Rainfall and river flows are predictors for β-glucuronidase positive *Escherichia coli* accumulation in mussels and Pacific oysters from the Dart Estuary (England)

Carlos J. A. Campos, Simon Kershaw, Ron J. Lee, Owen C. Morgan and Kevin Hargin

ABSTRACT

Rainfall and river flows are environmental variables influencing the microbial status of bivalve mollusc harvesting areas. This study investigated spatial and temporal relationships between rainfall, river flows and concentrations of *Escherichia coli* in mussels (*Mytilus* spp.) and Pacific oysters (*C. gigas*) from three harvesting areas in the Dart Estuary over the period 1996–2009. Mussels growing on the riverbed were found to be more contaminated than oysters growing in the water column. A step change in the levels of the microbial indicator was identified in both species from all harvesting areas. The highest levels of *E. coli* were detected when total rainfall exceeded 2 mm and water levels in the main tributaries exceeded the mean flow. The magnitude of response in levels of *E. coli* to these hydrological events varied between species and monitoring points, but was consistently higher between the 3rd and 4th days after the rainfall event. This lag time is assumed to result from catchment topography and geology determining peak levels of runoff at the headwaters 12–24 h after rainfall events. It is considered that future risk management measures may include sampling targeting hydrograph events.

Key words | *Crassostrea gigas*, *Escherichia coli*, *Mytilus* spp., official controls, rainfall, river flow

Carlos J. A. Campos (corresponding author)
Simon Kershaw
Ron J. Lee
Owen C. Morgan
Centre for Environment, Fisheries & Aquaculture Science (Cefas),
Aquatic Health and Hygiene Division, Food Safety Group,
Weymouth Laboratory, Weymouth,
Dorset DT4 8UB,
UK
E-mail: *carlos.campos@cefas.co.uk*

Kevin Hargin
Food Standards Agency (FSA),
125 Kingsway,
London WC2B 6NH,
UK

INTRODUCTION

A better knowledge of the effect of environmental factors influencing the microbial quality of bivalve shellfish in harvesting areas will contribute to measures to reduce contamination of these food items at source. Rainfall is recognisably one of the main factors affecting the uptake of viral (Pommepuy *et al.* 2008) and bacterial (Martinez-Urtaza *et al.* 2004) pathogens and their indicators in bivalves (Brock *et al.* 1985; Lee & Morgan 2003; Campos & Cachola 2007) and growing waters (Mallin *et al.* 2001; Chigbu *et al.* 2004). Outbreaks of viral gastroenteritis in developed countries are more frequent in winter months when norovirus is more common in the community and rainfall may predominate (Berg *et al.* 2000; Lees 2000).

Although most of the scientific literature indicates increased microbial contamination of bivalves following rainfall events, some studies suggest that the overall microbial status of some bivalve harvesting areas may actually improve following heavy rainfall (Lee & Morgan 2003), possibly due to dilution effects in estuaries able to quickly disperse contaminants and/or suspension of filter-feeding activity under exposure to lower levels of salinity (Younger *et al.* 2003).

Statutory controls intended to limit the placement on the market of contaminated bivalves in force within the European Union (EU) require that competent authorities should examine the quantities of pollutants released during the different periods of the year, according to the seasonal variations of rainfall readings (European Communities 2004). This is one of the requirements commonly referred to as the 'sanitary survey', which aims to inform the sampling programme for microbiological monitoring of bivalve

doi: 10.2166/wh.2011.136

harvesting areas (EU Working Group on the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas 2007). In the USA, rainfall variability is considered an important element of the sanitary survey for the purposes of determining the sampling strategy for microbial monitoring of growing waters (USFDA & ISSC 2007). In a number of countries, including the USA and Canada, rainfall conditions may determine water quality criteria for opening and closure of conditionally approved areas (CFIA *et al.* 2003; USFDA & ISSC 2007).

Rivers are major routes of microbial contamination from surface or sub-surface runoff impacting on coastal areas. Despite being recognised as an important predictor of microbial contamination of bivalve harvesting areas (Brock *et al.* 1985; Pommepuy *et al.* 2006, 2008), river flows are not explicitly mentioned in the EU legislation on official controls of bivalve molluscs intended for human consumption. In contrast, the establishment of performance standards for conditionally approved or conditionally restricted growing areas in the USA can be based both on the amount of rainfall in the vicinity of the growing area or on the river stage (USFDA & ISSC 2007).

Few published studies have considered the contribution of river flows in informing official public health controls for bivalve mollusc fisheries (Brock *et al.* 1985; Fiandrino *et al.* 2003). This is surprising since a considerable body of literature has shown that catchment-scale microbial dynamics determining bathing water compliance is often determined by hydrograph events (e.g. Crowther *et al.* 2001, 2002; Kay *et al.* 2005).

In the present study, data from the official classification monitoring programme in England and Wales was used to examine spatial and temporal relationships between levels of rainfall and river flows and levels of *Escherichia coli* (the EU statutory indicator of faecal contamination) in two species of bivalves from three harvesting areas in an English estuary.

MATERIALS AND METHODS

Dart Estuary

The Dart is a macro-tidal estuary (range: 4.3 m on mean spring tides and 1.8 m on mean neap tides at Dittisham) with semi-diurnal tides (Imray, Laurie, Norie and Wilson Ltd 2008) situated on the southwest coast of England (Figure 1). Pacific oysters (*Crassostrea gigas*) and mussels (*Mytilus* spp.) are produced in four intertidal areas in the middle reaches of the estuary. Of these, three were selected for this study on the basis of availability of *E. coli* monitoring data.

Oysters grow in bags supported above the riverbed on trestles; and some are grown in bags deposited on the foreshore. Mussels are harvested from the riverbed; some are also grown in bags deposited on the foreshore. Both species are harvested by hand, during periods of low water. These harvesting areas are above the 2 m depth contour at Chart Datum (Imray, Laurie, Norie and Wilson Ltd 2008; approximately the level of lowest astronomical tide).

The tidal length of the Dart Estuary is 19 km and the water residence time is estimated to be seven days (Uncles

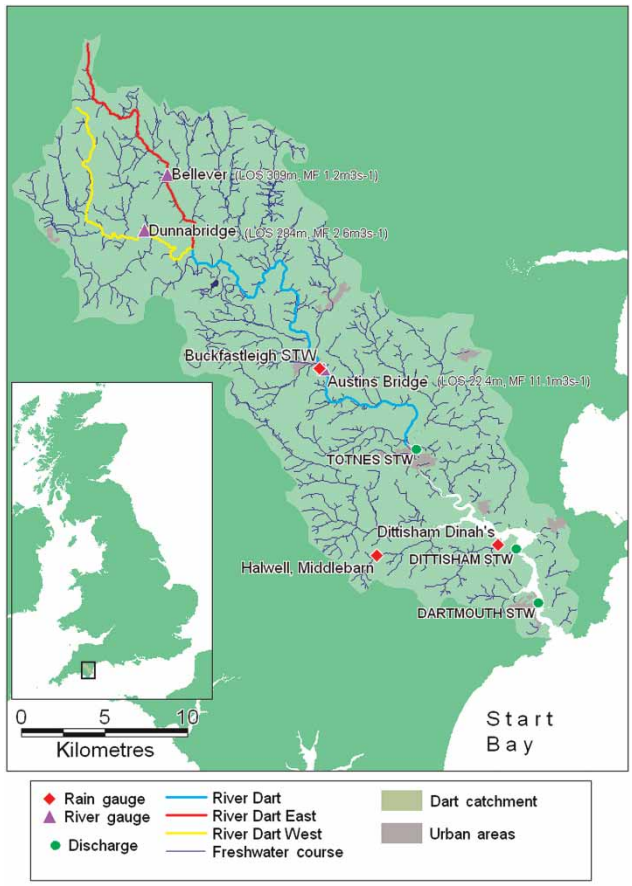

Figure 1 | Dart catchment showing gauging stations and significant sewage treatment works.

et al. 2002). The estuary is ebb dominant with a flow ratio indicating the emergence of a freshwater plume at its mouth (Halcrow Group Ltd 2002; Thain et al. 2004). On flood tides, mean flow velocities at the mouth of the estuary are 0.6 and 0.3 m s^{-1} on spring and neap tidal ranges, respectively (Thain et al. 2004). Tidal currents of up to 1 m s^{-1} occur towards the upper tidal limit on springs (Odling-Smee Oberman Associates Ltd, unpublished).

The estuary drains a catchment of approximately 470 km^2 from the headwaters of the River Dart on the slopes of Dartmoor National Park to Dartmouth, where it enters the sea. The catchment is essentially rural and includes upland moorland and natural and improved grassland, steep-sided wooded river valleys and low-lying, undulating land in the lower reaches. Cattle and sheep production takes place in moorland and grassland.

The resident human population in the catchment is over 41,400 people (Office for National Statistics, unpublished). Human population in the catchment doubles during the summer holiday season (Dartmouth Tourist Information Centre, personal communication).

Microbiological analyses and hydrometrics

European Union legislation stipulates that microbial monitoring of bivalves must be based on the levels of *E. coli* (European Communities 2005). This bacterium is present in the intestinal flora of warm-blooded animals and, for this reason, is a reliable indicator of contamination of faecal origin. Levels of β-glucuronidase-positive *E. coli* in Pacific oysters and mussels monitored over the period November 1991–March 2009 were analysed for this study. Local authority sample collection and transport after 2009 were conducted in accordance with Cefas protocols (Cefas 2009). The enumeration method is the five tube, three-dilution most probable number (MPN) test, ISO 16649-3 (ISO 2004). This involves inoculation of tubes of minerals modified glutamate broth medium, incubation of these tubes at 37 °C ± 1 °C for 24 h and subculture to tryptone bile glucuronide agar with incubation at 44 °C ± 1 °C for 20–24 h for determination of the MPN index from the number of positive tubes. Levels of the indicator of faecal contamination are reported as the MPN of *E. coli* 100 g^{-1} of flesh and intravalvular liquid (FIL).

Total daily rainfall (mm) records from Buckfastleigh WWTW, Halwell and Dittisham Dinah's and average daily river flow records from Bellever, Dunnabridge and Austin's Bridge representative of the Dart catchment (Figure 1) were obtained from the Environment Agency.

Time series river flow data were separated into base flow and high flow components using Water Engineering Time Series PROcessing tool developed by Willems (2004, 2009).

Statistical analyses

For statistical analyses of *E. coli* results, censored MPNs were given double and half values. Probability plots were generated for $\log_{10}$-transformed MPNs of *E. coli* for each species/monitoring point. These datasets were tested for normality using the Anderson-Darling test (significance: $p < 0.05$). Cumulative sum (CUSUM) analysis summation of the deviations of the observations from the mean plotted as a function of time of MPNs of *E. coli* in bivalves was carried out to identify step changes in time series data. CUSUM functions have been successfully applied as quantitative techniques for evaluating changes in water quality related to human activity and environmental variability (Cluis 1983; Nicholls 2001).

Descriptive statistics (minimum, maximum, median and geometric mean [calculated as the antilog of the mean of $\log_{10}$-transformed MPNs]) were calculated for datasets before and after step changes. Differences between geometric means of *E. coli* before and after step changes were tested for significance using the Mann–Whitney test (significance: $p < 0.05$). Standard deviation of $\log_{10}$-transformed MPNs of *E. coli* and 95% confidence interval for the mean were calculated as measures of dispersion of *E. coli* levels. Kurtosis and coefficient of skewness were calculated as measures of distribution of *E. coli* levels.

Relationships between rainfall and levels of *E. coli* and river flows and levels of *E. coli* were analysed using Spearman's rho coefficient (significance $p < 0.05$ and $p < 0.01$) since most datasets showed departures from normal distribution. Scatterplots were computed for statistically significant relationships between variables. Locally weighted scatterplot smoothing (LOWESS) lines were superimposed on scatterplots to enhance visual information on the distribution of data. Side-by-side box and whisker plots were

computed to compare the distributions of levels of *E. coli* in bivalves.

Seasonal variations of levels of *E. coli* were analysed by amalgamating MPNs of *E. coli* by season considering spring (March–May), summer (June–August), autumn (September–November) and winter (December–February). One-way analysis of variance (ANOVA) was used to test differences between seasons followed by a Tukey HSD test (honestly significant difference; significance: $p < 0.05$) to identify which means were significantly different from one another.

CUSUM analysis was carried out using Microsoft Excel. All other analyses were carried out using Minitab 15.

RESULTS

One step change was detected in the levels of *E. coli* in mussels and Pacific oysters from each harvesting area. Levels of the microbial indicator were higher in mussels than in oysters, before and after step changes (Table 1).

Before step changes, the spatial trend of contamination of harvesting areas was: C > A > B (Pacific oysters) and A > C > B (mussels). After step changes, levels of *E. coli* in bivalves were significantly ($p < 0.05$) lower than those before changes and medians of the indicator were equivalent between harvesting areas of the same species. In bivalves from most harvesting areas, levels of the indicator changed from a negative skewed distribution before the change to a positive skewed distribution after the change. One sample of mussels from harvesting area A had a concentration of *E. coli* within the range determining prohibition of harvesting for human consumption (MPN > 1,800,000 100 g^{-1} FIL).

Hydrographs for the River Dart showed an increase in mean flows from November to a peak in December. Hyetographs for the three gauging stations across the catchment indicated that the wettest month varied between October and January.

Medians of the microbiological indicator in mussels from harvesting areas A and C increased more than 1 $\log_{10}$ between September and December, whilst in mussels from harvesting area B, this difference was 0.7 $\log_{10}$ (Figure 2). However, no statistically significant variations were detected in levels of *E. coli* by month or when data was amalgamated by season.

Median levels of rainfall monitored in the three gauging stations suggested a decreasing gradient across the catchment. Levels of *E. coli* in bivalves from the three harvesting areas were higher during high river flows than those during low river flows (Figure 3). However, the difference was less than 1 $\log_{10}$.

Statistically significant positive correlations were detected between the amount of daily/cumulative rainfall and river flows and levels of *E. coli* in bivalves. The relationships varied in relation to the number of days between the sampling occasion and the rainfall event. The relationships also varied between gauging stations.

Overall, the highest correlation coefficients were found between levels of the microbiological indicator in mussels from harvesting area A and cumulative rainfall when sampling was undertaken 7 days after the rainfall event (Table 2; Figure 4). A higher number of positive relationships was found for river flows than for rainfall (Tables 2 and 3).

In most species, the highest relationships were detected when the sampling occasion occurred 3 days after the rainfall event (Table 2) or with increased water levels in watercourses 1–2 days before sampling (Table 3). When cumulative river flows are considered, levels of the microbiological indicator were higher when sampling occurred 3–4 days after an increase in water levels in watercourses.

Higher correlation coefficients between variables were detected for rainfall recorded in Buckfastleigh than rainfall recorded in Halwell and Dittisham (Table 2). Similarly, river flows recorded at Bellever and Dunnabridge were found to be more associated with levels of *E. coli* in bivalves than those recorded at Austins Bridge.

Between three and ten samples of mussels and less than four samples of Pacific oysters returned levels above the class B threshold (MPN of *E. coli* >4,600 100 g^{-1} FIL) (European Communities 2004) when flows in the River Dart exceeded the mean flow level.

DISCUSSION

The higher levels of *E. coli* detected in mussels compared to those detected in Pacific oysters from the Dart Estuary are consistent with the pattern of contamination found in harvesting areas where both species are commercially

Table 1 | Summary statistics of levels of *E. coli* in bivalves from three harvesting areas in the Dart Estuary

					MPN *E. coli* 100 g^{-1} flesh and intravalvular liquid								
										95% CI for mean			
Bed name	**Species**	**n**	**Date of first sample**	**Date of last sample**	**Min.**	**Max.**	**Median**	**Geometric mean**	**log_{10} SD**	**Lower**	**Upper**	**Skewness**	**Kurtosis**
Before step change													
Harvesting area A	*C. gigas*	40	19 April 1999	9 July 2002	310	13,000	1,300	1,474	0.43	1,071	2,027	0.42	−0.78
Harvesting area B	*C. gigas*	134	27 November 1991	26 June 2002	20	24,000	1,100	945	0.55	761	1,173	−0.32	0.11
Harvesting area C	*C. gigas*	78	1 July 1997	3 December 2002	140	17,000	1,850	1,597	0.41	1,290	1,977	−0.36	0.05
Harvesting area A	*Mytilus* spp.	47	19 April 1999	3 December 2002	500	24,000	4,300	4,241	0.38	3,277	5,490	−0.23	−0.41
Harvesting area B	*Mytilus* spp.	70	27 November 1997	18 February 2003	310	24,000	3,500	2,971	0.42	2,358	3,743	−0.09	−0.49
Harvesting area C	*Mytilus* spp.	71	20 March 1996	26 June 2002	130	50,000	3,500	3,249	0.46	2,522	4,186	−0.34	0.57
After step change													
Harvesting area A	*C. gigas*	69	23 July 2002	25 February 2009	20	5,400	310*	306	0.52	228	390	0.23	0.25
Harvesting area B	*C. gigas*	76	9 July 2002	10 March 2009	20	17,000	310*	362	0.57	258	465	0.69	0.73
Harvesting area C	*C. gigas*	64	21 January 2003	10 March 2009	<20	11,000	310*	314	0.58	218	416	0.34	1.01
Harvesting area A	*Mytilus* spp.	67	21 January 2003	10 March 2009	110	>1,800,000	725*	907	0.68	602	1,298	2.61	11.03
Harvesting area B	*Mytilus* spp.	68	19 March 2003	10 March 2009	70	17,000	750*	1,026	0.59	720	1,396	0.21	−0.81
Harvesting area C	*Mytilus* spp.	69	9 July 2002	25 February 2009	70	31,000	725*	725	0.54	611	1,118	0.80	0.81

n, number of samples; CI, confidence interval; SD, standard deviation; Mann–Whitney test.
*Statistically significant; $p < 0.05$.

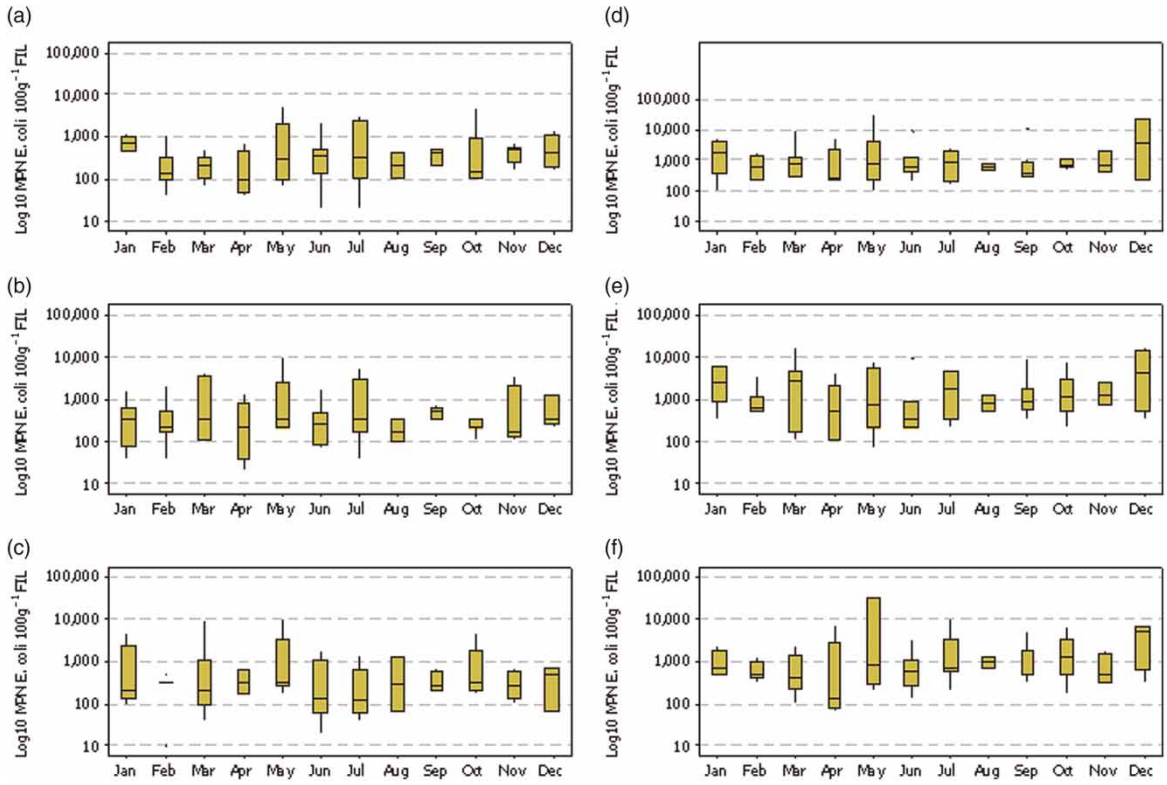

Figure 2 | Levels of *E. coli* in Pacific oysters: (a) harvesting area A, (b) harvesting area B, (c) harvesting area C; and mussels (d) harvesting area A, (e) harvesting area B, (f) harvesting area C, throughout the year.

harvested in England and Wales (Berry & Younger 2009). These inter-species differences in *E. coli* contamination may be attributed to the effects of different growing methods which in turn may be influenced by local hydrodynamic factors; hence the transport of contamination across and between harvesting areas or due to species-specific physiological mechanisms influencing retention of contaminants (Younger *et al*. 2003).

In December 2002, the level of treatment at Dartmouth & Kingswear WWTW was upgraded to include UV disinfection. Following this, between 2003 and 2004, the level of treatment was upgraded at Dittisham WWTW (membrane biological reactor) and Totnes WWTW (UV disinfection). These wastewater treatment works are considered to have a significant or potentially significant impact on the Dart designated Shellfish Water (Environment Agency 2007, 2008). Moreover, various initiatives have taken place in the Dart catchment to tackle inputs of diffuse pollution to the estuary (Devon Wildlife Trust 2006; McGonigle 2008). These initiatives will have contributed to the decrease in the levels of faecal contamination in the estuary. Significant reductions in the contamination of bivalve molluscs were seen in all areas/species combinations between July 2002 and February 2003; that is, in some area/species combinations the step change occurred before the sewage improvements and the latter diffuse pollution measures. This may have been due to natural fluctuation in the *E. coli* results producing a series of low results in late 2002 prior to the subsequent reductions due to the sewage improvements. Most significantly, the changes in the distributions of levels of *E. coli* in Pacific oysters and mussels indicate site-specific alterations in the microbial status of bivalve mollusc harvesting areas. The continued occurrence of very high *E. coli* results in bivalve molluscs after the step change indicates that the harvesting areas continue to be vulnerable to periods of increased microbial contamination.

The results from correlation analyses indicate that the microbial quality of bivalves in the Dart Estuary is governed

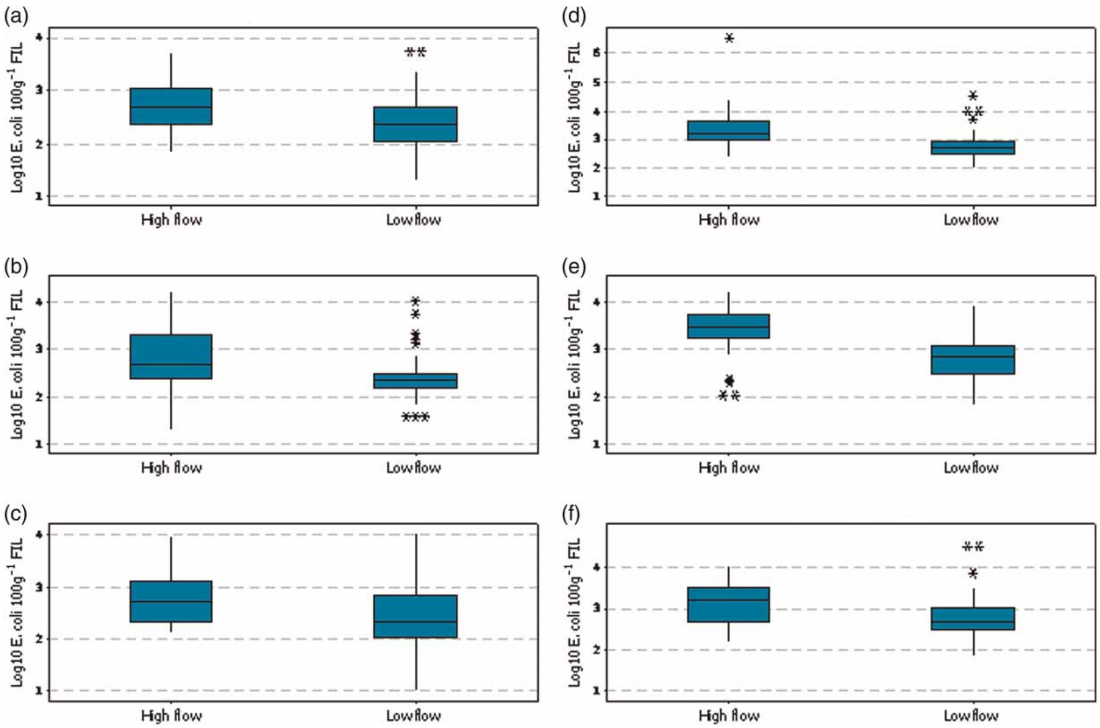

Figure 3 | Levels of *E. coli* in Pacific oysters: (a) harvesting area A, (b) harvesting area B, (c) harvesting area C; and mussels: (d) harvesting area A, (e) harvesting area B, (f) harvesting area C, during high and low river flows.

by rainfall and that it is also associated with river flows. The interrelationship between these two explanatory variables means that it is difficult to determine whether rainfall effects other than those mediated via the river systems contribute significantly to the *E. coli* contamination in bivalve molluscs. Such rainfall effects would include the operation of estuarine combined sewer overflows (CSOs), direct land runoff into the estuary, and re-suspension of contaminated sediment within the estuary itself. River-flow related effects would include the operation of riverine CSOs, land runoff into the river systems and re-suspension of contaminated river sediment.

Increased levels of *E. coli* in bivalves from all monitoring points under high river flow conditions suggest that storm water runoff is contributing to a significant proportion of *E. coli* accumulated by bivalves. Similar dependence of storm runoff events has been found in watercourses draining other steep-sided and mixed land use coastal catchments in the United Kingdom (Crowther *et al.* 2002; Kay *et al.* 2005). The higher correlation coefficients obtained between *E. coli* results and river flows recorded at Bellever and Dunnabridge than those obtained at Austins Bridge were unexpected as the latter is lower down the catchment and is expected to be representative of flows passing the other two locations.

Elevated concentrations of total coliforms, *E. coli* and enterococci have been detected in estuarine water following rainfall events, in particular in samples collected in the proximity of agricultural fields (Allen, unpublished). Most of these fields correspond to areas of the catchment identified as of potentially high risk of diffuse pollution from agricultural land (Marlow 2006). As in many parts of the UK, grassland on impermeable geological formations in the Dart catchment means that most of the pollution will be transported as surface or sub-surface runoff (Mawdsley *et al.* 1995). Most headwaters draining peat and non-peat moorland are characterised by lower levels of sulphate, nitrate and chloride than those found in lower catchment farmland and coniferous forests (Evans *et al.* 2001). Sharp rainfall events in the summer usually result in very little runoff in the Dart catchment. In contrast, winter floods tend to be more critical as a greater percentage of water

Table 2 | Spearman's rho coefficients between rainfall recorded at three gauging stations and MPNs of *E. coli* 100 g^{-1} FIL in bivalves from three monitoring points in the Dart Estuary for data periods after step changes

		MPN *E. coli* 100 g^{-1} FIL					
Rainfall		**Harvesting area A (*C. gigas*)**	**Harvesting area A (*Mytilus* spp.)**	**Harvesting area B (*C. gigas*)**	**Harvesting area B (*Mytilus* spp.)**	**Harvesting area C (*C. gigas*)**	**Harvesting area C (*Mytilus* spp.)**
Buckfastleigh STW	Time						
Daily	Day of sampling	0.114	0.244*	0.305*	0.260*	0.123	0.213*
	-1 day	0.188	0.398**	0.384**	0.385**	0.362**	0.216*
	-2 days	0.329**	0.508**	0.403**	0.391**	0.382**	0.416**
	-3 days	0.528**	0.668**	0.599**	0.595**	0.629**	0.609**
	-4 days	0.323**	0.573**	0.336**	0.451**	0.419**	0.367**
	-5 days	0.221*	0.468**	0.266**	0.396**	0.471**	0.338**
	-6 days	0.125	0.354**	0.266**	0.435**	0.408**	0.260*
	-7 days	0.079	0.273*	0.125	0.181	0.330**	0.108
Cumulative	-2 days	0.200*	0.367**	0.420**	0.351**	0.295**	0.262*
	-3 days	0.270*	0.459**	0.477**	0.414**	0.392**	0.344**
	-4 days	0.385**	0.58**	0.559**	0.558**	0.515**	0.494**
	-5 days	0.445**	0.656**	0.543**	0.591**	0.523**	0.536**
	-6 days	0.453**	0.693**	0.568**	0.618**	0.579**	0.536**
	-7 days	0.448**	0.707**	0.551**	0.625**	0.586**	0.545**
Halwell, Middlebarn							
Daily	Day of sampling	−0.001	0.212*	0.196*	0.180	0.094	0.199*
	-1 day	0.207*	0.302**	0.371**	0.212*	0.367**	0.137
	-2 days	0.344**	0.485**	0.462**	0.327**	0.212*	0.405**
	-3 days	0.431**	0.625**	0.551**	0.486**	0.546**	0.488**
	-4 days	0.282**	0.565**	0.262*	0.294**	0.284**	0.340**
	-5 days	0.119	0.398**	0.240*	0.398**	0.360**	0.293**
	-6 days	0.029	0.371**	0.193*	0.379**	0.423**	0.178
	-7 days	−0.033	0.215*	0.139	0.179	0.232*	0.065
Cumulative	-2 days	0.113	0.333**	0.323**	0.274*	0.295**	0.206*
	-3 days	0.194	0.412**	0.398**	0.332**	0.283**	0.291**
	-4 days	0.287**	0.524**	0.483**	0.454**	0.431**	0.417**
	-5 days	0.352**	0.636**	0.469**	0.481**	0.409**	0.454**
	-6 days	0.371**	0.670**	0.494**	0.522**	0.473**	0.455**
	-7 days	0.369**	0.695**	0.493**	0.547**	0.511**	0.459**
Dittisham							
Daily	Day of sampling	0.154	0.221*	0.236*	0.154	0.095	0.181
	-1 day	0.242*	0.392**	0.358**	0.291**	0.304**	0.123
	-2 days	0.344**	0.432**	0.415**	0.374**	0.295**	0.369**

(*continued*)

Table 2 | (*continued*)

Rainfall		MPN *E. coli* 100 g^{-1} FIL					
		Harvesting area A (*C. gigas*)	Harvesting area A (*Mytilus* spp.)	Harvesting area B (*C. gigas*)	Harvesting area B (*Mytilus* spp.)	Harvesting area C (*C. gigas*)	Harvesting area C (*Mytilus* spp.)
	-3 days	0.526**	0.608**	0.556**	0.606**	0.568**	0.478**
	-4 days	0.432**	0.564**	0.346**	0.429**	0.437**	0.480**
	-5 days	0.240*	0.482**	0.188*	0.387**	0.397**	0.270*
	-6 days	0.157	0.404**	0.291**	0.484**	0.431**	0.313**
	-7 days	0.098	0.344**	0.173	0.290**	0.366**	0.192
Cumulative	-2 days	0.170	0.343**	0.302**	0.270*	0.228*	0.190
	-3 days	0.281**	0.458**	0.400**	0.390**	0.312**	0.316**
	-4 days	0.392**	0.564**	0.515**	0.531**	0.466**	0.449**
	-5 days	0.478**	0.655**	0.500**	0.568**	0.489**	0.516**
	-6 days	0.512**	0.698**	0.510**	0.609**	0.565**	0.542**
	-7 days	0.519**	0.724**	0.519**	0.644**	0.608**	0.563**

Number of samples and corresponding sampling periods are given in Table 1.

*Statistically significant ($p < 0.05$).

**Statistically significant ($p < 0.01$).

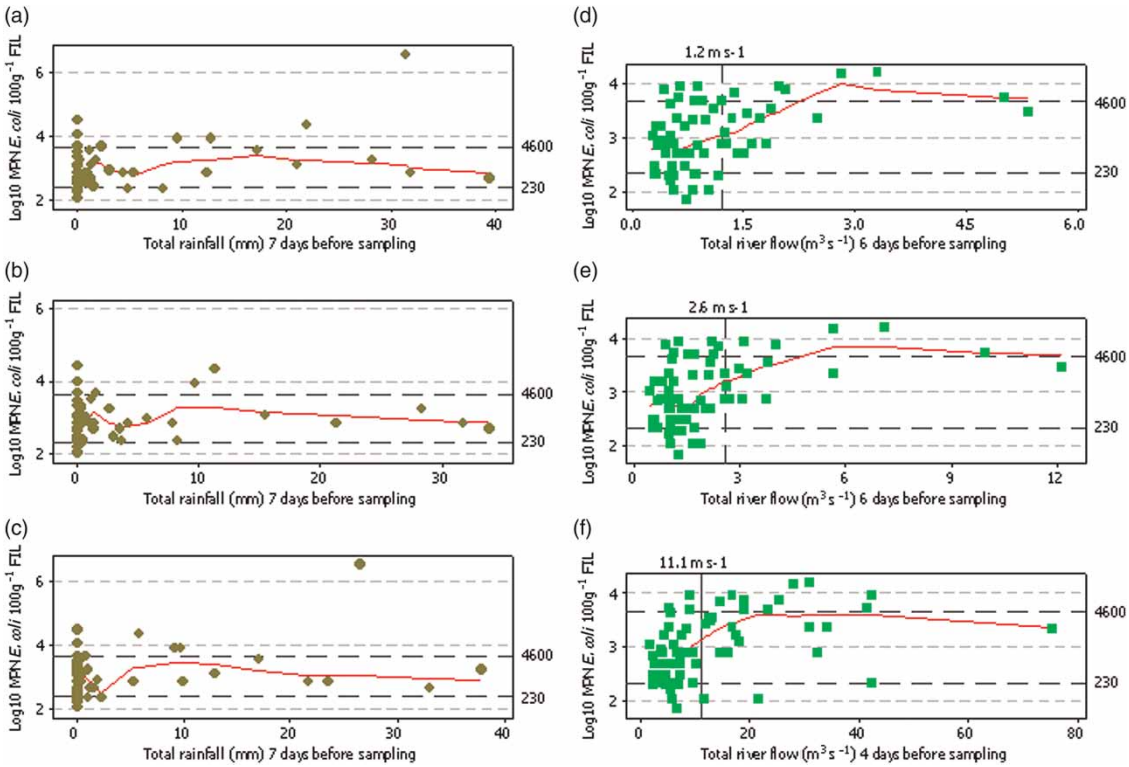

Figure 4 | Relationship between levels of *E. coli* in mussels from harvesting area A and cumulative rainfall: (a) Buckfastleigh STW, (b) Halwell, Middlebarn, (c) Dittisham; or river flow: (d) Bellever, (e) Dunnabridge, (f) Austins Bridge, and period of time before sampling. Only graphs for relationships yielding the maximum correlation coefficients are shown.

Table 3 | Spearman's rho coefficients between river flow recorded at three gauging stations and MPNs of *E. coli* 100 g^{-1} FIL in bivalves from three monitoring points in the Dart Estuary for data periods after step changes

		MPN *E. coli* 100 g^{-1} FIL					
River flow		**Harvesting area A (*C. gigas*)**	**Harvesting area A (*Mytilus* spp.)**	**Harvesting area B (*C. gigas*)**	**Harvesting area B (*Mytilus* spp.)**	**Harvesting area C (*C. gigas*)**	**Harvesting area C (*Mytilus* spp.)**
Bellever	Time						
Daily	Day of sampling	0.345**	0.489**	0.437**	0.540**	0.425**	0.321**
	-1 day	0.429**	0.520**	0.477**	0.598**	0.488**	0.460**
	-2 days	0.407**	0.499**	0.431**	0.573**	0.520**	0.425**
	-3 days	0.373**	0.521**	0.443**	0.564**	0.521**	0.396**
	-4 days	0.314**	0.456**	0.349**	0.511**	0.478**	0.377**
	-5 days	0.265*	0.417**	0.286**	0.520**	0.416**	0.300**
	-6 days	0.172	0.336**	0.205*	0.397**	0.418**	0.234*
	-7 days	0.196	0.348**	0.206*	0.360**	0.390**	0.192
Cumulative	-2 days	0.404**	0.528**	0.473**	0.571**	0.456**	0.378**
	-3 days	0.421**	0.549**	0.501**	0.608**	0.496**	0.414**
	-4 days	0.415**	0.556**	0.493**	0.612**	0.526**	0.432**
	-5 days	0.405**	0.557**	0.476**	0.611**	0.522**	0.419**
	-6 days	0.403**	0.552**	0.478**	0.619**	0.523**	0.424**
	-7 days	0.375**	0.547**	0.462**	0.612**	0.527**	0.417**
Dunnabridge							
Daily	Day of sampling	0.416**	0.523**	0.447**	0.571**	0.447**	0.378**
	-1 day	0.483**	0.543**	0.463**	0.602**	0.487**	0.500**
	-2 days	0.477**	0.517**	0.436**	0.575**	0.511**	0.501**
	-3 days	0.466**	0.552**	0.468**	0.592**	0.530**	0.472**
	-4 days	0.39**	0.485**	0.334**	0.529**	0.463**	0.442**
	-5 days	0.366**	0.469**	0.308**	0.552**	0.477**	0.382**
	-6 days	0.277*	0.370**	0.196*	0.420**	0.428**	0.315**
	-7 days	0.284**	0.361**	0.180	0.371**	0.378**	0.283**
Cumulative	-2 days	0.454**	0.552**	0.474**	0.589**	0.465**	0.424**
	-3 days	0.481**	0.569**	0.482**	0.607**	0.498**	0.457**
	-4 days	0.482**	0.577**	0.488**	0.622**	0.516**	0.481**
	-5 days	0.474**	0.571**	0.470**	0.612**	0.518**	0.475**
	-6 days	0.481**	0.571**	0.474**	0.627**	0.534**	0.479**
	-7 days	0.457**	0.558**	0.453**	0.617**	0.523**	0.465**
Austins Bridge							
Daily	Day of sampling	0.429**	0.494**	0.425**	0.573**	0.435**	0.400**
	-1 day	0.456**	0.488**	0.419**	0.565**	0.446**	0.449**
	-2 days	0.432**	0.480**	0.380**	0.545**	0.495**	0.437**

(*continued*)

Table 3 | (*continued*)

River flow		MPN *E. coli* 100 g^{-1} FIL					
		Harvesting area A (*C. gigas*)	Harvesting area A (*Mytilus* spp.)	Harvesting area B (*C. gigas*)	Harvesting area B (*Mytilus* spp.)	Harvesting area C (*C. gigas*)	Harvesting area C (*Mytilus* spp.)
	-3 days	0.405**	0.470**	0.379**	0.545**	0.482**	0.418**
	-4 days	0.372**	0.456**	0.316**	0.498**	0.435**	0.418**
	-5 days	0.325**	0.374**	0.233*	0.434**	0.406**	0.329**
	-6 days	0.237*	0.297**	0.166	0.335**	0.349**	0.244*
	-7 days	0.247*	0.308**	0.176	0.327**	0.345**	0.244*
Cumulative	-2 days	0.454**	0.506**	0.441**	0.589**	0.459**	0.434**
	-3 days	0.460**	0.518**	0.433**	0.593**	0.469**	0.444**
	-4 days	0.454**	0.519**	0.436**	0.600**	0.486**	0.449**
	-5 days	0.444**	0.510**	0.415**	0.592**	0.475**	0.443**
	-6 days	0.436**	0.510**	0.405**	0.587**	0.471**	0.441**
	-7 days	0.411**	0.489**	0.385**	0.572**	0.465**	0.428**

Number of samples and corresponding sampling periods are given in Table 1.
*Statistically significant ($p < 0.05$).
**Statistically significant ($p < 0.01$).

runs off quickly as a result of water saturation in soil (Odling-Smee, Oberman and Associates Ltd, unpublished).

Soils along riverbanks are potentially significant sources of *E. coli* in urban (Solo-Gabriele *et al.* 2000) and rural areas (Jamieson *et al.* 2004). In the Dart Estuary, when neap tides are coincident with high river flows, water levels in the main tributaries tend to be above the Chart Datum for a complete tidal cycle, inducing strong ebb flows and increasing sediment transport out of the channels (Odling-Smee, Oberman and Associates Ltd, unpublished). These periods result in partial mixing conditions in the water column of the estuary (R. Thain, Britannia Royal Naval College, personal communication). At low water (LW) -1 h, a well-developed vertical density gradient consisting of a shallow layer of buoyant surface water and a homogeneous lower layer is apparent at the mouth of the estuary (Thain *et al.* 2004). Being macro-tidal and having long tidal length and residence time, the Dart Estuary will have its turbidity maximum in the upper reaches (see Uncles *et al.* 2002). This is consistent with higher correlation coefficients found between rainfall and *E. coli* in mussels growing in the most upstream harvesting area (harvesting area A), an area more likely to be impacted by re-suspended sediments than harvesting area C. The higher correlations between river flows and levels of *E. coli* in mussels from harvesting area B than those from other harvesting areas may be explained by the combined effect of water flows from the River Dart and a less significant watercourse discharging in the vicinity of the harvesting area.

In the middle reaches of the River Dart, water levels respond rapidly to rainfall resulting in short times to peak (typically less than 12 h) and high water levels, which fall quickly after the rainfall event has ceased. In the lower River Dart, flood peaks tend to be delayed, usually peaking between 12 and 24 h as a result of the lower topography of the catchment (Environment Agency 2004). This hydrological lag time would be added to the additional time between that point and the tidal limit, the time of travel of contamination within the tidal estuary, and the time for the uptake by the bivalves to reach a maximum to produce the maximum statistically significant correlations between rainfall and levels of *E. coli* in bivalves seen when the rainfall event occurred 72 h before the day of sampling. The fact that statistically significant correlations were obtained between rainfall in the upper catchment and microbiological contamination of bivalves suggests that a significant proportion of microbial contamination may have its origin in the upper reaches of the catchment.

Climate change projections for the UK assume changes in the proportion of rainfall received in winter relative to summer and, most significantly, an increase in the

proportion of winter rainfall that falls in five days or longer sequences of 'heavy' rain (Hulme *et al*. 2002). If these trends continue in the future, they are likely to modify the frequency of occurrence and magnitude of extreme levels of *E. coli* in bivalves. Sampling that targets hydrograph events may help competent authorities to better understand episodes of microbial contamination in bivalves, thereby reducing environmental uncertainty and define control measures necessary to maintain an adequate level of public health protection.

Finally, the present study evidences the contribution of sanitary surveys in informing management strategies to control the delivery of microbial contamination impacting bivalve mollusc harvesting areas. For example, control of the hydraulic characteristics of watercourses may be a feasible management strategy where it is not possible to relocate harvesting areas (Fiandrino *et al*. 2003). Measures proposed by the Environment Agency to manage flood risk in the Dart include improved flood mapping, development management, new flood warning and flood defence structures and maintenance of existing channels and defences (Environment Agency 2009). These could contribute to alleviate domestic flooding by rivers and help control delivery of diffuse microbial pollution to the River Dart.

CONCLUSIONS

Levels of rainfall and river flows were found to explain a very significant proportion of the spatial and temporal variations in the levels of *E. coli* accumulated by mussels and Pacific oysters commercially harvested in the Dart Estuary (England). A lag time (3–4 days) was detected in the response of *E. coli* contamination to the hydrological events, which could be explained by the time of travel between the gauging station and the tidal limit, the time of travel within the tidal estuary and the time for the uptake by the bivalves. The high correlations obtained for gauging stations in the upper catchment suggests that a significant proportion of microbial contamination accumulated by bivalves may have its origin in the upper reaches of the catchment. Sampling targeting hydrograph events may help authorities involved in official public health controls to better understand episodes of microbial contamination in bivalves.

ACKNOWLEDGEMENTS

This study was funded by the Food Standards Agency. We would like to thank Emily Allen, Peter Odling-Smee (Odling-Smee, Oberman and Associates Ltd.) and Richard Thain (Britannia Royal Naval College). Two anonymous reviewers provided helpful suggestions for improving the manuscript.

REFERENCES

Berg, D. E., Kohn, M. A., Farley, T. A. & McFarland, L. M. 2000 Multi-state outbreaks of acute gastroenteritis traced to fecal-contaminated oysters harvested in Louisiana. *J. Infect. Dis.* **181** (Suppl 2), S381–S386.

Berry, R. & Younger, A. 2009 Interspecies comparison of *E. coli* accumulation in bivalve shellfish using data obtained from official control monitoring under EU Regulation 854/2004. In *7th International Conference on Molluscan Shellfish Safety, Ifremer, 14–19 June 2009*, SYMPOScience.org, Proceedings Cemagref, Cirad, Ifremer, Inra, France.

Brock, R. L., Galbraith, G. R. & Benseman, B. A. 1985 Relationships of rainfall, river flow, and salinity to faecal coliform levels in a mussel fishery. *New Zeal. J. Mar. Fresh. Res.* **19**, 485–494.

Campos, C. J. & Cachola, R. A. 2007 Faecal coliform in bivalve harvesting areas of the Alvor Lagoon (Southern Portugal): Influence of seasonal variability and urban development. *Environ. Monit. Assess.* **133**, 31–41.

Cefas (Centre for Environment, Fisheries & Aquaculture Science) 2009 *Protocol for the Collection of Shellfish under the Microbiological Classification Monitoring Programme (EU Regulation 854/2004). England and Wales programme.* Available from: http://www.nrlcefas.org/content/statutory%20procedures/samplingprotocolforlocalauthorities.PDF (accessed 1 February 2011).

CFIA (Canadian Food Inspection Agency), Environment Canada (EC) & Department of Fisheries and Oceans (DFO) 2003 *Canadian Shellfish Sanitation Program: Manual of Operations*. Available from: http://www.inspection.gc.ca/english/fssa/fispoi/man/cssppccsm/cssppccsme.shtml (accessed 1 February 2011).

Chigbu, P., Gordon, S. & Strange, T. 2004 Influence of inter-annual variations in climatic factors on fecal coliform levels in Mississippi Sound. *Water Res.* **38**, 4341–4352.

Cluis, D. A. 1983 Visual techniques for the detection of water quality trends: Double-mass curves and CUSUM functions. *Environ. Monit. Assess.* **3**, 173–184.

Crowther, J., Kay, D. & Wyer, M. D. 2001 Relationships between microbial water quality and environmental conditions in

coastal recreational waters: The Fylde coast, UK. *Water Res.* **35** (17), 4029–4038.

Crowther, J., Kay, D. & Wyer, M. D. 2002 Faecal-indicator concentrations in water draining lowland pastoral catchments in the UK: Relationships with land use and farming practices. *Water Res.* **36**, 1725–1734.

Devon Wildlife Trust 2006 *A Profile of the River Dart catchment 2006*, 2nd edition. Devon Wildlife Trust, Exeter.

Environment Agency 2004 *South Devon Catchment Flood Management Plan.* Available from: http://publications.environment-agency.gov.uk/pdf/GESW1109BOUO-e-e.pdf (accessed 1 February 2011).

Environment Agency 2007 Directive (79/923/EEC) on the quality required of Shellfish Waters, Article 5 Programme, Dart. Environment Agency programme in accordance with paragraph 6 of the surface waters (Shellfish) Directions 1997, for the purposes for giving effect to Article 5 of the Shellfish Waters Directive 2006/113/EC.

Environment Agency 2008 Directive (79/923/EEC) on the quality required of Shellfish Waters, Article 5 Programme, Dart. Environment Agency programme in accordance with paragraph 6 of the surface waters (Shellfish) Directions 1997, for the purposes for giving effect to Article 5 of the Shellfish Waters Directive 2006/113/EC.

Environment Agency 2009 *South Devon Catchment Flood Management Plan: Summary Report December 2009*. Available from: http://publications.environment-agency.gov.uk/pdf/GESW1109BOUO-e-e.pdf (accessed 1 February 2011).

EU Working Group on the Microbiological Monitoring of Bivalve Mollusc Harvesting Areas 2007 *Microbiological Monitoring of Bivalve Mollusc Harvesting Areas, Guide to Good Practice: Technical Application*. Cefas - European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs. Issue 3.

European Communities 2004 Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. *Off. J. Eur. Communities* **L226**, 25.06.04, 83–127.

European Communities 2005 Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Communities* **L338**, 22.12.05, 1–26.

Evans, C. D., Cooper, D. M. & Gannon, B. 2001 A novel method for mapping critical loads across a river network: Application to the River Dart, southwest England. *Water Air Soil Poll.* **1**, 437–453.

Fiandrino, A., Martin, Y., Got, P., Bonnefont, J. L. & Trousselier, M. 2003 Bacterial contamination of Mediterranean coastal seawater as affected by riverine inputs: Simulation approach applied to a shellfish breeding area (Thau lagoon, France). *Water Res.* **37**, 1711–1722.

Halcrow Group Ltd. 2002 *Futurecoast*. A software from the Department of Environment, Food and Rural Affairs (Defra), UK.

Hulme, M., Jenkins, G. J., Lu, X., Turnpenny, J. R., Mitchell, T. D., Jones, R. G., Lowe, J., Murphy, J. M., Hassell, P., McDonald, R. & Hill, S. 2002 *Climate Change Scenarios for the United Kingdom: The UKCIP02 Scientific Report*. Tyndall Centre for Climate Change Research, School of Environmental Sciences, University of East Anglia, Norwich, UK. Available from: http://www.ukcip.org.uk/index.php?id=353&option=com_content&task=view (accessed 1 February 2011).

Imray, Laurie, Norie and Wilson Ltd 2008 *Devon and Dorset Coasts, 2300-9: River Dart*. Imray, Norie and Wilson, St Ives, UK.

ISO (International Organisation for Standardization) 2004 *Draft ISO TS 16649–3. Microbiology of Food and Animal Feeding Stuffs - Enumeration of β-Glucuronidase Positive Escherichia coli - Part 3: Most Probable Number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide Acid*. ISO, Geneva.

Jamieson, R., Gordon, R., Joy, D. & Lee, H. 2004 Assessing microbial pollution of rural surface waters: A review of current watershed scale modeling approaches. *Agric. Water Manage.* **70** (1), 1–17.

Kay, D., Wyer, M., Crowther, J., Stapleton, C., Bradford, M., McDonald, A., Greaves, J., Francis, C. & Watkins, J. 2005 Predicting faecal indicator fluxes using digital land use data in the UK's sentinel Water Framework Directive catchment: The Ribble study. *Water Res.* **39**, 3967–3981.

Lees, D. 2000 Viruses and bivalve shellfish. *Int. J. Food Microbiol.* **59**, 81–116.

Lee, R. J. & Morgan, O. C. 2003 Environmental factors influencing the microbiological contamination of commercially harvested shellfish. *Water Sci. Technol.* **47** (3), 65–70.

Mallin, M. A., Ensign, S. H., McIver, M. R., Shank, G. C. & Fowler, P. K. 2001 Demographic, landscape, and meteorological factors controlling the microbial pollution of coastal waters. In: *The Ecology and Etiology of Newly Emerging Marine Diseases, Hydrobiologia* (J. W. Porter, ed.). Kluwer Academic Publishers, The Netherlands.

Marlow, R. 2006 *The Use of GIS Risk Maps to Identify Potential Sources of Diffuse Pollution in the Dart Catchment*. Devon Wildlife Trust, The Dart Catchment Project. Available from: http://www.devonwildlifetrust.org/index.php?section=champions:cycleau:downloads (accessed 2 February 2011).

Martinez-Urtaza, J., Saco, M., de Novoa, J., Perez-Piñeiro, P., Peiteado, J., Lozano-Leon, A. & Garcia-Martin, O. 2004 Influence of environmental factors and human activity on the presence of *Salmonella Serovars* in a marine environment. *Appl. Environ. Microbiol.* **70** (4), 2089–2097.

Mawdsley, J. L., Bardgett, R. D., Merry, R. J., Pain, B. F. & Theodorou, M. K. 1995 Pathogens in livestock waste, their potential for movement through soil and environmental pollution. *Appl. Soil Ecol.* **2**, 1–15.

McGonigle, D. 2008 *Targeting Catchment Sensitive Farming Resources: A review of methods used in the Dart Catchment Project*. The Wildlife Trusts, Devon.

Nicholls, K. H. 2001 CUSUM phytoplankton and chlorophyll functions illustrate the apparent onset of dreissenid mussel impacts in Lake Ontario. *J. Great Lakes Res.* **27** (4), 393–401.

Pommepuy, M., Hervio-Heath, D., Caprais, M. P., Gourmelon, M., Le Saux, J. C. & Le Guyader, F. 2006 Fecal contamination in coastal areas: An engineering approach. In: *Oceans and Human Health: Pathogens in the Marine Environment* (S. Belkin & R. Colwell, eds.). Springer, New York.

Pommepuy, M., Le Guyader, F. S., Le Saux, J. C., Guilfoyle, F., Doré, B., Kershaw, S., Lees, D., Lowther, J. A., Morgan, O. C., Romalde, J. L., Vilariño, D. & Roque, A. 2008 Reducing microbial risk associated with shellfish in European countries. In: *Improving Seafood Products for the Consumer* (T. Børresen, ed.). CRC Press, Cambridge, UK.

Solo-Gabriele, H. M., Wolfert, M. A., Desmarais, T. R. & Palmer, C. J. 2000 Sources of *Escherichia coli* in a coastal subtropical environment. *Appl. Environ. Microbiol.* **66** (1), 230–237.

Thain, R. H., Priestley, A. D. & Davidson, M. A. 2004 The formation of a tidal intrusion front at the mouth of a macrotidal, partially mixed estuary: A field study of the Dart estuary. *Estuar. Coast. Shelf Sci.* **61**, 161–172.

Uncles, R. J., Stephens, J. A. & Smith, R. E. 2002 The dependence of estuarine turbidity on tidal intrusion length, tidal range and residence time. *Cont. Shelf Res.* **22**, 1835–1856.

USFDA (United States Food and Drug Administration) & ISSC (Interstate Shellfish Sanitation Conference) 2007 *National Shellfish Sanitation Programme, Guide for the Control of Molluscan Shellfish*. United States Food and Drug Administration, Interstate Shellfish Sanitation Conference, 2007 Revision. Available from: http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FederalStatePrograms/NationalShellfishSanitationProgram/default.htm (accessed 2 February 2011).

Willems, P. 2004 *WETSPRO: Water Engineering Time Series PROcessing Tool. Methodology (Short Reference Manual), Version 2.0*. Katholieke Universiteit, Leuven, Belgium.

Willems, P. 2009 A time series tool to support the multi-criteria performance evaluation of rainfall-runoff models. *Environ. Modell. Softw.* **24**, 311–321.

Younger, A. D., Lee, R. J. & Lees, D. N. 2003 Microbiological monitoring of bivalve mollusc harvesting areas in England and Wales: Rationale and approach. In: *Molluscan Shellfish Safety* (A. Villalba, B. Reguera, J. L. Romalde & R. Beiras, eds.). Consellería de Pesca e Asuntos Marítimos de Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela, Spain.

First received 27 July 2010; accepted in revised form 25 November 2010. Available online 25 April 2011