Review of current evidence to inform selection of environmental predictors for Active Management Systems in classified shellfish harvesting areas

FSA Project FS103001

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Non-Technical Summary

This study reviewed current evidence to inform selection of environmental predictors for Active Management Systems in classified shellfish harvesting areas.
The aims of this study were to: (1) undertake a literature review of the factors that influence faecal contamination of shellfish; (2) Establish relationships between *Escherichia coli* (*E. coli*) concentrations in rivers and shellfish in UK waters and select sites for further analysis; (3) Evaluate economic impacts of shellfish bed closures.

Review

An initial literature review identified factors that may contribute to accumulation of *E. coli* in shellfish. These included: rainfall, sewage discharges, agricultural discharges, wildlife, including birds, catchment land use, catchment topography, soil characteristics and 'flashiness'.
Other factors affect *E. coli* persistence in water. These include: solar radiation, temperature, salinity, pH, sediments and flocs. with most factors appearing to have the potential to influence *E. coli* concentrations in shellfish. Shellfish species show different rates of accumulation and depuration of *E. coli*.

Analysis

Analysis was undertaken on 12 shellfish harvesting areas for this report: Barrow, Blackwater, Burry, Conwy, Crouch, Fal, Helford, Menai East, Poole, North Kent, Taw-Torridge and Wash.
At a catchment level, higher concentrations of *E. coli* in shellfish were associated with the proportion of improved grassland in the catchment, high turbidity in main input rivers, river flow, and the size of the catchment.
Analysis of temporal data showed a weak association between *E. coli* in shellfish and river flow over time. Best fit to the statistical models occurred when river flow incorporated only a short (0 or 1 day) lag time.
There was high variability in bacterial loadings among individual monitoring points within estuaries, and the relative loading of individual monitoring points over time.
Across all estuaries, *E. coli* levels were not correlated with predicted loads from Sewage Treatment Works within a 1 km radius.
However, analysis on the Conwy found a positive association between rainfall, Combined Sewage Overflows (CSOs) and E. coli in shellfish. E. coli levels were higher if the CSOs had been active the previous week.
Particle track models were useful to assess where shellfish beds were in relation to risk from rainfall and CSO events.

Economics

Our analysis suggests that small enterprises could withstand a decrease in profits (i.e. shellfish bed closures) for about 4 weeks while medium enterprises could potentially endure 6 weeks. However, further research is required, as this was a relatively small study and aspects such as buyer/retailer behavior, elasticity of mussels or oyster prices were not considered.

Recommendations

In order to set up a trial AMS scheme, this report recommends:
Selection of 2 to 4 catchments covering variations in catchment type and *E. coli* loadings. Initial suggestions include:

- The Conwy, a relatively clean site where significant analytical infrastructure, monitoring and data already exists and
- The Fal which demonstrates consistently high *E. coli* loadings.

Monitoring and sampling should be conducted over a 2-3 year period, to account for variability in weather patterns over time. Monitoring should include the measurement and data collation of the following:

- Characterisation of catchment type and land use, rainfall, river flow, turbidity

- Monitoring and instrumentation of CSO operation (event time and volume)
- Water, shellfish and sediment microbiological samples, nitrogen
- Estuarine characteristics and processes such as tides, wind direction, temperature, salinity, bathymetry/Lidar data.

Routine sampling of *E. coli* levels and key explanatory variables should occur at least every two weeks and include enhanced sampling frequency during weather events expected to lead to increased risk of E. coli contamination, in order to fine-tune our understanding of i) the triggers for such events, and ii) the recovery time following an event.

The project should involve multiple partners, be multidisciplinary and build on the work undertaken in this desk study.

Executive summary

Shellfish production represents an important economic growth sector for the UK, with the Government encouraging industry to double production over the next few years. However, the sustainability of shellfish aquaculture is highly dependent on maintaining clean and healthy coastal waters, as microbial water quality and its relationship with pathogen load in shellfish is of particular importance with regards to protecting public health. Currently, classification and closure of shellfish beds is controlled by the Food Standards Agency (FSA) following advice from CEFAS. Areas with high microbiological contamination can be closed and remain closed for extended periods of time to protect public health. This results in an economic loss to industry. Shellfish bed closures, however, do not fully protect human health as they can only be triggered once shellfish testing has taken place. An alternative, more science-led approach, using all the available site information, is therefore required to replace the current system.

An alternative strategy is the introduction of an Active Management System (AMS) which would use environmental indicators to predict increased risk of faecal pollution. This would provide the potential for more responsive management including pre-emptive closure of shellfish areas during periods of elevated risk and more rapid re-opening after indicators have returned below threshold levels.

The aims of this study were to: (1) undertake a literature review of the factors that influence faecal contamination of shellfish; (2) Establish relationships between *Escherichia coli* (*E. coli*) concentrations in rivers and shellfish in UK waters and select sites for further analysis; (3) Evaluate economic impacts of shellfish bed closures.

__1. Literature review:__ Critical analysis of the sources of faecal indicator bacteria and *E. coli* in the terrestrial environment and subsequent transport to shellfish beds identified a range of factors which may contribute to the accumulation of *E. coli* in shellfish. These include rainfall, sewage discharges, agricultural discharges, wildlife, including birds, and catchment land use. Also included were catchment characteristics such as catchment topography, soil characteristics and how fast a catchment responds to rainfall or ‘flashiness’.

The review of *E. coli* concentrations and persistence in water and subsequent transport to shellfish assessed the importance of solar radiation, temperature, salinity, pH, sediments and flocs with most factors appearing to have the potential to influence *E. coli* concentrations in shellfish. This was also the case when assessing factors affecting uptake and depuration of *E. coli* in shellfish, although there were differences between the response of different shellfish species.

__2. Relationships between E. coli concentrations in rivers and shellfish in UK waters:__ Analysis of 12 harvesting areas (Barrow, Blackwater, Burry, Conwy, Crouch, Fal, Helford, Menai East, Poole, North Kent, Taw-Torridge and Wash**)** was reduced to 10 sites following analysis with the removal of Barrow and Menai East. Analysis demonstrated a weak relationship between river flow and *E. coli* accumulation in shellfish. There was evidence to suggest that generally either zero lag or a lag phase of 1 day between river flow and shellfish contamination show best fit to statistical models, compared with longer lag times (up to 7 days).

Further detailed temporal analysis for the Conwy river using rainfall data and combined sewer overflow (CSO) data from Llanrwst suggested a likely association between CSO operations, rainfall and shellfish *E. coli* data particularly when the CSO had been active the week prior to shellfish samples being taken.

Commercial shellfish harvesting areas are mostly associated with small catchments, with the exception of the Wash. When investigating shellfish *E. coli* contamination from each representative monitoring point (RMP) and the nearest sewage treatment works (STW), it was apparent that areas such as Blackwater, the Fal and Taw/Torridge were subject to high *E. coli* loadings. When considering spatial associations, the Barrow, Blackwater and the Wash were considered potentially be at risk from STWs within a 1 km radius. However,

following statistical analysis, it was concluded that STWs operating under 'normal operating conditions' do not pose a high faecal risk to shellfish in the studied estuaries.

Two dimensional particle track modelling was undertaken for the Conwy estuary and concluded that the location of the shellfish beds at the bottom of the Conwy made them vulnerable to contamination from high rainfall events and the activation of CSOs. The 'flashiness' of a catchment which gives an indication of runoff from the land and the response of rivers to a rain event was also investigated for the 12 UK harvesting areas. Disappointingly, however, the lack of available data to parameterise the models meant that this did not yield useful information. Estimates of how fast these estuaries respond to rain events, however, could be included into the model at a later date.

The effects of environmental factors on *E. coli* contamination in shellfish (90%ile over 2000-2017) were characterised by fitting a General Linear Mixed Effects Model with binomial error distribution to the data. The final predictive model demonstrated highly significant positive relationships between *E. coli* in shellfish with flow, turbidity and catchment area × percent improved grassland. In addition, mussels and oysters demonstrated different responses to these explanatory variables, but only in certain estuaries such as the Blackwater and Kent.

The various modelling approaches and scales undertaken in our study revealed three main points that determine how an AMS tool might be developed and deployed:

1. At a catchment level, certain characteristics led to higher concentrations of *E. coli* in shellfish. These are: proportion of improved grassland in the catchment and high turbidity in main input rivers; although a number of other variables are correlated with these, such as rainfall and flow.
2. Within an estuary, there is high variability in bacterial loadings at individual monitoring points. This variability may be down to a number of factors, partly governed by complex flow pathways within estuaries of water on ebbing and flooding tides, and water residence times within the estuary. Proximity to routine STW discharge points is not a risk factor.
3. Analysis of temporal data shows there are weak positive relationships with river flow, and stronger positive relationships with CSO events. However, *E. coli* levels at individual beds still show high variability which is not easy to predict.

Therefore, we suggest that catchment level characteristics can be used to broadly predict which estuaries may be at higher risk. Where those estuaries contain CSOs, this leads to increased risk particularly in association with rainfall events.

3. Economic impacts of shellfish bed closures: Several caveats must be taken into account when any economic considerations are discussed with reference to businesses and their potential loss of earnings, not least the small number of respondents to a survey, which adds further caution to any analysis. There can be changes in buyer/retailer behavior, elasticity of mussels or oyster prices and enterprises sell at different times of the year with some more affected if closure of shellfish areas occurs when sales are at their peak. Our analysis suggests that small enterprises could withstand a decrease in profits (i.e. shellfish bed closures) for about 4 weeks while medium enterprises could endure 6 weeks. Of note, however, is that during closures the buyers further up the supply chain may have sought product elsewhere and may not necessarily return once the beds re-open. An economic impact would therefore be critical area for further research in any pilot trial.

The impact of closures of shellfish areas on environmental effects have received little attention with the economic and social aspects of far greater concern to the industry. Shellfish harvesting, however, is directly related to environmental variables including temperature, length of daylight and height of low tide. Closure of shellfish harvesting areas for any significant amount of time may actually have ecological benefits although holding a larger amount of biomass on the harvesting areas would have to be managed correctly. It is possible that the loss of a fishery due to closure may have more of an impact in certain areas where currently shellfish seed is relayed and grown leading less shellfish and impacts on water quality, biodiversity, birds and other ecosystem services including tourism.

Recommendations

Overall there remains considerable uncertainty surrounding the flow of microbial contaminants from agricultural catchments through to the coastal zone. This currently limits the implementation of effective mitigation measures and the formulation of robust policies and legislation to protect human health and the wider environment. Additional research is therefore required to disentangle the complexity of bacterial, and other interactions along freshwater–saline gradients.

Recommendations for pilot test catchments would include analysis of 2 to 4 catchments covering variations in catchment type and *E. coli* loadings. The Conwy is a good candidate, as a relatively clean site where significant analytical infrastructure, monitoring and data already exists. The Fal demonstrates consistently high *E. coli* loadings, with potential links to rainfall, and is also a good candidate estuary. Monitoring and sampling should be conducted over a 2-3 year period, to account for variability in weather patterns over time.

The project should involve multiple partners, be multidisciplinary and build on the work undertaken in this desk study. FSA in conjunction with the water companies, shellfishermen, Councils (including LAGs), Public health officials, NRW/EA and other stakeholders should be included and in many cases could contribute to monitoring and data collection.

Monitoring should include the measurement and data collation of the following:
Characterisation of catchment type and land use, rainfall, riverflow; monitoring and instrumentation of CSO operation (event time and volume); water, shellfish and sediment microbiological samples, turbidity, nitrogen; estuarine characteristics and processes such as tides, wind direction, temperature, salinity. Additional data such as bathymetry/Lidar data may be required.

Routine sampling of *E. coli* levels and key explanatory variables should occur at least every two weeks and include enhanced sampling frequency during weather events expected to lead to increased risk of E. coli contamination, in order to fine-tune our understanding of i) the triggers for such events, and ii) the recovery time following an event.

Contents

1 Introduction

Shellfish represent an important growth sector in the UK, with aquaculture predicted to more than double over the next few years as encouraged in the United Kingdom Multiannual National Plan for the Development of Sustainable Aquaculture. The current value of UK shellfish aquaculture is estimated at c. £19.1 million. Improving the balance of environmental, societal and economic sustainability therefore presents a future challenge in light of issues such as climate change and the protection of public health.

The sustainability of shellfish aquaculture is highly dependent on maintaining clean and healthy coastal waters and microbial water quality is of particular importance, especially with regard to minimising risk of transfer of human pathogens to consumers. Currently, classification and closure of shellfish beds in England and Wales is based on periodic sampling of shellfish, usually by local authority representatives. Samples are tested for levels of the faecal bacteria *Escherichia coli* (*E. coli*) and faecal coliforms (FC) with results transferred from CEFAS to the Food Standards Agency (FSA) with advice on the appropriate classification. High levels of bacterial contamination can cause the closure of shellfish harvesting areas for extended periods of time to ensure the protection of public health (i.e. from food poisoning). This can have potentially detrimental socio-economic effects on commercial shellfish companies.

Active Management Systems (AMS) offer an alternative means of shellfisheries regulation based on the use of environmental parameters to predict the timing and location of elevated levels of faecal pollution, to inform decision thresholds for preventative closure of shellfish beds showing a high probability of contamination by faecal pollution. Beds would re-open and harvesting resume once the elevated levels had returned to ‘background’ levels, as determined by routine monitoring and end-point testing of shellfish to enable adaptive management of the shellfishery, and a possible reduction in the periods of closure. This fits with the FSA’s strategy of delivering risk-based controls whilst ensuring public health protection, and has the potential to deliver a more holistically sustainable system of regulation.

Preliminary evidence has indicated that elevated *E. coli* levels can be linked to physio-chemical factors such as suspended particulate matter, nutrients, rainfall, tidal movements, seasonal variations, temperature, UV and salinity. In addition, catchment characteristics including land use, diffuse and point sources of pollution and number of Sewage Treatment Works (STWs) and Combined Sewer Overflows (CSOs) all contribute to potential elevated *E. coli* levels. However, in order to develop a ‘predictive model’ for any particular catchment, clarification is required regarding ‘trigger’ thresholds (e.g. by rainfall, catchment and other conditions which have been shown to predict increases in pathogen load), interactions between environmental variables, and the impact of shellfish physiological characteristics on *E. coli* uptake and clearance in the presence of contamination. Within a UK setting, active management is likely to encompass the linking of environmental statistical models to physical estuarine/coastal and shellfish physiological parameters.

1.1 Project aims

This project aims to address the need to develop and validate an Active Management System (AMS) that demonstrates the use of predictive environmental indicators for elevated faecal indicator organisms such as *E. coli* in shellfish waters and shellfish. The study will assess the use of AMS in allowing commercial shellfisheries to be closed during times of elevated faecal pollution, and then rapidly re-opening for harvesting once elevated levels have returned to ‘normal’ levels.

The project objectives are as follows:

1. To review available evidence on the role of environmental factors influencing *E. coli* contamination in shellfish in relation to rainfall events, and to highlight knowledge gaps;
2. To identify and collate microbiological and environmental data from a variety of sources;
3. To analyse available data to determine statistical relationships to underpin an AMS;

4. To propose methodology for a ‘tool’ to analyse incoming data against predicted models of elevated faecal pollution;
5. To produce a list of sites for use in a ‘pilot’ study of AMS;
6. To make recommendations on possible sampling frequency and methodology for the field study sites to include species specific consideration;
7. To assess environmental, economic and societal impacts of AMS;
8. To produce a report that reviews current evidence to devise criteria for the selection and environmental, economic and financial assessment of active management systems in classified shellfish harvesting areas;
9. To deliver the main findings and recommendations to the ‘Project Team’.

2 Review of available evidence

2.1 Methods

Scope
Geographic range: Information from both UK and international studies (including from the EU) was included. The primary focus was on UK-based studies, although studies from across the EU and other geographically similar locations were included where appropriate.
Time period: No time restrictions were placed on searches in terms of year produced.
Language: Literature reported in English was prioritised, due to the time constraints of reporting needs. Relevant non-English literature was translated and included where possible.

Methods
Literature to be included: Articles and information were included from both published and ‘grey’ literature. Much of the information available was from commissioned reports to government agencies and NGOs.
Initial searches were conducted by the primary author, using the search facilities in ISI Web of Science, ISI Web of Knowledge, JSTOR, ScienceDirect, and government or regulatory websites such as UK Department for the Environment, Food and Rural Affairs (DEFRA), UK Food Standards Agency (FSA), UK Environment Agency (EA), and Natural Resources Cymru (NRW), as well as worldwide web searches using the ‘Google’ search engine.
Additional references, particularly for the grey literature, were taken from the reference list of primary studies, industry partners and relevant bodies contacted directly for information. These included: UK FSA, DEFRA, EA, NRW, UK Water Companies (Dwr Cymru, Anglian Water, United Utilities, UKWIR), SEAFISH, and Shellfish Association of GB.
Key search terms: The key search terms for the review included ‘shellfish’, and ‘rainfall’, ‘faecal’ or ‘fecal’ ‘coliforms’ and ‘*E. coli*’.
Inclusion criteria: The first 100 articles to be listed in each search were checked for relevance, and further filtered using combinations of additional search criteria such as, but not limited to, ‘marine’, ‘persistence’, ‘coastal’, ‘estuarine’, ‘environment’, ‘faecal indicator organisms (and combinations of)’ and ‘nutrients’. Abstracts and the main text were then examined for relevance for further inclusion in the review.

2.2 Results

2.2.1 Review statistics

The search was conducted in February 2017. A search library of 530 articles was returned from application of the search method (after removal of duplicate items), of which several articles met the inclusion criteria for review of extracts or the full text (Table 1.2.1.). No articles could not be sourced within the time available for review completion. Evaluation of the remaining articles excluded several articles that did not meet the search criteria (Table 2.2.1.); 108 articles were subsequently included in the review.

Table 2.2.1. Articles excluded from further evaluation in the review

Exclusion criteria	Number of articles
Duplicate item	27
Initial search of titles: spurious reference	63
Not in English, and no translation was available	0

2.2.2 Shellfish site classification in England and Wales

Currently, two classification systems are in place in England and Wales [1]:

1. The annual ('temporary') classification
2. The long-term classification (LTC)

For new shellfish harvesting and relaying sites, after the preparation of the Sanitary Survey (SS) and sampling plan, and a minimum of three months initial sampling, an initial annual/temporary classification is assigned until the site meets the criteria for an LTC. Additionally, harvesting sites failing to meet the LTC criteria are also automatically assigned an annual/temporary classification.

2.2.2.1 Basis for annual classification

Classification is based on *E. coli* contamination levels enumerated from shellfish samples harvested from appropriate designated representative monitoring points (RMPs), allocated in the classification zone Sanitary Survey. Selected points are designed to incorporate the full extent of the shellfishery, accounting for variations in tidal flows, locations of local pollutant sources, and other relevant factors [1]. Classification categories (based on the requirements of EC Regulation 854/2004) are shown in Table 2.2.2.

Table 2.2.2: Classification criteria for shellfish beds in England and Wales

Class	*E. coli* concentration	Treatment
A	≤ 230 *E. coli*/100g	Molluscs can be harvested for direct human consumption
B	90% of samples must be ≤ 4600 *E. coli*/100g; AND all samples must be less than 46000 *E. coli*/100g	Molluscs can be sold for human consumption: after purification in an approved plant; OR after re-laying in an approved Class A re-laying area; OR after an EC-approved heat treatment process
C	≤ 46000 *E. coli*/100g	Molluscs can be sold for human consumption only after re-laying for at least two months in an approved re-laying area followed, where necessary, by treatment in a purification centre, or after an EC-approved heat treatment process
Prohibited	> 46,000 *E.coli*/100g	

Adapted from: [1]

In addition to prohibition at *E. coli* values > 46,000/100g flesh, Annex II Chapter IIC of the Regulation allows closure of beds if the competent authority anticipate a risk to human health, regardless of the initial bed classification [2].

Seasonal classifications are permitted in England and Wales where at least two years' worth of data showing a clear seasonal trend is available, and where sample results within a designated transition period as well as during the designated 'active season' are compliant with the relevant (higher class) *E. coli* level classification [3].

2.2.2.2 *Basis for long-term classification (LTC)*

Proposals for LTC were introduced following a review of classification in 2002 and a public consultation in 2004 [3]. Subsequently, the LTC system has been implemented in England and Wales since 2nd May 2006, in addition to the existing annual (temporary) classification, aiming to respond pre-emptively to potential risks to human health, and provide a more stable method of classifying and maintaining shellfish beds in the UK. Designation as LTC last for five years (FSA, 2004). LTC is a statistical system classifying shellfish beds in England ~~and~~ Wales based on shellfish hygiene data trends for individual beds [4]. Eligibility is dependent on the following criteria ([1]):

- Minimum 40 results available over the previous five years;
- Minimum 90% compliance with 4,600 *E. coli*/100g;

Additionally, sites are not recommended for LTC where:

- They conform to LT class B, but show annual class C or seasonal B/C at the initial formal review;
- They conform to LT class B but which have returned prohibited level results (unless the result was waived due to it being associated with an exceptional event);
- They met the nominal compliance criteria, but were declassified with notes 2 and 7.

Certain other circumstances may also impact eligibility, after investigation by the FSA[1]:

- Possible inclusion: where known improvements have been made to sewage discharges, and available data can demonstrate a significant improvement in underlying water quality over a three year period;
- Possible exclusion/loss of status: where there is a persistent deterioration in water quality, and it impacts on E. coli threshold compliance;
- Possible opt out: where there have been a mixture of class A and B results over the previous five years, and harvesters wish to maintain some annual class A sales rather than include all years under class B sales.

Initial implementation was planned for class B beds only, since no class A or C beds within England and Wales met eligibility criteria at the time of initial implementation (i.e. could not provide a sufficient period of stable data). However, it was anticipated that other sites may have become eligible during that period, so a formal review was planned for two years into the five year period [5]. Meanwhile, all class A and C beds, and ineligible class B beds would continue to be classified on an annual basis, with a view to including as many beds as possible under an LT classification in the longer term, subject to eligibility (reviewed annually). Details in CEFAS [1] indicate that since this initial decision was made, A and C have not yet been included under a similar LTC tiered system to class B, but that this may still be subject to review in future should the efficiencies and benefits of the class B scheme be potentially transferable to other classes.

2.2.2.3 *Implementation of LTC*

Whereas the response to potential pollution incidents under the previous single annual classification was conducted on an ad hoc and reactive basis, the LTC was designed to employ a rapid response mechanism whereby certain thresholds of *E. coli* contamination within the class B bracket would trigger a series of investigations and control measures. The three proposed tiers of response (currently, for Class B only) are detailed in Table 2.2.3.

Table 2.2.3: Proposed implementation of LTC in England and Wales

Tier	Trigger value	Response	Further measures
First (Initial Investigations)	4,600 – 10,000 *E. coli* / 100 g flesh	Verification of results, followed by statistical assessment by CEFAS to determine if a significant change in the general level of contamination has occurred. The Local Authority and Environment Agency would be responsible for investigating the cause of the contamination.	Closure or downgrading unlikely. Increased sampling frequency unlikely. No Action State triggered.

		No assistance from Local Action Group required.	
Second (Formal Investigations)	10,000 – 18,000 *E. coli* / 100 g flesh	Formal investigation to identify the cause of contamination. Involvement of the Local Action Group expected (using the pre-determined Local Action Plan procedure). Statistical assessment by CEFAS to determine if a significant change in underlying water quality has declined.	Closure or downgrading unlikely. Increased sampling frequency unlikely. No Action State triggered. Local Authority required to determine what control measures are needed.
Third (Formal Investigations leading to an Action State)	> 18,000 *E. coli* / 100 g flesh	An Action State is activated by the Local Authority, in place for up to 3 months. The Local Action Group is notified and implements the Local Action Plan to assist the Local Authority in providing appropriate control measures (implemented throughout the course of investigations). Statistical testing to determine whether there is a downward trend in water quality.	Closures and downgrades may be applied where appropriate. Additional sampling to be carried out to monitor and determine causes of high E. coli levels. Bed re-opening as soon as E. coli falls below legal limits. Downgrading prior to annual review in extreme cases.

Adapted from: FSA (2004); FSA (2006b).

2.2.3 Species of interest

Sixteen species of shellfish are farmed or harvested from the wild in England and Wales (Table 2.2.4). Recent estimates of production yield and economic value show that mussels and Pacific oysters are the most important commercial species produced.

Table 2.2.4: Farmed and wild shellfish species in England and Wales (2016)

Species	Common name
Abalone	*Haliotis tuberculata*
American Hard Clam	*Mercenaria mercenaria*
Cockle	*Cerastoderma edule*
Manila Clam	*Tapes philippinarum*
Mussels	*Mytilus* spp.
Native Oyster	*Ostrea edulis*
Pacific Oyster	*Crassostrea gigas*
Palourde	*Tapes decussatus*
Peppery Furrow Shell	*Scrobicularia plana*
Razor Clams	*Ensis ensis*
Sand Gaper	*Mya arenaria*
Scallop	*Pecten maximus*
Slipper Limpet	*Crepidula fornicata*
Tapes Clams	*Tapes* spp.
Thick Trough Shell	*Spisula* spp.
Venus Clams	*Veneridae* Family

Adapted from: CEFAS (2017);

2.2.4 Sources of E. coli in the environment and transport to shellfish growing areas

Key sources of *E. coli* and other faecal indicator organisms (FIOs) within catchments impacting upon shellfisheries include sewage discharges and agricultural activities, with further potential inputs from boating activity in coastal environments and tributary watercourses, and wild bird and mammal populations. The transport of faecal pollutants from source to shellfish beds is commonly triggered by rainfall events, with speed of transport and magnitude of impact on shellfish beds further influenced by a combination of catchment characteristics (hydrography, topography, geology, and land use types and distributions). The literature search revealed several recent reviews of environmental factors influencing faecal contamination of water and shellfisheries – material summarised therein is presented in the following sections, with secondary references provided where appropriate.

2.2.4.1 Rainfall

Rainfall is the environmental variable most often associated with peaks in *E. coli* concentrations in shellfish waters and shellfish [6-8]. Elevated levels of FIOs or *E. coli* in shellfish or coastal waters in UK studies have been detected at between one and seven days after a rainfall event ([7]; Table 2.2.5). Numerous authors have found that severity of pollution increases with rainfall magnitude, and seasonal patterns of faecal bacterial contamination to coastal waters or shellfish have commonly been attributed to differences in daily mean or peak rainfall, or cumulative rainfall [9-34]. However, eventual shellfish contamination levels may depend on highly localised rainfall distributions [35], or the presence of other sources of runoff such as surface springs [36]. The subsequent interactions of precipitation and runoff with hydrogeology, topography, river networks, and different land uses within the catchment containing potential FIO sources are also important, and may have a greater influence than rainfall [7, 23,37-39]. Indeed, some studies found no relationship, or only a weak relationship between rainfall intensity/timing and *E. coli* loadings, owing to stronger influences of other environmental variables [40-44]. Two studies observed negative relationship between FIO concentration and rainfall. Jin et al. [45] found a significant negative relationship ($p<0.05$) between rainfall persistence and shellfish *E. coli* concentrations over time, attributed to the growth of *E. coli* populations during sunny periods when water temperatures increased. A report also attributed higher FC counts found during shorter duration rainfall events to the flushing of accumulated pollutants not diluted by a longer period of precipitation[46]. Therefore, the balance between the impacts of rainfall timing, duration and intensity should be considered when predicting changes in FIO contamination levels. While the majority of correlations between rainfall variables and FIO concentrations have been positive in UK studies (Table 2.2.5), the presence of some negative or non-significant correlations suggest that other environmental factors are important in determining final shellfish contamination levels.

Rainfall events determine which principal sources of faecal pollution dominate shellfish bed contamination, since different types of pollutant are mobilised under base and high flow conditions[6]. The two principal sources of faecal contamination in the UK to shellfish beds are thought to be sewerage related sources, and agricultural runoff (Sections 2.2.4.2 and 2.2.4.3 respectively). Under base flow conditions, continuous-flow sewage effluents tend to dominate pollutant loadings to shellfish waters; whereas agricultural faecal pollutants (which tend to be deposited or applied to land rather than directly into watercourses) generally remain *in situ*. Under high-flow conditions (i.e. after a significant rainfall event), agricultural sources become relatively more dominant as surface runoff and sub-surface flows transport FIOs to watercourses. At high flow, intermittent sewage discharges can also play an important role (Section 2.2.4.2). An additional source of potential contamination mobilised during rainfall-initiated high river flows is the 'background' reservoir of faecal bacteria bound to sediments which are re-suspended in the water column when subject to faster flowing water[6] (Section 2.2.4.5).

Table 2.2.5: UK studies exploring the relationship between rainfall and faecal pollution of water or shellfish

Rainfall variables	Response variable	Response	Reference
Daily	EC concentration (mussels / Pacific oysters)	Highest concentration when rainfall > 2 mm, and 3-4 days after rainfall event Magnitude of response varied between species and sampling points	Campos et al. (2011)
Daily (1-7 days); cumulative (1-7 days)	EC concentration (native oysters)	Mostly positive correlations (2 of 2 sites, all days after rainfall); some significant ($p < 0.05$) between days 3 and 7	Campos et al. (2012a)
Daily (1-7 days); cumulative (1-7 days)	EC concentration (*Tapes* clams)	Mixture of positive and negative correlations (1 site); none significant ($p > 0.05$)	Campos et al. (2012a)
Daily (1-7 days); cumulative (1-7 days)	EC concentration (native oysters)	Positive correlations (2 of 2 sites, all days after rainfall); some sig. ($p < 0.05$) between days 1 and 7	Campos et al. (2012b)
Daily; cumulative (7 days)	EC concentration (native and Pacific oysters)	N.s. correlation with daily rainfall Significant positive correlations ($p < 0.01$) with 7-day rainfall, both for whole year and winter season datasets	Campos et al. (2017)
Daily	EC concentration (seawater)	Contamination followed 1-3 days after heavy rainfall event	CBBC (1959)

Daily	EC concentration (Pacific oysters)	Positive correlation (Spearman's *rho* = 0.3 to 0.5, $p < 0.05$) Consistently higher EC concentration 1-2 days after rainfall event; detectable elevated EC 7 days after rainfall event	CEFAS (2011)
Daily	EC concentration (mussels / Pacific oysters)	No significant relationship	Kay (2015)
Average (7 days)	EC concentration (Mussels)	Positive correlation during summer; highly significant at one site but coefficients varied between sampling sites	Magill et al. (2013)

EC = *E. coli*; FC = faecal coliforms. [1] Modelled; validated against Met Office data.

2.2.4.2 Sewage discharges

In the UK, under base flow conditions, urban sewerage-related sources dominate *E. coli* pollution to shellfish beds, since runoff from diffuse sources of pollution (agricultural runoff, small private septic tanks, bird and deer colonies) tends towards low levels in these conditions [47]. The size of facility (population served, effluent outflow volumes) partially determine its contribution to pollutant loads, with large wastewater treatment works (WwTWs or STWs) capable of contributing > 90% total faecal bacterial load to receiving waters [6].

Under high flow conditions, intermittent discharges from combined sewer overflows (CSOs) and storm tank overflows (STOs) dominate over STWs as the key source of human contamination to receiving waters [6, 48, 44]. Delivery of large *E. coli* loadings can be especially important during the initial period of discharge where contaminated sediments may be mobilised in addition to polluted water, a phenomenon known as the 'first flush' effect [8]. Private discharges from septic tanks can provide a substantial source of FIOs where there are many, poorly maintained tanks situated close to shellfish beds and draining (perhaps accidentally) directly into watercourses or into malfunctioning soakaways [34, 40, 41, 49, 50].

Bacterial loadings of sewage effluents typically differ according to the level of treatment they undergo before discharge to receiving waters [51-55]. In the UK, treatment ranges from preliminary screening (typically least effective at reducing bacterial loads) to tertiary treatment (typically most effective at reducing bacterial loads) (Table 2.2.6). Kay et al [52] compared effluent samples from 162 sewage discharge sites in the UK and Jersey, and found marked, statistically significant reductions in geomean FIO concentrations after secondary and tertiary treatment compared to primary treated sewage, as well as significant differences in final effluent FIO concentrations between some secondary and tertiary treatments. Some treatment facilities become less effective at removing FIOs under high-flow conditions (e.g. from intense rainfall inputs or seasonally increased sewage volumes due to tourism)[6; 56; 33; 57]. Several authors have demonstrated that reviewing and upgrading facilities in response to changes in rainfall and human population patterns has been effective at reducing FIO pollution to receiving waters over time [51; 7], however facility improvements do not always fully explain a decline in FIO levels (e.g. [58]). Intermittent (CSO) facilities with inadequate capacity may remain a particular contamination risk, since storm water can mix with overflowing untreated sewage and flow directly into environmental waters (e.g. [59; 48; 60]). Septic tanks may also be numerous within rural catchments. Frequently, these are located close to watercourses, however, their overall contribution to FIO loadings at the catchment scale remains poorly understood.

In some catchments, recreational boating activity is likely to be important in direct delivery of faecal bacteria to (or close to) shellfish growing waters, especially during summer and other peak holiday occasions [61; 41]. Overboard discharges from pleasure crafts are not prohibited in the UK, and the relatively small number of pump-out facilities for on-board toilets are poorly utilised [59]. Even within a small harbour under relatively stable meteorological conditions, Guillon-Cottard et al., [61] measured FC levels in nearby mussels exceeded 4,600 FC $100g^{-1}$ on ten occasions within a one-year period (sampled fortnightly, across three sampling points).

Table 2.2.6 Typical faecal coliform loadings of sewage effluents from UK sewage treatment facilities

Level of treatment	Specific effluent types	Base-flow geomean	High-flow geomean
Untreated (raw)	Crude sewage discharges, storm sewage overflows	1.7×10^{7}	2.8×10^{6} (2.5×10^{6} to 3.5×10^{6})

Primary	Primary settled sewage, stored settled sewage, settled septic tank	1.0×10^7 (5.6×10^6 to 1.8×10^7)	4.6×10^6 (8.0×10^5 to 5.7×10^6)
Secondary	Trickling filter, activated sludge, oxidation ditch, trickling/sand filter, rotating biological contactor	3.3×10^5 (1.6×10^5 to 4.3×10^5)	5.0×10^5 (1.3×10^5 to 6.7×10^5)
Tertiary	Reedbed/grass plot, UV disinfection	1.3×10^3 (2.8×10^2 to 1.3×10^4)	1.3×10^2 (3.6×10^2 to 1.5×10^4)

Adapted from: Kay et al. (64)

2.2.4.3 Agricultural discharges

Agricultural sources can contribute significant loads of FIOs to shellfish growing areas, following transportation overland and/or via watercourses to the tidal limit. Sources include direct deposition of faecal matter to land or watercourses by livestock; application of farm yard manure (FYM), slurries, sewage sludge, dirty water and irrigation water to land; and accidental spills or runoff from manure storage facilities and animal housing [62; 8].

Under high river flow conditions in the UK, runoff from diffuse pollution sources plays a greater role in the contamination of shellfish beds than under base flow conditions [6], although agricultural runoff does not always dominate over intermittent or diffuse sewage sources, even when agriculture represents a large percentage of the catchment area [63]. The main source of agricultural contamination in the UK is from grazed grassland (particularly improved grassland) [8, 64]. Kay et al. [64] compared geomean FC loadings in watercourses over a range of UK catchments under base and high flow conditions and estimated an almost 10-fold difference between loadings in predominantly (≥ 75%) improved pasture compared to predominantly (≥ 75%) rough grazing catchments. Particularly high FC concentrations have been observed in watercourses used as frequent livestock crossing points, which cattle may preferentially use for defecation (e.g. [62; 65]). Peaks in *E. coli* concentrations have also been observed in response to manure application, with the effect persisting in adjacent ditch water for several weeks after application [37]. Hodgson et al. [66] found a significant effect of dairy slurry application method (shallow injection > surface broadcast) and season (summer/autumn > spring) on slurry FIO persistence; half-life of *E. coli* varied from 6.4 to 34.1 days. Predominant livestock type impacts on expected contamination loads, with differences in typical daily faecal loads shed by a range of domestic farm animals varying from 2.4×10^8 *E. coli* d^{-1} (chickens) to 1.8×10^{10} (sheep) [65]. One study reviewed in Magill et al., [65] estimated a 4 to 8 fold difference in FIO concentrations between higher vs. lower animal stocking densities; these also varied seasonally and between sub-catchments.

The application of sewage sludge to land should pose a relatively small contamination risk in the UK: since 2006, regulations stipulate that only treated sludge (with a 2-log microbial reduction, applicable to a limited number of land uses) or 'enhanced' treated sludge (with a 6-log reduction, applicable to a range of land uses) may be applied to crop land [59]. Agricultural land uses not spread with manures or irrigated with contaminated water (e.g. many arable and horticultural areas) tend not to contribute to diffuse FIO contamination (e.g. [67]). Illegal spills of dirty water or slurries are hard to quantify, but are identified as a known problem in some catchments (e.g. [12]).

2.2.4.4 Other land use and land management considerations

In addition to sewerage facilities and agriculture, several other types of land use have been associated with elevated or diminished FIO loadings in shellfish growing areas ([64; 68; 8; 69]). Consideration is given here to the spatial extent and distribution of wild bird and mammal populations, dog walking routes, urban hard surfaces, forestry, and wetlands.

Diffuse FIO contamination originating from wildlife can be hard to quantify, since relevant populations may be widely dispersed and relatively more mobile compared to domesticated livestock, thereby increasing both spatial and temporal heterogeneity of faecal loadings. Nevertheless, several studies identified in the literature review have attempted to attribute faecal contamination within catchments to various animal and/or human sources, although in some cases this is a qualitative assumption based on unexplained residual 'background' pollution remaining after deducting known human and domesticated animal FIO sources (e.g. [70-72]). Where quantitative source apportionment has been attempted, the importance of wildlife sources varies between studies. Hagedorn et al. [41] estimated that after human FIO sources, wild birds dominate faecal pollution in two US tidal creeks, followed by livestock, pets and wildlife. Isolated FCs [46] from a US nature reserve, originating from birds most frequently, followed by wild dogs, rodents and horses. Connell Jr. et al.[16] found that unspecified wildlife sources dominated in frequency over both domestic animal and human waste sources in a US river catchment.

Commonly, birds are identified as an important potential source of faecal contamination to shellfisheries [41; 46; 30; 72]. Risk is likely to vary seasonally, particularly in the case of migratory birds. Birds either reside on shore close to shellfisheries, or directly defecate into shellfish growing waters. Ultimately, impacts depend on the location of colonies relative to shellfish beds, total numbers of birds, and species present (as different bird species typically vary in their FC shedding, [8]). Impacts may be substantial, with 100 gulls estimated to be capable of shedding FIO loadings equivalent to a secondary-treated WwTW serving 10,000 population equivalents; and a variety of wild birds recorded shedding up to 10^9 thermotolerant coliforms per day [65].

Risks of contamination from other wildlife are not widely explored in the literature. Potential sources in the UK include marine mammals (e.g. seals), as well as a wide range of terrestrial mammals (e.g. deer, rodents, foxes, badgers) (e.g. [8]). Popular dog walking routes may also provide a source of FIOs, particularly when adjacent to watercourses or shellfish growing areas.

Knowledge of the extent of urban land cover is important not only in estimating sewage discharge impacts (Section 2.2.4.2), but also in determining the total area of impermeable surface within a catchment. While the exact empirical relationship between percentage impervious surface in a catchment and risk of contamination to shellfish production areas varies in the literature, it has been suggested that catchments with >10% impermeable surfaces are subject to periodic peaks in microbial pollution [6; 12].

Land uses implicated in reducing the risk of FIO contamination to shellfish production areas include forestry and wetlands. A comparison of FC data from 15 catchments across Great Britain by Kay et al. [64] indicated that low geomean FC concentrations were associated with catchments containing a large proportion of upland coniferous forest compared to catchments containing larger areas of urban and improved pasture land uses. However, in some cases forestry harvesting activity may contribute to FIO retention in watercourses due to potentially large quantities of sediment runoff, which faecal bacteria can subsequently adsorb to [8]; Section 2.2.4.5), or by harbouring deer populations. Wetland areas are associated in a number of studies with reducing FIO loadings to watercourses and shellfish growing areas, most typically where they occur in the lower reaches of a catchment, where they act as a buffer and natural purification system (e.g. [23]). However, they may also harbour large bird populations, acting in some cases as both a FIO source and sink (e.g. [73]; [31]).

2.2.4.5 Catchment physical characteristics

Catchment topography, geology and soil characteristics all influence the rate of transport of FIOs to watercourses under both base and high flow conditions [7; 8].

Catchment topography impacts on the variation seen in lag times of microbial contamination in shellfish [58; 6]. Compared to shallower slope profiles, steeper slopes increase water and sediment velocity, resulting in a greater concentration of FIOs further downstream after rainfall [74]. Catchment size impacts on FIO accumulation and survival: all other things being equal, increased catchment size is more likely to correlate with an increased number of sewage sources and a larger area of agricultural land.

Soil and geological characteristics of a catchment also contribute to the rate of transport (and survival probability) of *E. coli* prior to reaching shellfish growing waters. A number of soil characteristics determine initial *E. coli* survival upon release to land: temperature, pH, nutrient availability, particle size, moisture content, and the presence of competing or predatory microorganisms [75]. Soil characteristics including texture, structure, and saturation thresholds also influence both the likelihood of adsorption of FIOs to soil particles, and the dominant processes transporting faecal particles or bacteria downstream [23; 8]. Rainfall intensity and distribution interact with soil texture and underlying geology in determining the proportion and total quantity of contaminated water and sediments transported via surface runoff and sub-surface flows.

The hydrological regime of a catchment, including its 'flashiness', is determined by interactions between rainfall intensity and distribution, the physical catchment characteristics discussed above, and overlying land uses (vegetation cover and type), as well as the density and distribution of watercourses and water bodies (lakes, reservoirs) within the catchment (e.g. [37]). The resultant river flows impact on the total FIO load delivered to shellfish waters, and the speed of its delivery.

2.2.5 Influences on E. coli survival in water

While initial mobilisation of *E. coli* and other FIOs, triggered by rainfall events, can indicate the timing of peak FIO loadings in watercourses and shellfish waters, water FIO concentrations can show a disparity with the levels of *E. coli* found in shellfish flesh. One reason for this is the varying survival rates of faecal bacteria in watercourses and shellfish waters [76], often determined by complex interactions between a number of physical and physio-chemical variables. Factors influencing FIO survival in environmental waters revealed in the literature are discussed in the following sections.

2.2.5.1 Solar radiation

Solar radiation is frequently recognised in the literature as the dominant factor determining the survival of bacteria in seawater, having a typically bactericidal effect [8]. Its impact on survival is dependent on a number of interacting factors. Firstly, the level of solar radiation reaching the water surface is determined by the season, time of day and latitude. Secondly, its penetration of the water column is dependent on water depth; the degree of mixing of the water column; and the concentration of dissolved and particulate organic matter (e.g. sediments, phytoplankton) in the water column [6; 77].

At some locations, significant (>10-fold reduction) bactericidal effects have been detected at 3 m depth, and at down to 10 m at polar latitudes ([78-80]). A review of T_{90} values (the time required for 90% of bacteria to die off) by Campos et al. [6] revealed that FIO die-off rates vary widely between locations and overall environmental conditions, from c. 2 hours in seawater in very sunny weather or near midday, to up to 240 hours in brackish waters under highly turbid conditions. There is some debate in the literature over which wavelengths of light (PAR or UV) contribute most to bacterial die-off [8].

2.2.5.2 Salinity

Salinity is a major determinant of FIO survival in aquatic environments [8; 6]. Bacterial decay rates are generally faster in seawater than fresh water [81-83]. For example, Carlucci et al. [84] found that after 48 h exposure to water of different salinities in the range 0 to 100%, *E. coli* survival was 59.9% in fresh water, highest (74.5%) at 25% salinity, and lowest (8.2%) at 100% salinity. In a recent review paper, Maalouf et al. [76] reported survival of *E. coli* in seawater to vary between 5 h and 3.5 d, although Anderson et al. [85] recorded surviving coliforms (2% survival) at up to 8 d after exposure to water of 30‰ salinity.

2.2.5.3 pH

The measured optimal pH range for enteric bacterial survival varies somewhat in the literature, at between pH 5-7 [82; 83]. Measured thresholds at which rapid die-off starts vary, as do theories of whether highly alkaline or highly acidic conditions are more detrimental to FIO survival. An early study by Carlucci and Pramer [86] measuring *E. coli* die-off in seawater after 48 h over the pH range 5 to 9, recorded 58.3% survival at pH 5, declining rapidly to < 0.01% survival at pH 9. The typically higher pH range of sea water (pH 7.5 to 8.5, [82]) compared to that of freshwater (generally around or slightly below pH 7) may therefore partially explain the higher die-off rates in seawater compared to freshwater.

As a variable correlated against FIO concentrations, pH remains relatively unexplored in the literature. Where pH was measured, several studies found no significant correlation between pH and various bacterial concentrations in fresh water [87; 88]) or sea water ([12; 15; 73]). Mignani et al [24] explained this lack of clear relationship by proposing that pH is often involved in synergistic or antagonistic interactions with other variables affecting coliform concentrations, obscuring the effect of pH alone. Where authors found a significant relationship between pH and bacterial concentration in water, it was principally a negative correlation.

2.2.5.4 Temperature

While *E. coli* and other enteric bacteria experience temperature shock on excretion from the body of mammals, they quickly adapt to new temperatures in fresh water or seawater [8]. Assuming no other limiting factors are present, *E. coli* can grow at temperatures as low as 10°C, but can survive at temperatures below this. The relationship between *E. coli* survival and persistence and water temperature is not straightforward. In controlled trials, Solic and Krstulovic [83] observed an inverse exponential relationship between temperature (range 6 to 37°C) and FC T_{90}, with a c. 55% decline in T_{90} for each 10°C water temperature increment. However, other authors have noted an increase in bacterial stability at low temperatures [82]. FIOs are capable of dormancy at low temperatures, with the potential to form reservoirs of contamination (particularly within sediments) which can theoretically remain viable for some time. In field studies, the response of FIOs to water temperature has been varied, with differences attributed to sometimes complex interactions with other environmental variables.

2.2.5.5 Attachment and re-suspension within aquatic environments

Anthropogenic impacts associated with land management, industry and waste generation can have profound effects on ecosystem functioning in the downstream catchment and associated coastal zone. Transfer of macronutrients, sediment, and microbial pollutants (derived from human and animal waste) from land to sea are thought to have significant impacts upon aquatic environments. Bacteria attach to a range of surfaces within environmental waters (e.g. dissolved or suspended organic and inorganic matter; animals, and plants) [89; 6]. Bacterial 'reservoirs' can be harboured in sediments in riverine, estuarine and marine environments, where FIO concentrations may be between 100 and 1000 times greater than in the surrounding water column [90; 82; 8]. Sediments may additionally harbour large populations of dormant but viable bacteria, which may not be easily detected using standard enumeration methods [91, 92]. Burial in sediments protects adsorbed FIOs from bactericidal agents, including primarily UV light, but also high salinity, heavy metal toxicity and bacteriophage infection [9; 8.]

Persistence of faecal bacteria in sediments is associated with sediment composition and bacterial morphology, both of which affect adsorption capacity. Clay particles in particular are thought to facilitate adhesion by bacteria – although the role of aluminium present in some clay types as a bactericidal agent may reduce persistence and requires further exploration [91]. Sediments containing a minimum 25% clay significantly reduce bacterial decay rates compared to sediments containing no cohesive particles. Intertidal areas of low-energy, depositional systems (e.g. mud flats) subject to pollution events are especially likely to harbour faecal contaminants, since their composition tends towards clay and other very small mineral fractions [91, 92]. The organic matter (OM) content of sediments is an important contributor to bacterial survival, impacting nutrient availability and adhesion capacity [89; 93; 82].

Burial in, and adhesion to, sediments enables the integration of contamination over longer periods than in overlying waters, with FIOs surviving for up to an order of magnitude longer (up to 80 d) than in seawater [6; 36; 91]. Sediment-associated faecal bacteria and organic matter originating in any part of a catchment may be transported downstream and deposited in or near shellfish growing waters, where they are re-suspended during storm events or normal tidal cycles [89].

Turbidity and re-suspension of faecal bacteria in the water column (e.g. from wave action, storm flows, strong winds or boating activity) reduces FIO die-off largely through impairing light penetration through the water column [6]. Bacterial survival and growth may additionally be promoted by enhanced nutrient concentrations associated with suspended matter, and decay rates have been found to vary with suspended substrate [89; 90; 94]. However, increased mixing also exposes bacteria to more frequent changes in environmental stressors (e.g. temperature, predators), which may reduce survival. The net impact of turbidity on FIO survival varies between species, which have different physiological mechanisms for tolerating environmental stressors.

Estuarine environments frequently trap large quantities of fine sediment (i.e. clay and silt [95]). The amount, type and size distribution of sediment particles can have significant consequences for the sorption, accumulation and transport of pollutants [96], including microbial pathogens [8; 89]. In aquatic systems, association with flocs represents a medium for pathogen transport and survival and numbers of floc-associated *E. coli*, Salmonella spp., Vibrio spp. and coliforms are enriched several-fold when compared to the surrounding water[98,99] representing a significant public health risk. Flocs are multidimensional ephemeral fragile aggregates of primarily organic detritus, including extracellular polymeric substances (EPS) exuded from aquatic organisms, [100] inorganic particles such as clay and silt, and water and the main vehicle for the transport of organic material from the water column to the sediment. Flocs also act as a major reservoir for the persistence of human pathogens in aquatic systems [98; 99; 101] and the composition of flocculated material will reflect catchment type including elements from point and diffuse sources affecting the aquatic environment.

Evidence suggests that sediment particle size and distribution has a significant impact on the spatial variation and persistence of human pathogenic bacteria and viruses within estuarine environments. Anthropogenic disturbance and hydrodynamic processes such as wave action and tides can re-suspend sediments back into the water column contaminating the surrounding area significantly impacting microbial water quality. Under normal river flow conditions, particles are retained within the estuary as a result of sediment pumping. Storm events alter this scenario by discharging a larger volume of freshwater than is typical and increased river water is likely to increase water velocities on the ebbing tide which may cause the critical bed shear stress to be attained and sediment to be resuspended. In this case, material typically deposited during “normal” conditions, could be washed out of the system and transferred down the estuary and potentially out to sea, however, our knowledge and understanding of the interactions between these factors and their influence on estuarine processes and public health are poorly understood.

2.2.5.6 Predation and competition

Predation and competition for nutrients from other microorganisms are important in controlling *E. coli* and other FIO populations, within both the water column and sediments [68; 8]. However, disaggregating the impacts of predation and competition from other contributors to die-off is hampered by the complex ecological interactions between predators, competitors, FIOs and physiochemical conditions found in aquatic environments. Consequently, these variables remain relatively unexplored in the literature [6]. However, several authors have attempted to disentangle the effects of predator/competitor organisms from other factors in controlled experiments. Reported die-off rates of $1\log_{10}$ in the water column range from < 24 h to 4 d [102; 103].

2.2.5.7 Hydrography

The amplitude and frequency of tidal cycles, and prevailing tidal currents, impact on the distribution and concentrations of FIOs found in shellfish growing waters. Tidal patterns vary at scales from sub-daily to inter-annually, with consequent variations in FIO patterns.

Shallow estuarine waters and wave-dominated systems (e.g. sandy beaches) show different responses to peak and base levels of FIOs due to varying degrees of mixing. Where there is a greater degree of mixing (i.e. in wave-dominated systems), variability in FIO concentrations may be obscured by pollutant dispersion (both vertically and horizontally), and by higher bacterial die-off rates due to increased exposure of bacteria in the water column to bactericidal influences (e.g. solar radiation, predators)[6]. FIO distribution in shallow and depositional estuaries is largely determined by the re-suspension of contaminated sediments in the water column during storm conditions [36].

Tidal forcing (dilution) may be more important in reducing loadings to shellfish production areas than die-off of faecal bacteria in Atlantic coastal systems subject to strong currents [104]. However, relative effects over time vary depending on tidal stage (low or high, spring or neap), which interacts with the relative impacts of incoming freshwater or direct sewage outflow FIO sources. Several studies have observed significant differences in FIO concentration between low and high tides, both within shallow tidally-driven estuaries (e.g. [13]), and narrow tidal creeks (e.g. [105]). Both attributed differences to increased turbidity and re-suspension of contaminated sediments during or near to low tide. Kershaw et al. [106] observed a 100-fold diurnal variation in seawater *E. coli* levels over a 10-day experimental period. Riou et al. [48] found that pollutant plumes rapidly extended further on spring ebb tides compared to neap tides, impacting a larger number of shellfish production sites. However, neap tides often carried higher concentrations of FIOs due to reduced mixing and dilution. Variation in FIO loadings to individual shellfish beds within a production area might therefore be expected to differ depending on their locations relative to tidal currents and pollution sources carried by such currents.

2.2.6 Influences on *E. coli* uptake and elimination in shellfish

The concentration of faecal bacteria in the water column does not always show a straightforward linear relationship with shellfish flesh bacterial concentration [7], although the two variables are often positively correlated. Accumulation and clearance of FIOs by shellfish varies with species, surrounding environmental conditions, and feed particle characteristics.

Potential accumulation and clearance rates and maxima differ between species, driven by inter-species differences in feeding filtration rates [107; 108; 68]. Maximum filtration rates in natural environmental waters vary according to bivalve filter pump capacity and food concentration in water, but tend to range between 20 and 100 L per day [108; 68]. However, information remains scarce on the role of biological processes in FIO uptake and clearance in shellfish, including details of the possible role of preferential feeding in FIO uptake [6; 107]. The literature generally reports faster and greater accumulation of faecal contaminants in cockles and mussels compared to oysters and clams (e.g. [108; 6]). However, because of the slower filtration rate of oysters, they often retain pollutants for longer after uptake.

Shellfish feeding rates are controlled by the temperature and salinity of overlying waters, with inter-species differences in upper and lower tolerances for survival and feeding activity. Consequently, spatial variations in temperature and salinity can determine variations in FIO contamination levels in shellfish at both the local and regional scale [6].

Pumping rate tends to increase with temperature in all bivalve species, partially attributed to a corresponding reduction in water viscosity [109]. CEFAS [107] noted that since temperature tends to co-vary with season, day length, UV light levels, and annual shellfish biological life cycles, drawing firm conclusions on the influence of temperature on FIO uptake in environmental waters is difficult. Nevertheless, differences between species in uptake and clearance rates at different ambient seawater temperatures might be expected. Shellfish species differ in their tolerance to low temperatures, with mussels able to withstand temperatures down to -4°C and feed even during winter; other species tend to only feed at a few degrees above zero [108]. This potentially increases the susceptibility of mussels to FIO contamination throughout the year compared to other species. Cockles display a more complex relationship with temperature, appearing to reduce accumulation rate at higher temperatures to reduce the metabolic burden of rapid filtration [107].

Salinity impacts shellfish feeding rates to a lesser extent than temperature. As salinity declines, bivalve closure occurs, or opening is delayed. This process occurs at different thresholds depending on species, typically corresponding to their natural position on the shore [107]. *Mytilus* spp. usually feed at 20 to 35‰ salinity, with other UK species commonly feeding at or above 20‰. Native oysters prefer salinities above 16‰, Pacific oysters around 25‰, and scallops at or above 30‰.

While exposure to temperatures and salinities outside of the preferred natural range of shellfish can reduce metabolic activity (and therefore ability to accumulate and clear FIOs), feeding rates may decline even within the 'normal' range for feeding activity [7]. However, variation occurs within a population, and under sub-optimal conditions of temperature and salinity, at least some individuals within a shellfish bed will be able to accumulate FIOs rapidly [108].

Accumulation and clearance of FIOs on exposure to faecal pollution follows three distinct phases Kershaw et al., [108]. First, rapid uptake occurs, usually within 0 to 1 hour of exposure; significant variation in uptake rates is observed between individuals during this phase. During the second phase, typically lasting between 1 and 20 hours, contamination levels within the population becomes more homogenous. Finally, at c. 20 hours or more, all individuals within the population should have reached maximum contamination levels; the duration of this period depends on species physiology, water contamination levels, and food levels in water. After exposure to clean water (e.g. during depuration), shellfish are usually capable of clearing FIOs from their tissues within 48 h or less [107]. Kershaw et al. [108] commented that within UK environmental waters, assuming clearance after 48 h may be inappropriate, since typically shellfish are exposed to low-level chronic pollution punctuated with episodes of acute contamination. These authors subsequently investigated the impacts of subjecting cockles, mussels and Pacific oysters to prolonged (96 h) pollution of six concentrations in seawater (1 to 330 cfu 100ml^{-1}) [106]. All species at all contamination levels rapidly accumulated *E. coli* (within 18 h of exposure), concentrating faecal bacteria within their tissues at levels consistently higher than ambient concentrations (Table 2.2.7). High tissue concentrations were maintained throughout the exposure period, followed by rapid clearance (within 48 h) after removal of the pollution source. This study illustrates that (1) shellfish are capable of rapidly responding to environmental stimuli (both pollution and depuration), and that even under relatively low levels of chronic contamination, bivalves can maintain elevated levels of FIOs within their tissues. Consequently, shellfish sampling for regulatory purposes should take these factors into account when considering sampling strategies around potential pollution triggers (e.g. storm events). Accumulation factors for a range of shellfish identified in the literature are presented in Table 2.2.7. While inter-species differences in accumulation factors are reported in individual studies, CEFAS [107] concluded that overall, these are not significant at a given position within the water column or shellfish bed. However, since different growing methods place cultured shellfish at different positions in the water column, sampling strategies should take account of the likely impacts on contamination levels (i.e. exposure levels to pollutants, and pollution dynamics).

Table 2.2.7 Accumulation factors (obtained in the laboratory), uptake and clearance rates from various species of shellfish

Species	Indicator organism	Exposure period (h)	Accumulation factor	Reference
Clam spp.				
C. gallina	EC	72	1.6 [2]	Martinez-Manzanarez et al. (1991)
M. arenaria	EC	48	20 [3]	Cabelli and Heffernan (1970)
M. mercenaria	EC	48	6.5 – 8.5 [1]	Cabelli and Heffernan (1970)
M. mercenaria	EC	48	12.5 [1]	Cabelli and Heffernan (1970)
M. mercenaria	EC	24	3 [2]	Timoney and Abston (1984)
M. mercenaria	FC	168	2.7 (0.02 – 20.4) [3]	Burkhardt et al. (1992)
M. mercenaria	EC	168	2 (0.02 – 17.5) [3]	Burkhardt et al. (1992)
T. decussatus	FC	n.s.	0.5 – 9.7	Campos and Cachola (2007)
Venus spp.	FC	27	0.6 [1]	Beucher (1993)
Cockle spp.				
C. edule	FC	27	1.5 [1]	Beucher (1993)
C. edule	EC	96	330	Kershaw et al. (2013)

Mussel spp.	EC			
Mytilus spp.	EC	96	15.2	Kershaw et al. (2013)
Mytilus spp.	EC	n.s.	5.9	Lees et al. (1995)
M. edulis	EC	12	0.9 – 3.4 [1]	Kay et al. (unpubl. data)
M. edulis	EC	12	1 – 7.7 [1]	Kay et al. (unpubl. data)
M. edulis	EC	46	1.2 – 7 [1]	Plusquellec et al. (1990)
M. edulis	FC	n.s.	13.1	Plusquellec et al. (1983)
M. edulis	FC	27	1.2 [2]	Beucher (1993)
Oyster spp.				
C. gigas	FC [1]	27	0.8 [1]	Beucher (1993)
C. gigas	EC	12	0.9 – 10.3 [1]	Kay et al. (unpubl. data)
C. gigas	EC	12	1 – 14 [1]	Kay et al. (unpubl. data)
C. gigas	EC	96	11.7	Kershaw et al. (2013)
C. gigas	EC	n.s.	2.6 – 6.9	Lees et al. (1995)
C. virginica	FC	Not stated	3 – 6 [2] [3-16?]	Perkins et al. (1980)
C. virginica	FC	n.s.	4.4 ($\sigma^2 = 4$)	Burkhardt and Calci (2000)
O. edulis	FC	27	0.5 [1]	Beucher (1993)

Adapted from: Campos et al. (2013); CEFAS (2014); Kershaw et al. (2012).
EC = *E. coli*; FC = faecal coliforms. [1] Calculated as the log of the concentration of the organism in shellfish flesh divided by the corresponding log of the concentration in the overlying water. [2] Calculation method not stated. [3] Calculated as the geomean indicator concentration of the organism in shellfish flesh divided by the corresponding geomean concentration in the overlying water.

3 Identification and collation of data

Data on *E. coli* in shellfish were initially identified from the CEFAS database and from the CEFAS sanitary surveys. Initial discrepancies were highlighted between the CEFAS Hub data and the FSA classification data. All discrepancies and erroneous recommended monitoring points (RMPs) were corrected before site selection and before any analysis or preliminary testing of proposed methodology for the tool. The *E. coli* data in shellfish flesh data from the CEFAS classification monitoring site was then collated and errors corrected using the sanitary survey data. Following discussions with both the Environment Agency (EA) and Natural Resources Wales (NRW), the *E. coli* in shellfish data was provided to the FSA in Wales by NRW. This data was only for Wales, however, and duplicated what had already been acquired.

Rainfall data used was from the nearest UK Met. Office station at Rhyl for the Conwy preliminary analysis. Flow measurements were taken from EA and NRW 15 minute values held on the CEH NRFA database. (https://nrfa.ceh.ac.uk/).

Catchment characterisation used delineation of catchment boundaries from (http://lle.gov.wales/catalogue/item/WaterFrameworkDirectiveRiverCatchmentWaterbodiesCycle2/?lang=en for Wales and http://environment.data.gov.uk/ds/catalogue/index.jsp#/catalogue for England). The extent of each estuary catchment was marked according to those used in the CEFAS Sanitary Survey reports. Land cover classes were taken from the Centre for Ecology and Hydrology Land Cover Map 2007.

Data on combined sewer overflows, intermittent private discharges were collated from the sanitary survey data for all of England and Wales. Data for Wales was also provided from the Event Monitoring Data from Welsh Water. Welsh Water also supplied their network model (InfoWorks) for the Conwy catchment which was also used in preliminary analysis.

Water quality data was obtained from the Harmonised Monitoring Scheme database: https://data.gov.uk/dataset/historic-uk-water-quality-sampling-harmonised-monitoring-scheme-detailed-data.

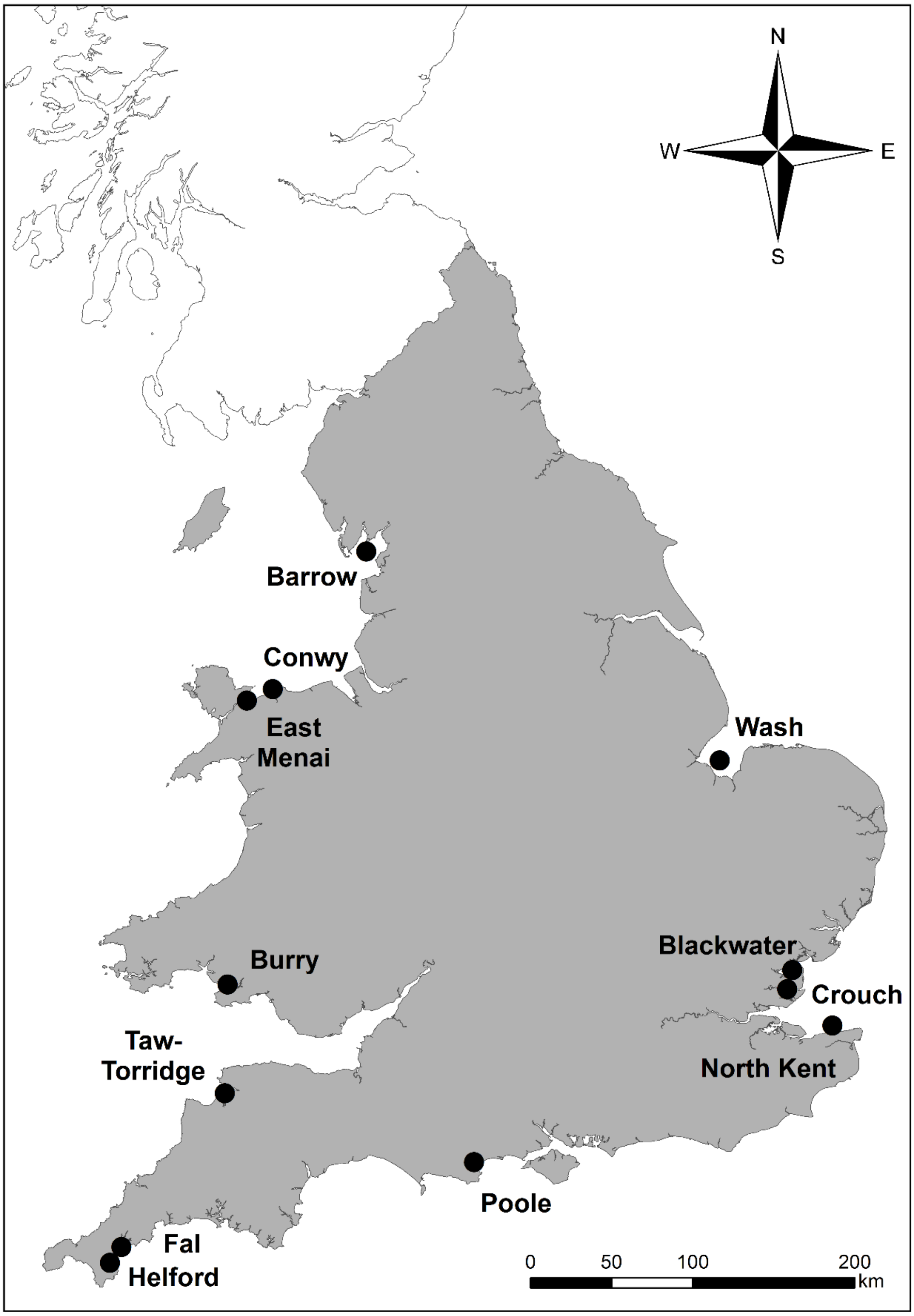
A range of other data is available that is held publicly both on *E. coli* (and other bacteria) in shellfish and also on *E. coli* (and other bacteria) in different catchments. For example, additional data in relation to ongoing projects in the Conwy estuary. In a number of instances the data have not been validated, or have not been collected using the consistent techniques, with no accredited cross reference untaken. For instance there are extensive data on *E. coli* in shellfish in Conwy which has be taken from homogenised shellfish tissue and then plating out of the resulting mixture [110]. The *E. coli* in shellfish data collected by CEFAS uses the most probable number method (MPN) (ISO/TS16649-3) test and though initial tests suggest that there is no difference between the two techniques there is no accreditation comparing these two approaches. In addition, there are also some data that uses the Impedance technique ([111]; ENISO 16140) and though there is comparison between MPN and Impedance (both methods have been calibrated against each other), there is no data-comparison between all three techniques. Impedance is more widely used in continental Europe.

Tidal cycles have not been taken into account in this report due to time limitations but may be a significant factor and may need to be included in taking any active management tool, as may wind direction, temperature and salinity.

3.1 Selection of sites for in-depth analysis

Twelve estuary areas were selected for more detailed analysis. These areas were chosen to represent variability in catchment size and catchment characteristics such as the proportion of improved grassland, arable and unimproved grassland which are known to contribute to nutrient and *E. coli* runoff into rivers. Other river characteristics were also considered, such as nitrate-N concentrations, turbidity, flow, flashiness, and long-term rainfall. There was also an aim to encompass a representative range of geographic locations around England and Wales in order to account for variability in overall geo-climatic conditions. The twelve estuaries are shown in Figure 3.1.

Figure 3.1. The location of the 12 sites used in the following analysis



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4 Analysis of available data

4.1 Relationship between *E. coli* counts in mussels and river flow.

Exploratory data analysis was undertaken by relating river flow and *E. coli* concentrations in mussels at the CEFAS RMP monitoring points in the 12 twelve estuary areas identified above. Not all rivers potentially impacting these areas are gauged, and in those cases flow data for a similar nearby gauged river was used if available. This can be justified if it is assumed that local variation in river flows (per unit area) is minor. The selected rivers by estuary area are shown in Table 4.1.

Table 4.1: Estuaries and rivers used for analysis of flow data

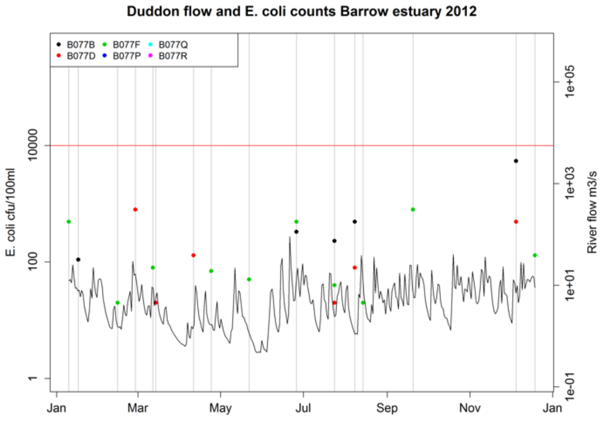
Estuary	River	NRFA_Code
Barrow	Duddon	74001
Blackwater	Blackwater	37010
Burry	Loughor	59002
Conwy	Conwy	66011
Crouch	Crouch	37031
Fal	Fal	48003
Frome/Poole	Frome	44001
Helford	Fal	48003
Taw	Taw	50001
Wash	Welland	31004
North Kent	Stour	40011
Menai	Conwy	66011

In some cases the river drains directly to an estuary with mussel beds, in others the link is less direct. A working hypothesis is that *E. coli* are mobilised during wet conditions. Under these conditions they may reach rivers in greater numbers from diffuse sources, including the activation of CSO discharges which may contain high concentrations of *E.coli*, and the possible remobilisation of *E. coli* from bed sediments through resuspension [89; 112]

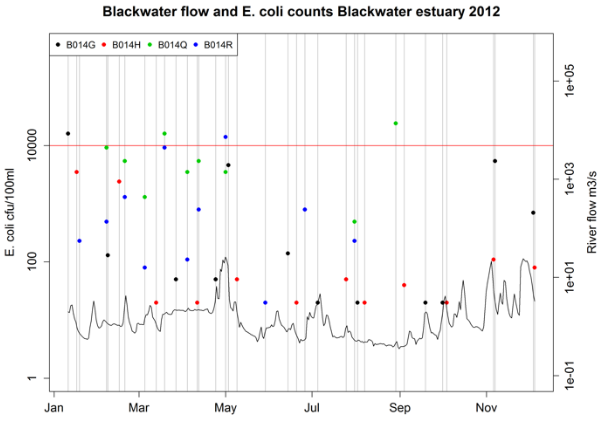
Figures 4.1 a-l show *E. coli* concentrations at sites within each estuary, together with river flow data for the example year 2012. The graphs show few clear patterns. *E. coli* counts are highly variable among beds within the same estuary, with the rank order of beds differing from one sample period to the next. There is no immediately obvious relationship between river flow and *E. coli* counts.

4.2 Within Estuary Variability

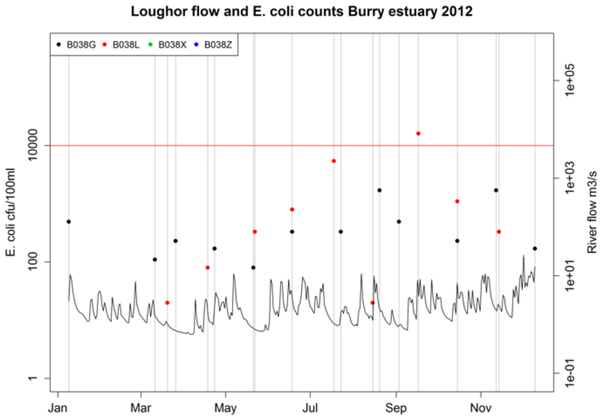
In the exploratory data analysis we examined within-estuary variability of *E. coli* concentrations. Paired plots between sites in the same RMP on a log scale and as raw data are shown in Figures A4.2 a-x. The associated correlation coefficient is tabulated in the upper right triangle of the plots. Each plotted point corresponds to a day on which a sample was taken at each of the sites being compared. In some cases there is no overlap in sampling days so no basis for a paired plot. The paired plots also include comparison of concentrations with a daily flow measurement in the associated river.


Duddon flow and E. coli counts Barrow estuary 2012
B077B B077F B077Q
B077D B077P B077R
E. coli cfu/100ml
River flow m3/s
Jan Mar May Jul Sep Nov Jan

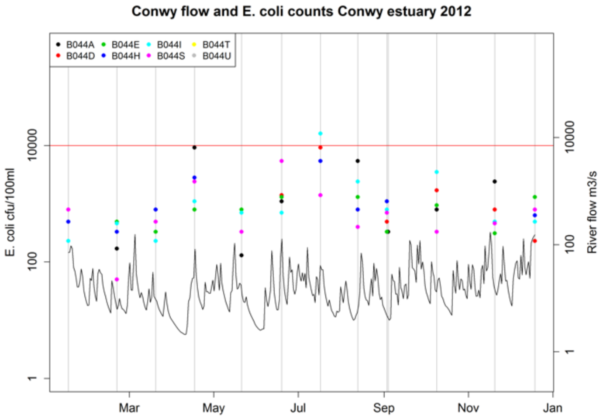
a


Blackwater flow and E. coli counts Blackwater estuary 2012
B014G B014H B014Q B014R
E. coli cfu/100ml
River flow m3/s
Jan Mar May Jul Sep Nov

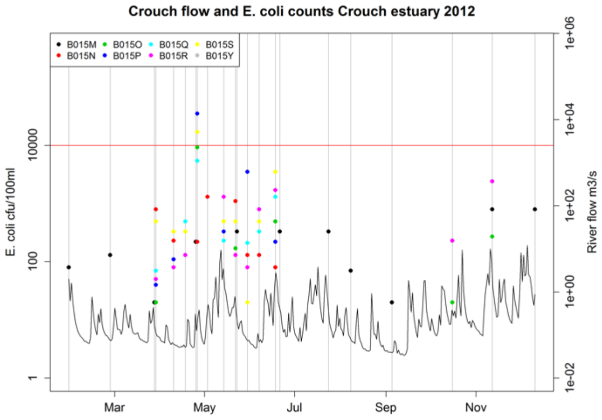
b


Loughor flow and E. coli counts Burry estuary 2012
B038G B038L B038X B038Z
E. coli cfu/100ml
River flow m3/s
Jan Mar May Jul Sep Nov

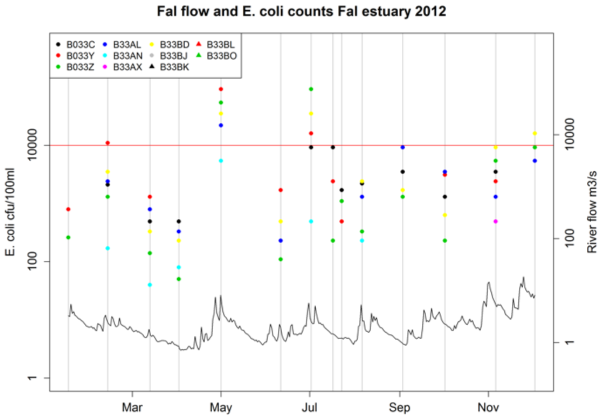
c


Conwy flow and E. coli counts Conwy estuary 2012
B044A B044E B044I B044T
B044D B044H B044S B044U
E. coli cfu/100ml
River flow m3/s
Mar May Jul Sep Nov Jan

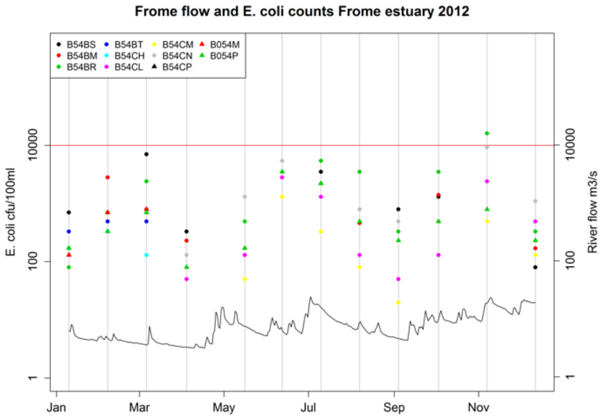
d


Crouch flow and E. coli counts Crouch estuary 2012
B015M B015O B015Q B015S
B015N B015P B015R B015Y
E. coli cfu/100ml
River flow m3/s
Mar May Jul Sep Nov

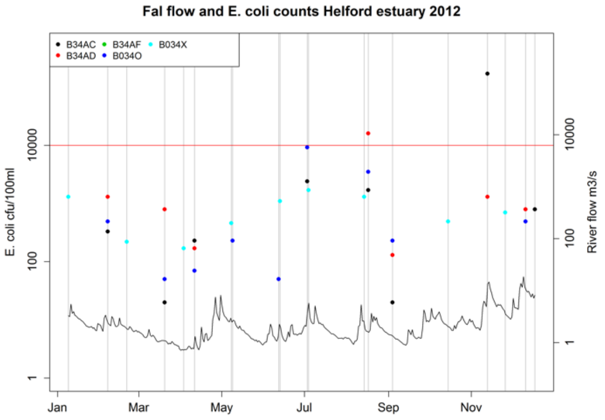
e


Fal flow and E. coli counts Fal estuary 2012
B033C B33AL B33BD B33BL
B033Y B33AN B33BJ B33BO
B033Z B33AX B33BK
E. coli cfu/100ml
River flow m3/s
Mar May Jul Sep Nov

f



g



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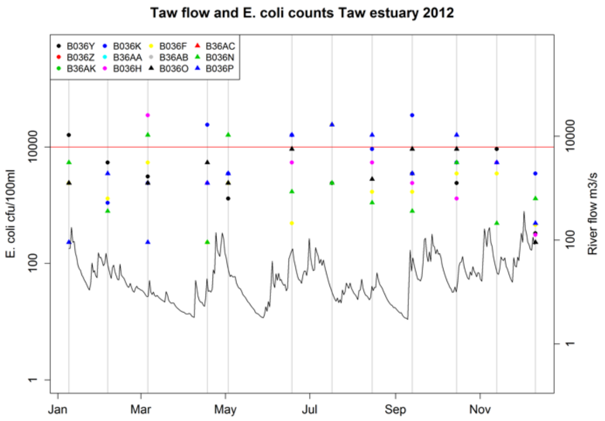

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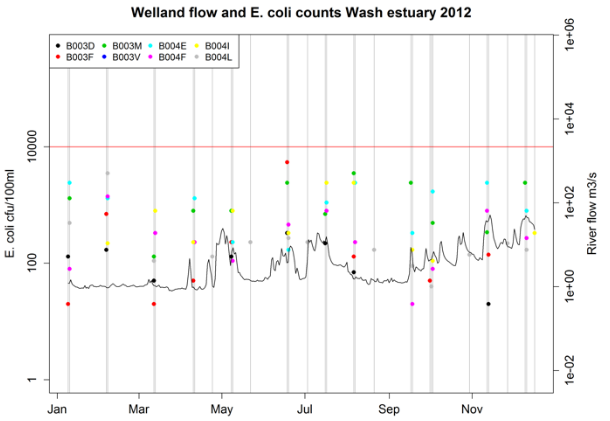

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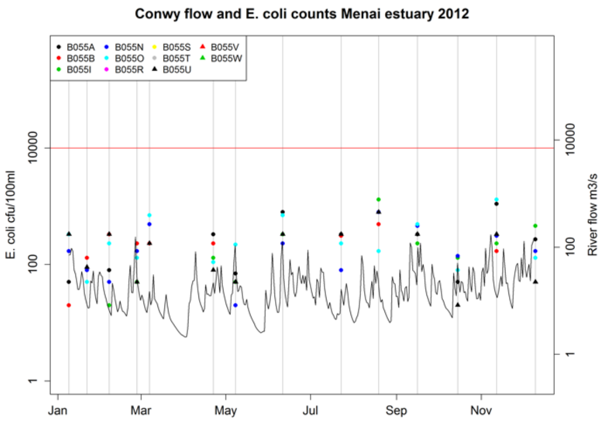

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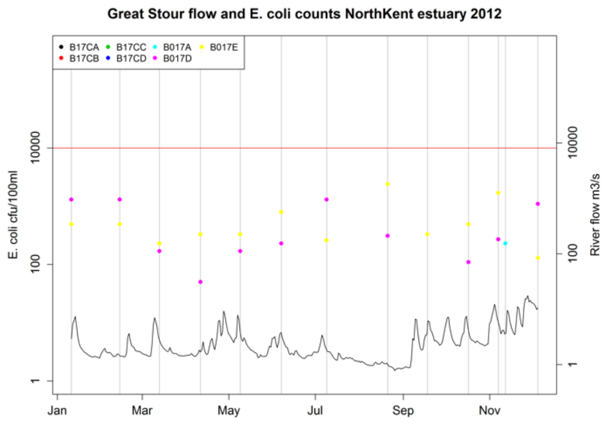
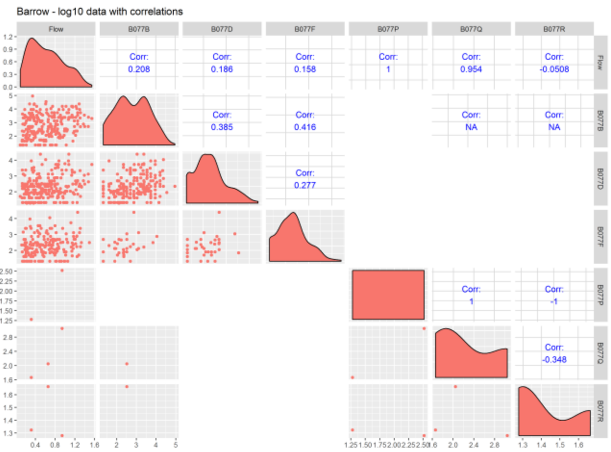
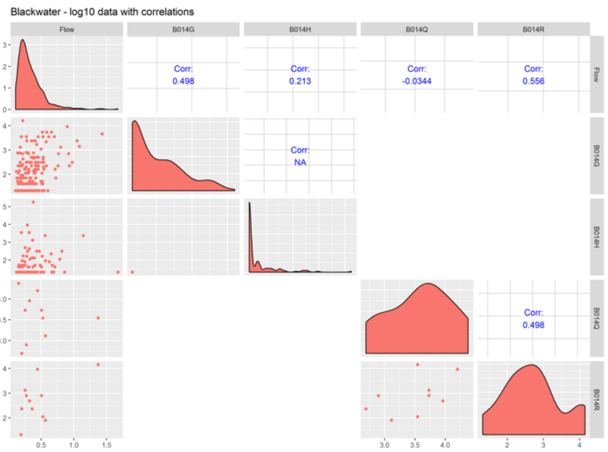


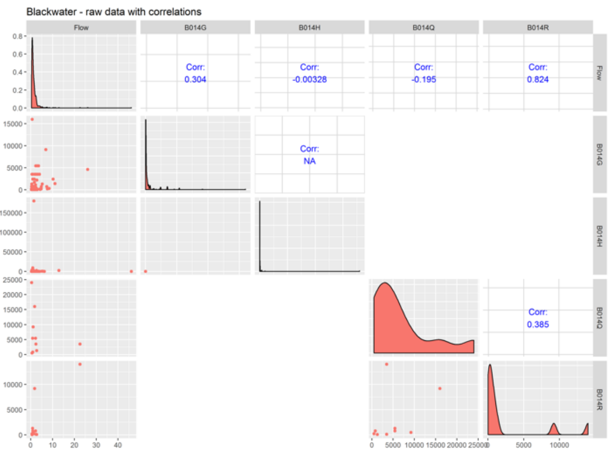
l

Figures 4.1 a – l. Annual time series plots of *E. coli* counts (CFU/100gms on the first y axis) and river flows (m^3/s) for the 12 selected shellfish areas. A red line has been drawn at 10000cfu/100gms in line with activation of investigations.

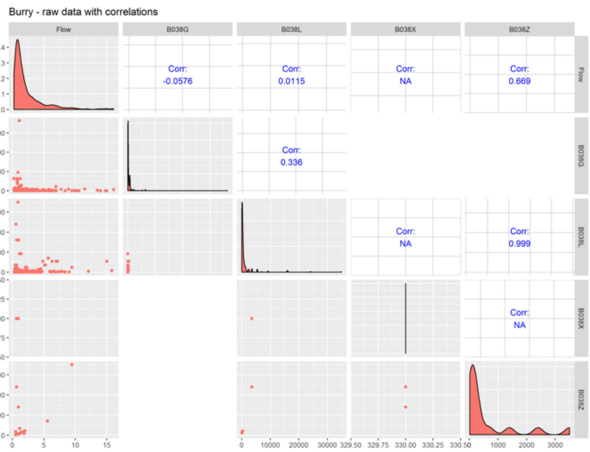

Barrow - log10 data with correlations
Flow
B077B
B077D
B077F
B077P
B077Q
B077R
Corr: 0.208
Corr: 0.186
Corr: 0.158
Corr: 1
Corr: 0.954
Corr: -0.0508
Corr: 0.385
Corr: 0.416
Corr: NA
Corr: NA
Corr: 0.277
Corr: 1
Corr: -1
Corr: -0.348
a

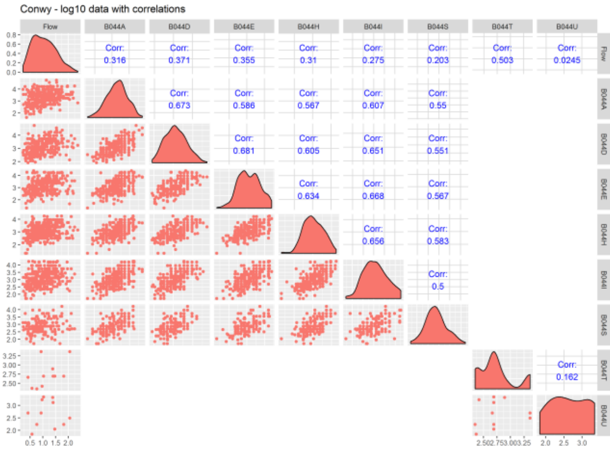

Barrow - raw data with correlations
Flow
B077B
B077D
B077F
B077P
B077Q
B077R
Corr: 0.0433
Corr: 0.0916
Corr: 0.147
Corr: 1
Corr: 0.949
Corr: -0.213
Corr: 0.0573
Corr: 0.0121
Corr: NA
Corr: NA
Corr: 0.054
Corr: 1
Corr: -1
Corr: -0.51
b


Blackwater - log10 data with correlations
Flow
B014G
B014H
B014Q
B014R
Corr: 0.498
Corr: 0.213
Corr: -0.0344
Corr: 0.556
Corr: NA
Corr: 0.498
c

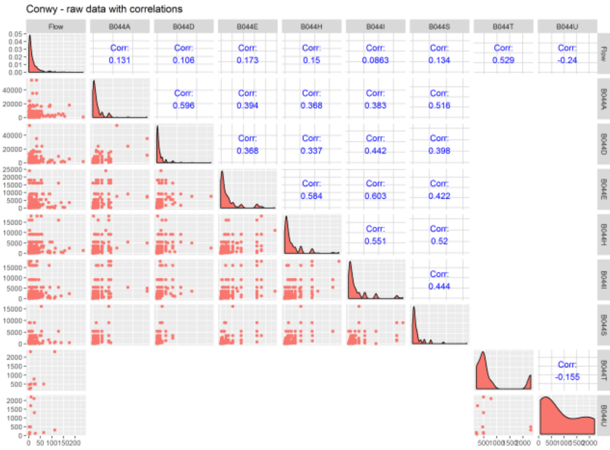

Blackwater - raw data with correlations
Flow
B014G
B014H
B014Q
B014R
Corr: 0.304
Corr: -0.00328
Corr: -0.195
Corr: 0.824
Corr: NA
Corr: 0.385
d


Burry - log10 data with correlations
Flow
B038G
B038L
B038X
B038Z
Corr: 0.0548
Corr: 0.193
Corr: NA
Corr: 0.537
Corr: 0.547
Corr: NA
Corr: 0.962
Corr: NA
e


Burry - raw data with correlations
Flow
B038G
B038L
B038X
B038Z
Corr: -0.0576
Corr: 0.0115
Corr: NA
Corr: 0.669
Corr: 0.336
Corr: NA
Corr: 0.999
Corr: NA
f


Conwy - log10 data with correlations

g


Conwy - raw data with correlations

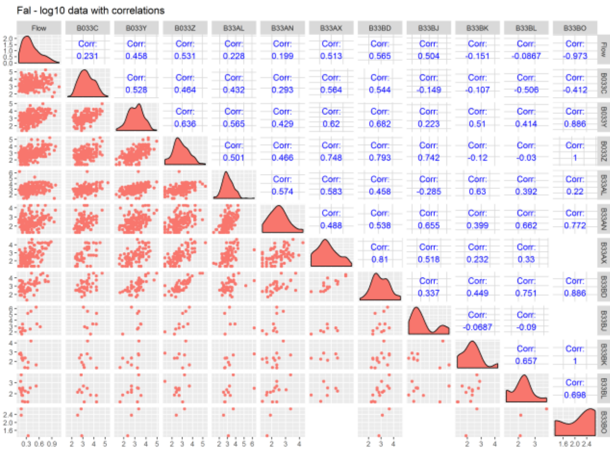
h


Crouch - log10 data with correlations

i


Crouch - raw data with correlations

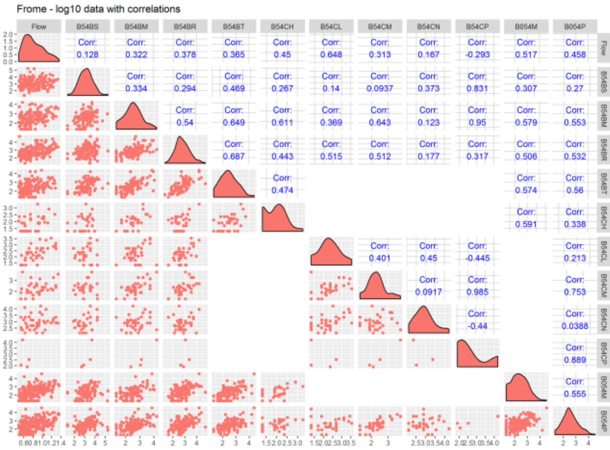
j


Fal - log10 data with correlations

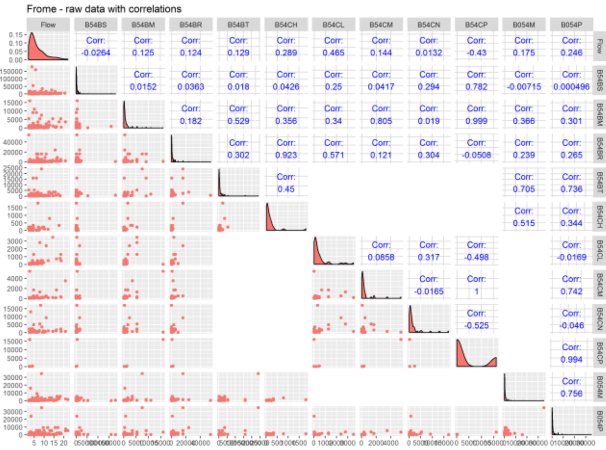
k


Fal - raw data with correlations

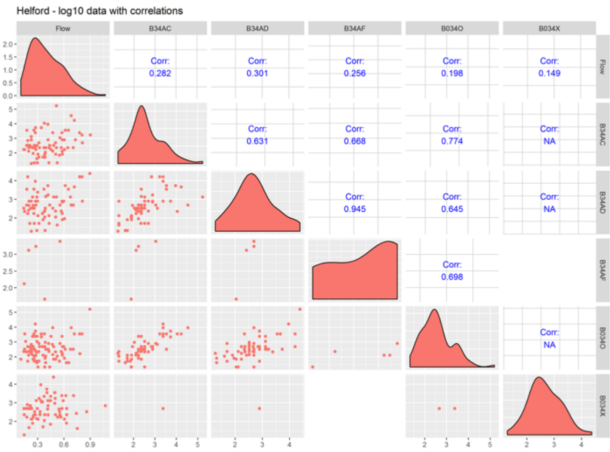
l


Frome - log10 data with correlations

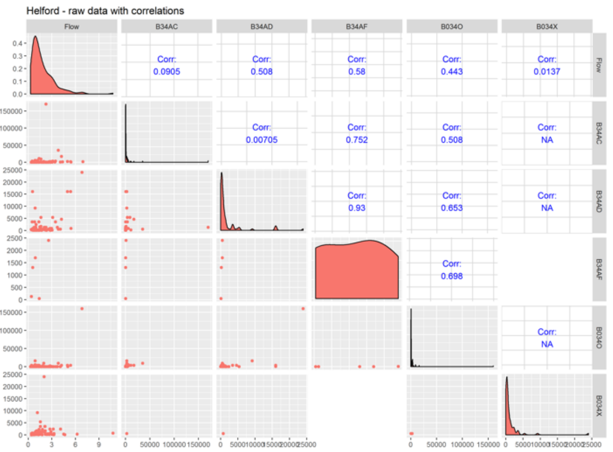
m


Frome - raw data with correlations

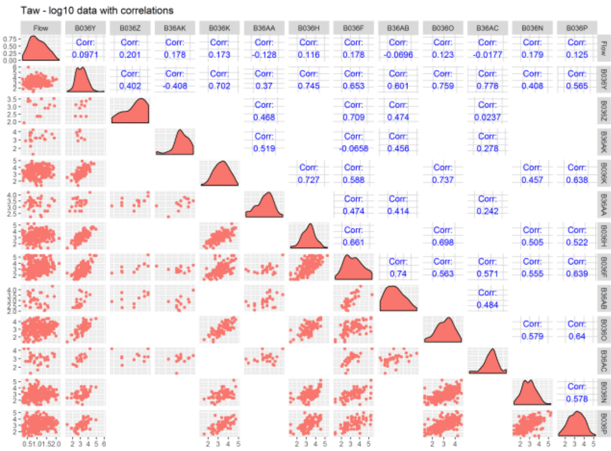
n


Helford - log10 data with correlations

o


Helford - raw data with correlations

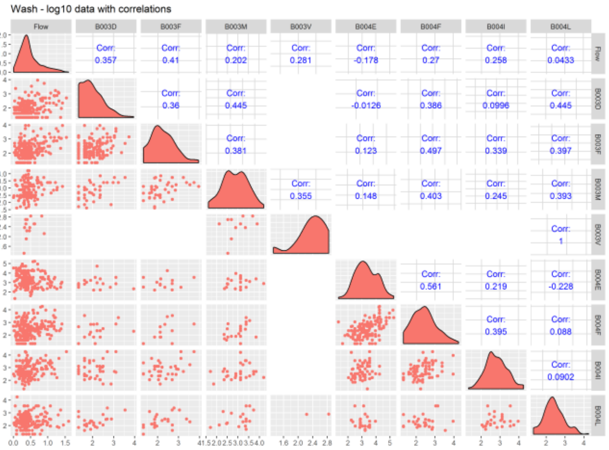
p


Taw - log10 data with correlations

q


Taw - raw data with correlations

r



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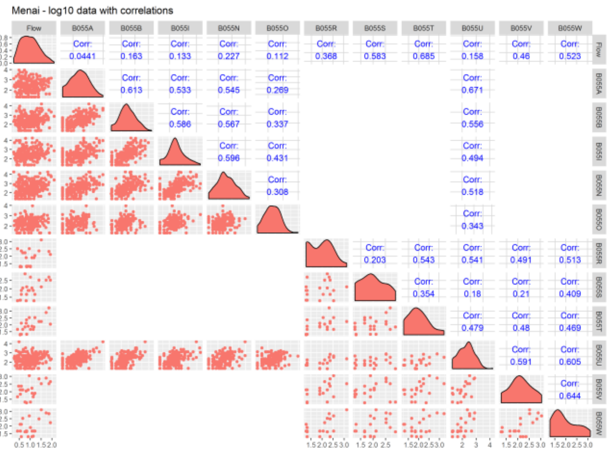

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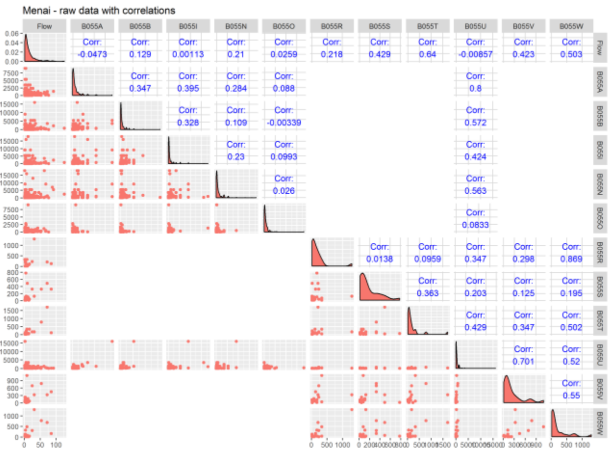
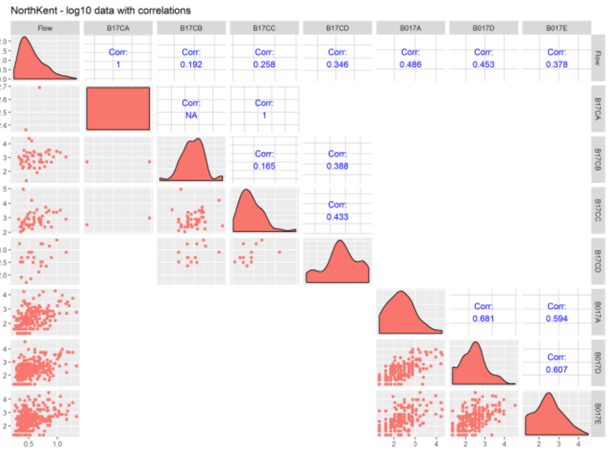


v

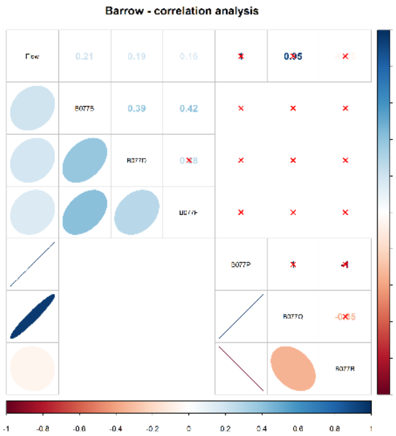


w

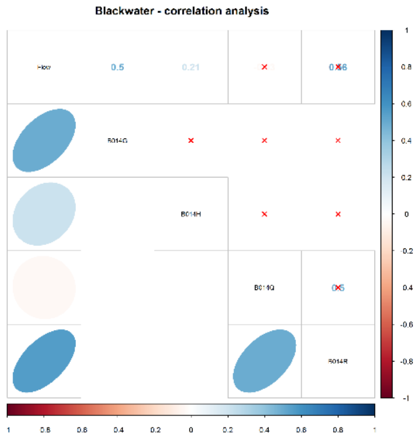
x

Figure A4.2.a-x. Paired plots of RMPs

Correlations tables associated with the paired plots of Figures A4.2a-x are shown in Figures A4.3a-l. Note that these correlations are based on varying numbers of points, which influences the significance which can be attached to the correlation values. The correlations are shown in the upper right triangle of the plots. Those which are not significant (p>0.05) are indicated by a red x. Lack of significance may be due to a lack of sufficient data points as well as to an apparent lack of association where there are many data points. The overall conclusion is that there are often weak relationships between flow and concentration, and between concentrations at sites within the same shellfish area suggesting that there are sometimes associations between beds on a site, e.g. in the Fal. However, the plots of the raw data suggest that the extreme high counts which are associated with bed closure are not closely associated with flow.



a



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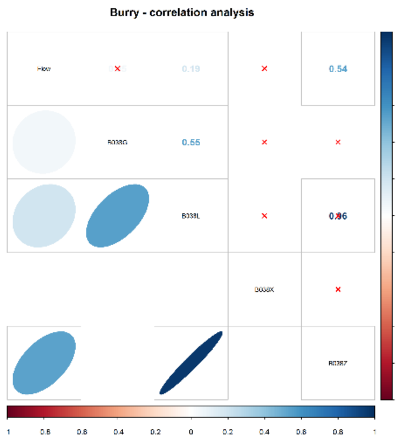

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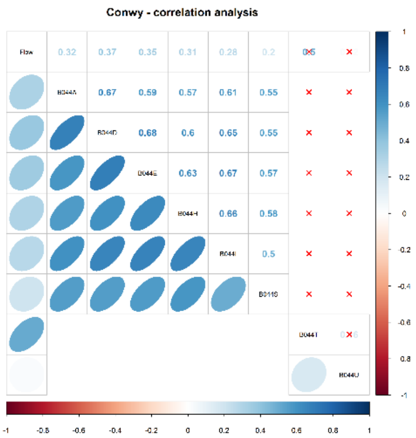

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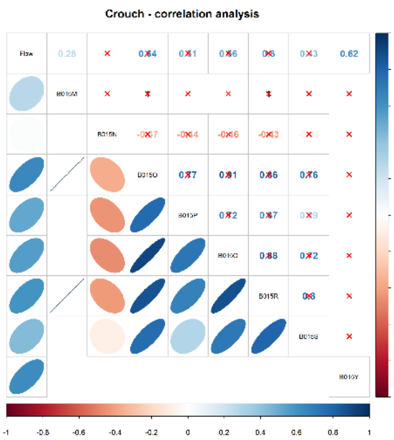

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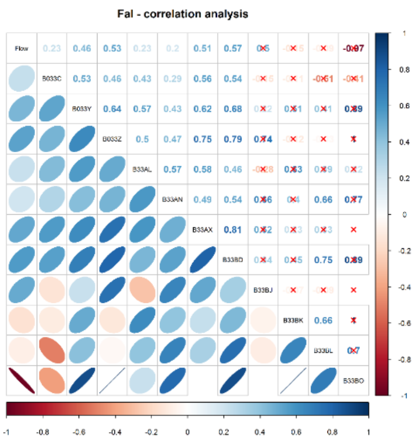

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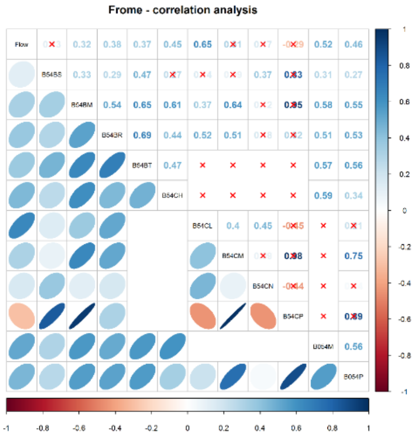

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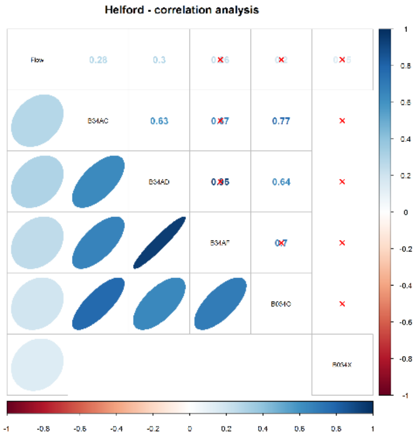

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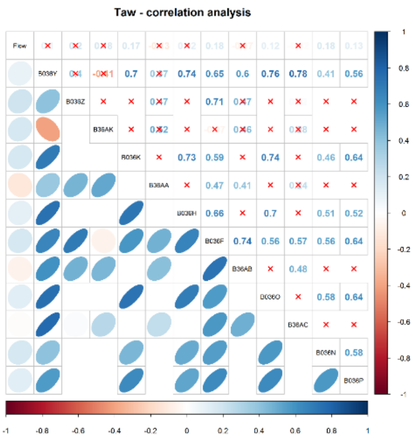

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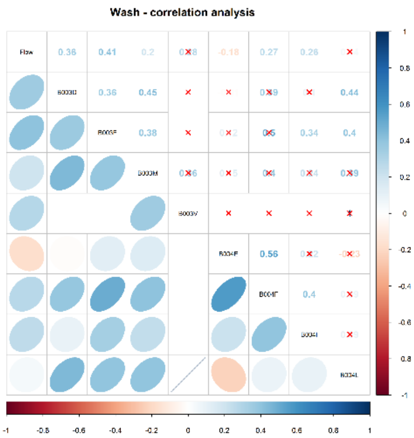

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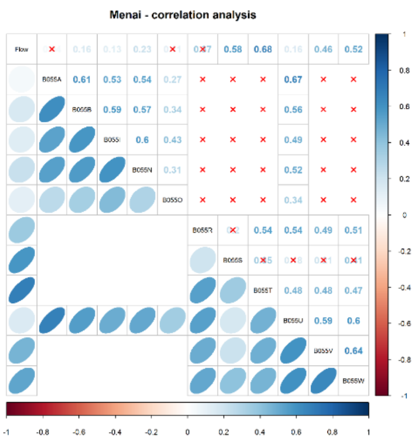

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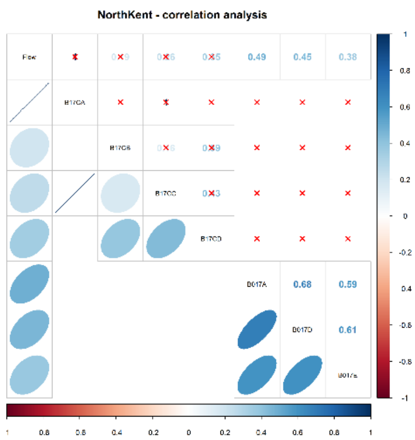


l

Figures 4.3a-l. Correlation tables of paired plots of Figures 4.2 a – x.

4.3 Analysis of variability with hydrological conditions

Figures A4.2 and A4.3 also show that there is a relationship between local river flows and *E. coli* counts in mussels, for logged plots, although this is often weak, and shows a large degree of scatter. This relationship is a likely cause of the within-area between-RMP spatial relationship for logged variables.

The weak but positive association between *E. coli* counts and flow at a log scale tends to demonstrate a relationship at low to medium flows and counts. However, our main interest is in the highest *E. coli* counts, which might lead to bed closure. Here, plots suggest very little association between high flows and high counts. Because of the high scatter of the data, to analyse any relationship statistically we classify both flows and counts into high and low classes. For flows the threshold chosen is the 80% quantile and for counts we choose 10,000 cfu/m^3. We then performed a 2x2 chi-squared test of association between these classes. The results of the test are shown in Table 4.2 (in Appendix 1).

The results indicate that for most areas the p-values of the chi-squared test are large, suggesting little association between high flows and high counts. However, for the Fal, many p-values are small suggesting an association. In many cases there are insufficient data to carry out the test since there are no instances of counts greater than 10,000 CFU/100mg at the site in question.

To assess possible lagged flow effects on E coli counts, regression analyses of logged *E. coli* counts on logged daily river flows was undertaken and included lags of 1 to 3 days. The results of the analysis are given in Tables 4.3 (Appendix 1).

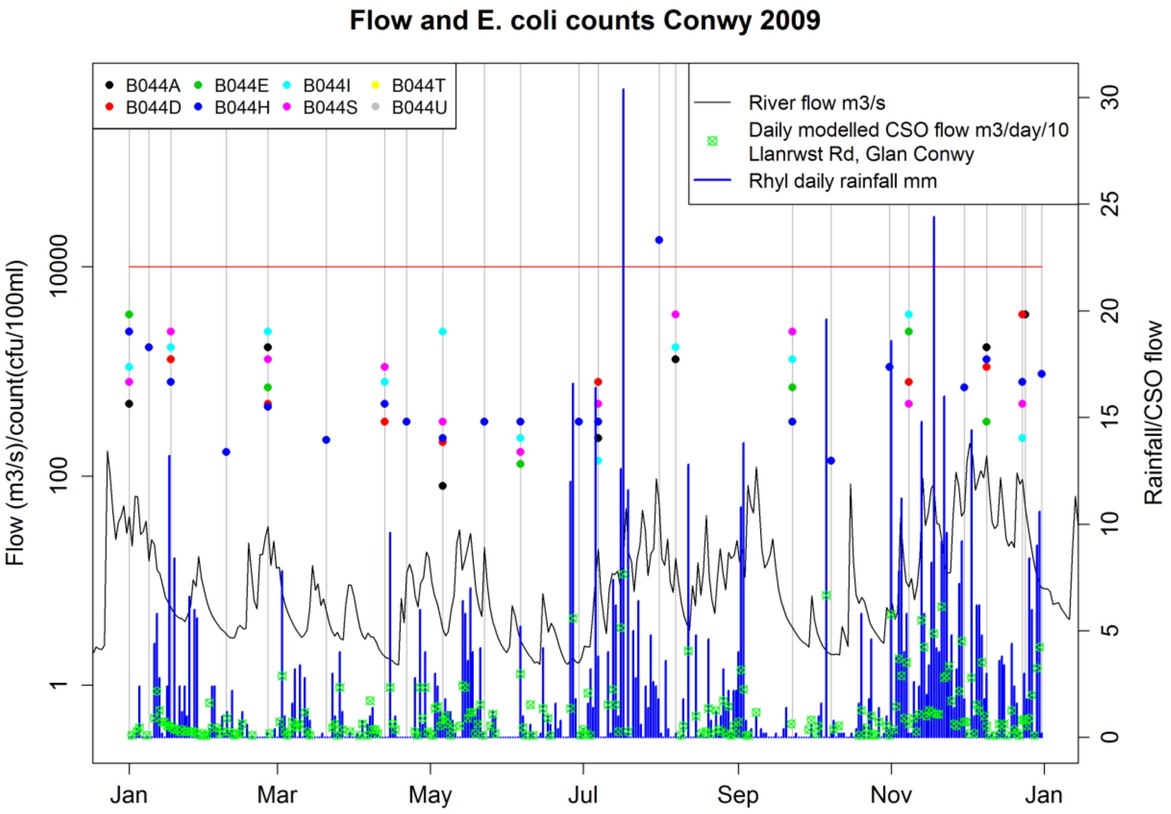
In each case a linear trend is also included in the regression equation (this is not presented). Only a single river flow variable is included, either not lagged or at a lag of 1 to 3 days. The regression analyses undertaken for these locations generally show best fits at either zero lag or a lag of 1 day, with poorer fits for longer lags. These tend to suggest a causal relationship between counts and either river flow, or environmental variables which are themselves related to river flow. Studies undertaken by CEFAS have indicated a relationship between counts of *E. coli* in shellfish and rainfall using between 2 and 7 days rainfall prior to sampling. However overall correlations were not strong indicating other environmental factors may be contributing to the *E. coli* counts in shellfish (Kershaw et al 2013, CEFAS report). Analysis undertaken in this study looked at lag time of up to a week with no strong correlations and as such the data is not presented here.

4.4 Further analysis undertaken for the Conwy Estuary, time-series analysis with modelled data on CSO operations

In addition to examining the relationship between counts and river flows, a further analysis for the Conwy considered rainfall and the operation of CSOs as possible simple environmental drivers. We used rainfall data from the nearest UK Meteorological Office station at Rhyl, 25km to the east of Conwy and at sea level.

WelshWater/Dwr Cymru provided the locations of CSOs in the Conwy estuary, and also the timing of their operation. Estimates of CSO discharges while operating were provided by the InfoWorks model, run by Arup for Welsh Water/Dwr Cymru. There are some 35 CSOs which might be considered as possible influences on *E. coli* numbers in mussels in the Conwy estuary. The frequency with which each CSO is activated varies between a few times a year to several times a month during normal weather conditions. Flow data from one particularly active CSO was considered as a potential driver (Llanrwst Rd Glanconwy).

Figure 4.3a – f shows annual time series of *E. coli* counts, daily flow, daily rainfall at Rhyl, and daily CSO discharge at Llanrwst Rd, for the years 2009 - 2014. Visual inspection suggests a possible association between CSO operations and rainfall.


Flow and E. coli counts Conwy 2009
B044A
B044E
B044I
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B044H
B044S
B044U
River flow m3/s
Daily modelled CSO flow m3/day/10
Llanrwst Rd, Glan Conwy
Rhyl daily rainfall mm
Flow (m3/s)/count(cfu/100ml)
Rainfall/CSO flow
Jan
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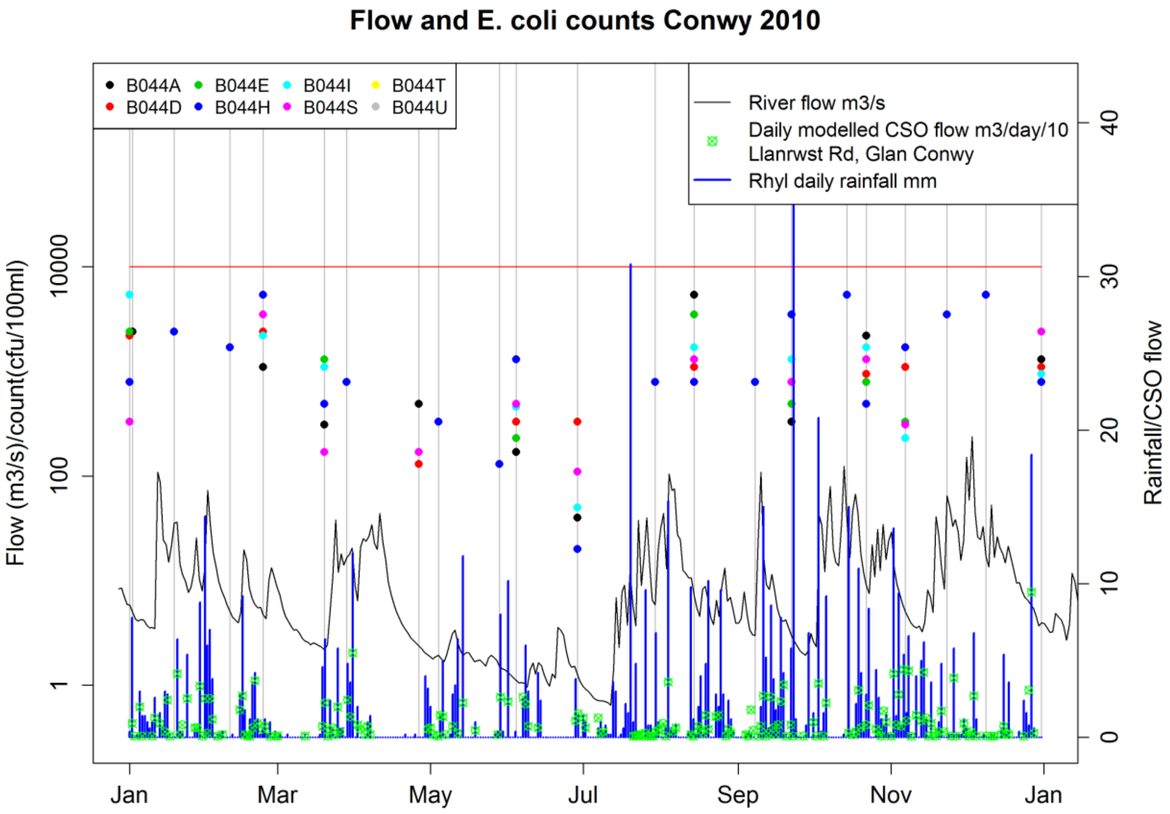
a


Flow and E. coli counts Conwy 2010
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B044D
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B044S
B044U
River flow m3/s
Daily modelled CSO flow m3/day/10
Llanrwst Rd, Glan Conwy
Rhyl daily rainfall mm
Flow (m3/s)/count(cfu/100ml)
Rainfall/CSO flow
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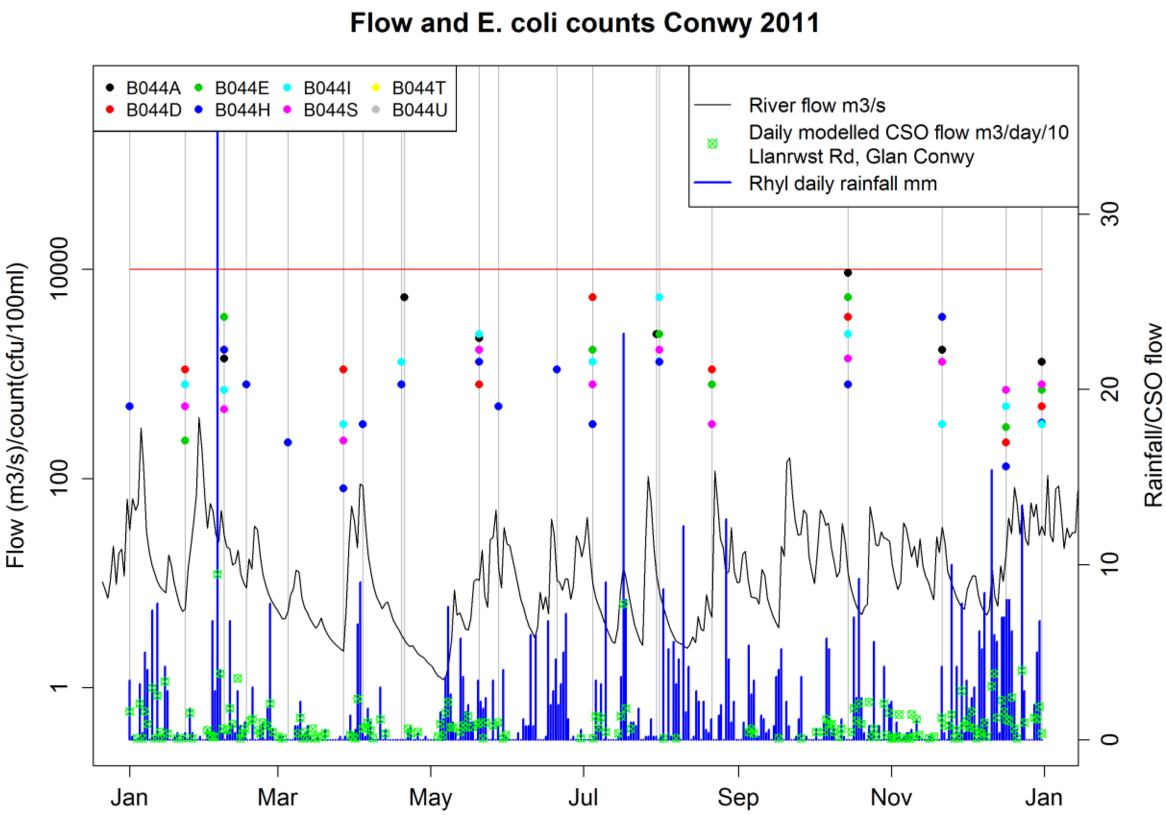
Flow and E. coli counts Conwy 2011
B044A B044E B044I B044T
B044D B044H B044S B044U
River flow m3/s
Daily modelled CSO flow m3/day/10
Llanrwst Rd, Glan Conwy
Rhyl daily rainfall mm
Flow (m3/s)/count(cfu/100ml)
Rainfall/CSO flow
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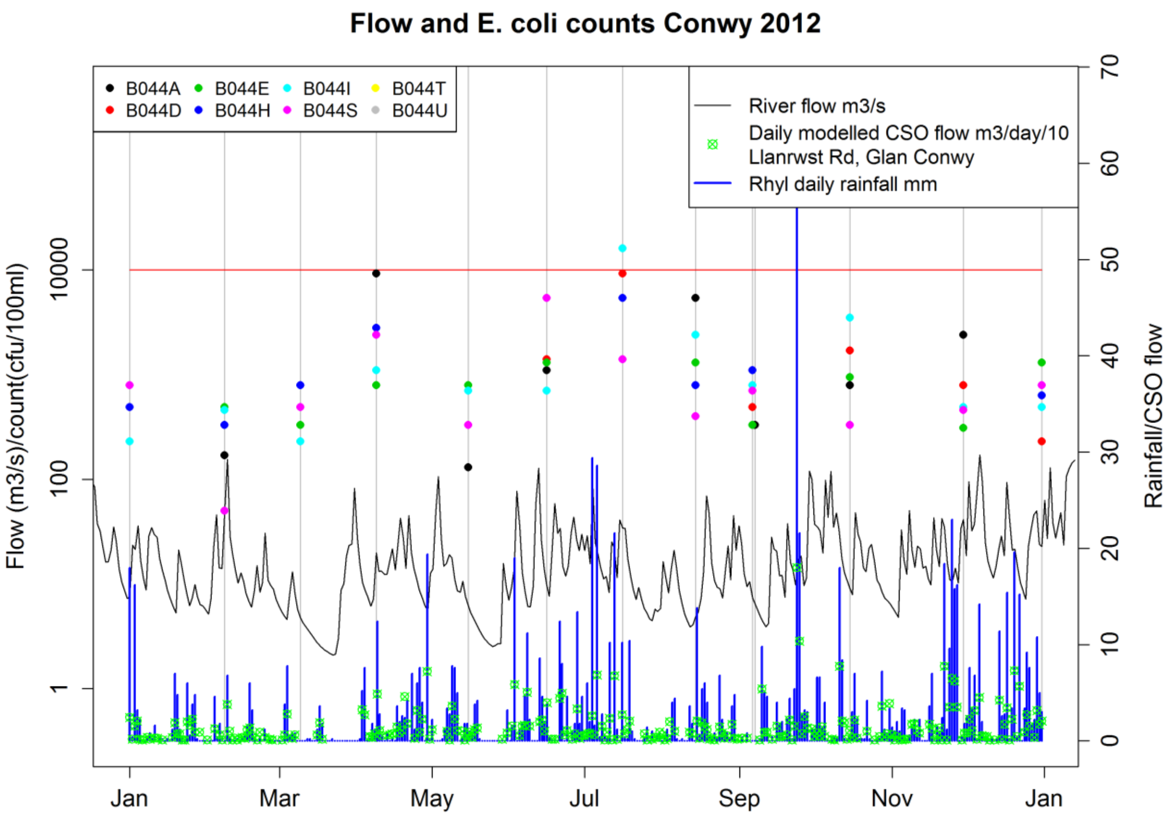
Flow and E. coli counts Conwy 2012
B044A B044E B044I B044T
B044D B044H B044S B044U
River flow m3/s
Daily modelled CSO flow m3/day/10
Llanrwst Rd, Glan Conwy
Rhyl daily rainfall mm
Flow (m3/s)/count(cfu/100ml)
Rainfall/CSO flow
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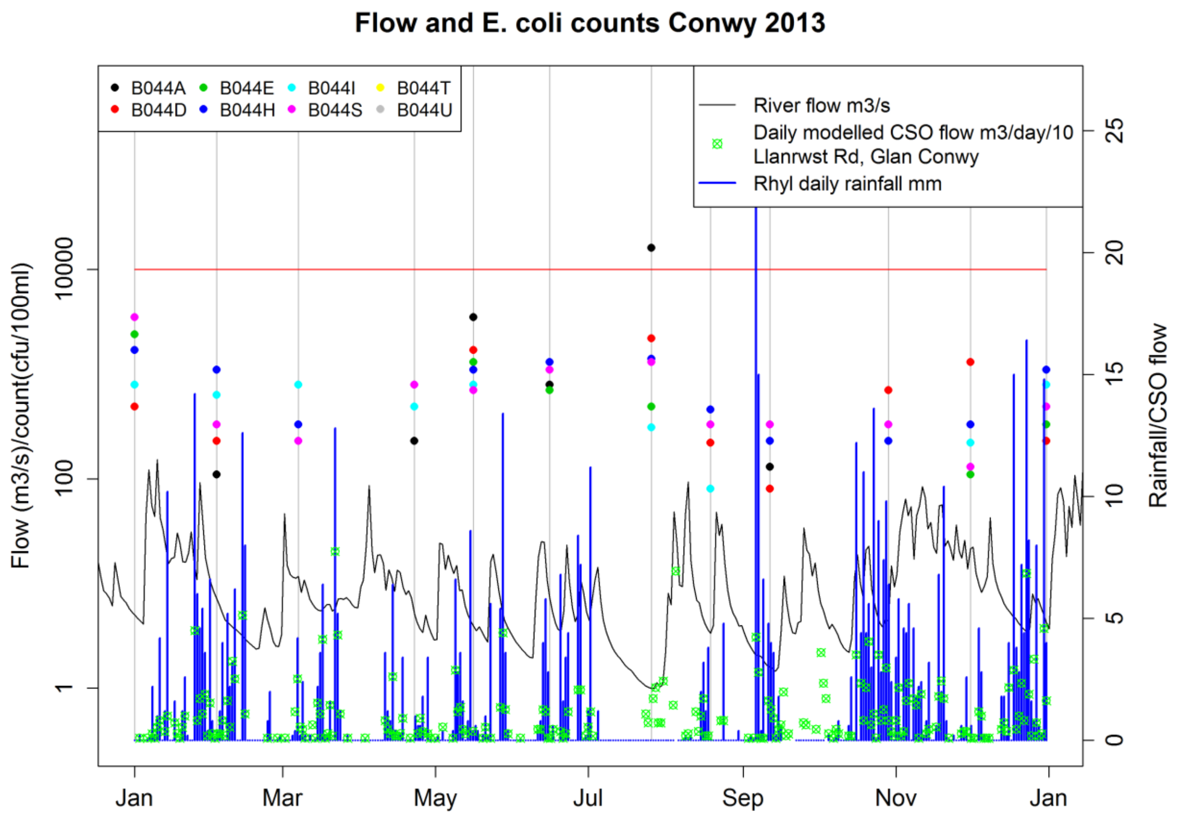

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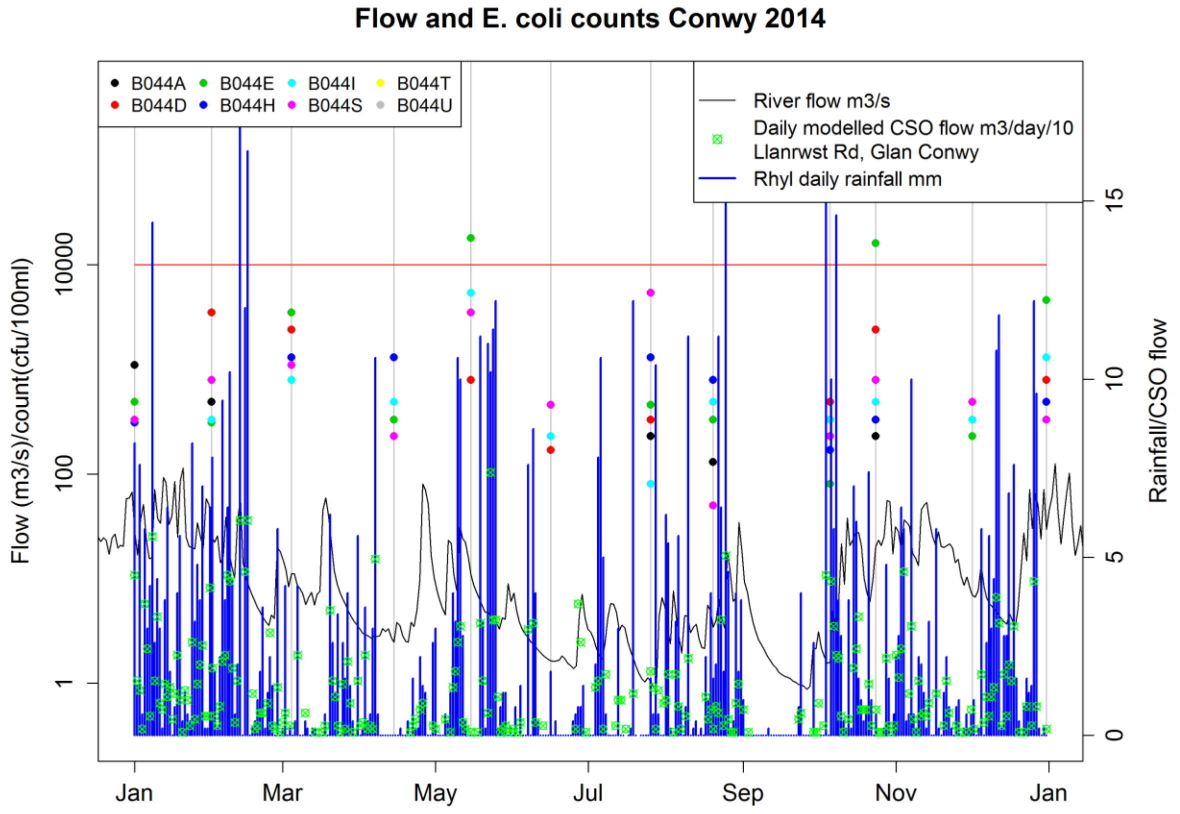
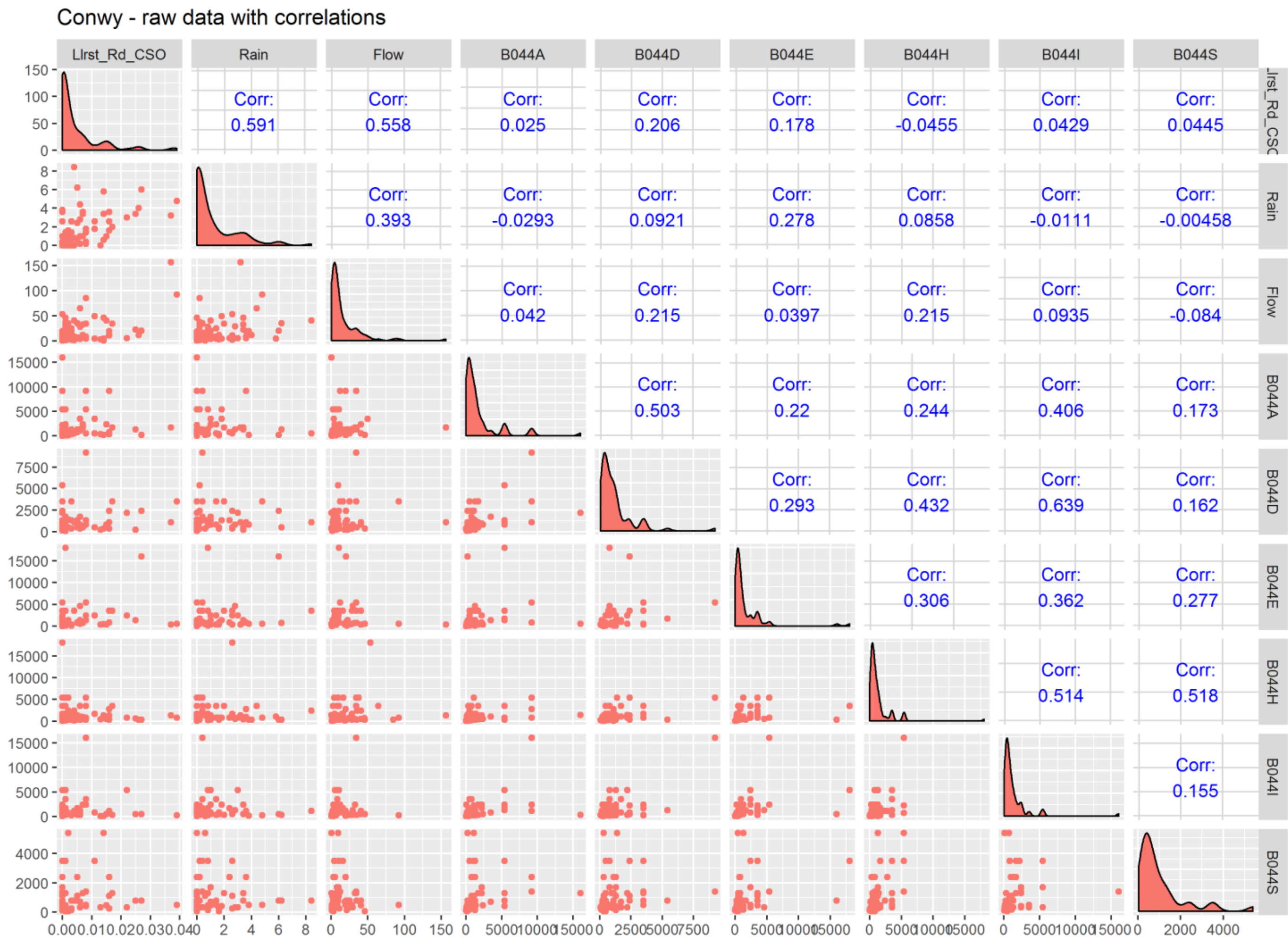


f

Figure 4.3a-f. Annual time series of *E. coli* counts in the Conwy estuary, daily flow in the river Conwy, daily rainfall at Rhyl, and daily CSO discharge at Llanrwst Rd, for the years 2009 - 2014.

Figure 4.4 a,b shows paired plots (logged and raw data) and correlations of daily CSO flow totals at Llanrwst Rd, daily rainfall at Rhyl, and *E. coli* counts in the Conwy estuary. Correlations are all low, suggesting no significant correlations.



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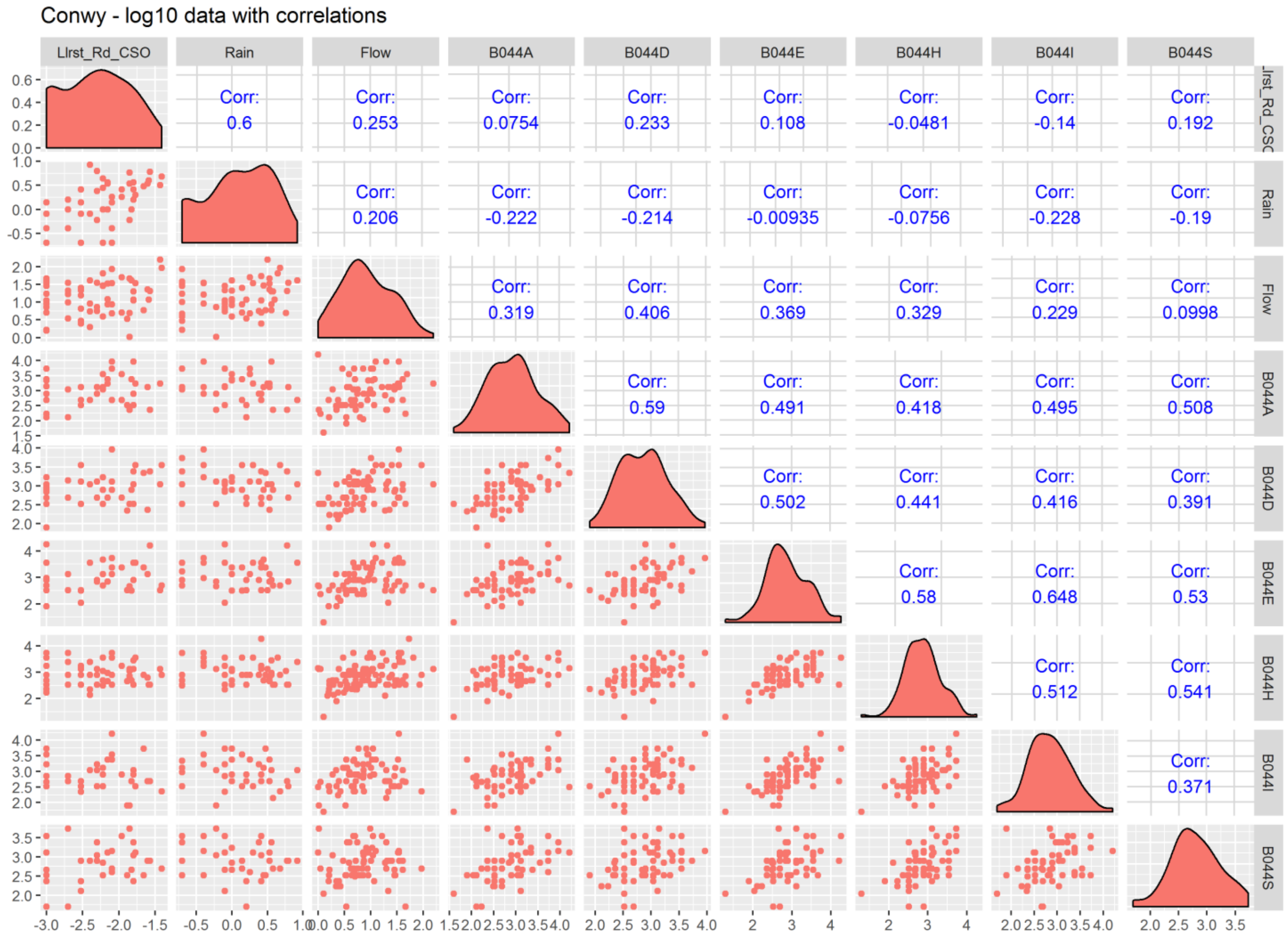


b

Figure 4.4a,b. paired plots (logged and raw data) and correlations of daily CSO flow totals at Llanrwst Rd, daily rainfall at Rhyl, and *E. coli* counts in the Conwy estuary

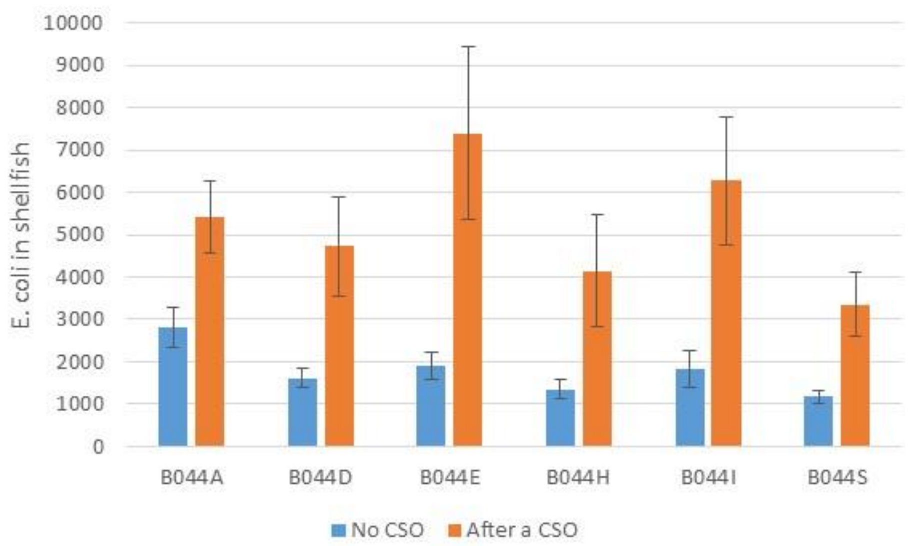


Figure 4.4.1 Conwy RMPs and CSO release. Average annual values for RMP *E. coli* during periods with out CSO release and during the week after a CSO release

Further analysis compared *E. coli* counts when there was no CSO activity at Deganwy pumping station the previous week, with counts when the CSO had been active (Figure 4.4.1, Table 4.4 Appendix 1). *E. coli* counts were consistently higher when the CSO had been active the previous week. Since CSO operation is related to rainfall, which is related to flow, and since flow is related to counts, at least on a log scale, a relationship between CSO operation and counts is not unexpected. Because of the correlation between potential drivers, a causal relationship between CSO operation and *E. coli* counts cannot be inferred. However, this apparent relationship might suggest interactive effects between multiple drivers

4.5 Discussion of initial analysis

In our analysis of the inter-relationship between *E. coli* counts in mussels, river flows, CSO discharges and rainfall, we assume the measured values are accurate. We are most confident of river flow and rainfall at the sites where they are measured, namely at Cwm Llanerch, Betws-y-Coed and Rhyl. *E. coli* counts are believed to be less reliable, and fine-scale spatial (1-10 m) and temporal variability (1-100 min) is unknown. In principle, local variability may be so high as to make reasonably accurate simulation unachievable. Additional information is needed on this fine scale variability.

Our results for the areas considered suggest that in general higher *E. coli* counts are weakly associated with higher local river flows, over the low to medium flow range. However, the chi-squared test suggests that in most areas there is little association between the highest flows and the highest counts. There is also little association among the highest counts at individual RMPs within each area. This suggests a background association between flow and counts at low to medium counts, but that the influence of other environmental sources needs to be quantified in order to simulate the occurrence of extreme high counts.

We know the main sources of *E. coli*, and in principle these can be tracked from source to shellfish, given sufficient information on the spatial and temporal distribution of sources and their trajectory. In addition to the trajectory, attenuation rates and the uptake characteristics of the shellfish need to be accounted for in generating accurate simulations of *E. coli* counts in shellfish, given the characteristics of the sources.

In the absence of detailed knowledge of trajectories, data mining can be used to explore relationships with potential environmental variables. Our analyses demonstrate the need to be clear on the focus of data mining. Relationships which hold at low to medium counts may break down at high counts because of multiple sources of *E. coli*.

Our analysis for the Conwy estuary suggests that while counts are loosely associated with flow in the Conwy river, rainfall and CSO activity at Llanrwst Rd, while themselves related, are poorly related to both flow and *E. coli* counts. Nevertheless, the analysis of weekly activity of Deganwy PS in the week prior to shellfish sampling clearly shows higher counts when there has been activity the previous week. This demonstrates the need to consider possible relationships between individual CSO operation and counts at individual sites, taking account also of *E. coli* loads in the CSO releases, and time lags. Some of this data is likely to be very poorly quantified. Empirical models might also consider indices of the state of the tide, and factors such as seasonality in deriving empirical relationships.

While we have found a poor relationship between river flow and counts at the higher end of the scale, we have not had access to time series of *E. coli* counts in river water. These could be either measured or modelled to provide potential drivers for an empirical model.

The generation of empirical simulation models without accounting explicitly for hydrodynamics is likely to be highly site specific, and require significant data mining, which itself requires a good database of extremes and the values of environmental variables shortly before the occurrence of these extremes. This might include such variables as the time of year, the state of the tide with respect to the operation of CSOs. Some driving variables are realistically never likely to be available, such as events associated with farm spillages. These will introduce uncertainty which cannot be eliminated.

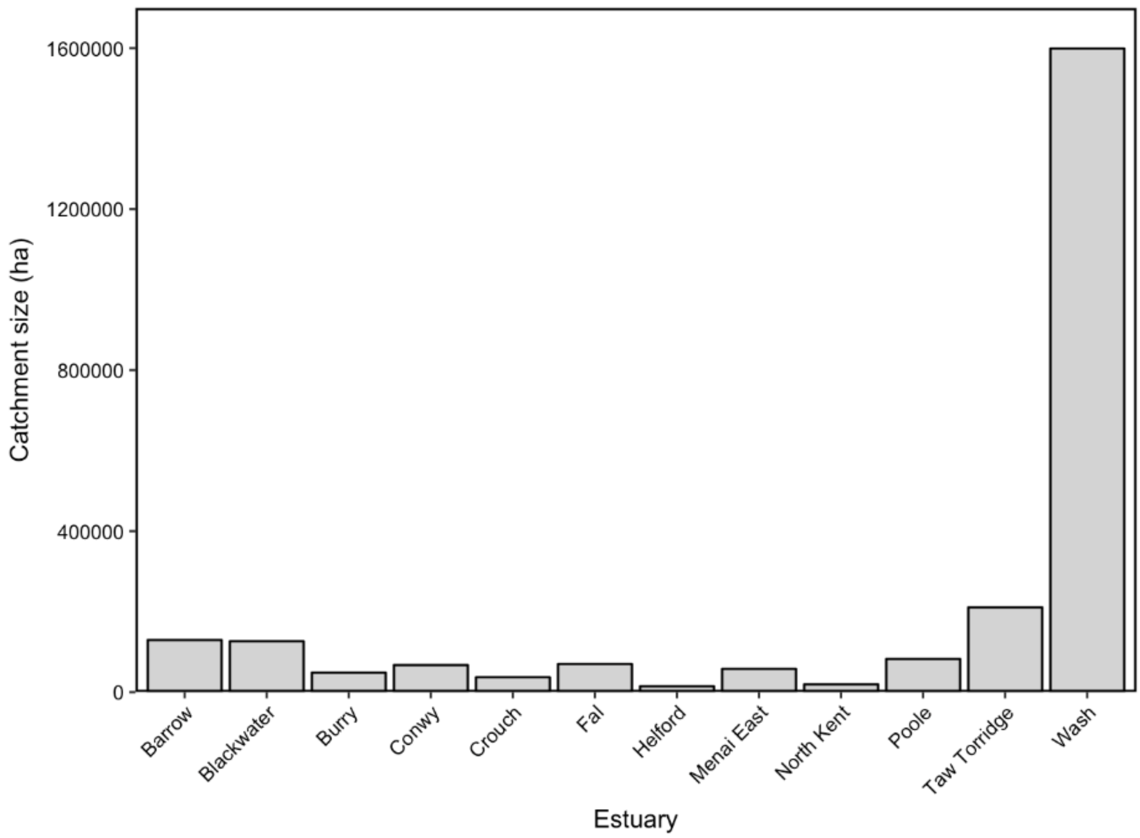
The likelihood is that a site-by-site regression tree model can be constructed which would account for a modest proportion of the variability in *E. coli* counts. In such a tree, a sequence of decisions is made as to whether a daily *E. coli* count is likely to be above or below a threshold value. The model is calibrated against measured values of the environmental variables, and the classification variable associated with the *E. coli* count.

4.6 Mapping of *E. coli* levels and CSOs.

Following the analysis undertaken above, spatial analysis was conducted using ArcMap 10.4 and data processing in R to visualise *E. coli* concentrations at each RMP in shellfish and the nearest CSO. Observations included catchment land cover maps with discharge data taken from the CEFAS sanitary surveys and from Welsh Water in Wales.

4.6.1 Catchment land cover.

Catchment land cover. For each estuary catchment, the area extent (ha) and proportional land cover type (%) was calculated. Catchment extents were taken from the Water Framework Directive data available at (http://lle.gov.wales/catalogue/item/WaterFrameworkDirectiveRiverCatchmentWaterbodiesCycle2/?lang=en for Wales and http://environment.data.gov.uk/ds/catalogue/index.jsp#/catalogue for England). The extent of each estuary catchment was marked according to those used in the CEFAS Sanitary Survey reports. Land cover classes were taken from the Centre for Ecology and Hydrology Land Cover Map 2007. Two categories of percentage covers were subset; i. 'Improved grassland' (LCM2007 class 3) and; ii. Combined cover of 'Arable', 'Improved grassland' and 'Semi-natural grassland' (LCM2007 classes 3 to 9).



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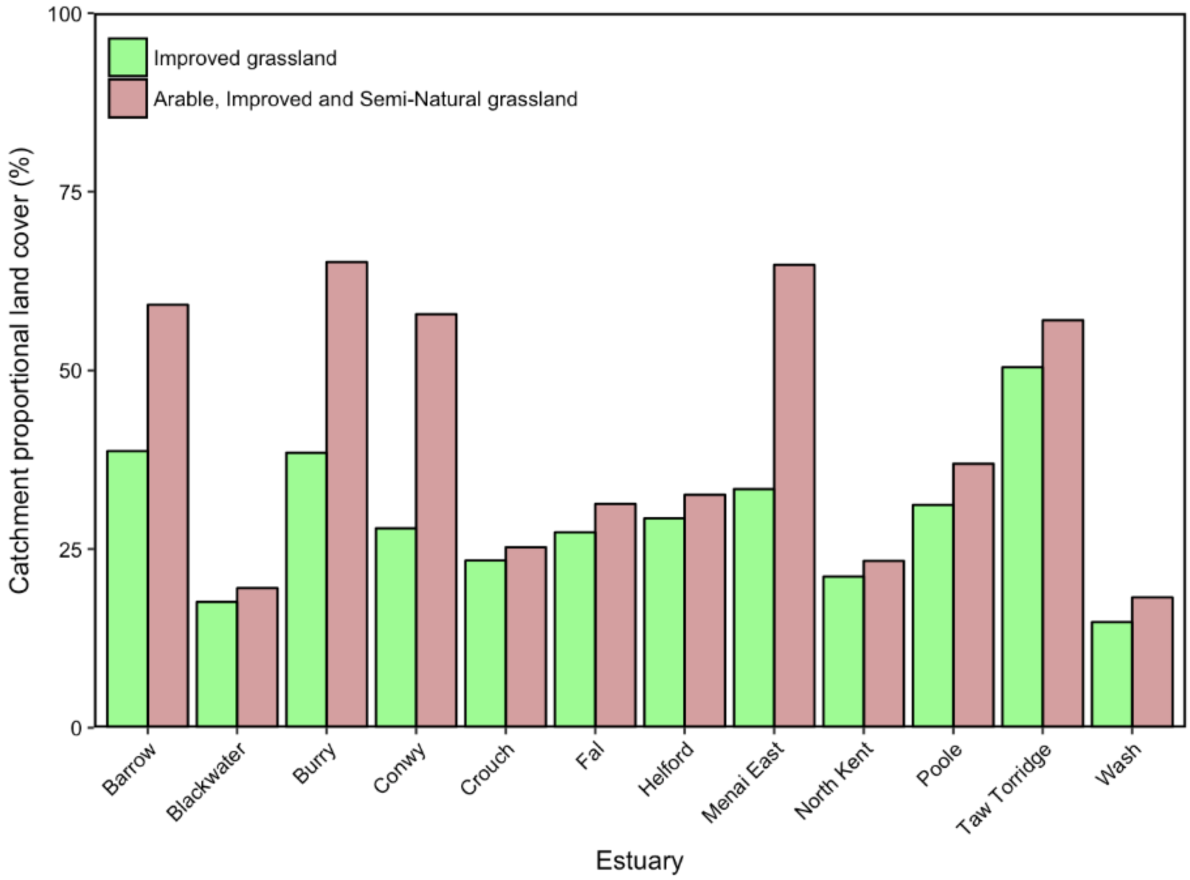


b

Figure 4.5a, b. *Top (a)* Catchment size in hectares for each estuary, and *Bottom (b)* the proportion of each catchment containing either improved grassland (green) or arable, improved and semi-natural grassland (brown).

The majority of the catchments are small (fig 4.5a), apart from the Wash which has a very large catchment. Improved grassland (fig 4.5b) covers a reasonably large proportion of total area in the selected catchments, ranging from roughly 15 – 50%. In the majority of catchments, it is the dominant of the three selected landcover categories (Arable, Improved and Semi-natural grassland). Although in Burry, Conwy and Menai it only makes up half of those categories.

4.6.2 RMPs and CSOs

For each location, maps were produced of i. the 'whole–catchment' with the associated RMPs and discharges, ii. an 'estuary-scale' map showing the likely position at which each outflow from a nearby STW enters the water, and iii. a 'loading map' with a surface overlay showing estimated *E. coli* levels across the estuary. Measurements of *E. coli* counts in shellfish at each RMP were used to interpolate between RMPs to generate the 'RMP loading map' for each estuary. Long-term monitoring of *E. coli* counts at each RMP were taken from the CEFAS RMP data. The 90th percentile between 2010 and 2017 were extracted for each RMP and used to construct the 'loading map'. The interpolated surface was created using the ArcGIS tool 'IDW', which interpolates a surface from points using an inverted distance weighted technique. Each surface was scaled between 0 and 10,000 cfu/day for a fair comparison between estuaries, and any areas with a bacterial count greater than 10,000 was assigned an 'at-risk' red overlay.

Barrow

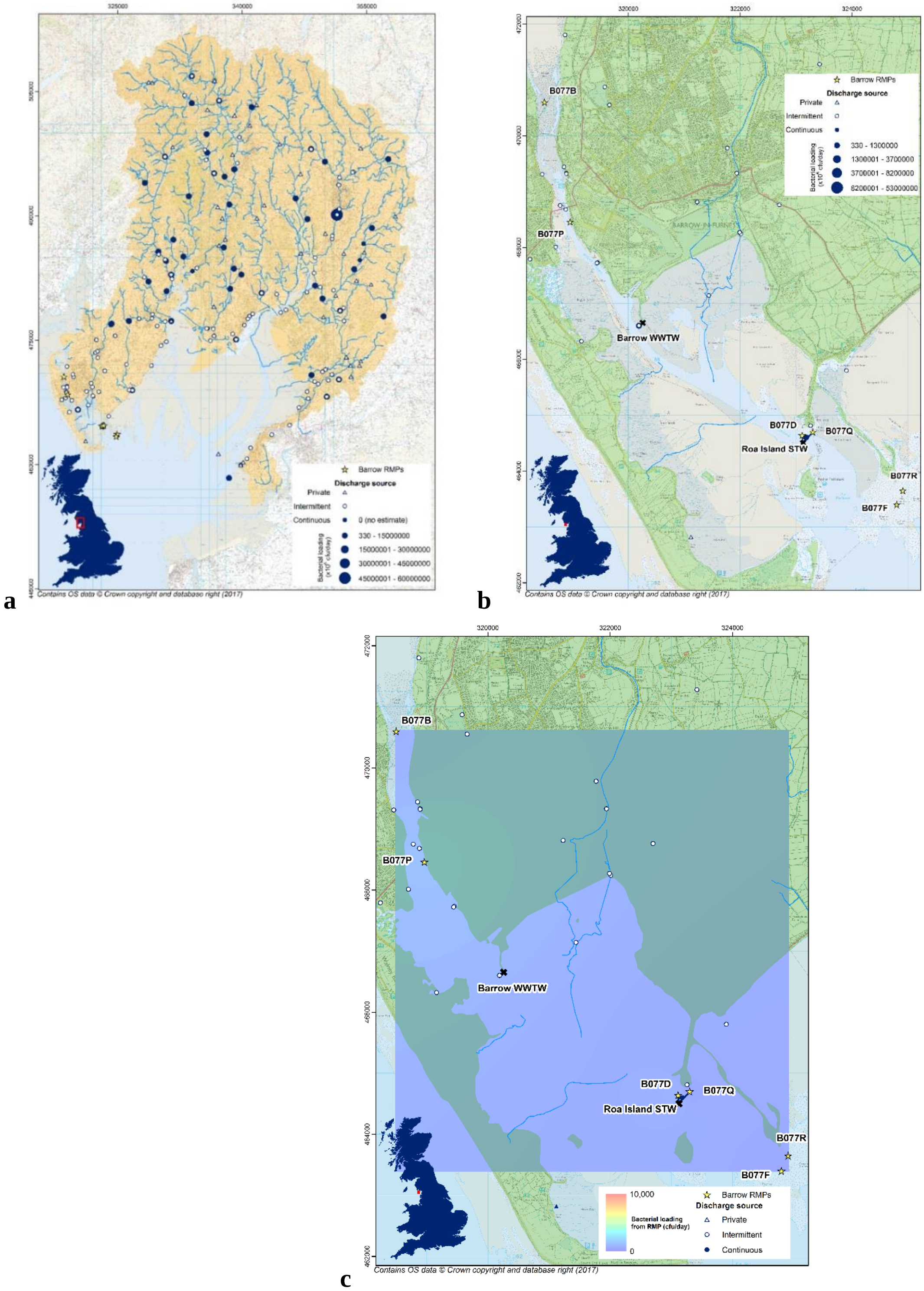
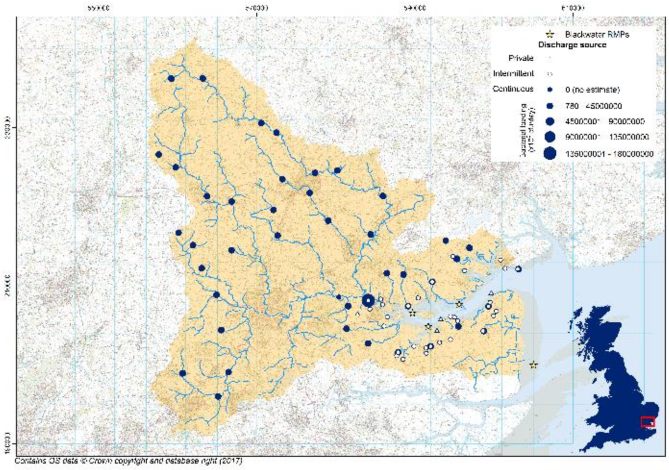


Figure 4.6 a,bc. Barrow whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Barrow RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Blackwater



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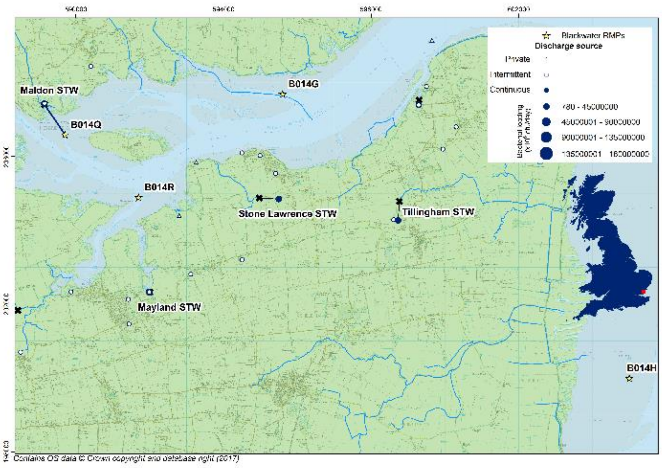

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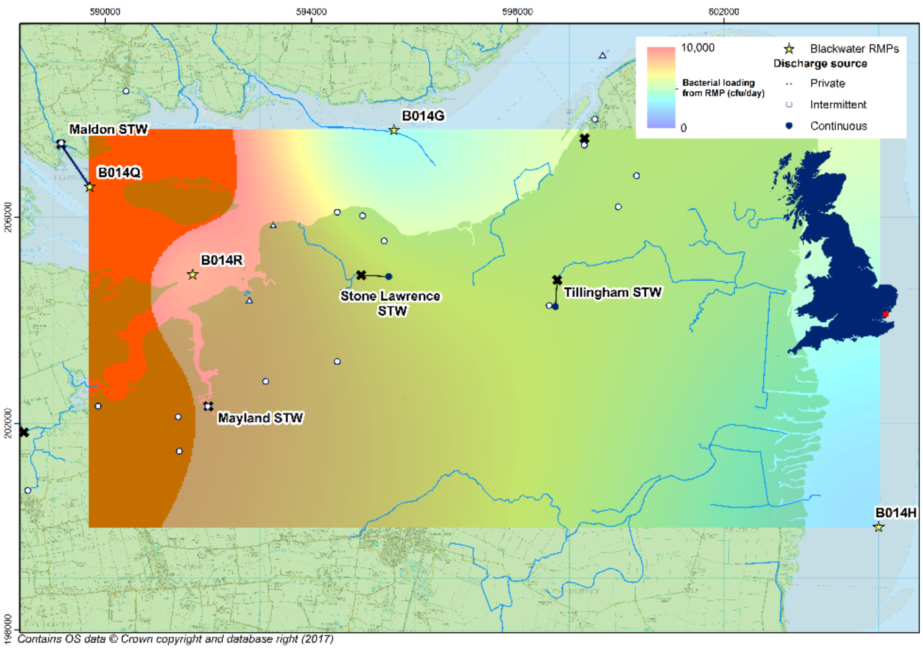
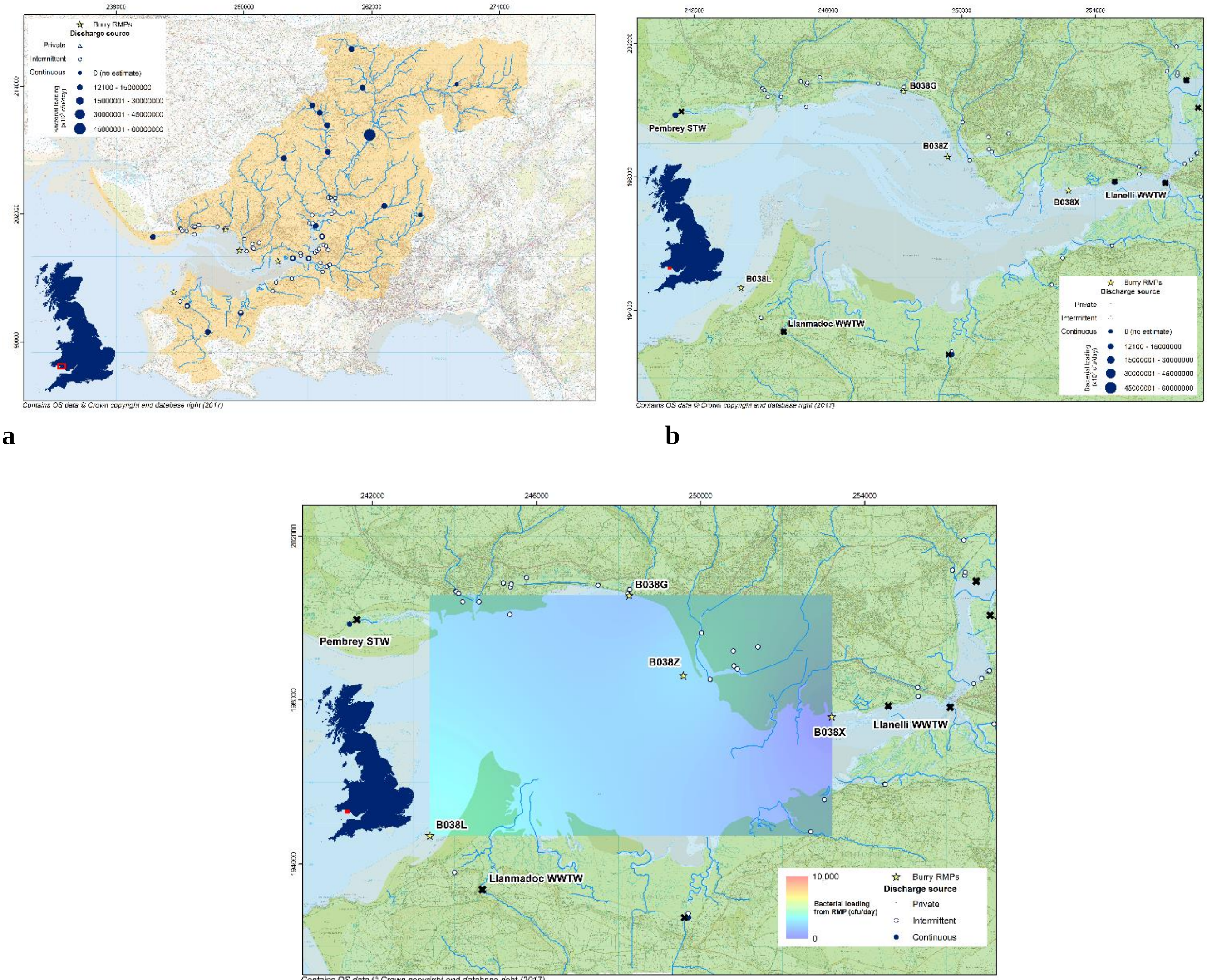


c

Figure 4.7 a,bc. Blackwater whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Blackwater RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

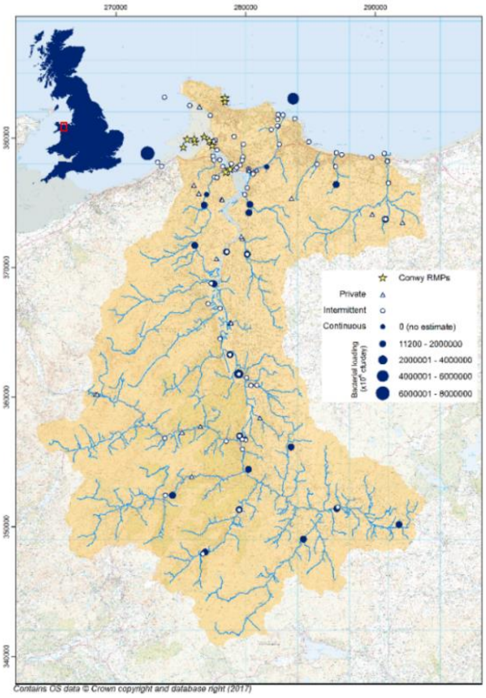
Burry



a b c

Figure 4.8 a,bc. Burry whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Burry RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Conwy



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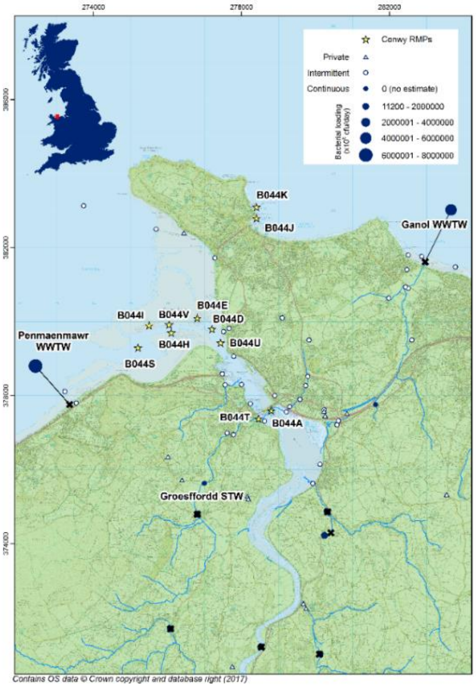

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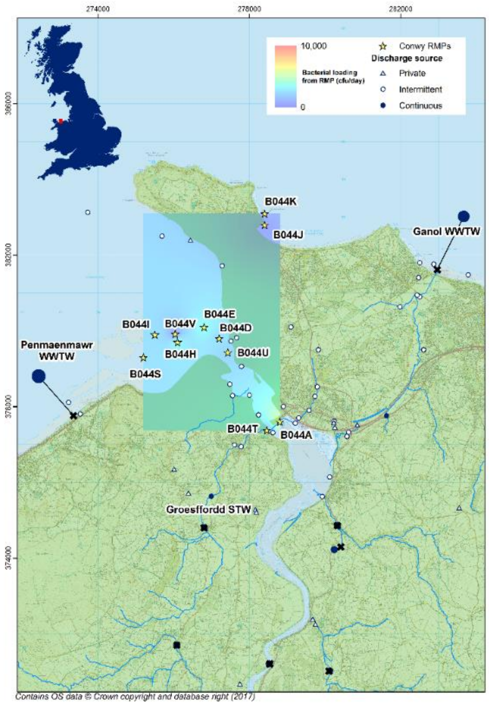

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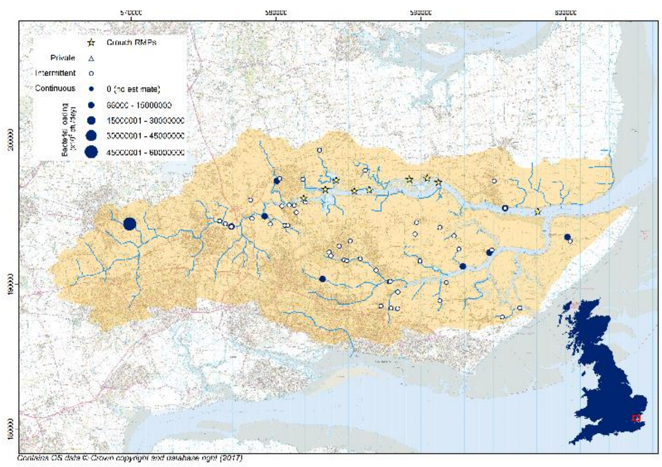
Figure 4.9 a,bc. Conwy whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Conwy RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Crouch



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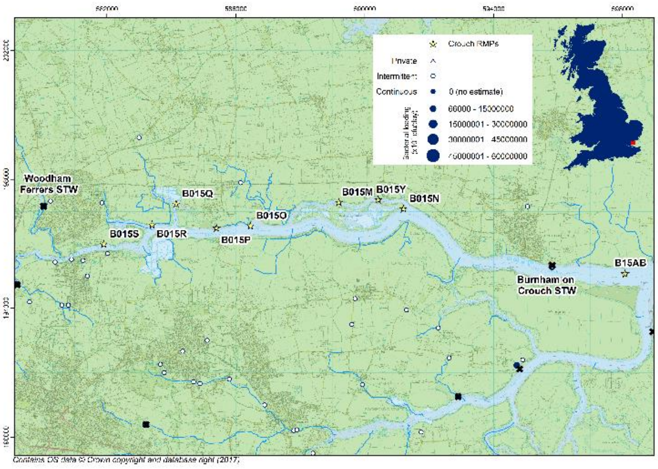

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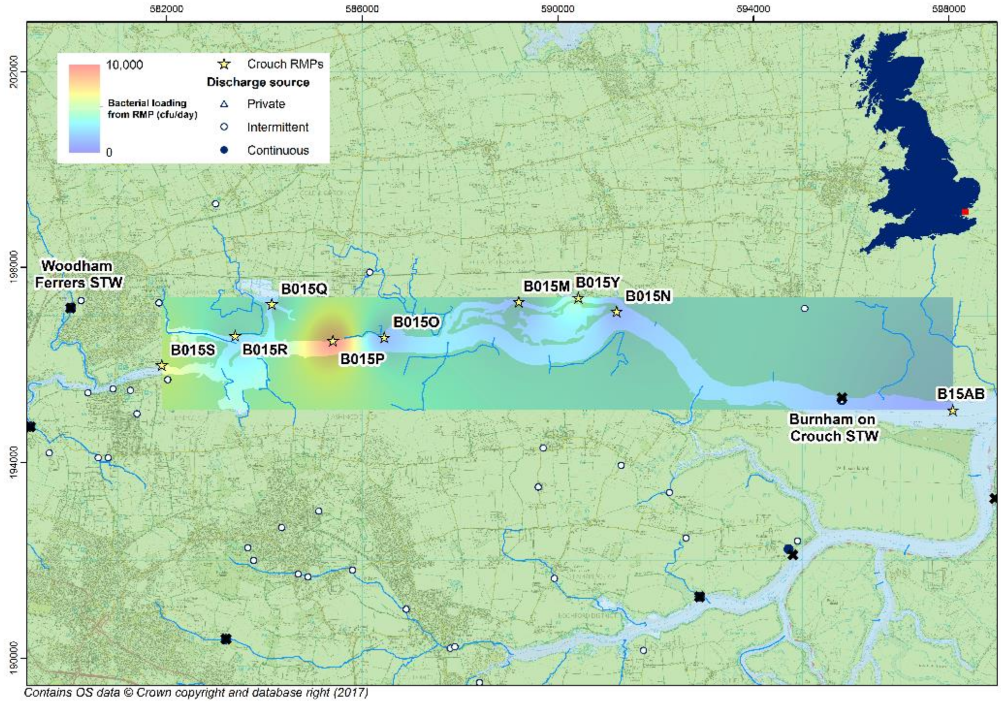

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Figure 4.10 a,bc. Crouch whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Crouch RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Fal

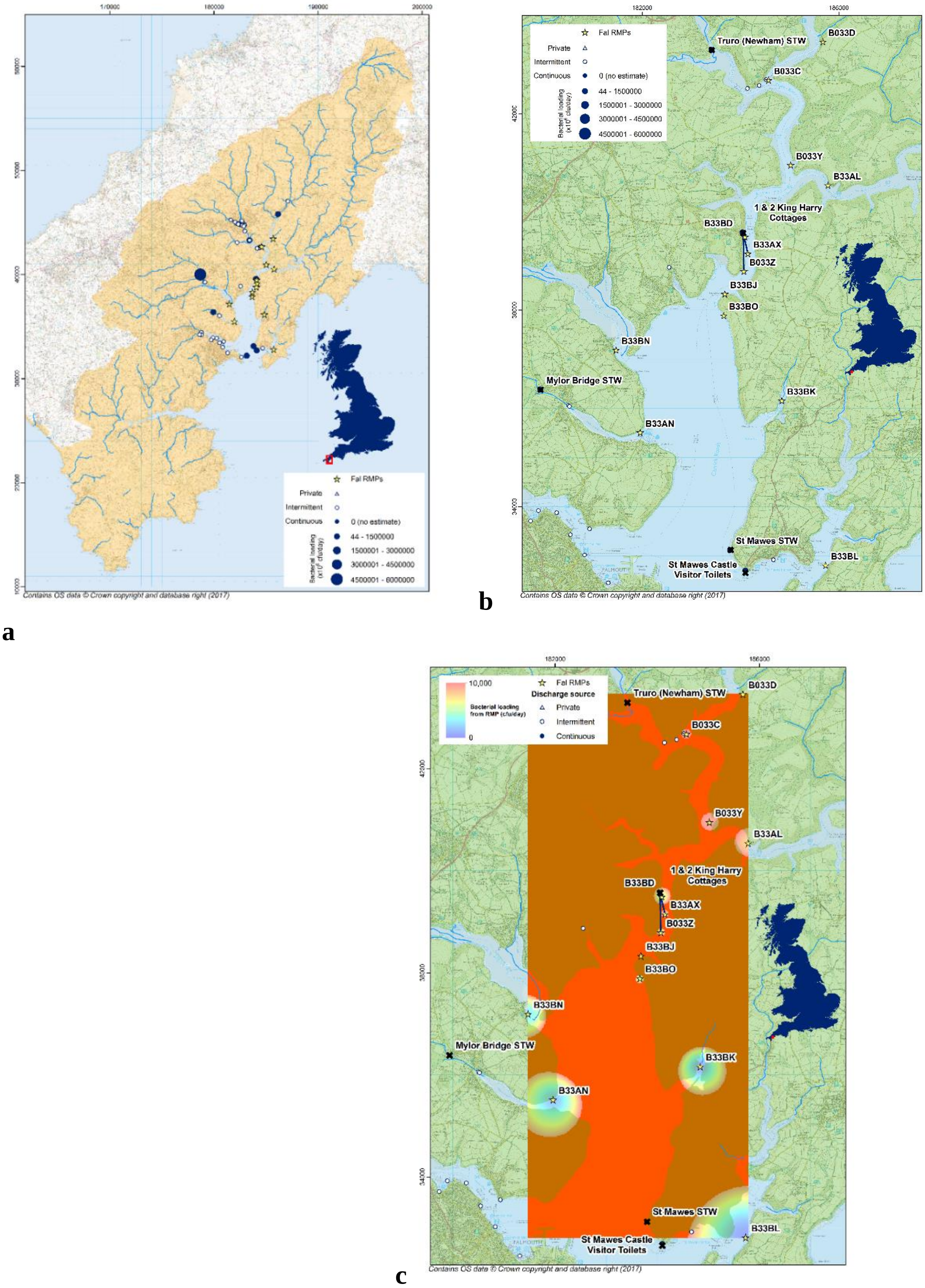


Figure 4.11 a,bc. Fal whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Fal RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Helford

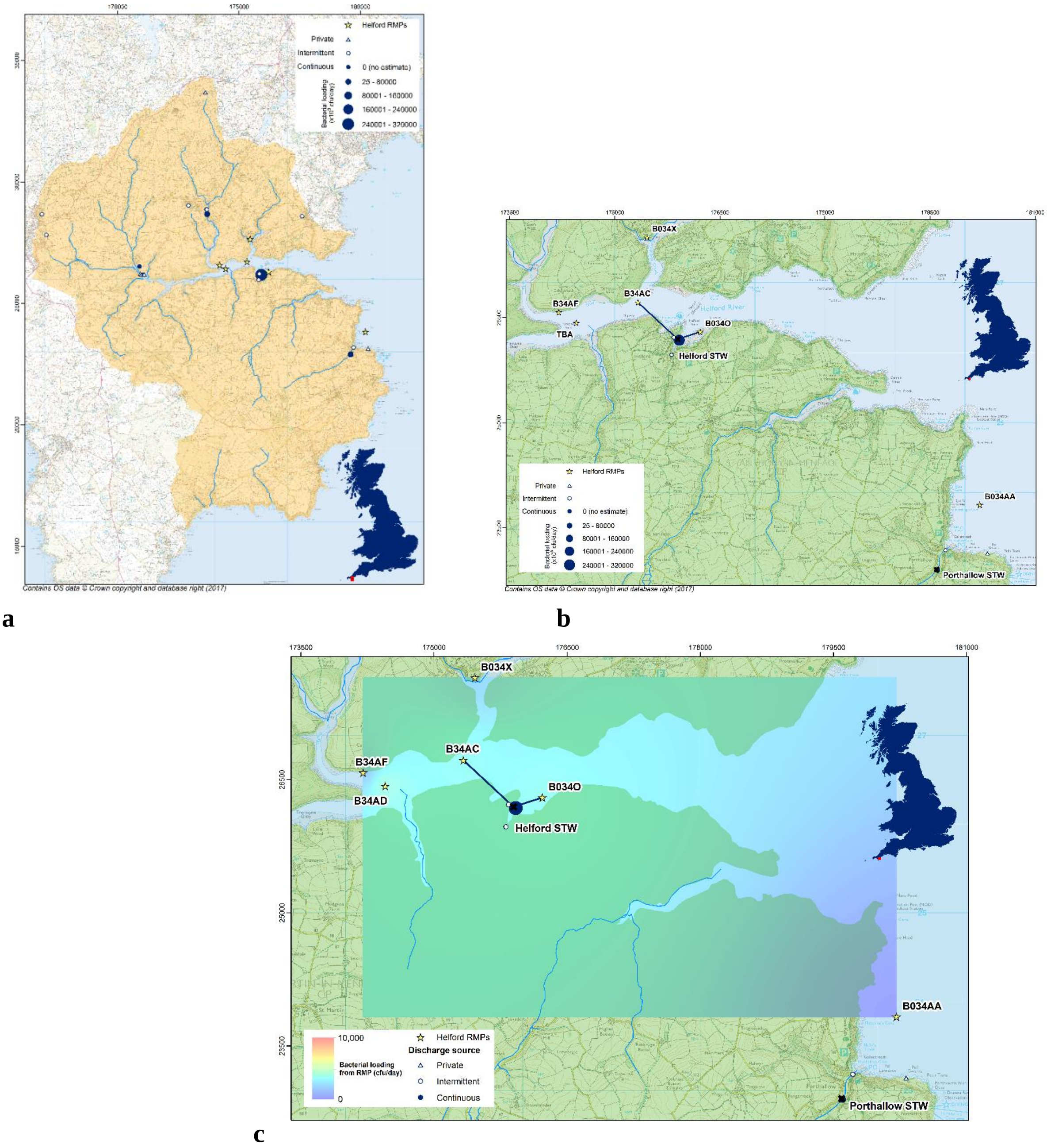
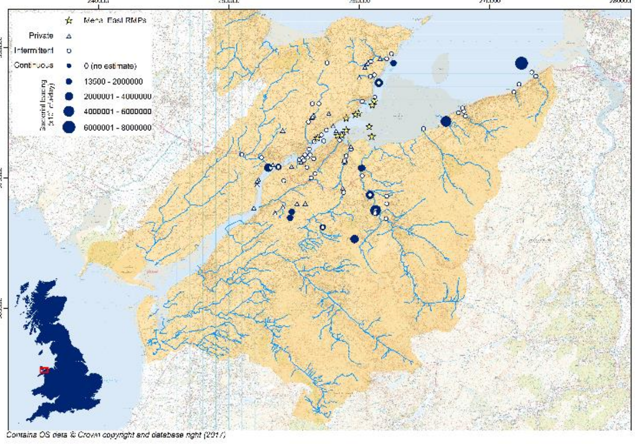


Figure 4.12 a,bc. Helford whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Helford RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Menai East



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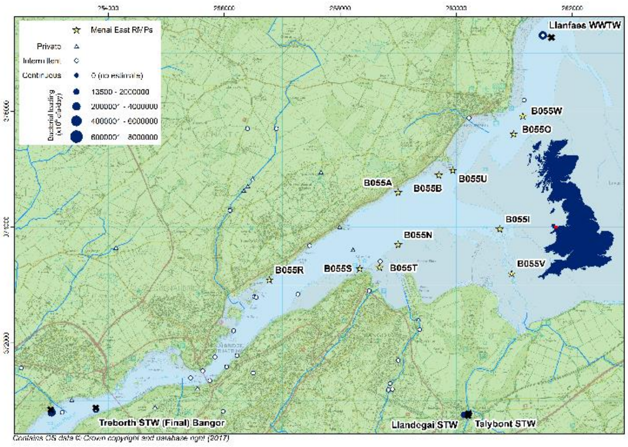

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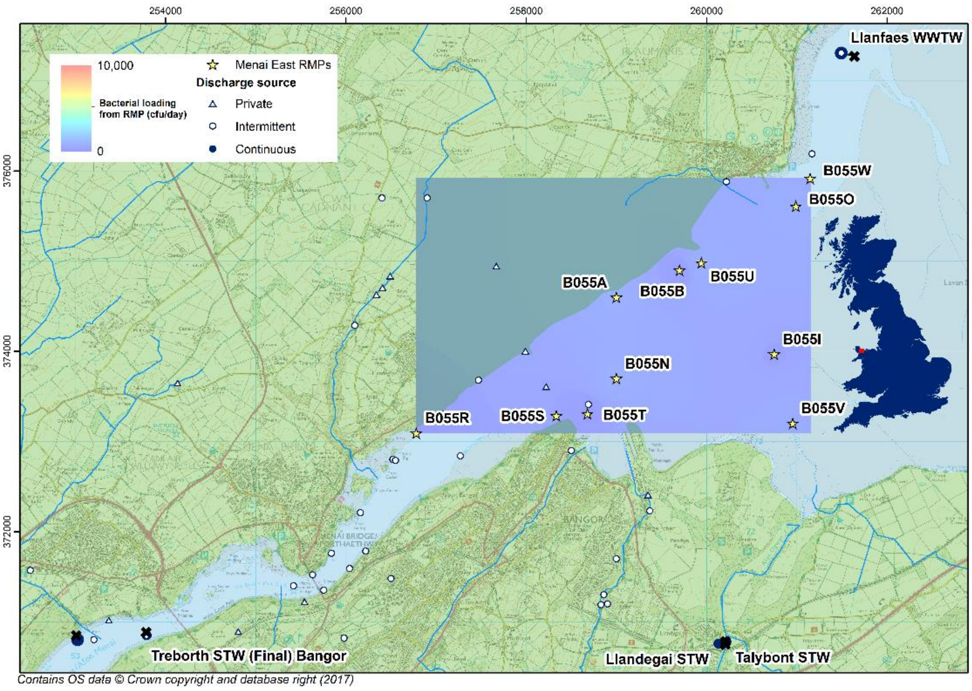
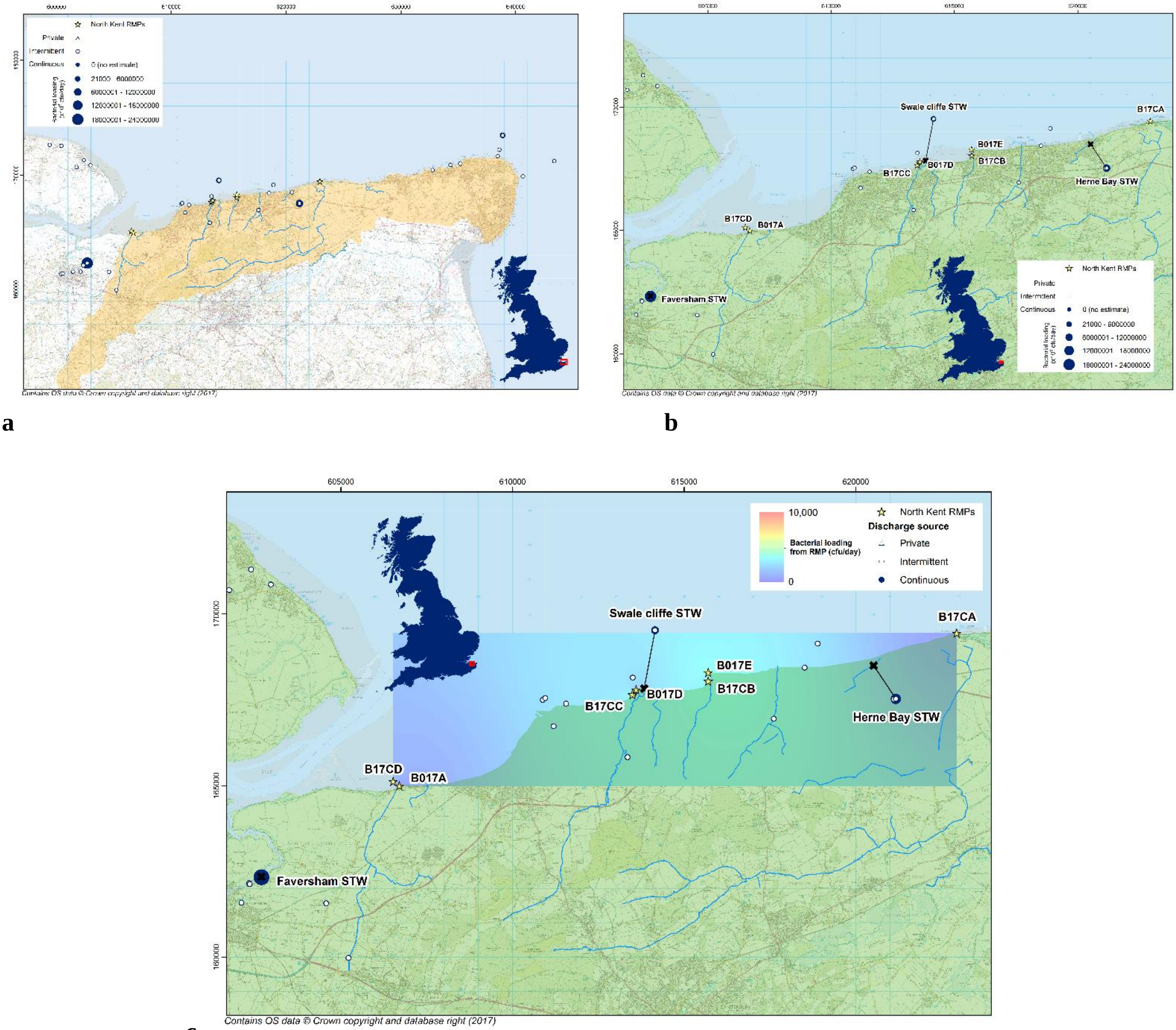


c

Figure 4.13 a,bc. Menai East whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Menai East RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

North Kent



a

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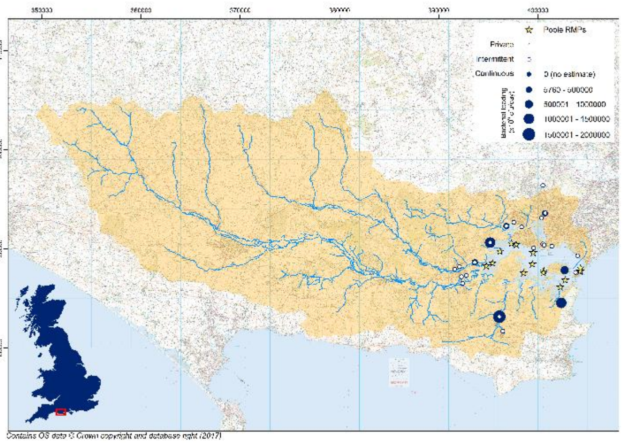
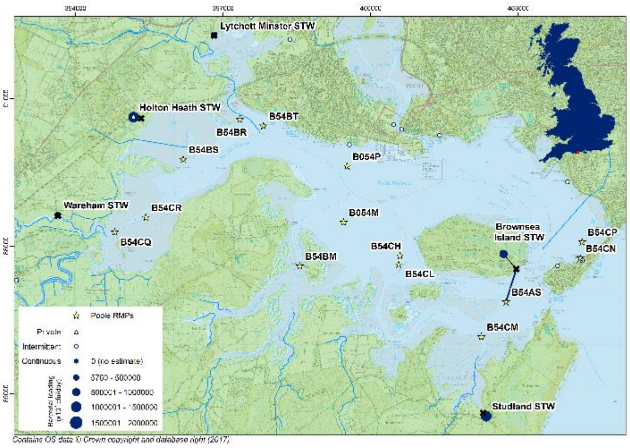
Figure 4.14 a,bc. North Kent whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **North Kent RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Poole



a



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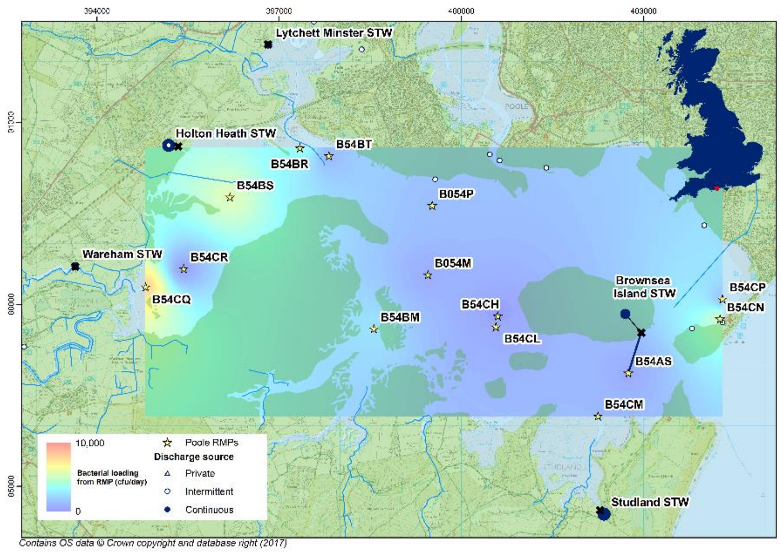
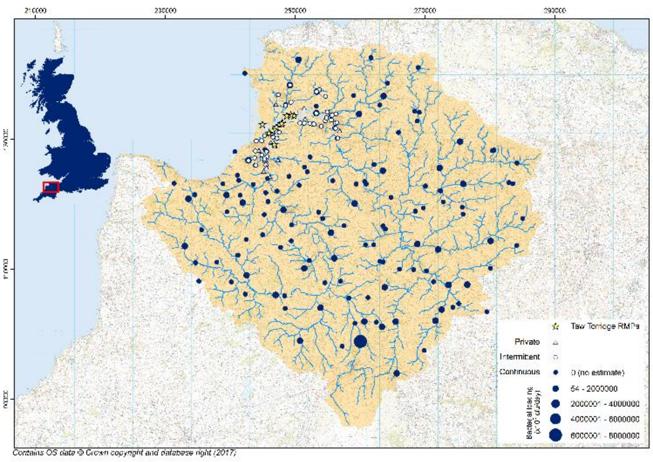


c

Figure 4.15 a,bc. Poole whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Poole RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Taw



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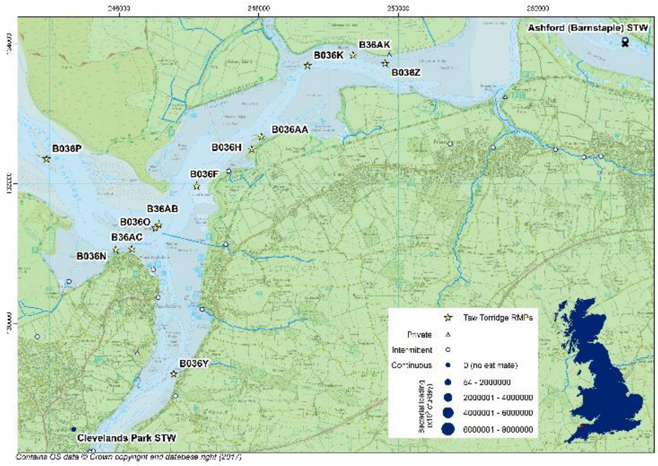

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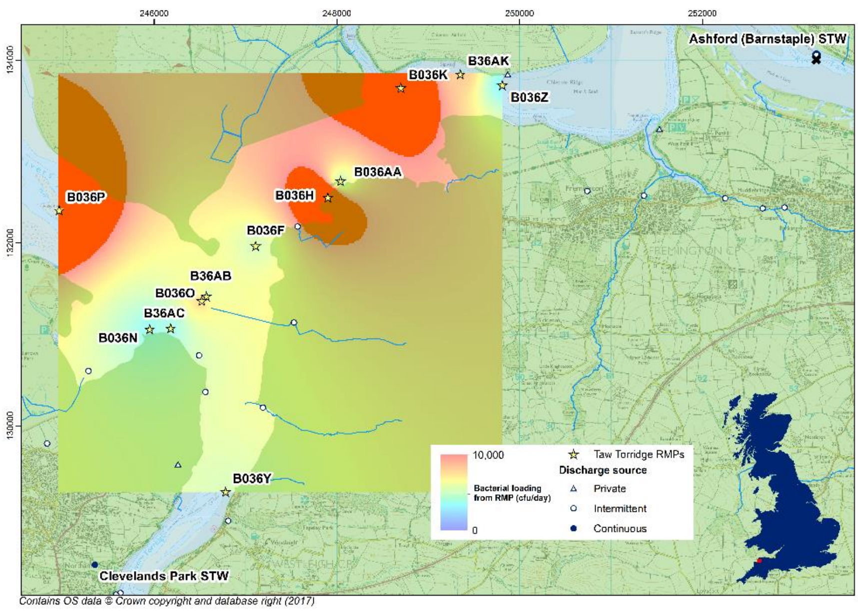


c

Figure 4.16 a,bc. Taw/Torridge whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The 'continuous' outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Taw/Torridge RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as 'at-risk'. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

Wash

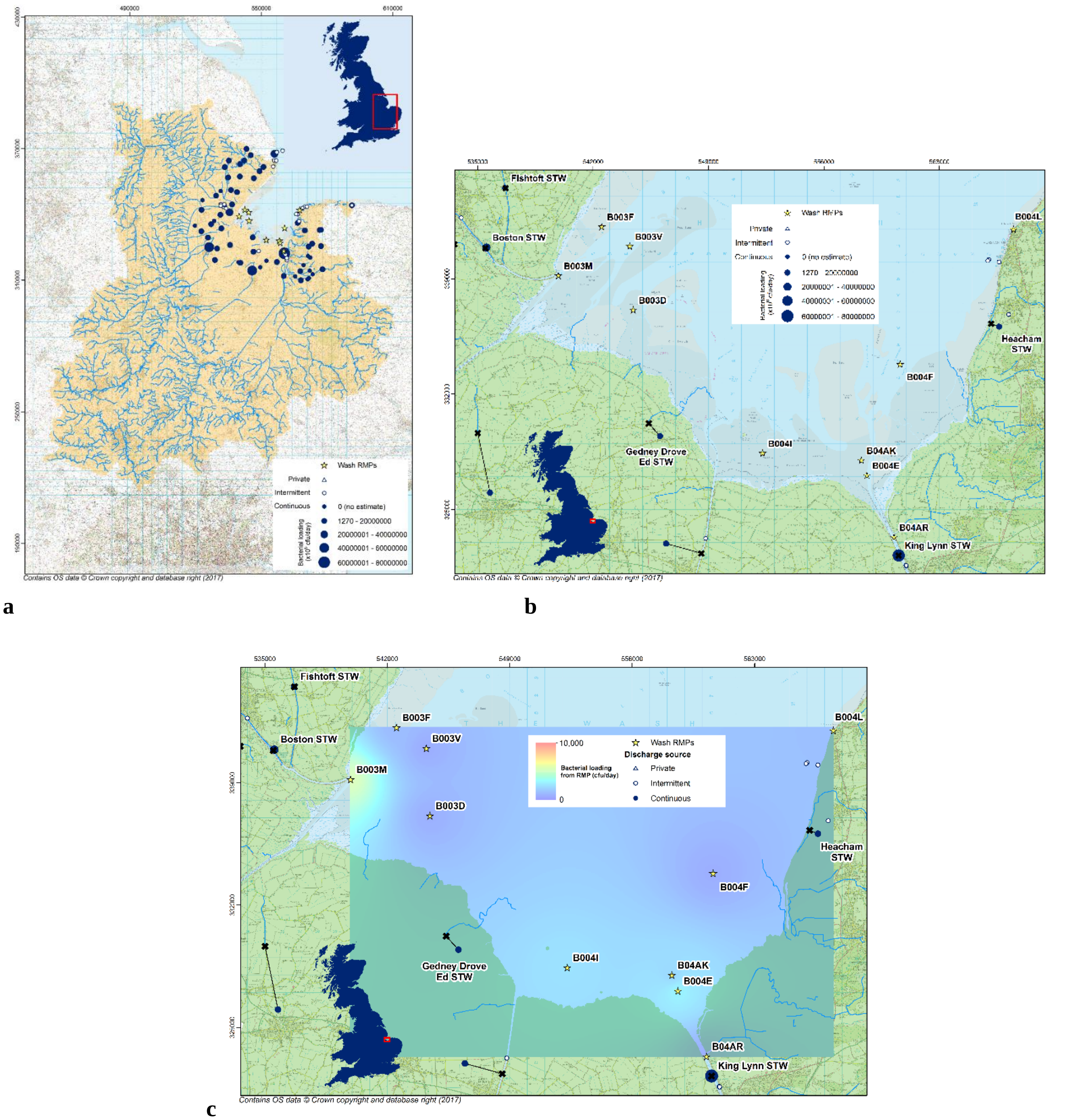


Figure 4.17 a,bc. Wash whole catchment (a). The entire catchment and river network for the estuary. The location of three sewage outflow types and RMPs are identified. The ‘continuous’ outflows have been scaled according to the amount of bacterial loading introduced by that outflow. **Estuary close-up (b)** A close-up perspective of the estuary showing all RMPs. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius. **Wash RMP loading map (c)** A close-up perspective of the estuary showing all RMPs. The coloured surface represents the estimated levels of *E. coli* across the estuary based on the 90th percentile between 2010 and 2017 at each RMP. Areas with a bacterial count greater than 10,000 cfu/day has been coloured red and is classified as ‘at-risk’. Black crosses show the likely position at which outflow from a nearby STW enters the water (entering either the nearest river or coastline). A thin black line connects the likely outflow point to its STW. A thick dark blue line connects an RMP to the likely outflow point if within a 1 km radius.

4.6.3 Cumulative risk factor.

Proximal CSO count. The number of sewage outflow points (continuous, intermittent and private sources) within a 1 km radius were counted for each RMP.

Cumulative risk factor. A 'cumulative risk factor' was assigned to each Representative Monitoring Point (RMP), to identify an expected risk to each RMP based on the bacterial loadings from continuous sewage treatment works (STWs) and their distance to each RMP. The 'cumulative risk factor' was calculated as:

$$\sum_i \frac{n_{bacteria}}{d^2}$$

where $n_{bacteria}$ is the estimated bacterial loading (cfu day^{-1}) at a given STW, taken from the sanitary survey of that area, and d is the linear distance (m) between that STW and the RMP. All STWs in a single estuary that had an estimated bacterial loading were used to calculate the 'cumulative risk factor'.

An additional 'cumulative risk factor' was assigned to RMPs within 1 km of the nearest likely position at which outflow from a STW enters a river or coast. Likely Outflow Point (LOP) position was identified by placing a point on the coastal high-water mark line or river line nearest to each STW. This point was considered the likely position at which outflow from a STW entered the water.

A 1km radius was chosen for the calculations for the risk factors. The cumulative risk figures indicate that Barrow (B077D and B77Q), Blackwater (B014Q and B014R) and predominantly the Wash B04AR have RMPs that are potentially at risk from STWs outfalls for mussels. There is a slight risk in Blackwater for Pacific oysters with regards to sewage discharges.

Summary:

In combination, the maps of RMP *E. coli* loading and the calculated risk factors based on estimated bacterial loading from STWs show there is little correlation between the two. For example, the Fal and the Taw have the highest RMP loads, but negligible risk factors from STWs. This suggests that, in general, outputs from STWs under normal operation do not pose a risk of high faecal contamination for shellfish. However, the approach does not take into account aspects such as water circulation, obstructing land masses, bathymetry, salinity and is calculated as a shortest distance.

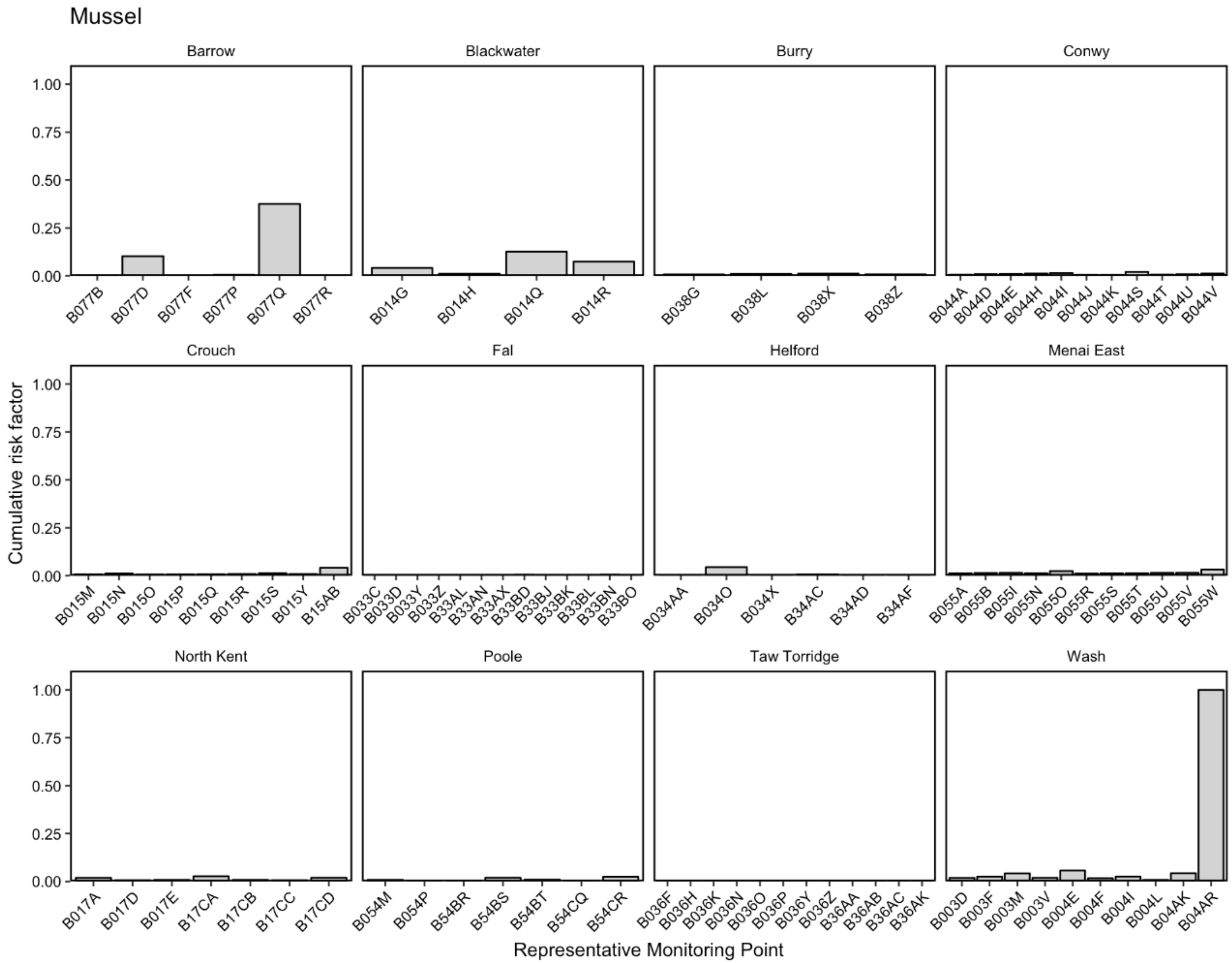


Figure 4.18. Cumulative risk factors (CRFs) for all RMPs, divided by Mussels. CRF has been scaled between 0 and 1, with 1 representing the greatest at-risk RMP.

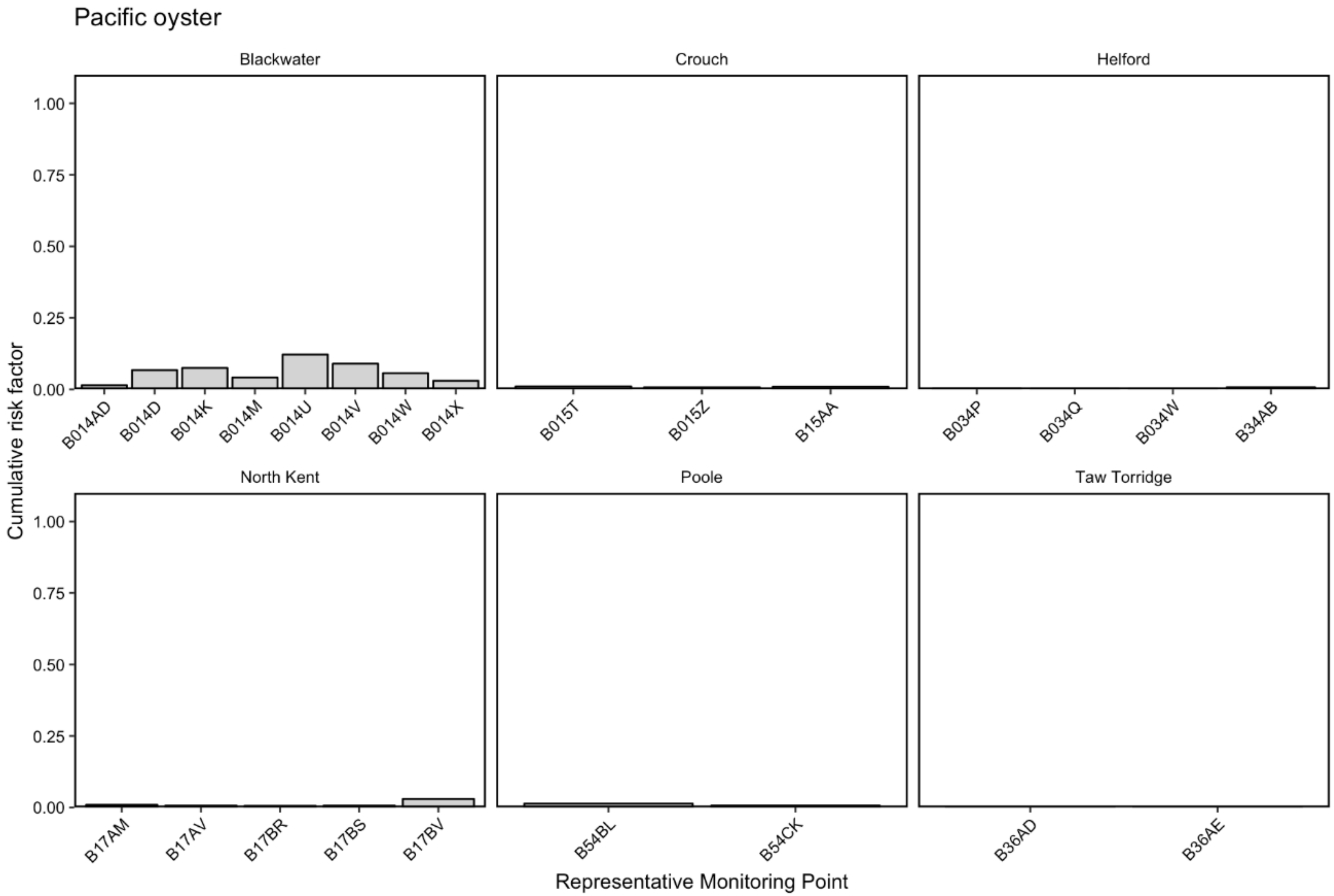


Figure 4.19. Cumulative risk factors (CRFs) for all RMPs, divided by Pacific Oysters. CRF has been scaled between 0 and 1, with 1 representing the greatest at-risk RMP.

5 Development of an active management tool

Further model exploration was undertaken in order to inform development of a tool. These models included hydrodynamic modelling at the estuary scale, and mixed models approaches to predict *E. coli* concentrations across multiple catchments.

5.1 Preliminary hydrodynamic estuarine modelling of Conwy and Menai Strait

A global challenge during the 21st century is to future proof estuarine systems against impacts of projected climate change and land use change. Communities in the UK are concerned about increased flood risk, together with the negative implications of poor water quality events that lead to public health risk, environmental degradation and socio-economic losses. Estuary impact models can help address this challenge, although there is a need to improve understanding of their limitations in terms of boundary forces and parameterisations.

The northwest Wales coastal region represents a dynamically complex and pristine environment on the west coast of the UK. Much of the Snowdonia mountain range drains into the Conwy estuary and Menai Strait, with annual rainfalls reaching around 3500 mm for the River Conwy (N.B. Smaller river sources drain directly into the Menai Strait, but are not gauged). The regional geology is largely impermeable, and this, coupled with large elevation gradients, leads to rapid flow responses to rainfall. Circulation in the Conwy estuary is inter-connected with that of the Menai Strait. This means contaminants that originate in the Conwy catchment can flow downstream and offshore connecting with the Menai region. The catchments are mainly rural, with low to moderate intensity agriculture generating relatively high nutrient runoff into the river network. Mussel shellfish beds are established in the Conwy estuary mouth, amongst a large expanse of shallow intertidal sand flats and deeper channels that cover the area between Conwy and the Menai Strait. The Strait is a fast-flowing tidal channel that is characterised by ebb-dominated (directed southwest) net flows (see [113]; and references therein). The Strait also houses significant mussel beds towards the north-eastern end. The Conwy estuary is characterised as an embayment type system that is macro-tidal, where, under mean conditions, the tidal volume exchange dominates over the river input. However, Robins et al. [114] showed that flows and mixing were controlled to a greater extent by river flow magnitude during storm conditions. This result implies that the transport of river-borne material (dissolved and particulate) through the estuary is largely determined by the river flow, with lesser modification due to the tide.

5.1.1 Model setup

The Telemac Modelling System (TELEMAC-2D, V7.0; *www.opentelemac.org*) was applied to the northwest Wales region, covering the Menai Strait and Conwy estuary (see Fig. 1). The model uses an unstructured-mesh bathymetric grid to drive a hydrostatic ocean model. The bathymetric mesh was created using BlueKenue®, and has a resolution of approximately 15 m within the estuary and Menai Strait, and coarser (50 - 500 m) offshore. Bathymetric data comprises Admiralty data (EDINA 2008), LIDAR data in intertidal regions (available from Natural Resources Wales for the Conwy and Bangor University for the Menai Strait), Multibeam surveys in the Menai Strait (conducted by Bangor University in 2014), and single-beam echosouder surveys of the sub-tidal Conwy channel (conducted by Bangor University in 2003). The model is based on the depth-averaged shallow water Saint-Venant equations of momentum and continuity, derived from the Navier–Stokes equations [115]. The classical k-ε turbulence model has been adapted into vertically averaged form to include additional dispersion terms [116]; a constant internal friction coefficient of 3×10^{-2} m was implemented in Nikuradse's law of bottom friction [115]. Turbulent viscosity has been set constant with the overall viscosity (molecular + turbulent) coefficient equal to 10^{-6}.

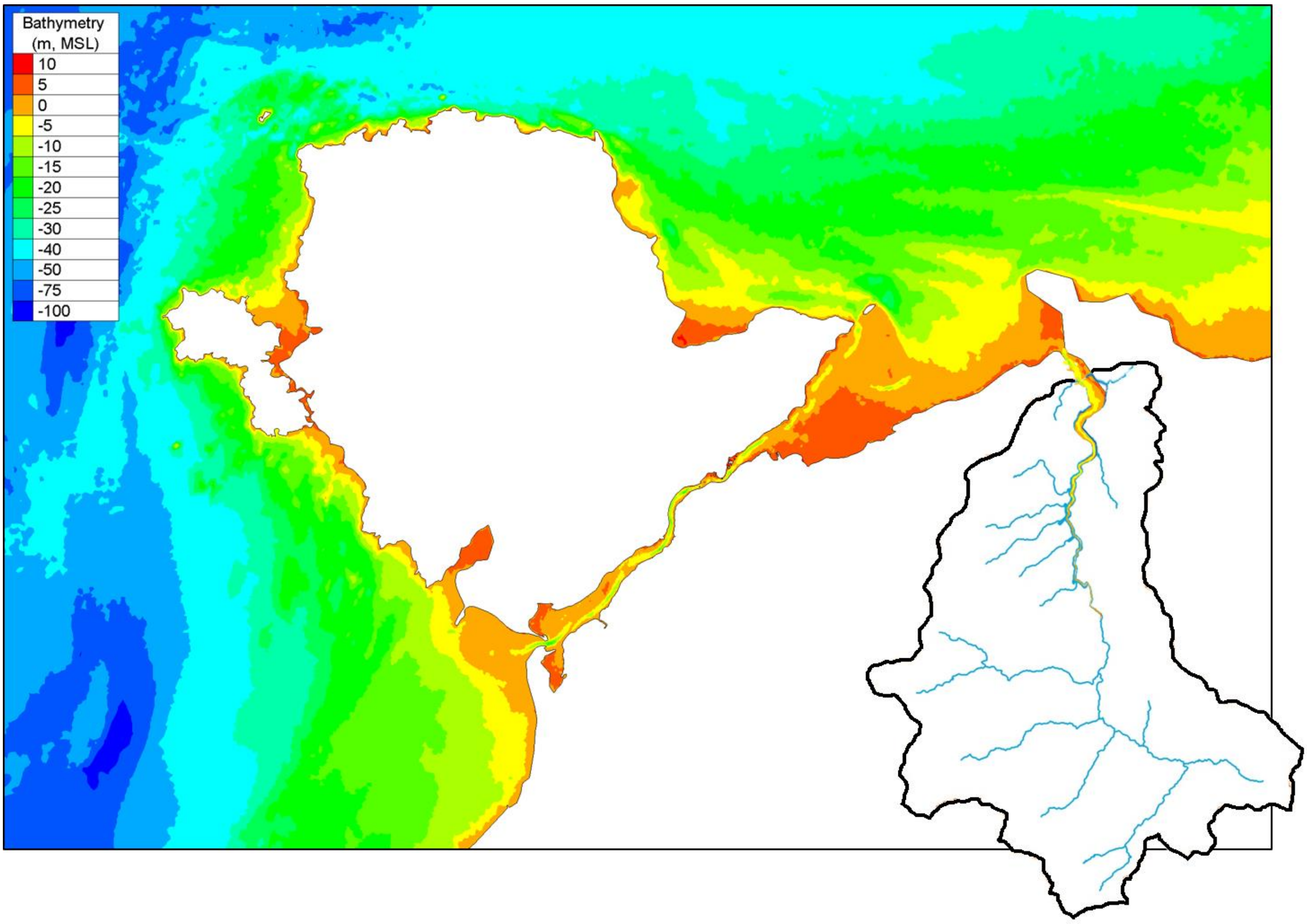


Figure 5.1. Map showing the bathymetry of the northwest Wales region encompassed within the TELEMAC model. The Conwy catchment boundary and main river tributaries are also shown (this region was modelled with a revier-routing model: CASCADE).

5.1.2 Model simulations

The northwest Wales model was initially run for a period of several days to create a steady-state salinity balance in the Conwy estuary, under minimum river flow conditions and a realistic tidal regime. Tidal boundary forcing was driven by the Poseidon Global Inverse Solution, TPXO, which provides the amplitude and phase of 13 harmonic constituents over the global ocean with a resolution of 15 arc-minutes. The tidal signal at the offshore boundary of the domain is reconstructed by TELEMAC from these datasets. The steady-state salinity distribution was then used as initial conditions for subsequent simulations. Next, the model simulated a typical month (30 days), with constant low river flows (of 10 m^3/s, which represents mean summer conditions) and no atmospheric forcing. This simulation (Run-0) enables us to visualise the baseline tidally-generated residual flows that are expected at the coast and offshore. Furthermore, these residuals give us an idea how dissolved and particulate material in the water column are likely to be dispersed.

Run-0 calculated strong (up to 0.1 m/s) ebb-dominant tidal residuals in the deeper channels near the estuary mouth, i.e., directed out of the estuary with the ebb flows (Fig. 2). Hence, during all river flow conditions, from drought to storm, ebb-dominant channels near the estuary mouth will be generated (their strength determined by river flow rates) and encourage offshore dispersal of material. Weaker flood-dominant residual flow was calculated on the surrounding tidal flats (Fig. 2). This pattern of ebb-dominant channels and flood-dominant flats is ubiquitous with intertidal estuaries such as those around the UK coast. Further up-estuary in the Conwy, the tidal-pumping effect leads to flood-dominant residual flows. Although we have not analysed residual flows in the Conwy estuary itself, since they are particularly sensitive to river flow, which was held constant for this simulation.

Several strong (0.05 to 0.1 m/s) eddy systems were predicted around the Great Orme and in Penrhyn Bay. Rather than acting as a barrier to dispersal, flows around the Great Orme are such that contaminants are likely to be transported quickly around the peninsula, in either direction (Fig. 5.2). Further west towards the Menai Strait, distinct residual pathways (up to 0.1 m/s) are present in the deeper channels, notably clockwise flows around Puffin Island and the northern Menai Strait (Fig. 3). These residuals are flanked by eddies in the coastal bays. As the Strait narrows, south of Beaumaris, residual flow is markedly strong and ebb-dominant, i.e., directed south-westwards; hence material in the water column here is likely to be transported in that direction (Fig. 5.3).

Next, we ran the model with realistic river flows derived from 15-minute river flow measurements at Cwm Llanerch which is at the tidal limit in the River Conwy and at the precise point of the model boundary (Fig. 4). The simulation (Run-1) started on 21 October 2013, to coincide with a particularly wet period and neap tides. By applying the CASCADE catchment-routing model over the same period in October 2013, two CSO events were triggered in the catchment at Betws-y-coed and Llanrwst waste water treatment works. Note that these two CSO events were idealised, but represent a likely short event. The resultant particle (bacterial or viral) concentration time series (shown in Fig. 4) was used as boundary forcing for conservative tracer input into the estuary model. Finally, we repeated this simulation, but we forced the run (Run-2) with spring tides, rather than neaps (i.e., we advanced the tidal prediction in the model by 7 days).

For the neap tide release (the initial few days of Run-1), the tidal influence within the estuary (the tidal excursion) was at its minimum because the tides were weakest. This means that the estuarine turbidity maximum – the point in the estuary where tidal-dominance meets river-dominance – was further towards the estuary mouth compared with spring tide conditions. Consequently, the dispersal of the simulated bacterial or viral concentrations was quickly transported towards the estuary mouth and offshore. By day 5, small traces of the particle concentration (<0.1 mg/l) were widely dispersed around the coastal zone surrounding the mouth – with most dispersal eastwards of the great Orme, as predicted from Run-0 (Fig. 5a). In contrast, for the spring tide release (during the initial few days of Run-2), the tidal excursion was maximal and the estuarine turbidity maximum was further upstream in the estuary, compared with Run-1. This ‘increased tidal-pumping’ effect restricted the downstream dispersal of the simulated particle concentrations, as seen in the day 5 snap-shot shown in Fig. 5.5.

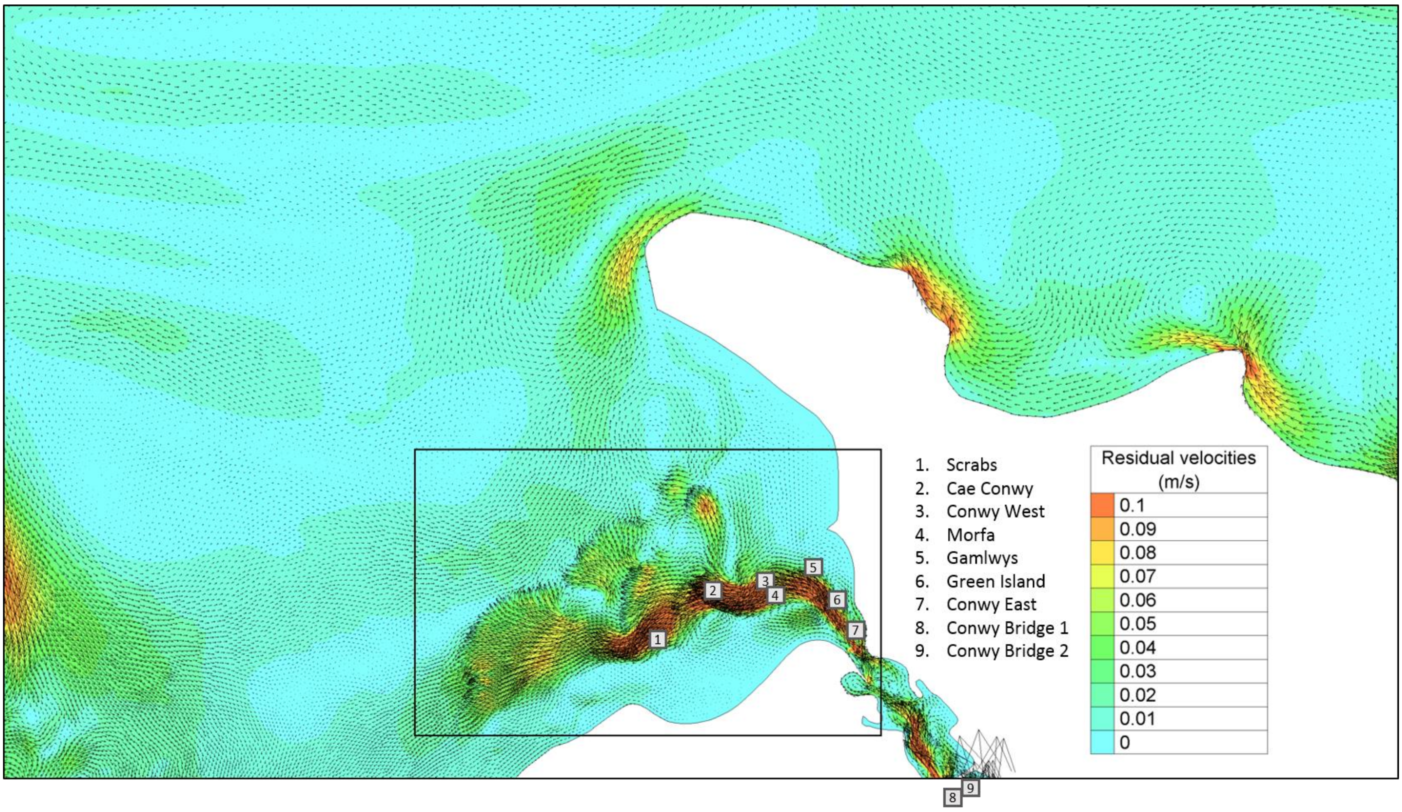
Summary

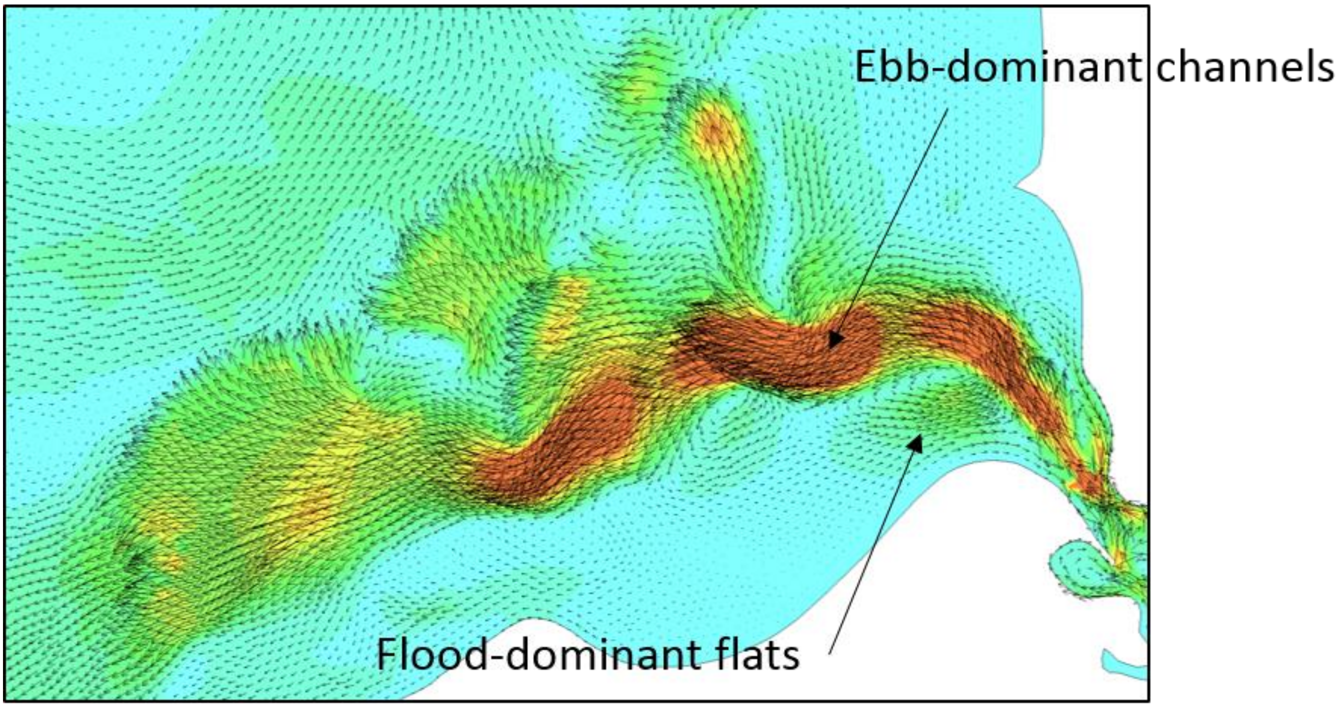
Our preliminary case study is the northwest Wales coastal region, encompassing the Menai Strait and Conwy estuary. We chose this region because we have sufficient high-resolution bathymetric data to parameterise the model domain, and there is an important shellfish industry in the region and also tourist beaches. Both the shellfish and tourist industries require good knowledge of water quality levels, and would also benefit from more accurate predictions of water quality following periods of increased risk of water quality degradation. This is a region with strong and complex tidal circulation that also responds rapidly to storm events. We applied a coupled catchment-coastal modelling system to the region, covering the Conwy catchment, Conwy estuary, and surrounding coastal region. We performed a set of semi-idealised simulations that were forced with realistic tides and river flows during a high rainfall period. Using the catchment model, we generated idealised particle concentrations at the river boundary of the estuary model. Our results show clearly the sensitivity of the region to the occurrence of a storm (and subsequent CSO triggering an increased bacterial concentration) relative to the lunar tidal cycle. During spring tides, bacteria or viral loads are retained within the estuary due to the relatively long tidal excursion. In contrast, during weaker neap tides, the same river forcing lead to more offshore dispersal of the bacterial or viral concentration.

Hydrodynamic models have been used to run simulations of water quality condition under a variety of storm event scenarios over harvesting areas in France [48; 117]. The two dimensional model included currents, dispersion and decay rates to simulate microorganism behaviour [117] and concluded that the location of shellfish beds near the coast made them vulnerable to both small and large rainfall events with the larger events including the overflows of CSOs. The results agree with other studies for coastal areas [50] and also for studies using hydrodynamics to assess the impact of bacteria or viral particles including Norovirus fluxes over shellfish areas [119; 117].

A more comprehensive study is required to better understand the water quality risk following storm events. Information on CSO trigger patterns, that is now emerging in the Conwy catchment and surrounding water network, could be used to simulate a range of bacterial or viral release scenarios – from sources within the catchment and also along the Northwest Wales coast. These scenarios, simulated for a range of realistic storm events (generating different rainfall and wave patterns) would enable water quality risk maps to be produced, with levels of model uncertainties clearly portrayed. Finally, the modelling methodology can be applied to other coastal systems around the UK.

a





b

Figure 5.2a,b: Residual tidal flows around the Conwy estuary mouth. The inset shows that the deeper channels are characteristically ebb-dominated, whereas the shallower tidal flats are flood-dominated.

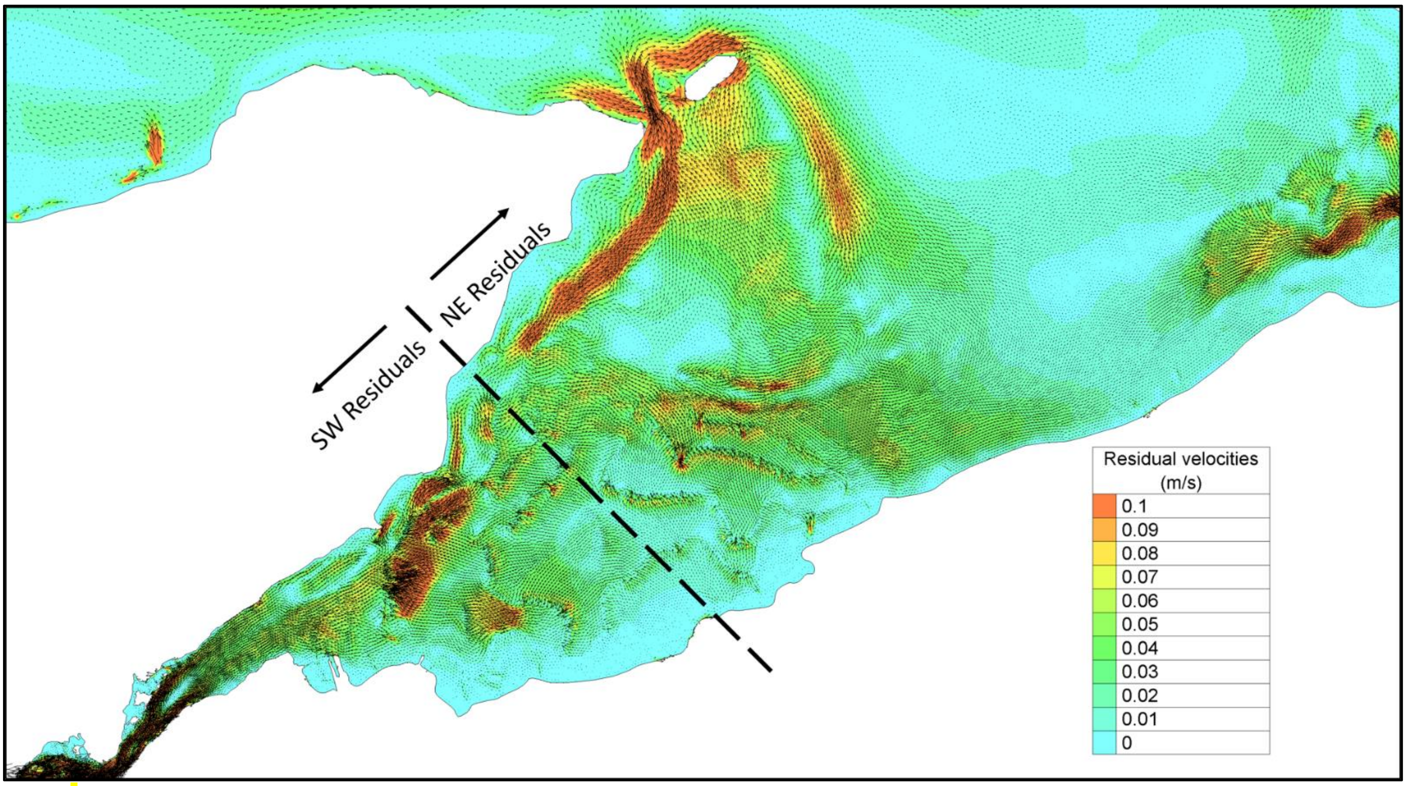


Figure 5.3. Residual tidal flows around the north-eastern Menai Strait. There is a clear partition zone shown between the ebb-dominated main channel and the flood-dominated north-eastern channel.

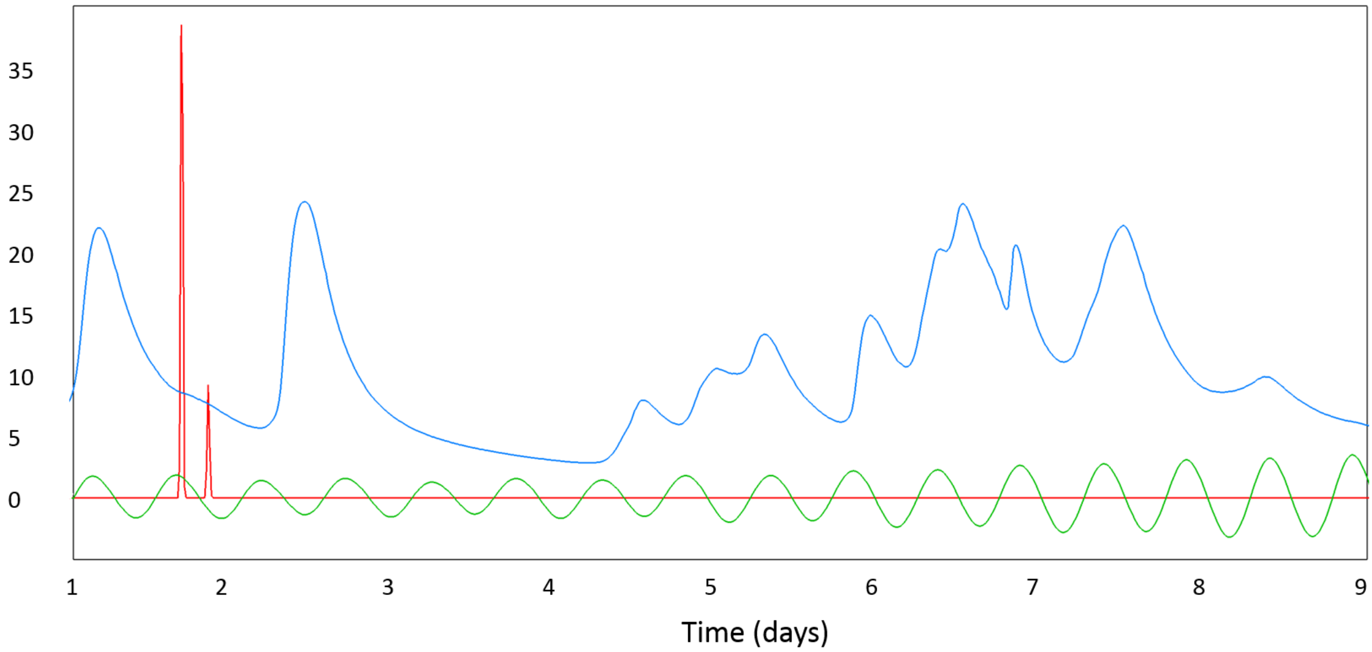


Figure 5.4. Time series showing the boundary forcing for the CSO release simulation (Run-1). The blue curve shows measured River Conwy flow data at the river-estuary boundary (in m^3/s divided by 5; i.e. peak flows are ~125 m^3/s). The red curve shows simulated viral concentrations (mg/l divided by 10) at the river-estuary boundary from idealised CSO events at Llanrwst (larger peak) and Betws-y-Coed (smaller peak). The viral simulations were generated by a catchment river-routing model. The green curve shows predicted tidal elevations at the offshore boundary (in m). The simulation started on 21 October 2013, during neap tide conditions.

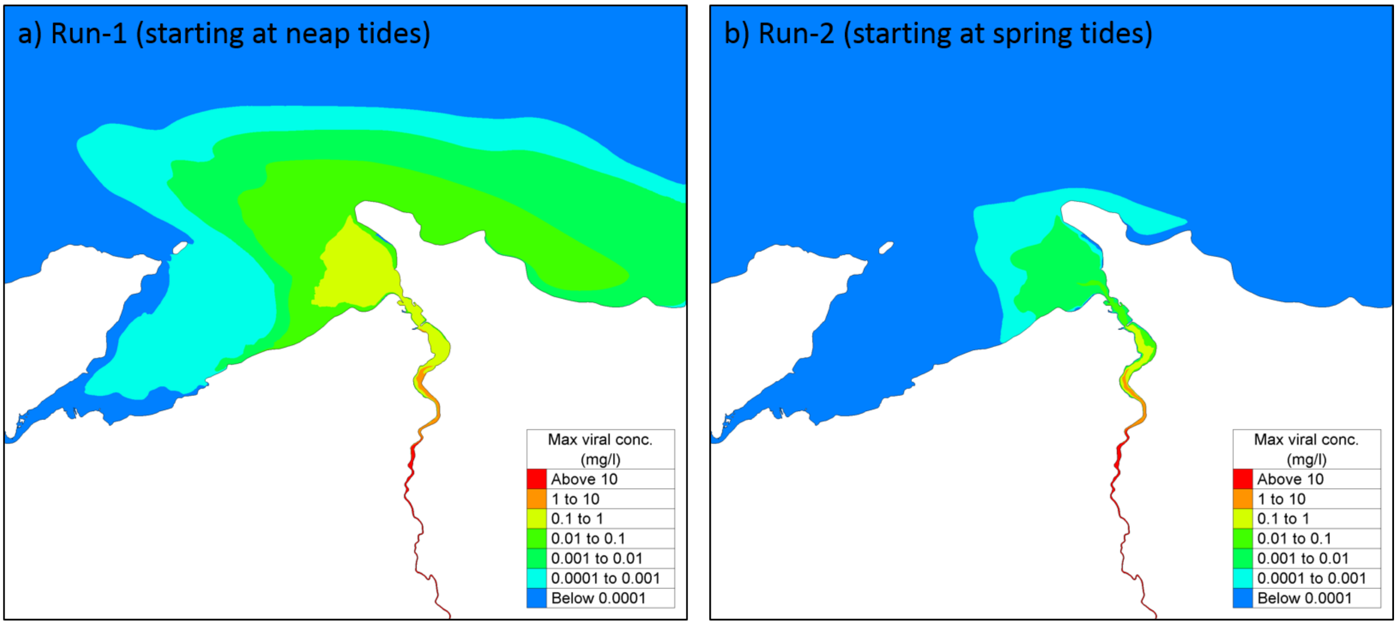


Figure 5.5. Contour maps showing the spatial dispersal of the FIO loads (in mg/l) after 5 days simulation, for (a) Run-1 where peak viral loading occurred during spring tides, and for (b) Run-2 where peak viral loading occurred during neap tides.

5.2 Understanding the factors contributing to high *E. coli* loads across multiple estuaries

Analysis of a combination of RMP-level and catchment-level factors was undertaken, in order to ascertain whether certain characteristics governing risk of high *E. coli* levels were generalizable across a range of settings.

5.2.1 Characterisation of river flows in selected UK catchments.

Rainfall and River flow data were used to assess the flashiness of rivers. Estuarine mixing is controlled by and sensitive to both tidal stirring and river flows. Mixing in some estuaries around the UK is controlled to a greater extent by tidal forces, particularly macro-tidal and hyper-tidal states (see [120] for a UK-wide characterisation of coastal tidal states). However, during high rainfall events, river flow contributions to estuarine mixing can dominate. Although the intensity of the rainfall event and the characteristics of the catchment and geology will determine the flashiness of the river flow that eventually enters the estuary. Consequently, some systems will be more sensitive to river flows than others (and river flows may be the controlling mixing force, rather than the tides). For example, the Conwy catchment is largely steep and impermeable, resulting in relatively flashy river hydrograph shapes (i.e., lasting several hours). Moreover, the Conwy estuary is relatively small, meaning that the estuarine mixing is sensitive to river flow conditions during high rainfall. Indeed, the estuary is entirely flushed by river flow during storm events. In contrast, catchments draining into the Humber estuary are larger and less steep than the Conwy, resulting in a comparatively slower hydrograph shape (i.e., lasting several days) entering the (much larger) Humber estuary. These combined factors means that the Humber system is relatively insensitive to fluctuations in rainfall (see [114] for further details).

5.2.2 Storm hydrograph shape: River flashiness

This work characterised the storm hydrograph shapes for the rivers around the UK that connect with the 12 shellfish regions chosen. Data from river gauging stations around the UK was attained (Jim Freer at Bristol University) and time series data were available dating back to the 1960s, inclusive, at 15-minute intervals, thereby enabling flood hydrographs during this period to be isolated and their shape analysed. An example of the analysis method is presented below for the River Conwy. Flow time series spanned 36-year series from 1980 to 2015, and we isolated ~2000 separate discharge events, based on our criteria of having a volume discharge larger than the mean volume discharge of all discharge events during the series. The selected discharge events ranged in peak magnitude from 27 $m^3 s^{-1}$ to 550 $m^3 s^{-1}$ (mean = 179 $m^3 s^{-1}$, standard deviation = 99 $m^3 s^{-1}$), and each event generally lasted between 12 to 24 hours.

So that we could examine each of the hydrograph shapes, relative to one another, the events were fitted, after scaling, to the curve of a two-parameter gamma probability density function, defined by:

$$f(x;k,\theta) = \frac{x^{k-1}e^{-\frac{x}{\theta}}}{\theta^k\Gamma(k)} \quad \text{[for } x > 0 \text{ and } k,\theta > 0] \tag{1}$$

where x is time, k and θ describe the shape and scale of the curve, respectively, and $\Gamma(k)$ is the gamma function evaluated at k. Events that were very close to one another (e.g., peak flows < 6 hours apart) were not analysed. Prior to fitting the curve, each hydrograph was shifted to originate at [0, 0] and scaled so that the integral of the hydrograph equalled one, which defines a gamma probability density function. We scaled in both time and magnitude so that the original hydrograph shape was unaltered. Finally, the standard deviation, σ, of each fitted gamma curve were calculated, defined as $= \sqrt{k\theta^2}$. We use σ as a measure of the flashiness of each hydrograph; the smallest values of σ being most flashy and largest values being least flashy.

<u>Results</u>

Rainfall and river flow statistics for the main rivers draining into case study estuaries/regions have been presented in Table (5.1.) Further, for six of the rivers, the above method of analysis of the general river hydrograph shape, i.e., river flashiness, has been conducted. This enables us to determine the estuary's relative sensitivity to rainfall and river flows.

Of the six rivers analysed within our 12 regions, the River Conwy is the most flashy (σ = 0.21) and the rivers entering the Carrick Roads are the least flashy (Fal: σ = 2.15, Kenwyn: σ = 3.43) (Table 1). Note from Table 1 that the Conwy catchment has a low Base Flow Index (BFI = 0.21) and consequently high runoff and fast-responding rivers, whereas the catchments draining into Carrick Roads have higher ground water flows (BFI = 0.65 – 0.67) resulting in slower-responding rivers. Expanding this analysis nationally would be very relevant to the continuation of this project. Knowledge of how sensitive each estuarine system is to river flow variations would greatly improve coastal water quality impact studies, initially at the model parameterisation stage, but also for operational forecast modelling and climate change or land use change studies.

Table 5.1. Rainfall and river flow statistics for the case study regions.
Rivers Helford and Caseg are un-gauged so no analysis can be performed.

Region	Rivers	Station ID	Mean annual rainfall (mm)	Mean river flow (m^3/s)	River storm flashyness (σ)	Base Flow Index
Duddon Estuary	River Duddon	74001	2218	5.9	0.46	0.3
Blackwater estuary, Essex	River Blackwater	37010	587	1.3	-	0.57
Burry Inlet, Carmarthenshire	River Loughor	59002	1563	2.2	0.53	0.44
Conwy, Conwy	River Conwy	66011	2183	19.8	0.21	0.28
Crouch estuary, Essex	River Crouch	37031	582	0.4	-	0.29

Carrick Roads, Cornwall	River Fal River Kenwyn River Kennal	48003 48005 48007	1272 1130 1344	2.1 0.38 0.51	2.15 3.43 -	0.67 0.65 0.67
Helford ria, Cornwall	River Helford	-	-	-	-	-
Menai East, Gwynedd	River Caseg	-	-	-	-	-
North Kent, Kent	River Medway	40003	764	11.1	0.92	0.4
Poole Harbour, Dorset	River Stour	43009	873	7.6	-	0.32
Taw & Torridge, Devon	River Taw River Torridge	50001 50002	1173 1196	18.2 15.5	- -	0.43 0.39
The Wash, East Anglia	R. Witham (Chelmer) River Nene Great Ouse (Brampton)	37008 32001 33020	 597 634 598	 1.1 9.3 0.83	- - -	 0.56 0.51 0.28

Summary: Flashiness gives a good indication as to the runoff from land and response of rivers. Conwy demonstrates high runoff and fast response to rainfall. Combined with other aspects of an active management tool, this could be useful in assessing response time of each catchment. However there are significant data gaps when trying to model flashiness, as it is dependent on gauged flow data and long time-series leading to an incomplete picture across these catchments (as demonstrated by lack of data in table 5.1). Preliminary analysis suggested that flashiness was in fact highly correlated with base-flow index. Relationships between base-flow index and *E. coli* counts were then explored, but were found not to be significant. As a result, these variables were omitted from further analysis.

5.3 Statistical Analysis

Statistical analysis was undertaken on the 12 selected catchments, and the majority of beds contained within each of them, numbering 131 beds in total.

5.3.1 Methods

All statistical analyses were done using the program R version 3.3.1 (R Development Core Team 2016). For subsequent analysis, the 90^{th} percentile of the bacteria counts were calculated for each bed for the period between 2010 and 2017. To characterize the effects of environmental factors on bacteria counts we fitted a generalized linear mixed effects model (GLMM) using a binomial error distribution. The explanatory variables initially included a set of variables describing the surrounding catchment landuse and river properties that drain into the sampling site, including catchment area, percent improved grassland, percent arable + improved grassland + unimproved pasture, mean annual rainfall (mm), flow rate, nitrate as nitrogen (NO_3-N) and turbidity. Initial explanatory and response variable data were assessed for outliers in the response and collinearity among the explanatory variables. The GLMM was fitted by first scaling the selected explanatory variables then fitting a full-model with all possible two-way interactions. Model selection was then performed using the drop1 function in the MASS package of R which compares models with individual terms dropped using Akaike information criterion (AIC). If dropping a model parameter produced a lower AIC compared to not dropping a model parameter a new GLMM was fitted without the model parameter with additional calls to drop1 to assess whether dropping additional parameters improved the model fit.

5.3.2 Results

90% Percentile bacteria counts ranged from 25.16 to 190,000 (mean = 1681.8, SD = 1670.6) (Fig 5.2). Assessment of the explanatory variables showed high correlation between percent of arable pasture and mean rainfall, flow rate, percent improved grass and nitrate as nitrogen (Fig 2). Additionally, we found correlations between nitrate as nitrogen and mean rainfall. To avoid violations of independence, percent improved grassland was kept and the other correlative variables excluded from further analyses. The initial GLMM model therefore consisted of bacteria counts as the response variable and catchment area, percent arable pasture, and turbidity with their two-way interactions as the explanatory variables. Model reduction using backward model selection and AIC resulted in a final GLMM of 90%percentile bacteria as the response and the explanatory variables consisting of the additive effects of catchment area, percent improved grassland and turbidity and the interactive effect of catchment area and percent improved grassland. Species nested in estuary and beds nested in estuary served as the random effects part of the model to account for unequal variance in bacteria across species and across estuaries. The final model indicates a significant positive relationship between turbidity, and flow and 90%ile bacteria counts (p-value = <0.001). We also found marginally non-significant positive relationships between bacteria counts and catchment area. There was a highly significant interaction between catchment area * percent improved grassland (Table 1).

Table 5.2. Terms retained in the final model, and their significance (*** = highly significant).

	Parameter	ChiSq	Df	Pr(>Chisq)
	Improved Grass	1.8843	1	0.1698474
	Flow	14.1840	1	0.0001658 ***
	Turbidity	42.7295	1	6.286e-11 ***
	Area2	0.1893	1	0.6634621
	Catchment area * percent improved grassland	15.8819	1	6.742e-05 ***

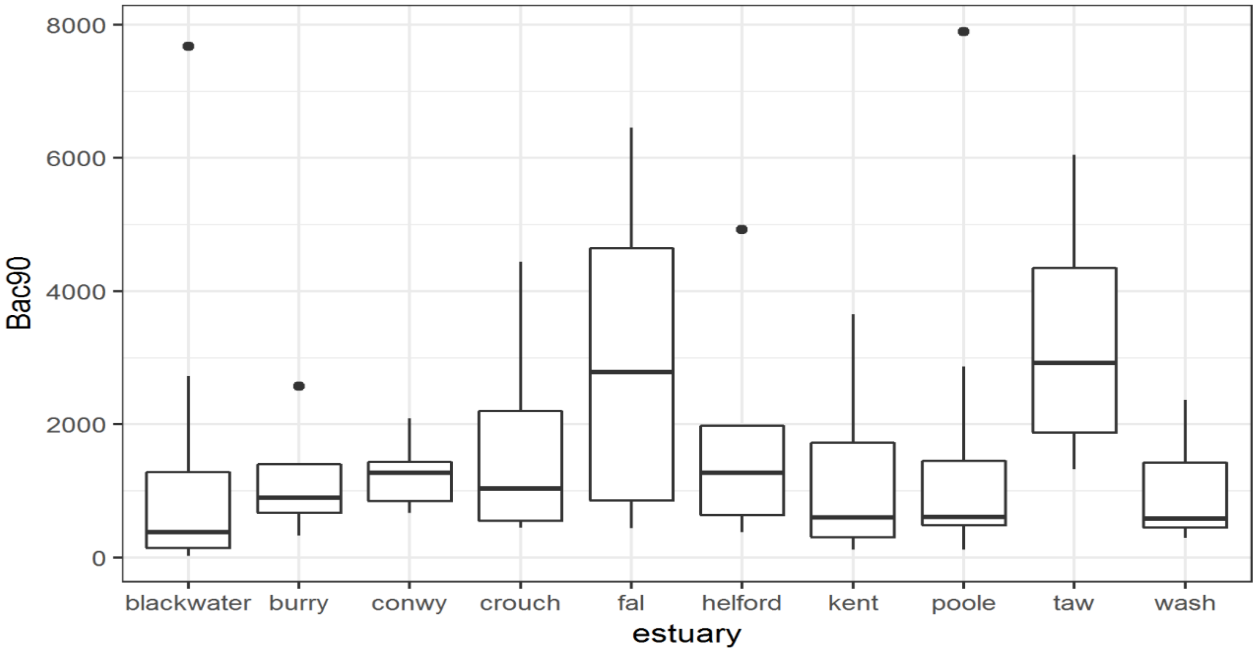


Figure 5.6. Boxplot of 90%ile *E. coli* counts in each estuary. Note, 3 outliers of 190,000 and 32,000 in the Fal, and 15,000 in the Taw have been excluded from this diagram.

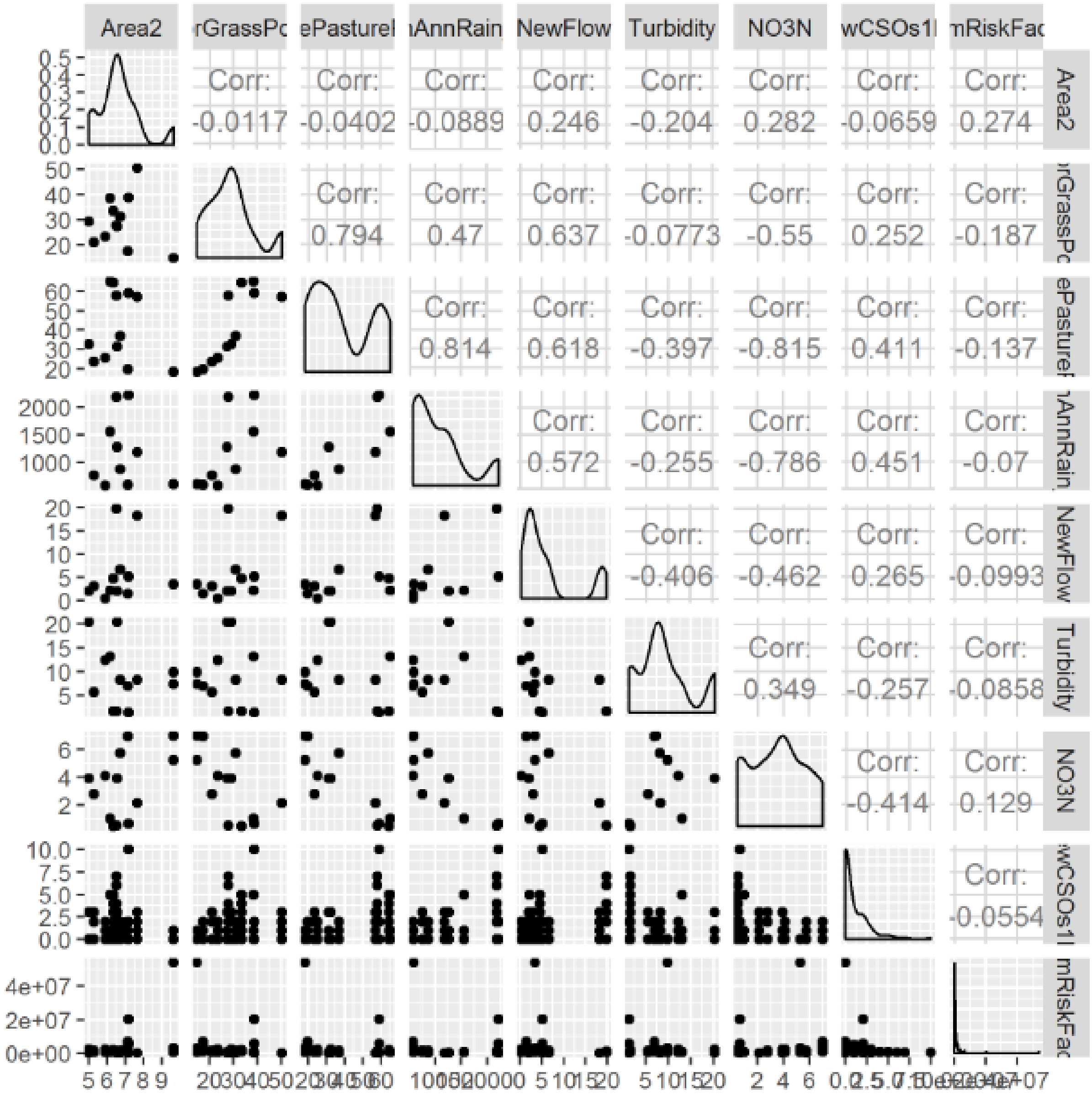


Figure 5.7. Correlation matrix of initial explanatory variables

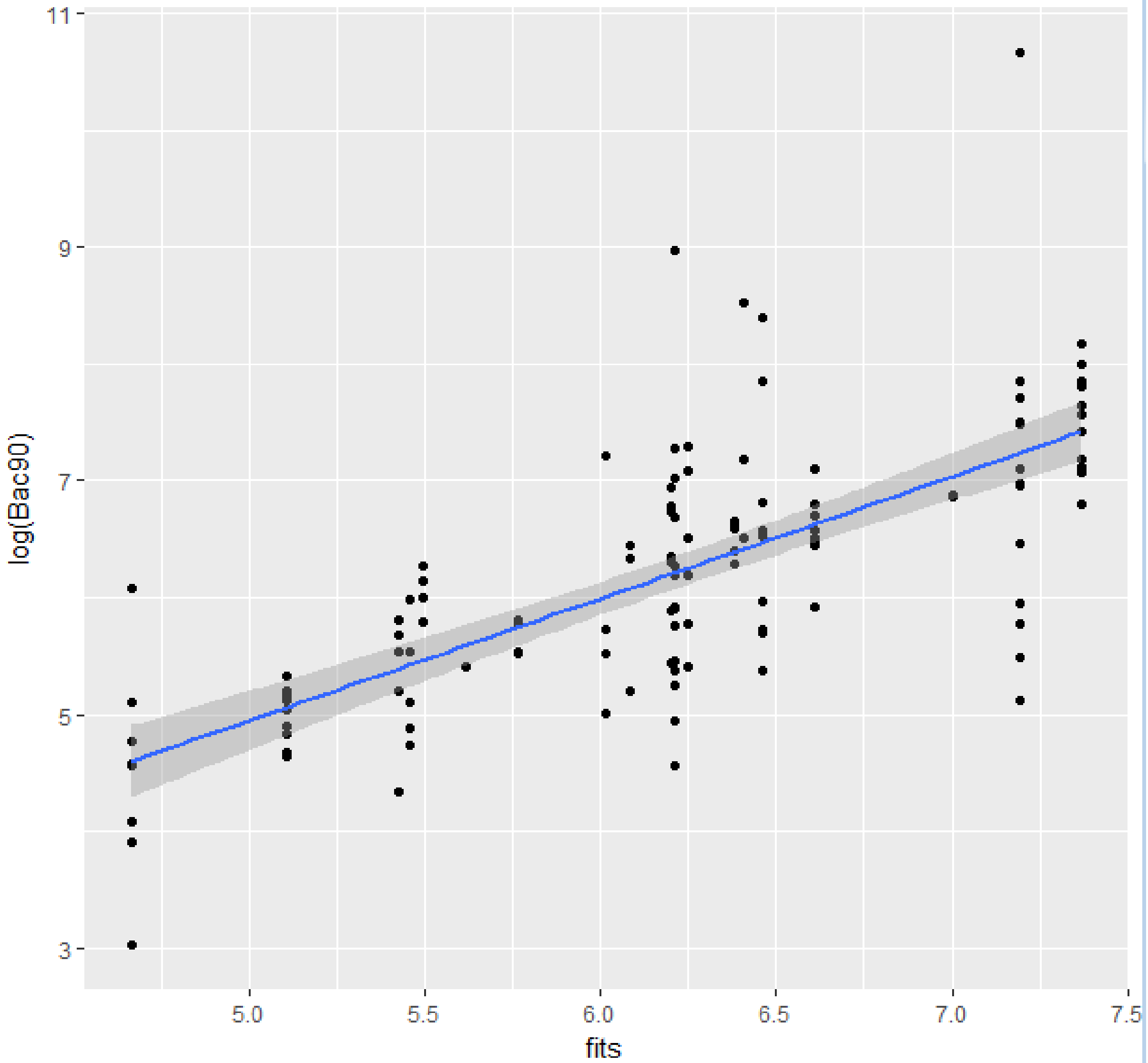


Figure 5.8. Fitted values vs log(observed 90%ile) *E. coli* loads. Overall model adjusted R2=0.5093.

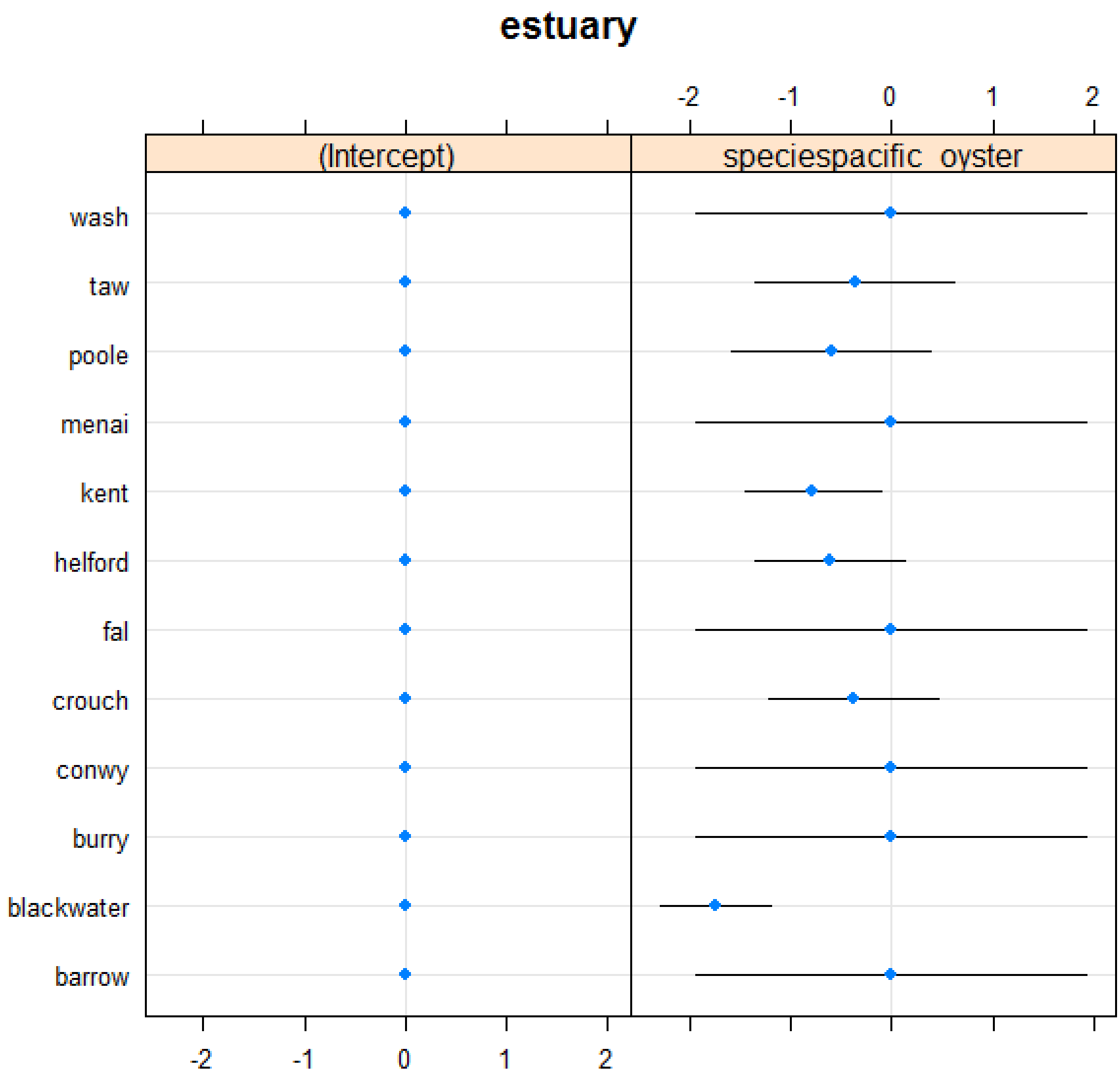


Figure 5.9. Graph showing that slope of relationship differs for Pacific Oyster compared with Blue Mussel, but only for certain estuaries. Most notably for the Blackwater and for Kent.

5.3.3 Summary:

This cross-site analysis confirms the lack of relationship between RMP *E. coli* counts and STW loads 'cumulative risk factor', identified in section 4. It also shows that certain catchment-level characteristics can act as predictors at an estuary level of increased propensity for high *E. coli* loads. Although a number of factors were correlated, these main catchment characteristics were: high proportion of improved grassland, river turbidity, high river flow, and catchment area, with a significant interaction between catchment size and improved grassland. Turbidity was the strongest explanatory variable – the reason for this is unclear. It may be that similar catchment land uses contribute to both high *E. coli* transfer into water courses and high sediment loads in water courses. However, there is also evidence that suspended solids provide nuclei for bacteria to attach onto. Therefore *E. coli* persist longer and travel further in water with high suspended solids/turbidity.

5.4 Factors to consider when developing a tool.

Researchers in Tasmania have developed a Decision support system to aid in deciding whether to close a shellfish harvesting area or not ([121]). The system uses data from collected environmental samples and data from weather stations as well as data relating to the status of the shellfish harvesting areas. The study used linear regression to learn conditions that may predict a closure and from that have developed machine learning algorithms to identify the cause of shellfish farm closure and predict opening/closure. The variables used consisted of the level of coliforms in water, rainfall recorded for the preceding 7 days from the closest weather station ([122]; [123]) and salinity of the sample. Interestingly, cumulative ranking expressed as vectors highlights that catchments are highly variable and in this study some zones responded to rain whereas in others salinity was the prime cause for closure. The researches also highlight that each location is also characterised by a number of other attributes such as whether the areas are North or South, if the influence is coastal or oceanic and what type of land use surrounds the river catchment flowing to the shellfish areas. However, this study is reliant on the number of coliforms in water samples rather than the number in shellfish samples. Antecedent rainfall and river flow have been correlated well with high faecal contamination in water ([124]; [125]; [126]) and have been used to develop regression or neural network models to predict bathing water quality but were found to be quite site specific. Stidson *et al* [126] used decision trees for bathing water compliance by considering multiple factor data which proved relatively successful in predicting past events and can evolve and improve via the incorporation of new datasets. However as with all models additional data such as seasonal data and more environmental variables would be required to cover more areas leading to site specific models.

The various modelling approaches and scales undertaken in our study revealed three main points which have a bearing on how a tool might be developed:

1. At a catchment level, certain characteristics lead to higher concentrations of *E. coli* in shellfish. These are: proportion of improved grassland in the catchment; high turbidity in main input rivers; although a number of other variables are correlated with these, such as rainfall and flow.

2. Within an estuary, there is high variability in loadings at individual monitoring points. This variability may be down to a number of factors, partly governed by complex flow pathways within estuaries of water on ebbing and flooding tides, and water residence times within the estuary. Proximity to routine STW discharge points is not a risk factor.

3. Analysis of temporal data shows there are weak positive relationships with river flow, and stronger positive relationships with CSO events. However, *E. coli* levels at individual beds still show high variability which is not easy to predict.

Therefore, we suggest that the catchment level characteristics can be used to broadly predict which estuaries may be at higher risk. Where those estuaries contain CSOs, this leads to further increased risk. Our analysis suggests that it is most likely to be active CSOs which present the main risk, rather than routine STW discharges or private discharges. Although the majority of our analyses focused on river flow, it is periods of intense rainfall which trigger CSO operation and overland flow from fields. Therefore, antecedent rainfall should also be considered. River flow most likely acts as a convenient proxy for rainfall events. However, it should be noted that even for an individual bed, and even when considering rainfall, it is very difficult to predict high *E. coli* loads. Any model would have to be bed-specific, rather than estuary specific. Selecting the bed most prone to closure would provide some indication of risk to other beds, but would not guarantee giving advanced warning for all other beds.

Text alerts (Bowes and Pyke [127]) is a system that is guidance that is used in certain areas of the country. This system is particularly designed to aid in shellfish management related to the release of untreated waste through CSOs which can increase *E. coli* concentrations by 4.5x ([117]) on harvesting beds. The system highlights the importance of local knowledge and gives examples of required parameters in assessing risk to shellfish beds. The CSO alert system operates by sending a text to harvesters (or those registered on the system) of a CSO spill event. At that stage it is down to the harvester to assess local tides, geography and metrological conditions and determine the direction of the sewage plume. There is however limited information on the volume of discharge. This system combined with other aspects of an active management strategy such as flowmeters on CSOs would greatly aid areas where CSOs are of potential concern.

Knowledge gaps in taking forward any pilot include some regular sampling of shellfish integrated with water sampling at the shellfish area and higher up the catchment. Data on CSOs including volume of SCO at any particular time would allow preliminary hydrodynamical models to assess likely risk. Data to also be included would be tides, (Springs and Neaps), wind direction and temperature. Regular turbidity and salinity measurements would also aid in gaining a better understanding of *E. coli* in shellfish in any pilot project.

Within the timescales of this study we were not able to trial approaches such as regression tree models. However, we recommend that the next phase of the work uses the variables identified above for a number of selected beds. This would ideally consider two different beds in one estuary, to see how variable the models would be within an estuary, and also comparing beds across at least two estuaries to determine the additional variability that comes from different catchment contexts.

6 List of sites

Initial data looked at all sites in Wales and England that had data for mussels. This was then narrowed using several criteria. The 12 areas listed in section 3 where chosen as areas with mussels and where possible oysters species. Where there was a series of data over at least the last 5 years and where there were differences in *E. coli* counts between the different RMP areas. In addition, sites were also chosen where there were bed closures due to unusually high levels of *E. coli*.

Subsequent data analysis in sections 4 and 5 reduced the 12 shellfish sites to 10 as both Barrow and Menai had low *E. coli* levels and relatively low riverine influence. The 10 sites suggested for potential further work are: Blackwater, Burry, Conwy, Crouch, Fal, Helford, Poole, North Kent, Taw-Torridge and Wash.

7 Sampling frequency and methodology for AMS field sites

Weekly shellfish sampling can occur following closure of a harvesting area through an action state however where an AMS is in place this may not be feasible. Of utmost importance is the protection of public health and ensuring a good product is placed on the market with minimal impact on the shellfish industry. Suggestions from other countries such as the US and New Zealand, the shellfish areas would be closed during a significant rainfall event with water testing initiated immediately following the rainfall event. Once the levels in the water have started to decline, shellfish samples would be taken and the closure lifted once both water and shellfish samples return to acceptable levels.

There are however differences in the amount of time required for shellfish to naturally reduce levels of *E. coli* in flesh, depending on the shellfish species, and environmental variables such as temperature, salinity and state of tide. Further, as stated in Section 2, faster accumulation rates of FIOs have been reported in mussels and cockles compared to oysters and clams. However, oyster and clams also retain pollutants for longer.

Bivalves can continue to maintain *E. coli* levels above ambient seawater levels but usually once removed from any contaminating source, the bivalves are clean within 48 hours of depuration commencing. Suggestions of sampling therefore could include initial water testing for levels of *E. coli* in the water and sediment as soon as turbidity had decreased to ‘normal’ levels and rain fall has ceased. Once the levels of FIOs in the water had returned to ‘base’ levels, shellfish could be monitored every 2 days until at least 2 clear readings were recorded and where no further increase in turbidity or any further rain event had occurred.

Where rain events are frequent it would be possible to test shellfish earlier. As stated in Section 2, it is often the ‘first flush’ that carries high numbers of bacteria concentrations can be very high where there has been little rain for a number of weeks (depending on catchment and other environmental variables). This may also explain the association between 0 and 1 day rainfall lag and *E. coli* concentrations demonstrated in Section 4.

8 Environmental, Economic and Societal Impacts of AMS.

The bivalve shellfish industry in England and Wales represents a strategic sector in the UK being mainly dominated by mussels and Pacific Oysters (95% and 4% of tonnage respectively; 82% and 15% of value) [128]. Wales has the highest tonnage of mussels producing between 7 – 10,000 tonnes annually. There is a strategic drive to double aquaculture and in particular shellfish production (Multiannual national plan for the development of sustainable aquaculture) within the UK in line with the Blue Growth agenda of the European Commission. However there are several issues that represent challenges for the expansion of shellfish aquaculture such as water quality and contamination of shellfish with faecal indicator bacteria and possible closure of shellfish harvesting areas.

Shellfish are an important part of the economy, lifestyle and heritage of rural coastal communities. Closures even though only temporary can cause significant economic hardship and loss of revenue [128] with social impacts on mental, physical, cultural and economic well-being of fishermen and their communities [130]. Various studies have indicated however that there is significant heterogeneity in the impact of the actual closure with aspects such as the level of harvest, tidal activity, size, frequency and timing/season of pollution closures all demonstrating dependence on local conditions and highlight the lack of knowledge and difficultly in management of shellfish areas in coastal waters.

High levels of FIOs in bivalve shellfish in England and Wales from areas classed as Long-Term class B, (e.g. *E. coli*), can lead to a triggering of an action state and the closure of the shellfish area and is distinct to the closure of areas due to the implementation of a Class C state. The re-opening of a bed will then be reconsidered once *E. coli* levels fall below legal limits following additional sampling. As described earlier in this report, the action state can remain in place for up to 3 months with opening during that period following the collection of at least 2 consecutive satisfactory laboratory results from each affected monitoring point. These samples must be taken at least 7 days apart leading to, at the absolute minimum, 2 weeks closure of the affected area.

Economic analysis undertaken for a previous report [131] which considered the effect of shellfish harvesting closures due to Norovirus has been used to generate information relating to potential impacts of AMS on shellfish enterprises. The report interviewed 11 shellfish enterprises around England and Wales and divided these into 2 groups, small-scale enterprises (incomes less than £1M and production less than 1,000 tonnes per year) and medium scale enterprises (incomes greater than £1M and production less than 1,000 tonnes per year). The interview gathered information on three main criteria, Cost Indicators (Variable costs, Fixed costs, Opportunity Costs and Average Wage), Profit Indicators (Production and Incomes) and Profitability Indicators (Total Capital, Net Profit and Rate of Return) from which equations were derived in order to quantify the performance of the producers under different scenarios. Two main scenarios were used to generate information as to the length of time before annual profit in the 2 shellfish categories tended to zero. Scenario 1 modelled the worst case with shellfish harvesting areas closed for over 3 months with no resale of unsold stocks e.g. shellfish buyers move elsewhere for shellfish leaving the product unsold in the harvesting area, whereas scenario 2 assumes that as soon as the area is open, product can be sold and up to 50% of the product held during the closed period is also sold. Both the scenarios were based during a period of shellfish harvesting by the enterprises.

Different technical characteristics and economic structures were identified between the small and medium scale enterprises varying from different extent of production sites to different business investments required. Costs also varied in relation to amount of production, transport of shellfish, fuel for boats with fixed costs considered in the model being maintenance, depreciation and other annual costs. Of the 14 shellfish enterprises that replied to the interviews, 6 concentrated on mussels and/or 7 on oysters. All information was analysed and models produced.

The results from the model must be taken in context of the variability of the industry, their buyers, the environment and particularly the small number of respondents to the survey (14%) and are the consequences for an ‘average’ small or medium scale enterprise. However, results indicate for scenario 1 that for small scale enterprises a closure of 10 weeks could occur before the enterprise would to experience economic loss whereas a closure for 15 weeks would affect medium scale enterprises which would be over the 3 month maximum closure unless water quality issues remained. For scenario 2 where production and sale of product resumed directly after 3 months, as well as sale of product held during the closure period, economic models for both small and medium enterprises indicate that although profitability would be affected in both cases, the ‘average’ enterprises would not necessarily enter an economic loss scenario with potential bankruptcy.

There are several caveats that need to be included when taking the results into consideration and generation of a more comprehensive survey. These include buyer/retailer behaviour, elasticity of mussel and oyster prices and potential wastage of the stock if above market size. Enterprises also sell at different times of the year and maybe more affected if production was affected when sales were at their highest. In addition, enterprises apparently invest a large portion of their profit into new capital and technology every year and hence a reduction in profits (increasing number of weeks closed) would mean the 10 and 15 week closures suggested above would not be sustainable in following years and should be reduced to 4 and 6 weeks respectively. This reduction would allow for additional investment and reducing the risk of bankruptcy. It should be noted that only 8 small scale and 3 medium scale enterprises responded to the survey leading to large uncertainty in the value of any results and would be a critical area for further research in any pilot trial. Overall though a precautionary approach is suggested in terms of public health and economic sustainability of shellfish enterprises.

The impact of closures of shellfish areas on environmental effects have received little attention with the economic and social aspects of far greater concern to the industry. Shellfish harvesting however is directly related to environmental variables including temperature, length of daylight and height of low tide. Closure of shellfish harvesting areas for any significant amount of time may actually have ecological benefits although holding a larger amount of biomass on the harvesting areas would have to be managed correctly. It is possible that the loss of a fishery due to closure may have more of an impact in certain areas where currently shellfish seed is relayed and grown leading less shellfish and impacts on water quality, biodiversity, birds and other ecosystem services including tourism.

9 Conclusions and recommendations

Overall there remains considerable uncertainty surrounding the flow of microbial contaminants from agricultural catchments through to the coastal zone limiting the implementation of effective mitigation measures and the formulation of robust policies and legislation to protect human health and the wider environment. Additional research is therefore required to disentangle the complexity of bacterial, and other human health pathogens such as viral interactions within water, sediments, nutrients and flocs along freshwater–saline gradients. This requires, and would benefit from multidisciplinary multi-partner and interagency working, including stakeholders involved in wastewater treatment and riverine, estuarine and coastal environments looking at reservoirs of microbial pathogens, their suspension and potential reactivation to determine mitigation actions and improve water quality and food security.

Recommendations for pilot test catchments would include analysis of 2 to 4 catchments covering variations in catchment type and *E. coli* loadings. The Conwy is a good candidate for one of the test catchments, as a relatively clean site where significant analytical infrastructure, monitoring and data already exists and can be used for comparison. The Fal demonstrates consistently high *E. coli* loadings, with potential links to rainfall, and may also be a good candidate estuary. Monitoring and sampling should be conducted over a 2-year period in the first instance, ideally within a 3 year project to account for variability in weather patterns over time.

The project should involve multiple partners, be multidisciplinary and build on the work undertaken in this desk study. FSA in conjunction with the water companies, shellfishermen, Councils (including LAGs), Public health officials, NRW/EA and others associated with the chosen catchments should all be included and in some instances statutory monitoring can be used reducing the burden of sampling. In addition, shellfishermen can be included in the project to aid in the sampling effort and where feasible to hold monitoring instrumentation in the vicinity of their shellfish areas.

Monitoring should include the measurement and data collation of the following:
Characterisation of catchment type and land use, rainfall, riverflow; monitoring and instrumentation of CSO operation (event time and volume); water, shellfish and sediment microbiological samples, turbidity, nitrogen; estuarine characteristics and processes such as tides, wind direction, temperature, salinity. Additional data such as bathymetry/Lidar data may be required. Routine sampling of *E. coli* levels and key explanatory variables should occur at least every two weeks and include enhanced sampling frequency during weather events expected to lead to increased risk of E. coli contamination, in order to fine-tune our understanding of i) the triggers for such events, and ii) the recovery time following an event.

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11 Appendix 1

<u>Table 4.3 Chi-squared test of association</u>

Area	Code	Chisq test p value	p>10000 given flow > .8 quantile	p>10000 given flow < .8 quantile
Barrow	B077B	**0.020**	0.150	0.050
Barrow	B077D	1.000	0.020	0.010
Barrow	B077F	0.820	0.030	0.010
Barrow	B077P			0.000
Barrow	B077Q			0.000
Barrow	B077R			0.000
Blackwater	B014G	1.000	0.000	0.010
Blackwater	B014H	1.000	0.000	0.010
Blackwater	B014Q	1.000	0.200	0.200
Blackwater	B014R	0.920	0.200	0.000
Burry	B038G	1.000	0.230	0.240
Burry	B038L	0.420	0.000	0.030
Burry	B038X			0.000
Burry	B038Z		0.000	0.000
Conwy	B044A	0.160	0.150	0.080
Conwy	B044D	**0.010**	0.150	0.040
Conwy	B044E	0.160	0.120	0.060
Conwy	B044H	0.260	0.050	0.010
Conwy	B044I	0.430	0.080	0.040
Conwy	B044S	0.400	0.040	0.000
Conwy	B044T		0.000	0.000
Conwy	B044U		0.000	0.000
Crouch	B015M	0.710	0.070	0.010
Crouch	B015N		0.000	0.000
Crouch	B015O		0.000	0.000
Crouch	B015P	0.480	0.500	0.000
Crouch	B015Q		0.000	0.000
Crouch	B015R	0.390	0.500	0.000
Crouch	B015S	0.480	0.500	0.000
Crouch	B015Y	0.850	0.170	0.040
Fal	B033C	**0.020**	0.320	0.160
Fal	B033Y	**0.000**	0.220	0.050
Fal	B033Z	**0.000**	0.280	0.040
Fal	B33AL	**0.000**	0.230	0.050
Fal	B33AN	1.000	0.030	0.030
Fal	B33AX	**0.000**	0.370	0.040
Fal	B33BD	**0.000**	0.430	0.020
Fal	B33BK			0.080

Fal	B33BL			0.000
Fal	B33BO			0.000
Frome	B054M	1.000	0.000	0.010
Frome	B054P	1.000	0.000	0.010
Frome	B54BM	1.000	0.000	0.020
Frome	B54BR	0.740	0.060	0.030
Frome	B54BS	1.000	0.090	0.080
Frome	B54BT	1.000	0.000	0.030
Frome	B54CH		0.000	0.000
Frome	B54CL		0.000	0.000
Frome	B54CM		0.000	0.000
Frome	B54CN	1.000	0.000	0.060
Frome	B54CP			0.250
Taw	B036F	1.000	0.250	0.250
Taw	B036H	1.000	0.120	0.110
Taw	B036K	0.980	0.150	0.170
Taw	B036N	0.830	0.060	0.080
Taw	B036O	0.250	0.040	0.100
Taw	B036P	0.500	0.110	0.160
Taw	B036Z		0.000	0.000
Taw	B36AA	1.000	0.000	0.030
Taw	B36AB	0.380	0.170	0.000
Taw	B36AC	1.000	0.000	0.070
Taw	B36AK	1.000	0.000	0.110
Wash	B003D		0.000	0.000
Wash	B003F		0.000	0.000
Wash	B003M	1.000	0.000	0.020
Wash	B003V		0.000	0.000
Wash	B004E	0.120	0.100	0.240
Wash	B004F	**0.030**	0.050	0.000
Wash	B004I	1.000	0.030	0.020
Wash	B004L	1.000	0.000	0.010

Table 4.2. Chi-squared test of E. coli counts above and below 10000CFU/mg shellfish flesh with 0.8 quantile flow. Figures in bold indicate where p values are low suggesting an association

Table 4.3. Regression Analysis. Bold figures indicate where p value <0.05 and where R^2 > 0.35

Region	RMP	Lag (Days)	Regression coefficient	Standard Error Regression Coefficient	p value	model r2	Degrees of freedom
Barrow	B077B	0.000	0.360	0.120	0.000	0.070	121.000
Barrow	B077B	1.000	0.420	0.110	0.000	0.120	121.000
Barrow	B077B	2.000	0.340	0.110	0.000	0.080	121.000
Barrow	B077B	3.000	0.260	0.110	0.020	0.050	121.000
Barrow	B077D	0.000	0.320	0.120	0.010	0.070	121.000
Barrow	B077D	1.000	0.360	0.110	0.000	0.100	121.000
Barrow	B077D	2.000	0.260	0.110	0.020	0.060	121.000
Barrow	B077D	3.000	0.200	0.110	0.070	0.040	121.000
Barrow	B077F	0.000	0.010	0.120	0.940	0.090	98.000
Barrow	B077F	1.000	0.020	0.120	0.840	0.090	98.000
Barrow	B077F	2.000	0.030	0.120	0.810	0.090	98.000
Barrow	B077F	3.000	0.060	0.120	0.590	0.090	98.000
Blackwater	B014G	0.000	1.130	0.190	0.000	0.230	120.000
Blackwater	B014G	1.000	1.230	0.180	0.000	0.290	120.000
Blackwater	B014G	2.000	1.140	0.170	0.000	0.280	120.000
Blackwater	B014G	3.000	1.190	0.180	0.000	0.270	120.000
Blackwater	B014H	0.000	0.670	0.190	0.000	0.160	114.000
Blackwater	B014H	1.000	0.510	0.180	0.010	0.130	114.000
Blackwater	B014H	2.000	0.470	0.200	0.020	0.110	114.000
Blackwater	B014H	3.000	0.510	0.200	0.010	0.120	114.000
Blackwater	B014Q	0.000	-0.150	0.450	0.740	0.060	7.000
Blackwater	B014Q	1.000	-0.180	0.550	0.750	0.050	7.000
Blackwater	B014Q	2.000	-0.170	0.530	0.760	0.050	7.000
Blackwater	B014Q	3.000	-0.210	0.990	0.840	0.050	7.000
Blackwater	B014R	0.000	1.050	0.570	0.100	0.310	8.000
Blackwater	**B014R**	**1.000**	**1.440**	**0.600**	**0.040**	**0.430**	**8.000**
Blackwater	**B014R**	**2.000**	**1.400**	**0.560**	**0.040**	**0.440**	**8.000**
Blackwater	B014R	3.000	1.820	0.930	0.090	0.330	8.000
Burry	B038G	0.000	0.570	0.140	0.000	0.200	112.000
Burry	B038G	1.000	0.600	0.150	0.000	0.200	112.000
Burry	B038G	2.000	0.540	0.140	0.000	0.180	112.000
Burry	B038G	3.000	0.510	0.130	0.000	0.180	112.000
Burry	B038L	0.000	0.310	0.200	0.120	0.050	116.000
Burry	B038L	1.000	0.330	0.200	0.100	0.050	116.000
Burry	B038L	2.000	0.380	0.200	0.060	0.060	116.000
Burry	B038L	3.000	0.370	0.190	0.050	0.060	116.000
Burry	B038Z	0.000	0.990	0.420	0.040	0.340	12.000
Burry	B038Z	1.000	0.910	0.470	0.070	0.270	12.000

Burry	**B038Z**	**2.000**	**1.050**	**0.350**	**0.010**	**0.460**	**12.000**
Burry	**B038Z**	**3.000**	**0.990**	**0.400**	**0.030**	**0.370**	**12.000**
Conwy	B044A	0.000	0.390	0.080	0.000	0.190	148.000
Conwy	B044A	1.000	0.420	0.080	0.000	0.210	148.000
Conwy	B044A	2.000	0.400	0.090	0.000	0.180	148.000
Conwy	B044A	3.000	0.320	0.080	0.000	0.150	148.000
Conwy	B044D	0.000	0.400	0.070	0.000	0.230	150.000
Conwy	B044D	1.000	0.420	0.070	0.000	0.230	150.000
Conwy	B044D	2.000	0.420	0.070	0.000	0.230	150.000
Conwy	B044D	3.000	0.350	0.070	0.000	0.170	150.000
Conwy	B044E	0.000	0.450	0.080	0.000	0.200	148.000
Conwy	B044E	1.000	0.460	0.080	0.000	0.190	148.000
Conwy	B044E	2.000	0.450	0.090	0.000	0.170	148.000
Conwy	B044E	3.000	0.420	0.080	0.000	0.160	148.000
Conwy	B044H	0.000	0.340	0.060	0.000	0.140	180.000
Conwy	B044H	1.000	0.350	0.060	0.000	0.150	180.000
Conwy	B044H	2.000	0.350	0.070	0.000	0.130	180.000
Conwy	B044H	3.000	0.340	0.070	0.000	0.140	180.000
Conwy	B044I	0.000	0.270	0.070	0.000	0.150	149.000
Conwy	B044I	1.000	0.300	0.070	0.000	0.170	149.000
Conwy	B044I	2.000	0.300	0.080	0.000	0.160	149.000
Conwy	B044I	3.000	0.310	0.070	0.000	0.170	149.000
Conwy	B044S	0.000	0.190	0.070	0.010	0.050	151.000
Conwy	B044S	1.000	0.210	0.070	0.010	0.050	151.000
Conwy	B044S	2.000	0.210	0.080	0.010	0.050	151.000
Conwy	B044S	3.000	0.170	0.070	0.020	0.040	151.000
Conwy	B044T	0.000	0.250	0.200	0.230	0.290	8.000
Conwy	B044T	1.000	0.270	0.200	0.210	0.310	8.000
Conwy	B044T	2.000	0.230	0.180	0.250	0.280	8.000
Conwy	B044T	3.000	0.050	0.230	0.840	0.150	8.000
Conwy	B044U	0.000	0.090	0.360	0.800	0.020	8.000
Conwy	B044U	1.000	0.220	0.360	0.550	0.060	8.000
Conwy	B044U	2.000	0.130	0.330	0.700	0.030	8.000
Conwy	B044U	3.000	0.300	0.370	0.450	0.080	8.000
Crouch	B015M	0.000	0.550	0.140	0.000	0.160	93.000
Crouch	B015M	1.000	0.520	0.160	0.000	0.120	93.000
Crouch	B015M	2.000	0.490	0.150	0.000	0.120	93.000
Crouch	B015M	3.000	0.470	0.140	0.000	0.130	93.000
Crouch	B015N	0.000	0.000	0.280	0.990	0.300	7.000
Crouch	B015N	1.000	-0.030	0.240	0.890	0.300	7.000
Crouch	B015N	2.000	0.160	0.220	0.490	0.350	7.000
Crouch	B015N	3.000	0.130	0.240	0.590	0.330	7.000

Crouch	**B015O**	**0.000**	**1.280**	**0.470**	**0.020**	**0.500**	**8.000**
Crouch	**B015O**	**1.000**	**1.310**	**0.330**	**0.000**	**0.670**	**8.000**
Crouch	**B015O**	**2.000**	**1.610**	**0.370**	**0.000**	**0.700**	**8.000**
Crouch	**B015O**	**3.000**	**1.290**	**0.370**	**0.010**	**0.610**	**8.000**
Crouch	B015P	0.000	1.010	0.730	0.210	0.290	6.000
Crouch	**B015P**	**1.000**	**1.320**	**0.480**	**0.030**	**0.580**	**6.000**
Crouch	**B015P**	**2.000**	**1.710**	**0.650**	**0.040**	**0.560**	**6.000**
Crouch	B015P	3.000	0.900	0.740	0.270	0.240	6.000
Crouch	B015Q	0.000	0.850	0.430	0.100	0.450	6.000
Crouch	**B015Q**	**1.000**	**0.860**	**0.320**	**0.040**	**0.590**	**6.000**
Crouch	**B015Q**	**2.000**	**1.080**	**0.440**	**0.050**	**0.540**	**6.000**
Crouch	B015Q	3.000	0.890	0.410	0.070	0.490	6.000
Crouch	**B015R**	**0.000**	**1.380**	**0.490**	**0.020**	**0.540**	**8.000**
Crouch	**B015R**	**1.000**	**1.360**	**0.360**	**0.010**	**0.660**	**8.000**
Crouch	**B015R**	**2.000**	**1.750**	**0.360**	**0.000**	**0.760**	**8.000**
Crouch	**B015R**	**3.000**	**1.550**	**0.300**	**0.000**	**0.790**	**8.000**
Crouch	B015S	0.000	0.900	0.660	0.220	0.240	6.000
Crouch	B015S	1.000	1.070	0.480	0.070	0.460	6.000
Crouch	B015S	2.000	1.300	0.680	0.100	0.380	6.000
Crouch	**B015S**	**3.000**	**1.430**	**0.470**	**0.020**	**0.610**	**6.000**
Crouch	**B015Y**	**0.000**	**1.370**	**0.310**	**0.000**	**0.420**	**27.000**
Crouch	**B015Y**	**1.000**	**1.100**	**0.280**	**0.000**	**0.360**	**27.000**
Crouch	**B015Y**	**2.000**	**1.430**	**0.250**	**0.000**	**0.550**	**27.000**
Crouch	**B015Y**	**3.000**	**1.360**	**0.190**	**0.000**	**0.660**	**27.000**
Fal	B033C	0.000	0.830	0.170	0.000	0.280	84.000
Fal	B033C	1.000	0.840	0.160	0.000	0.300	84.000
Fal	B033C	2.000	0.740	0.170	0.000	0.260	84.000
Fal	B033C	3.000	0.720	0.180	0.000	0.230	84.000
Fal	B033Y	0.000	1.180	0.170	0.000	0.260	143.000
Fal	B033Y	1.000	1.110	0.160	0.000	0.260	143.000
Fal	B033Y	2.000	0.950	0.170	0.000	0.200	143.000
Fal	B033Y	3.000	0.820	0.180	0.000	0.140	143.000
Fal	B033Z	0.000	1.530	0.180	0.000	0.330	142.000
Fal	**B033Z**	**1.000**	**1.510**	**0.170**	**0.000**	**0.350**	**142.000**
Fal	B033Z	2.000	1.420	0.170	0.000	0.320	142.000
Fal	B033Z	3.000	1.320	0.190	0.000	0.250	142.000
Fal	B33AL	0.000	0.840	0.160	0.000	0.180	128.000
Fal	B33AL	1.000	0.800	0.160	0.000	0.180	128.000
Fal	B33AL	2.000	0.700	0.160	0.000	0.150	128.000
Fal	B33AL	3.000	0.630	0.180	0.000	0.110	128.000
Fal	B33AN	0.000	0.310	0.220	0.160	0.020	114.000
Fal	B33AN	1.000	0.310	0.210	0.150	0.020	114.000

Fal	B33AN	2.000	0.310	0.210	0.140	0.020	114.000
Fal	B33AN	3.000	0.210	0.230	0.360	0.010	114.000
Fal	B33AX	0.000	1.430	0.230	0.000	0.260	113.000
Fal	B33AX	1.000	1.410	0.220	0.000	0.270	113.000
Fal	B33AX	2.000	1.230	0.230	0.000	0.210	113.000
Fal	B33AX	3.000	1.140	0.240	0.000	0.180	113.000
Fal	B33BD	0.000	1.180	0.230	0.000	0.330	60.000
Fal	**B33BD**	**1.000**	**1.200**	**0.220**	**0.000**	**0.350**	**60.000**
Fal	**B33BD**	**2.000**	**1.150**	**0.210**	**0.000**	**0.350**	**60.000**
Fal	B33BD	3.000	1.110	0.230	0.000	0.290	60.000
Fal	B33BJ	0.000	-0.620	1.560	0.700	0.420	9.000
Fal	B33BJ	1.000	-0.330	1.570	0.840	0.420	9.000
Fal	B33BJ	2.000	-0.240	1.600	0.880	0.410	9.000
Fal	B33BJ	3.000	-0.840	1.540	0.600	0.430	9.000
Fal	B33BK	0.000	-0.440	1.050	0.690	0.090	10.000
Fal	B33BK	1.000	-0.460	1.010	0.660	0.090	10.000
Fal	B33BK	2.000	-0.420	1.020	0.690	0.090	10.000
Fal	B33BK	3.000	-0.720	0.960	0.470	0.120	10.000
Fal	B33BL	0.000	-0.490	0.840	0.570	0.030	13.000
Fal	B33BL	1.000	-0.570	0.880	0.530	0.030	13.000
Fal	B33BL	2.000	-0.370	0.900	0.690	0.010	13.000
Fal	B33BL	3.000	-0.650	0.820	0.440	0.050	13.000
Frome	B054M	0.000	1.380	0.260	0.000	0.230	106.000
Frome	B054M	1.000	1.580	0.240	0.000	0.300	106.000
Frome	B054M	2.000	1.500	0.230	0.000	0.300	106.000
Frome	B054M	3.000	1.300	0.250	0.000	0.220	106.000
Frome	B054P	0.000	0.940	0.190	0.000	0.140	147.000
Frome	B054P	1.000	0.940	0.190	0.000	0.150	147.000
Frome	B054P	2.000	0.880	0.180	0.000	0.140	147.000
Frome	B054P	3.000	0.800	0.190	0.000	0.110	147.000
Frome	B54BM	0.000	0.840	0.220	0.000	0.110	144.000
Frome	B54BM	1.000	0.920	0.210	0.000	0.140	144.000
Frome	B54BM	2.000	0.750	0.200	0.000	0.100	144.000
Frome	B54BM	3.000	0.650	0.210	0.000	0.080	144.000
Frome	B54BR	0.000	0.900	0.200	0.000	0.130	134.000
Frome	B54BR	1.000	0.940	0.190	0.000	0.150	134.000
Frome	B54BR	2.000	0.890	0.190	0.000	0.150	134.000
Frome	B54BR	3.000	0.800	0.200	0.000	0.110	134.000
Frome	B54BS	0.000	0.440	0.230	0.060	0.030	128.000
Frome	B54BS	1.000	0.490	0.230	0.030	0.040	128.000
Frome	B54BS	2.000	0.450	0.220	0.040	0.040	128.000
Frome	B54BS	3.000	0.340	0.230	0.130	0.020	128.000

Frome	B54BT	0.000	0.820	0.260	0.000	0.140	97.000
Frome	B54BT	1.000	0.890	0.250	0.000	0.150	97.000
Frome	B54BT	2.000	0.810	0.240	0.000	0.150	97.000
Frome	B54BT	3.000	0.670	0.250	0.010	0.110	97.000
Frome	B54CH	0.000	1.370	0.360	0.000	0.280	36.000
Frome	B54CH	1.000	1.430	0.330	0.000	0.340	36.000
Frome	B54CH	2.000	1.290	0.300	0.000	0.340	36.000
Frome	B54CH	3.000	1.370	0.320	0.000	0.330	36.000
Frome	**B54CL**	**0.000**	**1.610**	**0.310**	**0.000**	**0.430**	**36.000**
Frome	**B54CL**	**1.000**	**1.650**	**0.300**	**0.000**	**0.470**	**36.000**
Frome	B54CL	2.000	1.390	0.330	0.000	0.340	36.000
Frome	B54CL	3.000	1.400	0.340	0.000	0.330	36.000
Frome	B54CM	0.000	0.890	0.370	0.020	0.160	35.000
Frome	B54CM	1.000	0.950	0.360	0.010	0.180	35.000
Frome	B54CM	2.000	0.820	0.350	0.030	0.140	35.000
Frome	B54CM	3.000	0.850	0.370	0.030	0.150	35.000
Frome	B54CN	0.000	0.310	0.320	0.340	0.030	41.000
Frome	B54CN	1.000	0.430	0.320	0.180	0.050	41.000
Frome	B54CN	2.000	0.310	0.310	0.320	0.030	41.000
Frome	B54CN	3.000	0.310	0.320	0.330	0.030	41.000
Frome	B54CP	0.000	-2.860	9.510	0.810	0.080	1.000
Frome	B54CP	1.000	-3.050	7.900	0.770	0.130	1.000
Frome	B54CP	2.000	-4.080	7.180	0.670	0.240	1.000
Frome	B54CP	3.000	-3.620	9.840	0.780	0.120	1.000
Helford	B034O	0.000	0.410	0.250	0.100	0.050	88.000
Helford	B034O	1.000	0.460	0.240	0.060	0.050	88.000
Helford	B034O	2.000	0.300	0.230	0.200	0.030	88.000
Helford	B034O	3.000	0.240	0.230	0.300	0.030	88.000
Helford	B034X	0.000	0.360	0.240	0.130	0.050	64.000
Helford	B034X	1.000	0.260	0.230	0.260	0.030	64.000
Helford	B034X	2.000	0.110	0.240	0.640	0.010	64.000
Helford	B034X	3.000	-0.050	0.250	0.830	0.010	64.000
Helford	B34AC	0.000	0.700	0.300	0.020	0.070	71.000
Helford	B34AC	1.000	0.800	0.290	0.010	0.100	71.000
Helford	B34AC	2.000	0.570	0.290	0.050	0.050	71.000
Helford	B34AC	3.000	0.370	0.290	0.200	0.020	71.000
Helford	B34AD	0.000	0.540	0.290	0.060	0.070	65.000
Helford	B34AD	1.000	0.610	0.270	0.030	0.090	65.000
Helford	B34AD	2.000	0.450	0.260	0.080	0.070	65.000
Helford	B34AD	3.000	0.160	0.260	0.550	0.030	65.000
Helford	B34AF	0.000	1.270	1.460	0.480	0.400	2.000
Helford	B34AF	1.000	1.250	1.310	0.440	0.440	2.000

Helford	B34AF	2.000	1.150	1.310	0.470	0.410	2.000
Helford	B34AF	3.000	1.060	1.320	0.510	0.380	2.000
Menai	B055A	0.000	0.050	0.120	0.700	0.000	110.000
Menai	B055A	1.000	0.000	0.110	0.970	0.000	110.000
Menai	B055A	2.000	0.030	0.100	0.760	0.000	110.000
Menai	B055A	3.000	0.030	0.100	0.800	0.000	110.000
Menai	B055B	0.000	0.250	0.100	0.010	0.100	114.000
Menai	B055B	1.000	0.300	0.090	0.000	0.130	114.000
Menai	B055B	2.000	0.190	0.090	0.030	0.090	114.000
Menai	B055B	3.000	0.110	0.090	0.200	0.060	114.000
Menai	B055I	0.000	0.160	0.120	0.190	0.080	108.000
Menai	B055I	1.000	0.230	0.110	0.040	0.100	108.000
Menai	B055I	2.000	0.150	0.100	0.140	0.090	108.000
Menai	B055I	3.000	0.120	0.100	0.250	0.080	108.000
Menai	B055N	0.000	0.210	0.070	0.000	0.050	332.000
Menai	B055N	1.000	0.230	0.070	0.000	0.060	332.000
Menai	B055N	2.000	0.130	0.060	0.020	0.040	332.000
Menai	B055N	3.000	0.060	0.060	0.290	0.030	332.000
Menai	B055O	0.000	0.220	0.120	0.060	0.050	110.000
Menai	B055O	1.000	0.210	0.110	0.070	0.050	110.000
Menai	B055O	2.000	0.180	0.100	0.080	0.050	110.000
Menai	B055O	3.000	0.190	0.100	0.070	0.050	110.000
Menai	B055R	0.000	0.370	0.260	0.180	0.160	18.000
Menai	B055R	1.000	0.190	0.240	0.450	0.100	18.000
Menai	B055R	2.000	0.110	0.290	0.710	0.080	18.000
Menai	B055R	3.000	0.160	0.250	0.520	0.090	18.000
Menai	**B055S**	**0.000**	**0.590**	**0.190**	**0.010**	**0.380**	**19.000**
Menai	B055S	1.000	0.440	0.180	0.020	0.290	19.000
Menai	B055S	2.000	0.300	0.220	0.190	0.150	19.000
Menai	B055S	3.000	0.160	0.210	0.470	0.100	19.000
Menai	**B055T**	**0.000**	**0.690**	**0.170**	**0.000**	**0.510**	**19.000**
Menai	**B055T**	**1.000**	**0.530**	**0.160**	**0.000**	**0.420**	**19.000**
Menai	B055T	2.000	0.490	0.190	0.020	0.330	19.000
Menai	B055T	3.000	0.320	0.200	0.130	0.210	19.000
Menai	B055U	0.000	0.210	0.100	0.040	0.030	133.000
Menai	B055U	1.000	0.220	0.100	0.020	0.040	133.000
Menai	B055U	2.000	0.150	0.090	0.100	0.020	133.000
Menai	B055U	3.000	0.040	0.090	0.690	0.000	133.000
Menai	B055V	0.000	0.430	0.200	0.050	0.210	20.000
Menai	B055V	1.000	0.320	0.180	0.090	0.170	20.000
Menai	B055V	2.000	0.180	0.210	0.400	0.070	20.000
Menai	B055V	3.000	0.130	0.200	0.540	0.060	20.000

Menai	B055W	0.000	0.590	0.230	0.020	0.260	20.000
Menai	B055W	1.000	0.310	0.220	0.170	0.110	20.000
Menai	B055W	2.000	0.230	0.250	0.370	0.060	20.000
Menai	B055W	3.000	0.210	0.240	0.380	0.060	20.000
NorthKent	B017A	0.000	0.780	0.210	0.000	0.200	73.000
NorthKent	B017A	1.000	0.700	0.200	0.000	0.190	73.000
NorthKent	B017A	2.000	0.700	0.210	0.000	0.180	73.000
NorthKent	B017A	3.000	0.610	0.210	0.010	0.150	73.000
NorthKent	B017D	0.000	0.820	0.190	0.000	0.230	115.000
NorthKent	B017D	1.000	0.700	0.170	0.000	0.210	115.000
NorthKent	B017D	2.000	0.700	0.170	0.000	0.220	115.000
NorthKent	B017D	3.000	0.660	0.170	0.000	0.210	115.000
NorthKent	B017E	0.000	0.820	0.170	0.000	0.300	115.000
NorthKent	B017E	1.000	0.710	0.160	0.000	0.290	115.000
NorthKent	B017E	2.000	0.660	0.160	0.000	0.270	115.000
NorthKent	B017E	3.000	0.590	0.160	0.000	0.250	115.000
NorthKent	B17CB	0.000	0.480	0.310	0.120	0.070	45.000
NorthKent	B17CB	1.000	0.490	0.270	0.080	0.090	45.000
NorthKent	B17CB	2.000	0.410	0.280	0.140	0.070	45.000
NorthKent	B17CB	3.000	0.270	0.270	0.330	0.040	45.000
NorthKent	B17CC	0.000	0.490	0.320	0.130	0.090	43.000
NorthKent	B17CC	1.000	0.460	0.300	0.130	0.090	43.000
NorthKent	B17CC	2.000	0.460	0.290	0.120	0.090	43.000
NorthKent	B17CC	3.000	0.370	0.280	0.190	0.080	43.000
NorthKent	B17CD	0.000	0.330	0.310	0.300	0.290	21.000
NorthKent	B17CD	1.000	0.310	0.280	0.270	0.290	21.000
NorthKent	B17CD	2.000	0.330	0.270	0.230	0.300	21.000
NorthKent	B17CD	3.000	0.330	0.250	0.200	0.310	21.000
Taw	B036F	0.000	0.510	0.110	0.000	0.190	150.000
Taw	B036F	1.000	0.510	0.110	0.000	0.190	150.000
Taw	B036F	2.000	0.410	0.110	0.000	0.150	150.000
Taw	B036F	3.000	0.370	0.110	0.000	0.140	150.000
Taw	B036H	0.000	0.330	0.120	0.010	0.160	123.000
Taw	B036H	1.000	0.280	0.110	0.010	0.150	123.000
Taw	B036H	2.000	0.190	0.120	0.100	0.130	123.000
Taw	B036H	3.000	0.150	0.110	0.200	0.120	123.000
Taw	B036K	0.000	0.190	0.120	0.120	0.180	119.000
Taw	B036K	1.000	0.150	0.110	0.180	0.170	119.000
Taw	B036K	2.000	0.070	0.110	0.570	0.160	119.000
Taw	B036K	3.000	0.060	0.110	0.610	0.160	119.000
Taw	B036N	0.000	0.330	0.100	0.000	0.180	127.000
Taw	B036N	1.000	0.320	0.100	0.000	0.180	127.000

Taw	B036N	2.000	0.310	0.100	0.000	0.180	127.000
Taw	B036N	3.000	0.250	0.100	0.010	0.160	127.000
Taw	B036O	0.000	0.360	0.120	0.000	0.210	126.000
Taw	B036O	1.000	0.350	0.120	0.000	0.210	126.000
Taw	B036O	2.000	0.300	0.120	0.010	0.190	126.000
Taw	B036O	3.000	0.240	0.110	0.040	0.180	126.000
Taw	B036P	0.000	0.430	0.110	0.000	0.250	127.000
Taw	B036P	1.000	0.410	0.110	0.000	0.250	127.000
Taw	B036P	2.000	0.350	0.110	0.000	0.220	127.000
Taw	B036P	3.000	0.270	0.110	0.010	0.200	127.000
Taw	B036Y	0.000	0.170	0.110	0.130	0.020	136.000
Taw	B036Y	1.000	0.160	0.110	0.140	0.020	136.000
Taw	B036Y	2.000	0.060	0.110	0.560	0.000	136.000
Taw	B036Y	3.000	0.020	0.110	0.880	0.000	136.000
Taw	B036Z	0.000	0.230	0.240	0.370	0.130	12.000
Taw	B036Z	1.000	0.210	0.250	0.430	0.110	12.000
Taw	B036Z	2.000	0.090	0.230	0.690	0.080	12.000
Taw	B036Z	3.000	0.140	0.250	0.590	0.090	12.000
Taw	B36AA	0.000	-0.150	0.160	0.350	0.060	35.000
Taw	B36AA	1.000	-0.180	0.150	0.240	0.070	35.000
Taw	B36AA	2.000	-0.200	0.140	0.150	0.090	35.000
Taw	B36AA	3.000	-0.220	0.140	0.140	0.090	35.000
Taw	B36AB	0.000	-0.110	0.210	0.620	0.060	32.000
Taw	B36AB	1.000	-0.220	0.210	0.310	0.090	32.000
Taw	B36AB	2.000	-0.370	0.190	0.050	0.160	32.000
Taw	B36AB	3.000	-0.330	0.190	0.100	0.130	32.000
Taw	B36AC	0.000	-0.070	0.210	0.730	0.030	34.000
Taw	B36AC	1.000	-0.110	0.210	0.620	0.040	34.000
Taw	B36AC	2.000	-0.150	0.200	0.460	0.050	34.000
Taw	B36AC	3.000	-0.140	0.200	0.500	0.040	34.000
Taw	B36AK	0.000	0.210	0.310	0.510	0.180	17.000
Taw	B36AK	1.000	0.000	0.310	0.990	0.160	17.000
Taw	B36AK	2.000	-0.040	0.300	0.900	0.160	17.000
Taw	B36AK	3.000	-0.020	0.300	0.960	0.160	17.000
Wash	B003D	0.000	0.390	0.120	0.000	0.090	99.000
Wash	B003D	1.000	0.390	0.110	0.000	0.110	99.000
Wash	B003D	2.000	0.350	0.110	0.000	0.100	99.000
Wash	B003D	3.000	0.360	0.110	0.000	0.110	99.000
Wash	B003F	0.000	0.680	0.130	0.000	0.230	98.000
Wash	B003F	1.000	0.550	0.120	0.000	0.170	98.000
Wash	B003F	2.000	0.470	0.120	0.000	0.140	98.000
Wash	B003F	3.000	0.430	0.120	0.000	0.120	98.000

Wash	B003M	0.000	0.090	0.110	0.410	0.090	119.000
Wash	B003M	1.000	0.120	0.120	0.340	0.090	119.000
Wash	B003M	2.000	0.160	0.120	0.200	0.090	119.000
Wash	B003M	3.000	0.170	0.120	0.160	0.100	119.000
Wash	B003V	0.000	0.430	0.450	0.360	0.160	11.000
Wash	B003V	1.000	0.290	0.440	0.520	0.120	11.000
Wash	B003V	2.000	0.270	0.410	0.530	0.120	11.000
Wash	B003V	3.000	0.490	0.390	0.230	0.200	11.000
Wash	B004E	0.000	-0.160	0.120	0.180	0.030	77.000
Wash	B004E	1.000	-0.220	0.110	0.060	0.050	77.000
Wash	B004E	2.000	-0.170	0.120	0.160	0.030	77.000
Wash	B004E	3.000	-0.100	0.120	0.410	0.010	77.000
Wash	B004F	0.000	0.450	0.110	0.000	0.150	100.000
Wash	B004F	1.000	0.450	0.110	0.000	0.160	100.000
Wash	B004F	2.000	0.470	0.110	0.000	0.180	100.000
Wash	B004F	3.000	0.500	0.100	0.000	0.200	100.000
Wash	B004I	0.000	0.230	0.140	0.090	0.030	100.000
Wash	B004I	1.000	0.180	0.130	0.170	0.020	100.000
Wash	B004I	2.000	0.210	0.130	0.110	0.030	100.000
Wash	B004I	3.000	0.230	0.130	0.080	0.030	100.000
Wash	B004L	0.000	0.020	0.110	0.860	0.010	131.000
Wash	B004L	1.000	0.050	0.100	0.660	0.010	131.000
Wash	B004L	2.000	0.070	0.110	0.500	0.010	131.000
Wash	B004L	3.000	0.050	0.100	0.610	0.010	131.000

Table 4.4. E. coli counts with CSO discharges

Year	RMP	Geom mean no CSO previous week	Geom mean CSO previous week
2004	B044A	1579.39	2732.39
2005	B044A	2042.28	5979.93
2006	B044A	2277.96	2420.14
2007	B044A	2541.17	3476.03
2008	B044A	2443.5	5400
2009	B044A	672.57	1700
2010	B044A	612.92	
2011	B044A	1705.77	2400
2012	B044A	883.52	9200

2013	B044A	585.77	
2014	B044A	455.87	5400
2004	B044D	938.56	1505.54
2005	B044D	979.39	4563.54
2006	B044D	1035.03	3001.48
2007	B044D	1629.59	4371.48
2008	B044D	1303.47	9200
2009	B044D	703.31	1100
2010	B044D	643.17	
2011	B044D	1190.74	1300
2012	B044D	621.42	9200
2013	B044D	512.24	
2014	B044D	642.56	790
2004	B044E	611.2	6196.77
2005	B044E	1016.11	4812.74
2006	B044E	898.31	2218.58
2007	B044E	2039.85	4016.6
2008	B044E	1106.94	16000
2009	B044E	746.36	330
2010	B044E	590.01	
2011	B044E	1009.26	2400
2012	B044E	616.66	5400
2013	B044E	506.86	
2014	B044E	717.32	18000
2004	B044H	780.74	5047.77
2005	B044H	1013.94	2134.14
2006	B044H	820.65	1315.21
2007	B044H	1670.05	1437.43
2008	B044H	838.67	16000
2009	B044H	644.59	953.94
2010	B044H	835.42	5400
2011	B044H	532.49	1300
2012	B044H	954.4	5400
2013	B044H	646.66	
2014	B044H	582.78	3500
2004	B044I	1424.05	2482.55
2005	B044I	1364.55	3065.24
2006	B044I	1145.19	2089.75
2007	B044I	1165.24	1804.92
2008	B044I	907.51	5400
2009	B044I	936.76	
2010	B044I	748.81	

2011	B044I	729.81	5400
2012	B044I	703.53	16000
2013	B044I	454.93	
2014	B044I	383.47	5400
2004	B044S	572.58	3163.86
2005	B044S	490.85	1094.88
2006	B044S	742.71	1159.78
2007	B044S	987.67	3476.03
2008	B044S	932.28	5400
2009	B044S	841.75	
2010	B044S	562.6	
2011	B044S	686.13	1700
2012	B044S	585.64	1400
2013	B044S	529.06	
2014	B044S	468.86	3500