.

Figure 2: Map of rural /urban mixed catchment showing sampling locations (★) and discharges.



Prediction of manure applications and rainfall/runoff data

The manure spreading practices have been characterised using GIS-based survey data, including the Farm Manure Practice data for 1996/97. By linking these with the DEFRA Agricultural Census data an assessment of the most likely manure practice in each catchment and hence the potential risk for organic manure run-off has been be made.

A modelling component has been used to estimate the occurrence of pollutants in run-off from the agricultural land within two catchments of interest. This model simulates the amount of run-off occurring, the run-off pathways (i.e. surface or subsurface) and the pollutant load in the run-off at the field scale. The model utilises the databases of land use, soil type, sub-surface drainage, manure/dirty water applications and meteorological data. The run-off estimates obtained were then used in conjunction with the manure/sewage sludge application database to estimate pollutant loads. This approach has enabled the influence of sewage sludge applications to be identified.

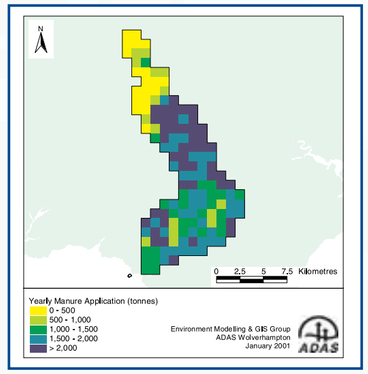


Figure 3: Manure application to land - rural catchment.

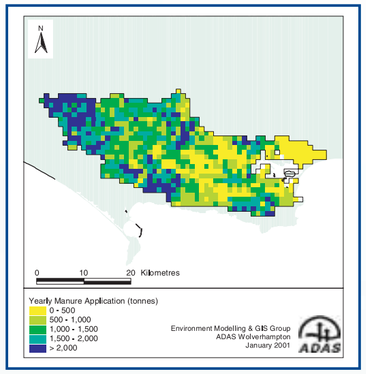


Figure 4: Manure application to land - rural/urban mixed catchment.

Comparison of run-off pollution and shellfisheries contamination

Time-specific, predictions of likely pollution incidents have been compared with the routine *E. coli* monitoring data from the shellfish production areas. Comparison with known point source input incidents has also been made. Using this approach a linkage between land run-off and pollution events in shellfish production areas has been established.

Establishment of procedures in the laboratory

The following test methods to distinguish between human and animal contamination were examined:

Oligonucleotide probing of FRNA bacteriophage genotypes

Detection of bacteriophages to *Bacteroides fragilis*

PCR detection of human adenoviruses (types 40,41)

Multiplex PCR detection of HH1 genetic marker in *E. coli*

Gas chromatography mass spectrometry detection of sterols

Gas chromatography mass spectrometry detection of caffeine

These were evaluated using panels of wastes including sewage (crude, primary, secondary and UV treated); farm effluent in the form of dirty water; manure of intensively farmed animals (e.g. pigs, cattle and poultry); pooled individual faecal samples from free range animals (e.g. sheep); and pooled waste effluent from slaughterhouses.

Genotypification of FRNA bacteriophage into four serotypes MS2, SP (animal) and GA, Qß (human) and detection of human adenoviruses were selected as appropriate methods and applied to oysters (*Crassostrea gigas*) and mussels (*Mytilius edulis*).

Acknowledgement

The financial support for this work from the Food Standards Agency (FSA) is acknowledged.

Table 1: Example of evaluation of data from genotypification of FRNA bacteriophage in shellfish harvested from different classification areas.

Classification of harvesting area	Species	% GA (H)	% Qß	% MS2 (A)	% SP (A)	% plaque recovery
Prohibited	*C.gigas*	36.4	3	3	0	42.4
Prohibited	*C.gigas*	51.2	0	19.5	0	70.7
Prohibited	*C.gigas*	37.7	0	12.1	0	49.8
Prohibited	*C.gigas*	25.7	1	5.7	0	32.4
Prohibited	*C.gigas*	46.0	12.0	10.0	0	68.0
Prohibited	*C.gigas*	75.7	1.4	0.7	0	77.8
Prohibited	*M.edulis*	45.5	1.8	2.7	0	50.0
Prohibited	*C.gigas*	69.0	15.9	0	0.8	85.7
B	*C.gigas*	50.0	0	14.3	0	64.3
B	*C.gigas*	86.4	4.5	0	0	90.9
B	*C.gigas*	75.0	0	0	0	75.0
B	*C.gigas*	78.1	0	0	0	78.1
B	*C.gigas*	66.7	0	6.7	0	73.4
C	*C.gigas*	72.7	0	0	0	72.7
C	*M.edulis*	70.0	0	0	0	70.0

Intensive sampling of two selected catchments

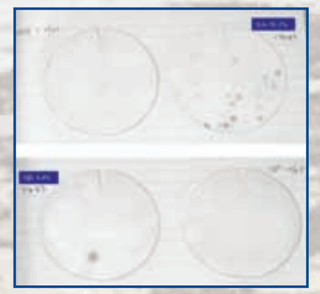
Figure 5: Automated sampling apparatus for storm surveys.

Water samples are being collected weekly throughout the catchments using automatic samplers. Water and shellfish are being sampled from the two estuaries weekly. This ongoing sampling programme began in October 2001 and it is anticipated that it will finish in April 2002. It is anticipated that this sampling regime will encompass periods of heavy rainfall and the autumn and spring manure application to land.

Figure 6: Commercial shellfish beds at the estuary of the rural catchment.

Figure 7: Experimental shellfish placed at the estuary of the rural / urban catchment.

Analysis of water and shellfish samples



Currently riverine and estuarine water samples are being enumerated for *E. coli* (membrane filtration) and FRNA bacteriophage (double agar overlay). Shellfish flesh is analysed for *E. coli* (3x5 tube MPN), FRNA bacteriophage (double agar overlay) and human adenovirus (PCR). FRNA bacteriophage plaques are transferred to nylon membrane and stored for nucleic acid hybridisation. Completion of this programme of work is scheduled for December 2002.

Figure 8: Typification of FRNA bacteriophage showing percentage bacteriophage genotypes.

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